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Water temperature and dietary histidine affect cataract formation in Atlantic salmon (*Salmo salar* L.) diploid and triploid yearling smolt

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Abstract

The aim of the present study was to investigate cataract development in diploid (2N) and triploid (3N) Atlantic salmon smolts and post-smolts at two water temperatures (10 and 16 °C) given diets with different histidine supplementation (LH, 10.4 and HH, 13.1 g kg^{-1}) before and after seawater transfer. In freshwater, a severe cataract outbreak was recorded in both ploidies reared at 16 °C. The cataract score was significantly higher in triploids compared to diploids, and the severity was lower in both ploidies fed the HH diet. The cataract development at 10 °C was minor. Low gill Na^+, K^+-ATPase activity in fish reared at 16 °C before seawater transfer was followed by osmoregulatory stress with elevated plasma electrolyte concentrations and high mortality in sea water. Both diploids and triploids reared at 10 °C developed cataracts during the seawater period, with higher severities in triploids than diploids and a reduced severity in the fish fed the HH diet. The findings of this study demonstrate the importance of environmental conditions in the husbandry of Atlantic salmon, and particularly triploids, with regard to smoltification and adjusted diets to mitigate cataract development in fresh and sea water.

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Introduction

The use of sterile farmed salmon is currently being tested as a countermeasure to protect the genetics of wild salmon, as it has demonstrated that escaped farmed salmon hybridize with wild conspecifics (Glover *et al.* 2012). Triploidization is the induction of sterility and currently the only alternative to produce sterile salmon with regard to commercial demands (Piferrer *et al.* 2009). However, the adoption of triploid Atlantic salmon in the industry has been restrained due to welfare issues (reviewed by Fraser *et al.* 2012; Benfey 2015) and impeded growth and higher mortality compared to diploids at high water temperatures (Galbreath *et al.* 1994; McGeachy, Benfey & Friars 1995; O'Flynn *et al.* 1997). Triploid salmon differ from diploids by containing fewer but larger cells in most organs that, dependent on cell shape, can result in a reduced cellular surface to volume ratio that represents the basis of physiological differences (Benfey 1999). Differences in gut morphology between diploid and triploid salmon suggest differences in utilization and metabolism between ploidies (Peruzzi, Hagen & Jobling 2015), and also differences in the susceptibility to cataracts (Wall & Richards 1992; Oppedal,

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Taranger & Hansen 2003; Leclercq *et al.* 2011; Taylor *et al.* 2013).

Cataract is defined as opacity of the lens and is common in eye pathology in fish (Hargis 1991). Causes of cataractogenesis in salmonids are multifactorial, but often related to environmental conditions (Bjerkås *et al.* 1996; Bjerkås *et al.* 2003). In particular, salmonids as visual feeders can be significantly impacted by severe cataract formation in terms of reduced feed intake and growth (Breck & Sveier 2001), secondary diseases and also mortality (Hargis 1991). Cataracts are affiliated with ethical and welfare issues in salmon aquaculture, but can also result in economic losses (Menzies *et al.* 2002). In particular, water temperature appears to have a major influence on cataract formation in Atlantic salmon (Bjerkås & Bjørnestad 1999; Bjerkås *et al.* 2001; Waagbø *et al.* 2010) and especially in triploids (Taylor *et al.* 2015). Further, the transition from fresh to sea water during parr-smolt transformation (smoltification) associated with osmoregulatory stress appears to be an important stage for cataract outbreaks (Iwata *et al.* 1987; Bjerkås *et al.* 2003; Breck & Sveier 2001; Breck *et al.* 2005a; Breck *et al.* 2005b; Remø *et al.* 2014). The consequence of osmotic imbalance in the lens is the formation of cloudiness that subsequently reduces vision (Rhodes *et al.* 2010; Tröβe *et al.* 2010). In contrast to cataract that appear earlier in fresh or later during the seawater stage, the osmotic induced cataracts are considered reversible (Hargis 1991). Albeit specific risk periods have been identified, cataract formation in Atlantic salmon has been observed during freshwater (Bjerkås *et al.* 1996) as well as in the first (Breck *et al.* 2005a) and second (Waagbø *et al.* 2010) year at sea, respectively.

Nutritional imbalances attributed to cataract formation in salmon farming have been extensively studied with the conclusion that the amino acid histidine is a key component for the prevention of cataracts in salmon (Breck *et al.* 2003; Bjerkås & Sveier 2004; Bjerkås, Breck & Waagbø 2006; Tröβe *et al.* 2010; Waagbø *et al.* 2010; Remø *et al.* 2014). However, the histidine requirement for growth (8 g kg^{-1}, Scott 1998; NRC 2011) does not meet the requirement for minimizing cataracts in commercial Atlantic salmon diets, above all not in triploid salmon (Taylor *et al.* 2015). The underlying mechanism for cataract mitigation is suggested to be through the histidine metabolite N-acetyl histidine (NAH), which has also been found in the heart and brain; however, in the salmon lens, NAH functions as an osmolyte (Rhodes *et al.* 2010) and may also function as an intracellular antioxidant (Remø *et al.* 2011). Lens NAH concentrations correlate positively with the dietary histidine concentration and negatively with cataract severity (cataract score) in Atlantic salmon (Breck *et al.* 2005a; Tröβe *et al.* 2010; Waagbø *et al.* 2010; Remø *et al.* 2014). The dipeptide anserine is the major histidine compound (imidazole) found in white muscle tissues in salmon and depends on the dietary histidine level (Remø *et al.* 2014). The roles of anserine are related to muscle buffering and burst swimming capacity in sea water, and the muscle concentration increases during smoltification and thereafter (Ogata, Konno & Silverstein 1998; Breck *et al.* 2005a). Nonetheless, more research is needed to develop best practice rearing protocols for farming triploid Atlantic salmon in order to ensure welfare and performance.

The present feeding study examined whether low or high levels of dietary histidine supplementation above the requirement for growth mitigate the effect of cataractogenesis in diploid and triploid Atlantic salmon smolt before and during smoltification at moderate and high temperatures.

Materials and methods

The experiment was conducted at the Institute of Marine Research (IMR), Matre, Norway, and approved by the Norwegian Animal Research Authority, performed according to prevailing animal welfare regulations (FOTS id 5283).

Fish stock and rearing conditions

Diploid and triploid eyed eggs were acquired from AquaGen (AquaGen AS, Postboks 1240, Sluppen, 7462 Trondheim). The eggs were incubated at 6 °C, and peak hatching took place on 11 January 2013. First feeding started on 5 March 2013 at 10.5 °C. On 7 March 2013, the temperature was raised to 13 °C. The fish were reared in heated water until 18 June 2013, when the temperature was changed to ambient (12 °C). The photoperiod was LD24:0 from first feeding to 1 October 2013, when it was changed to simulated natural photoperiod. The fish were vaccinated (Norvax®, MINOVA 6 vet. Intervet International B.V.) on 25 November 2013 and individually tagged

(Trovan transponder ID-100A Microtransponder, Trovan® BTS Scandinavia) on 5 February 2014.

Ploidy verification

Ploidy was determined based on red blood cell diameter (Benfey, Sutterlin & Thompson 1984; Peruzzi *et al.* 2005; Opstad *et al.* 2013). On 17 September 2013, 120 blood smears of each ploidy batch were taken. After air drying, the blood smears were photographed at a resolution of 4.396 pixels μm^{-1} using a Leica DMRE microscope (type 020-525.755) at 40× magnification and a Scion camera (CFW-1312C). Three pictures were taken from each blood smear. From the pictures 81-555 (average 286), blood cells from each fish were automatically size measured using ImageJ (https://rsb.info.nih.gov/ij/) and a slightly modified version of the ObjectJ (https://sils.fnwi.uva.nl/bcb/objectj/) project 'Elliptical oocytes' (https://sils.fnwi.uva.nl/bcb/objectj/examples/oocytes/Oocytes.htm). On average, the diploid fish had a major axis blood cell diameter of 16.2 ± 0.4 (SD), while the corresponding value for the triploid fish was 19.8 ± 0.5. There was no overlap in mean red blood cell size between fish from the diploid and triploid group and therefore the induction of triploidy was considered to be 100% successful.

Experimental design

On 5 March 2014, 938 diploid and 925 triploid PIT-tagged Atlantic salmon (1+) presmolt were randomly distributed into 32 quadratic tanks (455 L) with 16 tanks $ploidy^{-1}$ and 57–60 fish $tank^{-1}$. The fish were reared under simulated natural light conditions from tank stocking to experimental termination and at ambient temperature (5.3 °C) during the acclimation period until the experimental start on 27 March. Constant freshwater flow was supplied at 100% DO. The fish were fed in excess between the hours of 08:00 and 18:00. Previous and during the acclimation period, a commercial diet (Nutra Olympic 3 mm, Skretting AS; 9.0 g histidine kg^{-1}) was provided according to the manufacturers' tables and by automatic feeders (ARVO-TEC T Drum 2000, Arvotec). Feeding and photoperiod were controlled by customized computer software (SD Matre, Normatic AS). The experimental period lasted from 27 March until 24 June and 12 August 2014 for the 16 °C and 10 °C groups, respectively. Body weight, fork length and condition factor at the experimental start were 138.2 g ± 0.9; 22.7 cm ± 0.05; 1.16 ± 0.003 and 153.2 g ± 0.9; 23.6 cm ± 0.05 and 1.15 ± 0.00 (mean ± SEM; $n = 938$; 925) for diploids and triploids, respectively. Thereafter the fish were supplied with one of two experimental diets, differing in the amount of supplemented histidine. A low histidine diet (LH) containing 10.4 g (despite termed 'low histidine diet', the histidine concentration is above standard commercial diets) and a high histidine (HH) diet with 13.1 g histidine kg^{-1} (4.5 mm) formulated and manufactured by Cargill Aqua Nutrition, Florø, Norway. Feed ingredients, feed composition and amino acid profile are shown in Table 1. On 31

Table 1 Ingredients, proximate nutritional composition and amino acid composition of two experimental diets (4.5 mm) varying in the level of histidine: commercial (pre): 9.0, low (LH): 10.4; high (HH): 13.1 g kg^{-1}

	Experimental diet	
	LH	HH
Ingredients (g kg^{-1})		
Fish meal Euro	522	523
Soya protein concentrate	104	105
Fish oil	103	103
Rapeseed oil	100	100
Wheat gluten	42	43
Tapioca	89	89
Pea protein concentrate	11	11
MCP	13	13
Vitamin and mineral premix	3	3
Micro nutrients	10	5
Histidine	0	4
Proximate composition (g kg^{-1})		
Protein	481.0	476.0
Lipid	254.0	264.0
Starch	75.0	77.0
Total phosphorous	14.4	13.6
Amino acid composition (mg g^{-1})		
Hyp	3.3	3.1
His	10.4	13.1
Tau	4.3	4.2
Ser	20.6	20.6
Arg	28.7	28.7
Gly	27.6	26.8
Asp	44.4	44.7
Glu	73.0	73.1
Thr	20.4	19.2
Ala	26.9	26.2
Pro	22.7	22.5
Lys	39.3	36.6
Tyr	14.5	14.5
Met	16.2	12.9
Val	24.8	24.3
Ile	19.9	19.5
Leu	35.6	35.3
Phe	22.1	22.0
Σ amino acids	454.7	447.1

March 2014, the water temperature of all tanks was elevated by 1 °C day^{-1} until eight diploid and eight triploid tanks reached 10 °C (4 April 2014), the remaining 8 diploid and 8 triploid tanks were further elevated until 16 °C (10 April 2014; Fig. 1). Both ploidies (2N, 3N), experimental diets (LH, HH) and temperatures (10, 16 °C) were used in a full crossover design, resulting in eight experimental groups (2N10LH; 2N10HH; 3N10LH; 3N10HH; 2N16LH; 2N16HH; 3N16LH; and 3N16HH) with quadruplicate tank replicates.

On 14 May 2014, the water supply was switched from freshwater to sea water (henceforth referred as seawater transfer) with 35 g L^{-1} salinity and maintained DO (80–100%). The temperature was not changed during seawater transfer. Due to high mortality in the 16 °C groups after seawater transfer, the water temperature was lowered from 16 to 14 °C from 17 May until 5 June 2014 (Fig. 1).

Sampling procedures

Three samplings were conducted in the FW phase (7 weeks, 3 weeks, 2 days prior to change to SW) and four in the SW phase (3 days, 3 weeks, 6 weeks, 13 weeks after change to SW; Fig. 1). The fish were starved approximately 40 h prior to each sampling. As there was no treatment of temperature and diet at the experimental start, only one fish per tank ($n = 32$; 16 fish $ploidy^{-1}$) was sampled, as well as one whole fish for any unforeseen analyses. Length, weight and PIT tag identity were recorded from all fish. Thereafter three fish per tank and sampling point ($n = 12$ treatment $group^{-1}$ $sampling^{-1}$) were sampled and three additional whole fish $tank^{-1}$ were removed after 3 days (12 May) and after 6 weeks (24 June) in SW. The fish were killed by an overdosed bath of anaesthetics (Finquel®) followed by a single blow to the head. Length (to the nearest 1 mm), weight (to the nearest 1 g) and the PIT tag identity were recorded before blood was drawn from the caudal vessel using heparinized needles and syringes. White muscle samples were taken, and subsequently lenses carefully dissected. Gills (7 weeks, 2 days before as well as 3 weeks after change to SW) were dissected and all samples immediately frozen in liquid nitrogen before stored at −80 °C until further analysis.

At the experimental start (27 March) and 2 days before change to SW (12 May) as well as 6 weeks after change to SW (24 June) and at termination (12 August), all fish were measured for length, weight and PIT tag identity. Additionally, 200 diploid and 200 triploid fish were inspected for cataract by using a slit lamp microscope (HEINE® HSL 150 hand-held slit lamp, HEINE Optotechnik) at the experimental start, thereafter of 10 fish $tank^{-1}$ (40 fish $group^{-1}$) for the same dates. Cataracts were graded as cataract score according to its severity on a scale from 0 to 4 for each eye and 0–8 for each fish, respectively (Wall & Bjerkås 1999).

For lens and white muscle analyses, samples were pooled according to the following treatments in the initial sampling (1 sample $tank^{-1}$; 4 pooled

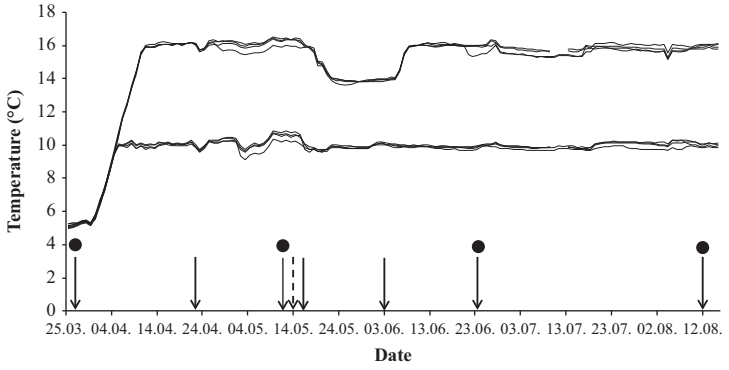

Figure 1 Experimental design to test the effect of water temperature (10 vs. 16 °C) and dietary histidine supplementation (LH: 10.4 vs. HH: 13.1 g kg^{-1}) on the performance of diploid and triploid Atlantic salmon in fresh and sea water. Solid arrows indicate sampling dates, and black dots above arrows indicate sampling dates when all PIT-tagged fish were additionally measured for weight and length. A single dashed arrow indicates the transfer to sea water. The water temperature was temporarily reduced in groups reared at 16 °C due to high mortality after seawater transfer.

samples ploidy^{-1}) and thereafter within each tank (3 samples tank^{-1}; 4 pooled samples replicate^{-1}).

Blood and plasma

Blood was drawn from the caudal vein and each sample centrifuged for 1 min at 11228 g at 6 °C (Eppendorf, 5415R) allowing plasma to separate.

Plasma chloride was determined with AVL 9180 Electrolyte Analyzer (Roche Diagnostics) using an ion-selective electrode. Plasma osmolality was determined by freeze point determination (Fiske micro-osmometer Model 210).

Amino acid and histidine imidazole determination

Amino analyser ninhydrin detection (Amersham Pharmacia Biotech) was used to analyse feed.

Muscle (free basic) and amino acid (total free) concentrations were analysed according to Breck *et al.* (2005a).

Lens histidine and NAH concentrations were analysed by reverse phase HPLC (Waters Corporation) according to a modified method (Breck 2004), originally described by O'Dowd *et al.* (1990).

Gill Na^+, K^+-ATPase activity

Gill Na^+, K^+-ATPase (NKA) activity was determined by the method of McCormick (1993). Briefly, this kinetic assay utilizes the hydrolysis of ATP, which is enzymatically coupled to the conversion of NADH to NAD^+ by pyruvate kinase and lactic dehydrogenase with or without the addition of ouabain, a specific inhibitor of NKA. Readings were done at 340 nm for 10 min at 25 °C and enzyme activity is expressed as μmol ADP * mg protein^{-1} * h^{-1}.

Calculations and statistical analysis

The specific growth rate (% growth day^{-1}) was calculated as $SGR = (e^q - 1)\ 100$ (Houde & Schekter 1981) where $q = [\ln(W_2) - \ln(W_1)]/(t_2 - t_1)$ (Bagenal & Tesch 1978), W_2 and W_1 are body weight at times t_1 and t_2, respectively. Fulton's condition factor was calculated as cf = 100 * weight [g]/fork length^3 [cm].

The data were statistically analysed using Statistica 11 with a significance level of 5% ($P < 0.05$). If not differently stated, the results are presented as mean ± SEM. Parameters were analysed by nested ANOVA designs considering temperature, ploidy and diet as fixed factors and nesting these in tank replicates as random factor. Significant nested ANOVAs were followed by factorial ANOVA designs with temperature, ploidy and diet as independent variables to detect possible interactions between the variables. Significant factorial ANOVAs were followed by Student–Newman–Keul (SNK) post hoc tests to detect possible differences between the individual groups. A one-way ANOVA with ploidy as independent variable was performed with data from the initial sampling point (27 March) when the variables temperature and diet were not present. Data were arcsine-transformed prior to statistical analysis when necessary.

Results

Growth

At the start of the experiment, triploid fish were significantly longer and heavier, but both triploid groups going to be held 16 °C had a lower condition factor than all other groups (SNK, $P < 0.05$). The development in length, weight and the condition factor during the experiment is presented in Table 2.

Whereas length growth was similar between ploidies at 16 °C, in freshwater, diploids had higher weight growth (SGR: 0.89 and 0.94% for LH and HH, respectively) than the remaining groups which had similar SGRs between 0.70% and 0.78% (factorial ANOVA, temperature × ploidy, $P < 0.001$; Table 3). Higher length growth (mm day^{-1}) in triploids at 10 °C resulted in a significant interaction between temperature and ploidy in freshwater (factorial ANOVA, $P < 0.001$). Mean weight and length of groups at 16 °C in sea water (Table 2) are masked due to high mortality (Table 4) and the SGR and growth in mm d^{-1} (Table 3) give therefore a better representation of performance during this period.

During the first 6 weeks in sea water, groups at 10 °C grew (SGR and mm d^{-1}) similarly to each other, but significantly better (SNK, $P < 0.05$) compared to all 16 °C groups (nested ANOVA, $P < 0.001$). Within groups at 16 °C, diploids maintained their weight, whereas triploid had

Table 2 Development of length (L), weight (W) and condition factor (CF) (mean±SEM) in diploid (2N) and triploid (3N) Atlantic salmon at 10 and 16 °C, fed two experimental diets differing in the level of histidine (LH: 10.4; HH: 13.1 g kg^{-1}). The experimental diets replaced a commercial diet on 30.03 and the water temperature was then elevated from ambient (5.3 °C) to either 10 or 16 °C (1 °C day^{-1}). The water inflow was changed from freshwater to sea water on 14.05. Groups at 16 °C were terminated on 24.06, while 10 °C groups were terminated on 12.08. Groups at 16 °C were terminated early on 24.06. Except for 27.03 (one-way ANOVA), an asterisk indicates statistical *P*-values from a nested ANOVA design.

	Sampling											
	27.03			12.05			26.06			12.08		
Group	L	W	CF	L	W	CF	L	W	CF	L	W	CF
2N10LH	22.7 ± 0.1^{a}	139 ± 3^{a}	1.17 ± 0.00^{a}	25.7 ± 1.7^{c}	190 ± 3^{b}	1.11 ± 0.01^{ab}	27.5 ± 1.9^{b}	221 ± 5^{ab}	1.04 ± 0.00^{ab}	32.6 ± 0.3^{c}	372 ± 9^{b}	1.05 ± 0.01^{b}
2N10HH	22.6 ± 0.0^{a}	135 ± 1^{a}	1.16 ± 0.01^{a}	25.6 ± 1.7^{c}	191 ± 2^{b}	1.12 ± 0.00^{ab}	27.4 ± 2.0^{b}	225 ± 3^{ab}	1.07 ± 0.01^{a}	32.4 ± 0.2^{c}	384 ± 11^{b}	1.10 ± 0.01^{a}
3N10LH	23.5 ± 0.0^{b}	153 ± 2^{b}	1.16 ± 0.01^{ab}	26.7 ± 1.8^{b}	213 ± 2^{a}	1.10 ± 0.00^{b}	29.0 ± 2.1^{a}	247 ± 3^{a}	0.99 ± 0.01^{b}	34.5 ± 0.3^{a}	422 ± 13^{a}	0.99 ± 0.01^{d}
3N10HH	23.5 ± 0.1^{b}	152 ± 2^{b}	1.16 ± 0.01^{ab}	26.7 ± 1.8^{b}	218 ± 4^{a}	1.13 ± 0.01^{a}	28.6 ± 2.1^{a}	246 ± 6^{a}	1.02 ± 0.01^{ab}	33.6 ± 0.2^{b}	402 ± 9^{ab}	1.02 ± 0.01^{c}
2N16LH	22.8 ± 0.1^{a}	139 ± 3^{a}	1.17 ± 0.01^{a}	26.5 ± 1.8^{b}	211 ± 6^{a}	1.10 ± 0.01^{b}	27.2 ± 1.9^{b}	206 ± 11^{b}	1.00 ± 0.02^{b}	–	–	–
2N16HH	22.8 ± 0.1^{a}	140 ± 2^{a}	1.16 ± 0.01^{a}	26.5 ± 1.8^{b}	216 ± 3^{a}	1.13 ± 0.01^{a}	27.1 ± 2.0^{b}	206 ± 11^{b}	1.00 ± 0.02^{b}	–	–	–
3N16LH	23.6 ± 0.1^{b}	153 ± 2^{b}	1.15 ± 0.01^{b}	27.2 ± 2.1^{a}	217 ± 4^{a}	1.04 ± 0.01^{c}	28.7 ± 1.9^{a}	220 ± 9^{ab}	0.91 ± 0.01^{c}	–	–	–
3N16HH	23.7 ± 0.1^{b}	156 ± 3^{b}	1.15 ± 0.00^{b}	27.3 ± 1.9^{a}	220 ± 8^{a}	1.06 ± 0.01^{c}	28.4 ± 1.4^{a}	212 ± 19^{ab}	0.90 ± 0.01^{c}	–	–	–
Effect	*P*-value											
Temperature*	–	–	–	**>0.001**	**>0.001**	**>0.001**	0.260	**>0.001**	**>0.001**	–	–	–
Ploidy*	**>0.001**	**>0.001**	**>0.001**	**>0.001**	**>0.001**	**>0.001**	**>0.001**	**0.015**	**>0.001**	**>0.001**	**0.008**	**>0.001**
Diet*	–	–	–	0.735	0.374	**0.007**	0.373	0.826	0.274	0.078	0.711	**0.006**
T × P	–	–	–	**0.044**	**>0.001**	**>0.001**	0.815	0.222	**0.004**	–	–	–
T × D	–	–	–	0.617	0.978	0.498	0.878	0.622	**0.027**	–	–	–
P × D	–	–	–	0.739	0.777	0.951	0.587	0.557	0.739	0.139	0.075	0.076
T × P × D	–	–	–	0.982	0.649	0.164	0.856	0.915	0.419	–	–	–

Superscripts denote significant differences (SNK, $P < 0.05$) between the groups for each parameter at each period.
P-values in bold indicate a significant effect of the respective variable or interaction.

Table 3 Specific growth rate and length growth (mean ± SEM) of diploid (2N) and triploid (3N) Atlantic salmon reared at 10 and 16 °C and fed two experimental diets differing in the level of histidine (LH: 10.4; HH: 13.1 g kg^{-1}). The experimental diets replaced a commercial diet on 30.03 and the water temperature was then elevated from ambient (5.3 °C) to either 10 or 16 °C (1 °C day^{-1}). The water inflow was changed from freshwater to sea water on 14.05. Groups at 16 °C were terminated on 24.06, while 10 °C groups were terminated on 12.08. Asterisk indicates statistical *P*-values from a nested ANOVA design.

	23.03–12.05		12.05–24.06		24.06–12.08	
Group	SGR	mm d^{-1}	SGR	mm d^{-1}	SGR	mm d^{-1}
2N10LH	0.70 ± 0.04^{b}	0.65 ± 0.02^{d}	0.38 ± 0.03^{a}	0.48 ± 0.03^{a}	1.09 ± 0.04^{ab}	1.05 ± 0.06^{b}
2N10HH	0.77 ± 0.03^{b}	0.67 ± 0.02^{cd}	0.41 ± 0.05^{a}	0.48 ± 0.02^{a}	1.13 ± 0.04^{a}	1.05 ± 0.05^{b}
3N10LH	0.74 ± 0.02^{b}	0.71 ± 0.01^{b}	0.33 ± 0.04^{a}	0.54 ± 0.01^{a}	1.11 ± 0.04^{ab}	1.15 ± 0.04^{a}
3N10HH	0.78 ± 0.02^{b}	0.70 ± 0.01^{bc}	0.31 ± 0.03^{a}	0.51 ± 0.03^{a}	1.03 ± 0.03^{b}	1.07 ± 0.01^{b}
2N16LH	0.89 ± 0.04^{a}	0.82 ± 0.02^{a}	0.03 ± 0.09^{b}	0.26 ± 0.05^{b}	–	–
2N16HH	0.94 ± 0.03^{a}	0.81 ± 0.01^{a}	-0.04 ± 0.06^{bc}	0.22 ± 0.04^{b}	–	–
3N16LH	0.76 ± 0.03^{b}	0.80 ± 0.02^{a}	-0.18 ± 0.08^{cd}	0.25 ± 0.06^{b}	–	–
3N16HH	0.76 ± 0.05^{b}	0.80 ± 0.04^{a}	-0.18 ± 0.03^{d}	0.17 ± 0.02^{b}	–	–
Effect	*P*-value					
Temperature*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	–	–
Ploidy*	**<0.001**	0.388	**<0.001**	0.899	0.104	0.277
Diet*	**0.006**	0.786	0.133	0.382	0.518	0.363
T × P	**<0.001**	**<0.001**	0.123	**0.045**	–	–
T × D	0.240	0.542	0.086	0.263	–	–
P × D	0.216	0.369	0.604	0.332	**0.014**	0.090
T × P × D	0.940	0.307	0.859	1.000	–	–

Superscripts denote significant differences (SNK, $P < 0.05$) between the groups at each period.
P-values in bold indicate a significant effect of the respective variable or interaction.

negative growth rates (Table 3). At both temperatures, specific growth rates in diploids were higher than in triploid groups. The condition factor decreased in all groups, but less in diploids than triploids, less at 10 °C than 16 °C and less in fish fed the high histidine diet (nested ANOVA, $P < 0.05$; Table 2). From week seven in sea water until termination (24 June to 12 August), all groups at 10 °C had stable SGRs (>1% d^{-1}). Among groups fed the high histidine diet, diploids had a significantly higher SGR compared to triploids, whereas the respective triploid group grew faster in length (Table 3) resulting in a lower condition factor (SNK, $P < 0.05$; Table 2). The condition factor at termination was significantly higher in diploids than triploids and also higher in both ploidies fed the high histidine diet (nested ANOVA, $P < 0.05$; SNK, $P < 0.05$).

Mortality

There was no mortality (Table 4) in the period between tank stocking and 2 days after seawater transfer (15 May). On 16 May, after 3 days in sea water, groups at 16 °C experienced significant mortality and triploids more than diploids. No mortality was recorded in any ploidy at 10 °C (factorial ANOVA, temperature × ploidy, $P < 0.001$). Mortality continued in the 16 °C treatments between the third and fifth week in sea water, however without an effect of ploidy (Table 4).

Smoltification and osmoregulation

Gill NKA enzyme activity was recorded 3 weeks (23 April) and 2 days (12 May) before, as well as 3 weeks after (3 June) seawater transfer. On 23 April, fish at 10 °C had generally higher gill NKA activity than fish at 16 °C (nested ANOVA, $P < 0.05$; Fig. 2a+b). Shortly before seawater transfer, the 10 °C groups expressed nearly threefold the amount of gill activity compared to their respective groups at 16 °C (nested ANOVA, $P < 0.0001$) and still twofold the amount after 3 weeks in SW (nested ANOVA, $P < 0.0001$; Fig. 2a+b).

Initial levels of plasma osmolality (mOsm; Fig. 2c+d) and chloride (Cl^{-}, Fig. 2e+f) were similar between ploidies and developed in a comparable pattern throughout the experiment. Two days before seawater transfer, the 16 °C groups had higher levels of plasma mOsm (nested ANOVA, $P = 0.022$). After 3 days in sea water, osmolality and chloride concentrations in fish at 16 °C peaked high compared to fish of the

Table 4 Mortality (%±SE) development between sampling points of diploid (2N) and triploid (3N) Atlantic salmon at 10 and 16 °C, fed two experimental diets differing in the level of histidine (LH: 10.4; HH: 13.1 g kg^{-1}). The experimental diets replaced a commercial diet on 30.03 and the water temperature was then elevated from ambient (5.3 °C) to either 10 or 16 °C (1 °C day^{-1}). The water inflow was changed from freshwater to sea water on 14.05. Groups at 16 °C were terminated early on 24.06. Unlike lower case, superscripts denote significant differences (SNK, $P < 0.05$) between the groups at each sampling point; bold *P*-values for temperature (T), ploidy (P), diet (D) and interactions indicate significant effects (factorial ANOVA, $P < 0.05$)

	Group							*P*-value					
Period	2N10LH	2N10HH	3N10LH	3N10HH	2N16LH	2N16HH	3N16LH	3N16HH	T	P	D	T × P	T × D	P × D	T × P × D
27.03–22.04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	–	–	–	–
23.04–11.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	–	–	–	–
12.05–15.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	–	–	–	–
16.05–02.06	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	17.6 ± 6.0^{c}	15.4 ± 6.2^{c}	49.2 ± 9.3^{b}	69.0 ± 6.0^{a}	**<0.001**	**<0.001**	0.449	**<0.001**	0.449	0.152	0.152
03.06–23.06	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	13.1 ± 4.4^{a}	17.9 ± 2.8^{a}	16.1 ± 5.0^{a}	14.9 ± 3.4^{a}	**<0.001**	0.971	0.584	0.973	0.584	0.416	0.416
24.06–12.08	0.0	0.0	0.7 ± 0.7	2.2 ± 2.2	–	–	–	–	–	0.199	0.700	–	–	0.700	–

Superscripts denote significant differences (SNK, $P < 0.05$) between the groups at each period.
P-values in bold indicate a significant effect of the respective variable or interaction.

respective 10 °C groups (nested ANOVA, $P < 0.0001$) and higher osmolality levels in triploids at 16 °C caused a significant interaction between temperature and ploidy (factorial ANOVA, $P = 0.035$).

A significant effect of temperature remained on both parameters after 6 weeks in sea water (nested ANOVA, $P < 0.05$). There were no differences recorded between ploidy and diet within the 10 °C groups after thirteen weeks in sea water.

From winter solstice (21 December) until the day before the temperature was gradually increased (26 March), the mean water temperature was 5.5 ± 0.5 °C (min 4.3 °C; max 6.4 °C; degree-days 523) and until seawater transfer (14 May), the 16 °C groups were reared at 220 degree-days more than the 10 °C groups (986 vs 1206 degree-days).

Cataract development

The observation of cataracts at the experimental start, with previously fed commercial diets and under ambient water temperature, was low and low graded, but differed statistically between ploidies (mean score 2N: 0.04 ± 0.02; 3N: 0.16 ± 0.05; $n = 200$ $ploidy^{-1}$; Fig. 3a, +b). The prevalence was 3% in the diploid and 11.5% in the triploid population and the mean score of affected individuals was 1.17 and 1.52 in diploids and triploids, respectively. In most affected fish in both ploidies, the location of cataracts was at the anterior cortex.

At the end of the freshwater period, groups at 16 °C developed significantly higher cataract scores (from 1.5 ± 0.3 (2N16LH) to 4.3 ± 0.1 (3N16LH)) compared to all groups at 10 °C (nested ANOVA, $P < 0.001$). Within groups at 16 °C, cataracts developed systematically more in triploids (mean score > 3) compared to diploids (mean score < 3) and also significantly more in groups fed the low histidine diet (factorial ANOVA, temperature × ploidy, temperature × diet, $P < 0.001$; SNK, $P < 0.05$). Groups at 10 °C developed minor cataracts and only 3N10LH (0.8 ± 0.1) had a significantly higher (SNK, $P < 0.05$) cataract score than 2N10HH (0.3 ± 0.1). The lenses of fish fed the low histidine diet at 16 °C predominantly revealed jelly-like or cloudy structures in the anterior cortex, which were not observed among fish reared at 10 °C.

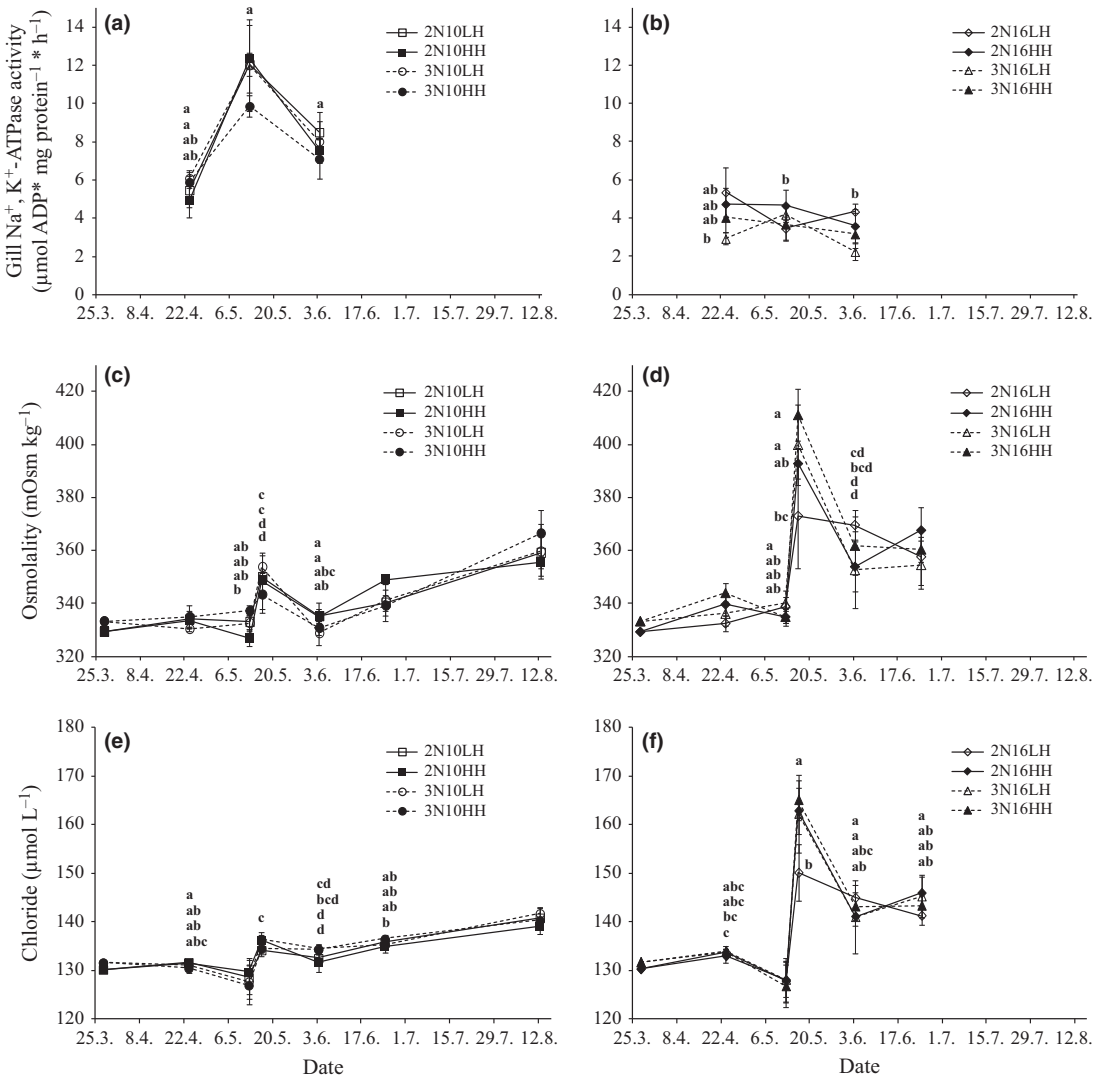

Figure 2 Development of gill Na^+, K^+-ATPase activity (a, b), plasma osmolality (c, d) and plasma chloride levels (e, f) (mean ± SEM) in diploid (2N) and triploid (3N) Atlantic salmon smolts and post-smolts (seawater change 14.05.) at 10 and 16 °C, fed two diets differing in the level of histidine (LH: 10.4 g kg^{-1}; HH: 13.1 g kg^{-1}). The experimental diets were replaced by a commercial diet on 30.03 and the water temperature was then elevated from ambient (5.3 °C) to 10 and 16 °C (1 °C day^{-1}), respectively. Different superscripts indicate significant differences (SNK, $P < 0.05$) between the groups, but within each parameter (a, b; c, d; e, f), at each sampling point.

During the first 6 weeks in sea water, diploids and triploids that fed the low histidine diet at 16 °C developed cataracts further (mean score > 5), whereas the conspecific groups that fed the high histidine diet halted cataract formation (2N) or only slightly increased (3N) (factorial ANOVA, ploidy × diet, $P < 0.05$). Among groups at 10 °C, cataracts increased significantly more in 3N10LH compared to the remaining groups. Triploids fed the HH diet and diploid fed the LH diet developed similar cataract severity, and only the cataract score in diploids fed the high histidine diet did not increase (factorial ANOVA, temperature × diet, $P < 0.05$). The anterior cortex was mostly affected in fish at 10 °C, whereas jelly and cloudy structures prevailed in fish at 16 °C, often in form of ring or star appearances. At termination, cataracts were systematically higher in triploids than diploids (nested ANOVA, $P < 0.001$) and groups fed the

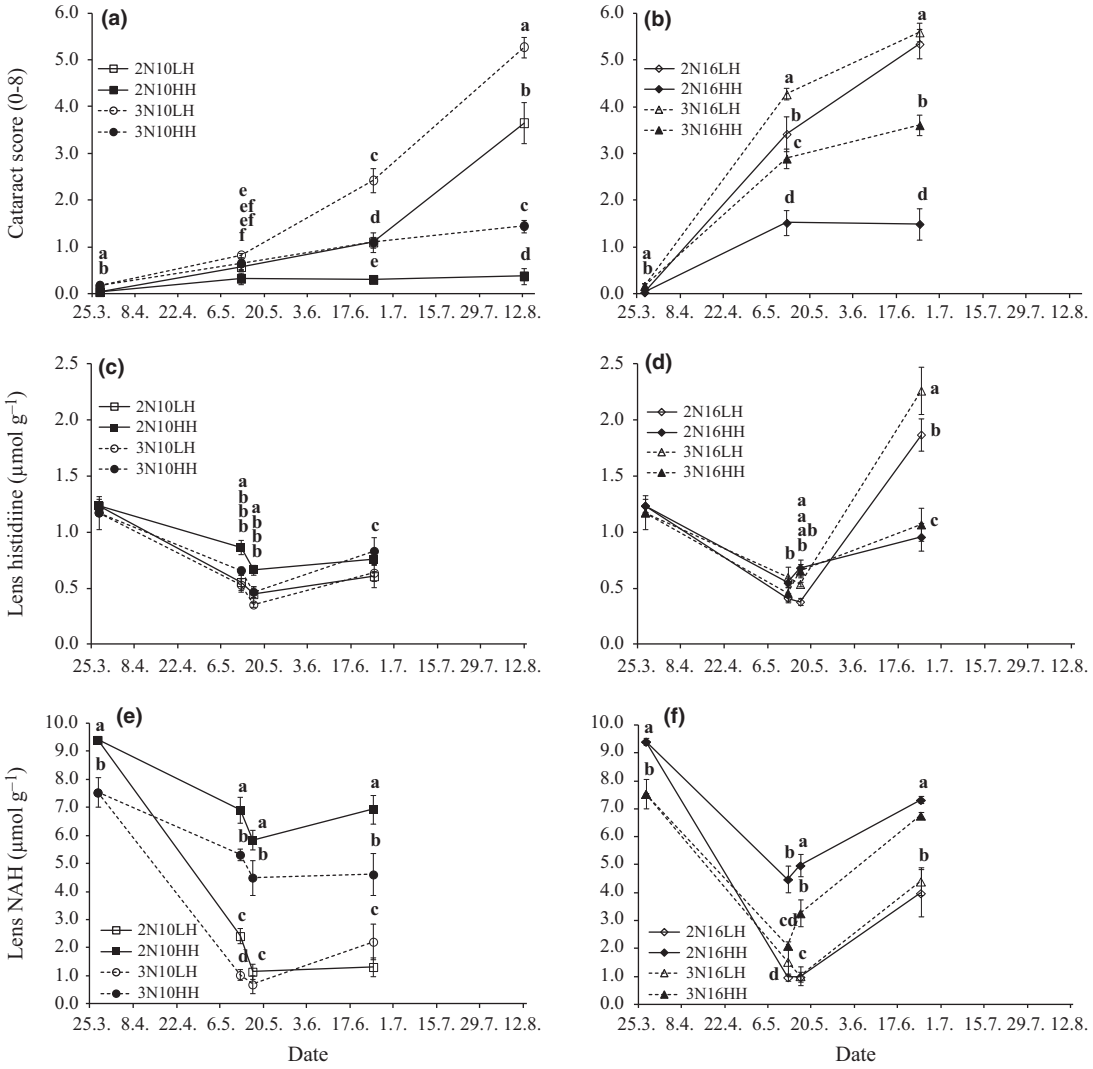

Figure 3 Development of cataract (a, b) (sum score 0-8; mean ± SEM), lens histidine (c, d) and lens N-acetyl-histidine (e, f) (mean ± SEM; $n = 4$ pooled samples) concentrations in diploid (2N) and triploid (3N) Atlantic salmon smolts and post-smolts (seawater change 14.05) at 10 and 16 °C, fed two diets differing in the level of histidine (LH: 10.4 g kg^{-1}; HH: 13.1 g kg^{-1}). On 30.03, the experimental diets replaced a commercial diet and the water temperature was then elevated from ambient (5.3 °C) to 10 and 16 °C (1 °C day^{-1}), respectively. Different superscripts indicate significant differences (SNK, $P < 0.05$) between the groups, but within each parameter (a, b; c, d; e, f), at each sampling point.

high histidine diet had lower cataract scores compared to groups fed the low histidine diet (nested ANOVA, $P < 0.001$). Distinct jelly structures, often star shaped, were found in both ploidies fed the low histidine diet, whereas groups fed the respective high histidine diet were moderately affected in the anterior or showed cataract formation with singular or multiple spots. In the last 7 weeks of the experiment, both ploidies fed the low histidine diet significantly increased in cataract score, whereas the groups fed the high histidine diet halted cataract formation (nested ANOVA, ploidy, diet, $P < 0.001$). Thus, diploid salmon fed the high histidine diet and reared at low temperature showed minor cataract development throughout the experiment.

Lens histidine and NAH

Lens histidine levels were similar between ploidies at the experimental start (2N: 1.24 ± 0.06 vs. 3N: 1.17 ± 0.15 µmol g^{-1}; Fig. 3c+d). At the

end of the freshwater period, all groups significantly decreased lens histidine concentrations, although groups at 10 °C and fed the high histidine diet less than groups at 16 °C and fed the low histidine diet (factorial ANOVA, temperature × diet, $P = 0.015$; ploidy × diet, $P = 0.012$). Shortly after seawater transfer, a continues decrease in lens histidine was recorded in the 10 °C groups, whereas fish at 16 °C stagnated when fed the low histidine diet and increased when fed the high histidine diet. Within both temperatures, fish fed the high histidine diet had higher concentrations of lens histidine compared to fish fed the respective low histidine diet (nested ANOVA, $P < 0.05$). After 6 weeks in sea water, lens histidine concentrations were similar between ploidies of the same diet at 10 °C (HH > LH), whereas diploids and triploids at 16 °C that were fed the low histidine diet contained more than twice as much lens histidine compared to the high histidine diet groups (SNK, $P < 0.05$).

Diploid lenses contained significantly more NAH at the first sampling (one-way ANOVA, $P < 0.05$; 2N: 9.41 ± 0.13 vs. 3N: 7.54 ± 0.52 μmol g^{-1}; Fig. 3e+f). Until the end of the freshwater period, concentrations significantly decreased in all groups, but less in groups fed the high histidine diet (nested ANOVA, $P < 0.05$). The effect of diet on lens NAH levels remained after 2 days in sea water and concentrations decreased slightly in fish at 10 °C, whereas the 16 °C groups moderately increased when fed the high histidine diet. After 6 weeks in sea water, lens NAH concentrations were similar between ploidies of the same diet at 16 °C and at groups fed the low histidine diet at 10 °C. Among groups fed the high histidine diet at 10 °C, diploid lenses contained significantly higher levels of NAH than triploids (nested ANOVA, diet, $P < 0.05$; factorial ANOVA, ploidy × diet, $P < 0.05$).

White muscle amino acid concentration

In March, the level of white muscle total free amino acids was higher in diploids than triploids. Triploids had significantly higher concentrations of anserine, whereas diploids were significantly elevated in the non-essential amino acids serine and glycine (Table 5).

The sum of white muscle free amino acids decreased by nearly 25% shortly before seawater transfer in all experimental groups due to significant reductions of essential (methionine and histidine) and non-essential amino acids (serine, proline, glycine, alanine) and nitrogenous compounds (β-alanine, urea and anserine, in groups fed the low histidine diet).

Before seawater transfer, both ploidies fed the high histidine diet had significantly higher levels of anserine and carnosine compared to groups fed the low histidine diet (factorial ANOVA, $P < 0.001$), which counterbalanced the sum of amino acids with an increase in non-essential amino acids (serine, glycine, alanine, hydroxyproline) and nitrogenous compounds (urea, L-α-amino-N-butyric acid).

The diet allocation reflected overall the concentration of histidine in the white muscle during freshwater. Diploid salmon contained more histidine than triploids at the experimental start; however, the difference was not statistically different (one-way ANOVA, $P = 0.053$). Prior to seawater transfer, all groups decreased in white muscle histidine, particularly groups fed the low histidine diet at 10 °C (factorial ANOVA, $P < 0.05$). The fastest growing group during freshwater diploids fed the high histidine diet at 16 °C decreased significantly less than the remaining groups and was able to maintain most of the initial tissue histidine content (Table 5).

Discussion

The present study demonstrated that triploid Atlantic salmon yearlings developed more severe cataracts at high temperatures and with insufficient dietary histidine from 7 weeks prior to thirteen weeks after seawater transfer. However, the diet with a higher histidine level successfully mitigated cataract outbreaks in both ploidies when reared at 10 °C. Additionally, the study has shown that high temperature had a negative effect on smoltification, survival and seawater performance in diploid, and more so in triploid salmon.

Growth and smoltification

In freshwater, both diploid groups reared at 16 °C grew faster than the remaining groups which grew similarly. This is in accordance with higher feed intake in diploids compared to triploid Atlantic salmon at high (19 °C) water temperatures in sea water (Hansen *et al.* 2015). The

Table 5 Free amino acid concentration in white muscle tissue (pooled mean ± SEM; $n = 4$ samples) in diploid and triploid Atlantic salmon at the experimental start (27.03) at ambient water temperature (5.3 °C) and at the end of the freshwater period (12.05) in 10 °C and 16 °C, fed diets with low (LH: 10.4 g kg^{-1}) or high histidine (HH: 13.4 g kg^{-1}) content

	Diploid					Triploid				
		12.05					12.05			
		10 °C		16 °C			10 °C		16 °C	
Amino acid (μmol g^{-1})	27.03	LH	HH	LH	HH	27.03	LH	HH	LH	HH
Essential										
Threonine	0.42 ± 0.04	0.37 ± 0.03^{a}	0.25 ± 0.02^{b*}	0.35 ± 0.02^{a}	0.22 ± 0.02^{b*}	0.41 ± 0.02	0.41 ± 0.02^{a}	0.26 ± 0.03^{b*}	0.38 ± 0.02^{a}	0.26 ± 0.01^{b*}
Valine	0.21 ± 0.01	0.19 ± 0.01^{b}	0.18 ± 0.02^{b}	0.27 ± 0.02^{a*}	0.22 ± 0.01^{ab}	0.20 ± 0.00	0.23 ± 0.01^{ab}	0.22 ± 0.02^{ab}	0.25 ± 0.00^{a}	0.24 ± 0.01^{ab}
Methionine	0.06 ± 0.00	0.03 ± 0.00^{d*}	0.04 ± 0.00^{bc*}	0.05 ± 0.00^{bc*}	0.05 ± 0.00^{abc*}	0.06 ± 0.00	0.04 ± 0.00^{c*}	0.05 ± 0.00^{bc}	0.05 ± 0.00^{ab}	0.06 ± 0.00^{a}
Isoleucine	0.08 ± 0.01	0.08 ± 0.00	0.07 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.00	0.09 ± 0.01	0.10 ± 0.00	0.09 ± 0.01
Leucine	0.20 ± 0.01	0.15 ± 0.00*	0.14 ± 0.00*	0.18 ± 0.00	0.16 ± 0.00*	0.19 ± 0.01	0.18 ± 0.01	0.17 ± 0.02	0.18 ± 0.00	0.16 ± 0.00
Phenylalanine	0.03 ± 0.00	0.06 ± 0.00^{b*}	0.07 ± 0.00^{b*}	0.08 ± 0.00^{a*}	0.07 ± 0.00^{ab*}	0.04 ± 0.00	0.07 ± 0.00^{ab*}	0.07 ± 0.00^{ab*}	0.08 ± 0.00^{a*}	0.08 ± 0.00^{ab*}
Lysine	0.14 ± 0.03	0.32 ± 0.02^{b*}	0.15 ± 0.01^{d}	0.49 ± 0.05^{a*}	0.28 ± 0.02^{bc*}	0.12 ± 0.03	0.30 ± 0.04^{b*}	0.18 ± 0.03 ^{cd}	0.35 ± 0.01^{b*}	0.23 ± 0.02^{bc*}
Histidine	0.75 ± 0.11	0.04 ± 0.00^{c*}	0.34 ± 0.12^{b*}	0.09 ± 0.02^{bc*}	0.61 ± 0.11^{a}	0.61 ± 0.06	0.05 ± 0.01^{c*}	0.25 ± 0.07^{bc*}	0.09 ± 0.01^{bc*}	0.31 ± 0.03^{bc*}
Arginine	0.04 ± 0.00	0.11 ± 0.01^{ab*}	0.06 ± 0.00^{c}	0.13 ± 0.02^{a*}	0.09 ± 0.00^{bc*}	0.04 ± 0.00	0.09 ± 0.01^{bc*}	0.07 ± 0.01^{bc*}	0.09 ± 0.00^{bc*}	0.06 ± 0.01^{c}
Tryptophan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-essential										
Serine	0.74 ± 0.04^{A}	0.35 ± 0.00^{a*}	0.22 ± 0.03^{b*}	0.19 ± 0.01^{b*}	0.09 ± 0.02^{c*}	0.55 ± 0.01^{B}	0.35 ± 0.01^{a*}	0.20 ± 0.01^{b*}	0.21 ± 0.02^{b*}	0.11 ± 0.01^{c*}
Glutamic acid	0.41 ± 0.01	0.42 ± 0.01^{c}	0.40 ± 0.02^{c}	0.61 ± 0.06^{ab*}	0.55 ± 0.04^{abc*}	0.47 ± 0.04	0.53 ± 0.03^{abc}	0.47 ± 0.01^{bc}	0.65 ± 0.03^{a*}	0.54 ± 0.06^{abc}
Glutamine	0.30 ± 0.01	0.62 ± 0.03^{d*}	0.65 ± 0.04 ^{cd*}	1.09 ± 0.09^{a*}	0.87 ± 0.05^{abc*}	0.30 ± 0.01	0.70 ± 0.02^{bcd*}	0.70 ± 0.04^{bcd*}	1.03 ± 0.10^{a*}	0.90 ± 0.05^{ab*}
Proline	0.71 ± 0.16	0.07 ± 0.01*	0.06 ± 0.00*	0.10 ± 0.01*	0.07 ± 0.01*	0.56 ± 0.13	0.09 ± 0.02*	0.07 ± 0.01*	0.08 ± 0.01*	0.06 ± 0.00*
Glycine	9.42 ± 0.25^{A}	3.82 ± 0.27^{a*}	3.16 ± 0.02^{c*}	2.01 ± 0.07^{d*}	1.39 ± 0.03^{e*}	7.69 ± 0.49^{B}	3.67 ± 0.04^{ab*}	3.34 ± 0.20^{bc*}	1.95 ± 0.10^{d*}	1.45 ± 0.05^{e*}
Alanine	4.56 ± 0.39	2.71 ± 0.18^{a*}	1.98 ± 0.04^{bc*}	2.21 ± 0.16^{b*}	1.74 ± 0.04^{cd*}	4.92 ± 0.10	2.80 ± 0.03^{a*}	2.30 ± 0.11^{b*}	1.97 ± 0.08^{bc*}	1.57 ± 0.03^{d*}
Tyrosine	0.06 ± 0.02	0.11 ± 0.02	0.11 ± 0.01	0.11 ± 0.02	0.11 ± 0.02	0.07 ± 0.01	0.12 ± 0.02	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.02
Aspartic acid	0.10 ± 0.01	0.16 ± 0.01*	0.14 ± 0.01*	0.16 ± 0.01*	0.15 ± 0.01*	0.12 ± 0.00	0.17 ± 0.01*	0.17 ± 0.01*	0.15 ± 0.02	0.15 ± 0.01
Hydroxyproline	0.77 ± 0.06	0.95 ± 0.10^{a}	0.64 ± 0.06^{bcd}	0.70 ± 0.08^{abc}	0.62 ± 0.04^{cd}	0.75 ± 0.07	0.90 ± 0.07^{ab}	0.82 ± 0.03^{abc}	0.59 ± 0.07^{cd}	0.41 ± 0.07^{d*}
Nitrogenous compounds										
β-Alanine	0.95 ± 0.07	0.59 ± 0.05^{a*}	0.22 ± 0.03^{bc*}	0.32 ± 0.05^{b*}	0.08 ± 0.00^{d*}	0.80 ± 0.08	0.69 ± 0.05^{a}	0.32 ± 0.07^{b*}	0.27 ± 0.02^{b*}	0.08 ± 0.01^{cd*}
1-Methyl-histidine	0.01 ± 0.00	0.01 ± 0.00*	0.01 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.01 ± 0.00	0.01 ± 0.00*	0.01 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*
Carnosine	0.20 ± 0.03	0.06 ± 0.01^{b}	0.30 ± 0.09^{ab}	0.17 ± 0.07^{ab}	0.45 ± 0.14^{a}	0.19 ± 0.05	0.08 ± 0.02^{b*}	0.23 ± 0.02^{ab}	0.08 ± 0.00^{b*}	0.24 ± 0.04^{ab}
Anserine	16.9 ± 0.0^{B}	14.7 ± 0.3^{b*}	18.5 ± 0.4^{a*}	15.1 ± 0.2^{b*}	18.0 ± 0.1^{a*}	17.4 ± 0.1^{A}	15.0 ± 0.7^{b*}	17.8 ± 0.5^{a}	14.8 ± 0.4^{b*}	18.1 ± 0.2^{a}
Taurine	0.59 ± 0.01	0.63 ± 0.02^{b}	0.65 ± 0.03^{b}	0.69 ± 0.04^{ab}	0.78 ± 0.05^{a*}	0.73 ± 0.08	0.65 ± 0.05^{b}	0.63 ± 0.01^{b}	0.64 ± 0.03^{ab}	0.63 ± 0.02^{ab}
O-phospho-ethanolamine	0.05 ± 0.00	0.03 ± 0.00^{b*}	0.03 ± 0.00^{ab*}	0.02 ± 0.00^{c*}	0.02 ± 0.00^{c*}	0.06 ± 0.00	0.03 ± 0.00^{a*}	0.03 ± 0.00^{ab*}	0.02 ± 0.00^{c*}	0.02 ± 0.00^{c*}
Urea	1.85 ± 0.34	1.42 ± 0.12^{a}	0.85 ± 0.04^{b*}	1.11 ± 0.09^{b*}	0.61 ± 0.05^{c*}	1.43 ± 0.10	1.49 ± 0.05^{a}	0.92 ± 0.04^{b*}	1.34 ± 0.06^{a}	1.05 ± 0.06^{b*}
L-α-amino-N-butyric acid	0.10 ± 0.00	0.18 ± 0.01^{a*}	0.09 ± 0.00 ^{cd}	0.14 ± 0.01^{b*}	0.08 ± 0.00^{d}	0.09 ± 0.01	0.19 ± 0.01^{a*}	0.10 ± 0.00^{cd}	0.12 ± 0.01^{bc*}	0.07 ± 0.01^{d}
Ammonia	4.23 ± 0.04	4.95 ± 0.08 ^{cd*}	4.79 ± 0.03^{d*}	5.35 ± 0.11^{ab*}	5.29 ± 0.07^{ab*}	4.16 ± 0.06	5.11 ± 0.04^{bc*}	4.90 ± 0.07^{cd*}	5.44 ± 0.08^{a*}	5.17 ± 0.02^{abc*}
Ornithine	0.01 ± 0.00	0.02 ± 0.00^{ab*}	0.01 ± 0.00^{c}	0.02 ± 0.00^{a*}	0.02 ± 0.00^{abc*}	0.01 ± 0.00	0.01 ± 0.00^{cd*}	0.01 ± 0.00^{bcd*}	0.01 ± 0.00^{cd*}	0.01 ± 0.00^{d}
ΣFree amino acids	43.9 ± 0.1	33.2 ± 0.0	34.2 ± 0.0	31.9 ± 0.0	32.8 ± 0.0	42.0 ± 0.1	34.1 ± 0.0	34.5 ± 0.0	31.0 ± 0.0	32.2 ± 0.0

Unlike upper case, superscripts denote significant differences between ploidy on 27.03 (t-test, $P < 0.05$); unlike lower case, superscripts denote significant differences between all experimental groups on 12.05 (SNK, $P < 0.05$); asterisks denote significant differences within ploidy between 27.03 and 12.05 (one-way ANOVA, $P < 0.05$); n.d., not detectable.

fact that diploids had higher SGRs and mm day^{-1} at 16 °C compared to 10 °C in the present study, while triploids only differed in mm day^{-1} between the temperatures may point to that the optimum temperature for growth in freshwater is different between diploids and triploids. Growth in freshwater has often been reported superior in triploids compared to diploids (Fjelldal & Hansen 2010; Leclercq *et al.* 2011; Taylor *et al.* 2012; Taylor *et al.* 2013), and in the present study, triploids were significantly heavier compared to diploids at experimental start. This is likely due to generally lower temperature in freshwater rearing that is mainly within the preferred range of triploids. Future studies should focus on determining the optimal temperature for growth in freshwater in triploid Atlantic salmon.

In the first period after seawater transfer, growth rates were lower in all groups compared to freshwater and also lower compared to the second period in sea water. Whereas groups at 10 °C grew moderately, groups at 16 °C only maintained (diploids) or even lost weight (triploids). Decreasing condition factors in Atlantic salmon in the weeks before and after smoltification can occur and are attributable to metabolic adjustments (Clarke, Shelbourn & Brett 1981; Sheridan 1989) and increased skeletal growth (Hoar 1988). Initial seawater performance in triploids has been identified to be inferior compared to diploids (Leclercq *et al.* 2011; Taylor *et al.* 2012). Additionally, the poor performance after seawater transfer in groups reared at 16 °C was concomitant with high mortality, especially in triploids. One reason may be insufficient gill NKA activity prior to seawater transfer and osmoregulatory disturbances in osmolality and chloride homeostasis in sea water. Therefore, all groups at 16 °C were removed after the first seawater period and fish killed. Similarly, Handeland *et al.* (2000) have recorded higher mortality during the first 18 days in sea water in diploid out of season Atlantic salmon at 18.9 °C (18%) compared to colder temperatures. In the same study, salmon transferred at 18.9 °C exhibited similar plasma chloride concentrations as in the present study and higher concentrations to groups reared at colder temperatures. Ion perturbations may also be due to osmoregulatory dysfunctions in other osmoregulatory organs and changes in epithelial permeability.

In contrast to growth and smoltification at 10 and 16 °C during the last 6 weeks in freshwater in the present study, earlier studies have shown: (i) normal growth (SGR ~ 1.5%; Grini *et al.* 2011) and low mortality (0–1.3%; Grini 2008) at both temperatures during the first 6 weeks after transfer to sea water, when the fish were maintained at the same temperatures after transfer; (ii) no mortality during the first 96 h in sea water at both temperatures, when the fish were transferred to 8.9 °C sea water (Fjelldal, Hansen & Huang 2011); and (iii) no mortality and normal growth (SGR 0.92–0.97%) at 16 °C in the first 6 weeks in sea water, when the fish were reared at 8.9 °C (Fjelldal *et al.* 2011). Further, Fraser *et al.* (2012) transferred underyearling diploid and triploid Atlantic salmon smolts from 16 °C and LD24:0 to 9 °C sea water and did not experience mortality due to insufficient hypo-osmoregulatory ability.

In contrast, groups at 10 °C displayed typical gill NKA peaks (~10–12 μmol ADP * mg protein^{-1} * h^{-1}) before seawater transfer with values comparable to Handeland *et al.* (2004), suggesting that the fish were transferred within the smolt window. No mortalities in either ploidy support this notion. During the first weeks after seawater transfer, growth can be impaired due to metabolic adjustments. The SGR in the present study of groups reared at 10 °C was 0.3–0.4% for the first 6 weeks in sea water, but higher (>1% day^{-1}) in the weeks thereafter until termination. These observations are in accordance with Handeland, Arnesen & Stefansson (2003) who measured lower growth rates in first month after seawater transfer (~0.35%); however, growth rates more than doubled during the second month at sea water. As the fish in the present study were only sampled 6 weeks after seawater transfer, it is possible that growth rates have recovered earlier.

Taylor *et al.* (2012) have found that underyearling triploid Atlantic salmon can undergo smoltification up to 4 weeks sooner than diploids, whereas yearling smolts reached smoltification simultaneously despite triploid fish were larger than diploids (Boeuf *et al.* 1994). In the present study, triploid and diploid yearling smolts at 10 °C seem to have a similar smolt development in contrast to those at 16 °C; despite similar gill NKA values and plasma ion concentrations, triploids had significantly higher mortality than diploids during the first period after change to sea water. It has been demonstrated that Atlantic salmon need approximately 250 degree-days before >90% of the smolts reach peak gill NKA enzyme activity (Handeland *et al.* 2004). From the experimental start until seawater

transfer, there was a deviation of 220 degree-days between groups at 10 and 16 °C that is likely to have resulted in the difference in smoltification status and hence the observed inadequate hypo-osmoregulatory ability at 16 °C. Increased freshwater temperature before smoltification has shown to accelerate smoltification and subsequently desmoltification (Duston, Saunders & Knox 1991). It is therefore possible that those fish have smolted before the first measurement of gill NKA activity. Overall, the high histidine diet had a positive effect on the condition factor in both ploidies and temperatures, suggesting beneficial effects on the metabolism.

The high mortality of 49–69% during the first period in sea water among the triploids at 16 °C shows that special care should be taken when rearing triploid smolts at elevated temperature such as in recirculation systems. There is a prominent need for development of best practice protocols for triploid smolt production in order to avoid such big losses in commercial farming. There is an increasing production of triploid smolts in Norwegian aquaculture in conjunction with more smolt farms changing from flow through to recirculation.

Temperature

In the present study, groups reared at 16 °C developed more prevalent and severe cataracts during the last weeks in freshwater, in contrast to groups that only developed negligible cataracts reared at 10 °C. Among both temperatures, triploids were generally more affected than diploids, but within both ploidies and temperatures, the diet with a higher concentration of histidine had a beneficial effect. Severe cataract outbreaks in freshwater have rarely been observed in Atlantic salmon (Bjerkås *et al.* 1996, 2001), most likely due to low or moderate water temperatures until transfer to sea water. The optimum temperature for growth in Atlantic salmon post-smolts is 13–14 °C for diploids of 70–300 g (Handeland, Imsland & Stefansson 2008). At higher temperature, feeding will cease as oxidative pressure increases, associated with shifts in the antioxidant defence system due to higher metabolic activity and energy demand. It may be possible that inclusion of dietary histidine in the low histidine diet was insufficient for fish at 16 °C due to increased metabolic activity.

The severe increase in lens opacities in groups at 16 °C during the final weeks in freshwater may be connected to osmoregulatory problems, but may also be connected to differences in metabolism at the two temperatures. Elevated temperatures up to the thermal optimum result in higher growth rates, and previous studies have shown a positive correlation between fish size or growth rate and cataract score, when the severity was mild or moderate (Waagbø *et al.* 1996; Bjerkås & Sveier 2004; Breck *et al.* 2005a; Taylor *et al.* 2015). In the present study, the altered growth rate between diploids reared at 10 and 16 °C may have contributed to the higher cataract score, whereas triploid groups did not grow as fast as diploids at 16 °C, although developed more cataracts compared to the respective groups at 10 °C.

The groups reared at 16 °C performed poorly after seawater transfer and were affected by high mortality within the first 6 weeks. Breck & Sveier (2001) transferred two groups of Atlantic salmon in a 4-week interval into sea water and found the later group not only to perform less well after transfer, but was also more affected by lens opacities. It is likely that not only growth performance, but also the high prevalence of cataracts in these groups is influenced by osmoregulatory stress. Defective osmoregulation during smoltification in sea water has been suspected to induce cataractogenesis (Bjerkås *et al.* 2003), and this may explain the high prevalence of cataracts in the groups reared at 16 °C in the present study.

In parallel, a distinction in the cataract score between ploidies was evident at each sampling point with triploids displaying higher cataract scores under the same treatment as diploids. Studies comparing cataracts between diploid and triploid Atlantic salmon are scarce (Wall & Richards 1992; Leclercq *et al.* 2011; Taylor *et al.* 2013, 2015), but show the same trend of triploids developing more and greater severity of cataracts under the same conditions as diploids. Leclercq *et al.* (2011) and Taylor *et al.* (2015) transferred diploid and triploid Atlantic salmon siblings as off-season smolts (<50 g) to sea water in January and did not observe cataracts until the first summer, when the cataract score was higher in triploids than diploids. This supports the importance of water temperature, especially during the periods before and after transfer to sea. Further, it was found that a histidine-enriched diet (17.4 vs. 12.6 g kg^{-1}) halted cataract progression in adult triploid salmon from April to September (8–15 °C) in the second year at sea when applied in February of the same year, whereas the

mean cataract score in the triploid group fed the respective other diet increased nearly twofold during the same time (Taylor *et al.* 2015). This indicates that enriched diets should preferably be applied throughout the entire production period, but at least with sufficient time prior to risk periods in order to create a buffer function. Hence, it may be possible that the severe cataract outbreak in freshwater in groups at 16 °C might have been preventable, if, in particular, the high histidine diet was applied some weeks before the temperature was increased.

Histidine and NAH

Supplementation of dietary histidine significantly reduced the severity of cataracts in both ploidies reared at 10 and 16 °C in freshwater, and in sea water for 13 and 6 weeks in groups at 10 and 16 °C, respectively. These findings are in line with previous studies showing that histidine supplementation reduces the outbreak and development of cataracts in the salmon lens in sea water in both diploids (Breck *et al.* 2005a; Waagbø *et al.* 2010; Remø *et al.* 2014) and triploids (Taylor *et al.* 2015). The dietary histidine concentrations used in the present study were both above the requirement for growth for Atlantic salmon (8 g his kg^{-1}; Scott 1998) and the high histidine diet was similar to the requirement to minimize cataract development for Atlantic salmon smolt after sea transfer.

Breck *et al.* (2005a) fed diets with two different levels of histidine (11.7 and 18.0 g kg^{-1}) for the last 6 weeks in freshwater (mean temperature 12.7 °C) to Atlantic salmon and found only negligible cataracts and low lens NAH levels before seawater transfer, irrespective of dietary histidine level. In the present experiment, both diploid and triploid smolt had high lens concentrations of NAH at the start of the experiment, showing that the synthesis of NAH can be initiated in freshwater. This may be a function of fish size or smoltification status and thus differs from previous studies where lens NAH concentration has been investigated in freshwater (Breck *et al.* 2005a,b; Remø *et al.* 2014). Despite similar lens histidine concentrations between ploidies at the experimental start, triploids had significantly lower lens NAH. During the freshwater period, lens NAH concentrations declined in all groups. The same pattern was found by Taylor *et al.* (2015) in adult diploid and triploid Atlantic salmon in sea water and may indicate a lower ability for acetylating NAH in triploids compared to diploids and hence make them more prone to cataract development. Indeed, triploid groups developed more cataracts compared to their diploid conspecifics until seawater transfer and lens NAH concentrations were, with one exception (2N16LH), lower. The present results suggest that both diploid and triploid salmon require a high dietary histidine concentration to sustain high lens NAH levels and reduce the risk of cataract development also in freshwater when reared at high temperature.

The period after seawater transfer is considered a risk period for cataract development (Breck *et al.* 2005a); however, both the prevalence and severity of cataracts can be minimized by increasing the dietary histidine concentration (Remø *et al.* 2014). After seawater transfer, NAH plays an important role as an osmolyte in the salmon lens (Breck *et al.* 2005b). In the present study, there was a trend of reduced lens NAH shortly after seawater transfer. Rhodes *et al.* (2010) suggested that NAH is the major osmolyte in the salmon lens due to positive correlations (*in vitro*, *in vivo*) of NAH and the osmolality of the external medium. Tröße *et al.* (2009) found that the efflux of NAH from Atlantic salmon lenses *ex vivo* under hypo-osmotic conditions is related to the dietary histidine content and the protein source. Further, NAH is the preferred source of utilization due to its superior efficiency in lens osmoregulation compared to efflux of other amino acids (Tröβe *et al.* 2010). Therefore, the reduced concentrations after 2 days in sea water may be an indication of NAH used as an osmolyte when transferred to sea water.

Six weeks after seawater transfer, lens NAH concentrations were higher in both ploidies fed the high compared to low histidine diet, and higher in diploids fed the high histidine diet compared to triploids. The triploid groups had higher cataract score compared to the respective diploid groups given the same diets. These results lead to the assumption that triploids have a higher dietary histidine requirement than diploids due to a higher mean cataract score under the same conditions, assuming that the requirements for other cataract mitigating nutrients are met.

Muscle imidazoles

In Atlantic salmon, anserine is quantitatively the most abundant white muscle imidazole. Anserine

is important for maintaining the intracellular pH homeostasis needed after strenuous exercise during the river migration into sea water (Abe 1987, 1995; Okuma & Abe 1992). In the present study, the muscle anserine concentration was significantly higher in triploids than diploids at the experimental start. At the end of the freshwater period, anserine concentrations, as well as its precursor carnosine, but not β-alanine, were elevated in groups fed the high histidine diet, irrespective of rearing temperature and ploidy. In previous studies, muscle anserine concentrations have been reported to be low in freshwater (Remø *et al.* 2014) and the synthesis being upregulated after seawater transfer (Ogata *et al.* 1998; Breck *et al.* 2005a). However, the present results show that high levels of anserine can already be synthesized in freshwater, prior to smoltification, and this may be explained by a the size difference of the fish studied. Thus, there may be a size threshold for initiating the anserine, as well as lens NAH synthesis, before the fish is transferred to sea and prior to smoltification. The concentration of muscle imidazoles has been shown to reflect the dietary histidine concentration after seawater transfer (Remø *et al.* 2014), but the present study also shows that the anserine concentration in freshwater is dependent on the dietary histidine level. Large smolt may therefore benefit from having higher dietary histidine concentrations also in freshwater.

Conclusion

In summary, this study demonstrated that the smoltification status of Atlantic salmon reared high water temperature in the weeks before seawater transfer is altered compared to ones reared at moderate temperature. Possible desmoltification in diploid and triploid Atlantic salmon results in insufficient hyperosmoregulatory ability before and high mortality after seawater transfer. Further, high water temperature also resulted in a major cataract outbreak that is more severe when fed a diet with low histidine supplementation. In contrast, moderate water temperatures and high histidine supplementation are successful in reducing the incidence and severity of cataracts in diploid and triploid Atlantic salmon in fresh and sea water. Despite both experimental diets contained histidine concentrations higher than the recommended level for growth, severe cataract formation developed in groups exposed to 16 °C, particularly groups fed the unsupplemented histidine diet and triploids were more affected than diploids. After 13 weeks in sea water, both ploidies kept at 10 °C and fed the unsupplemented histidine diet experienced severe cataract outbreaks. Lens NAH, reflecting dietary histidine, was related to the incidence and severity of cataracts.

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