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Solving bottlenecks in triploid salmon production - a way to strengthen the sustainability of the salmon aquaculture industry

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HAVFORSKNINGSINSTITUTTET
INSTITUTE OF MARINE RESEARCH

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Final report for project 216197

Bergen, januar 2016

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Project summary

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The current project aimed to study how triploid salmon are able to regulate their gene expressional out-put to a similar level as in diploids, and to elucidate the mechanisms involved in the induction of skeletal deformities and cataracts and the reduced thermal tolerance of triploid Atlantic salmon.

We show that triploid Atlantic salmon shows non-additive gene expression. Further the general mechanism of non additive expression does not seem to be due to hypermethylation of the whole genome. Although some specific changes in methylation seems to occur possibly related to protein turnover heart formation, bone/cartilage formation and micro RNA regulation. Our results show that the differences in gene regulation is very complex, and may differ between tissues. Further studies should focus on one tissue only.

Both postsmolt and adult triploids perform similarly or better than triploids at low temperatures and poorer at high temperatures. It is suggested that triploid Atlantic salmon post-smolts and adults have a similar energy metabolism during varying environments, but differ homeostatically in their physiological response to suboptimal conditions. Nonetheless similar or even better performance at lower water temperatures could favour the use of triploid Atlantic salmon not only in open net pen aquaculture in geographical areas with moderate maximum water temperatures, but also in closed flow-through systems with sufficient oxygen levels throughout the year.

In FW, a grave cataract outbreak was recorded in groups at 16 °C, although with a reduced severity in both ploidies when fed a high histidine diet. At 10 °C, ploidy and diet groups performed similarly to each other with respect to growth and mortality, however triploid status and low dietary histidin were associated with increased cataract score.

When fed 0.6% P from 3 g, triploids had lower vertebra ash content than diploids at 10 and 30 g, but had reach the level of diploids at 75 g size. The parallel increase in vertebra ash between diploids and triploids between 10 and 30 g, when feed 0.6% P throughout, points to that the period between 3 and 10 g is most critical for triploids. There was a tendency for triploids having lower vertebra ash content than diploids at 10 g, also when fed the a 1.2% P diet. These study shows that the period between 3 and 10 g seems critical for triploids with regard to dietary P and that available P in this period should be considerably above 0.6%.

Primary and secondary objectives of the project

Primary objective:
To increase the knowledge about the environmental sensitivity of triploid salmon and the rearing conditions and feeds which are necessary to maintain their welfare and production performance.

Secondary objective:
1. To compare the gene expressional and epigenetic control in triploid and diploid salmon
2. To obtain performance data on triploid postsmolts and adults over the normal temperature range of Atlantic salmon production.
3. Establish data on performance under hypoxic conditions and to elucidate the dependence between temperature and sensitivity to hypoxia in triploids.
4. Investigate the mechanisms behind the development of deformities and cataracts in triploids and whether these can be remedied with specialized diets or feeding regimes?

WP1: Do triploid salmon have a mechanism of "gene dosage compensation" and could this phenomena be related to the epigenetic mechanism of DNA methylation?

Problems related to triploidy in Atlantic salmon has been indentified such as low tolerance to high temperature in seawater, susceptibility to skeletal deformities, cataracts and heart deformations. Little is known about what may cause these phenotypes in triploids. The answer might be found in how they regulate their additional alleles. It is further unknown how triploid fish use or regulate the extra chromosomal copies. What is known is that the extra chromosomal copies do not seem to significantly cause an additive gene expressional response in fish. Likewise in plants where ploidisation is a common breeding technique, gene expression upon ploidsation is often non-additive. Plant studies have revealed epigenetic mechanisms behind this dosage compensation of gene expression including hypermethylation of DNA, histone acetylation and micro-RNA. It is unknown if such epigenetic mechanisms are initiated upon triploidsation in fish, but novel molecular techniques has enabled us to investigate if methylation of the genome could contribute to non-additive gene expressional regulation in triploids. To investigate whether nonadditive gene expression upon triploidy in Atlantic salmon could be regulated by increased methylation of the genome we first examined gene expression level of several genes in triploid and diploid Atlantic salmon. None of the investigated genes showed differential expression between triploid and diploids. Also we measured the DNA:RNA ratio and as expected it was significantly lower in triploid compared to diploid fish (Figure 1).

To explore local methylation effects, we measured the CpG methylation level of the housekeeping gene elongationfactor 1α in whole 700 day° embryos, but no differences could be detected between triploid and diploid samples (n=10 per group Figure 2).

This result pointed at no general change in methylation level between triploid and diploid Atlantic salmon. To search for methylation differences on a global level we performed RRBS (Reduced Representation Bilsulfite Sequencing) on samples from whole diploid and triploid embryos (700 day°). Obtained sequence material was mapped upon an early release of the salmon genome assembly. Initial results showed that global methylation level was similar between triploid and diploid Atlantic salmon. CpG islands and gene prediction revealed that in the sequenced material, only 1395 genes showed representative CpG islands. Of the 1395 genes 362 genes were more methylated (above 30% more) in triploid than diploids while 140 genes showed the opposite (Figure 3). In the group of overmethylated genes in triploids, we detected genes related to protein turnover, heart formation, bone/cartilage formation and micro RNA regulation. We could not in other stages of development conclude that the same genes were differentially methylated. This results suggest that the gene regulation is very complex and may differ in between tissues and further studies should focus on one tissue only. To conclude, triploid Atlantic salmon shows non-additive gene expression. Further the general mechanism of non additive expression does not seem to be due to hypermethylation of the whole genome. Although some specific changes in methylation seems to occur possibly related to protein turnover heart formation, bone/cartilage formation and micro RNA regulation.

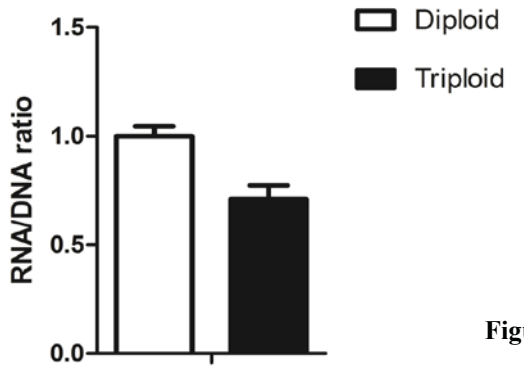

Figure 1. RNA/DNA ratio of diploid and triploid Atlantic salmon.

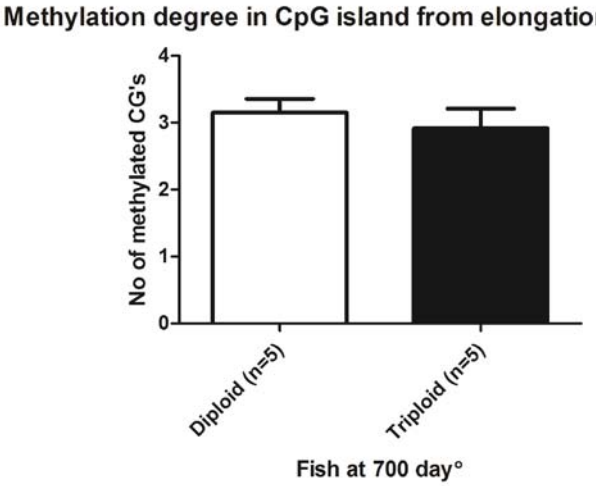

Figure 2. CpG methylation level of the housekeeping gene elongationfactor 1α in whol 700 day° Atlantic salmon embryos.

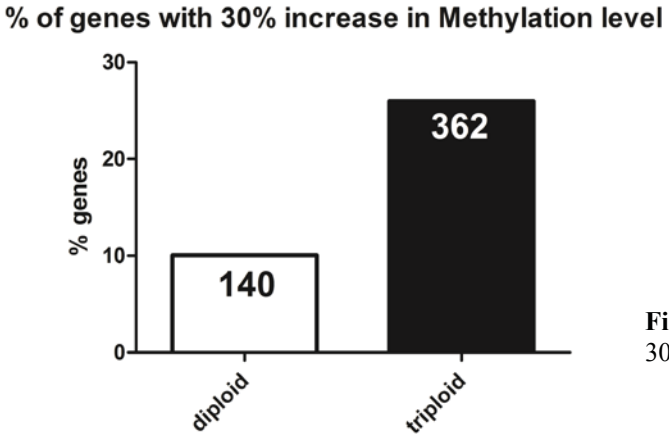

Figure 3. Percentage of overmethylated (more than 30%) genes in diploid and triploid Atlantic salmon.

WP2: Thermal tolerance and interaction with hypoxia in diploid and triploid Atlantic salmon

Triploid salmonids have been reported to be more sensitive to changes in the rearing environment and to environmental extremes than diploids. Based on the current literature and our own observations is seem most likely that this is linked to a reduced O_2 carrying capacity in triploids (Bernier et al., 2004), rather than ploidy effects on stress response (Benfey and Biron, 2000) or the cardiac output (Benfey et al., 2009). A reduced aerobic capacity in triploids may be related to that their red blood cells exhibit increased mean corpuscular volume (MCV) as a result of their extra chromosomal set, which decreases the surface-to volume ratio. Increased mortality in triploid trout has been reported when the temperature is at its highest (Ojolick et al., 1995). At higher temperatures, the metabolic scope is reduced, and the energy available for the animal to grow, digest food, support locomotion etc is lowered. This may partly explain the increase in mortality at higher temperatures. To date, the major limitation in successfully defining optimal environmental requirements for triploids has focused on single parameter effects at the time, thus there is a clear need for a study based on a multivariate approach to determine interactions between defined risk variables to avoid compromised welfare.

Two experiments was conducted to establish the temperature tolerance and to elucidate the dependence between temperature and sensitivity to hypoxia of small (0.3 kg) and large (2.2 kg) diploid and triploid Atlantic salmon in seawater. In both experiments, diploids and triploids were reared in triplicate 3 m tanks (totally 6 tank) on an alternating thermal regime in the range 3 to 18 ^{o}C, and subjected to hypoxic condition at 6 and 18 ^{o}C. Except for the hypoxic periods, the oxygen saturation of the outlet was kept at 100%. Oxygen consumption rate (VO_2), survival and appetite was measured, and fish was sacrificed for blood and tissue sampling. Lengths and weights of all individuals were recorded at start and termination, and of all fish that are sacrificed during the samplings in between.

Based on these experiments two manuscripts are in progress:

Sambraus F., Olsen, RE., Remen, M., Torgersen, T., Hansen, T. Fjelldal, PG. 2015. Water temperature and oxygen: The effect of triploidy on performance and metabolism in farmed Atlantic salmon (Salmo salar) postsmolts. Aquaculture (in review):

The experimental design of the small fish experiment is shown in Figure 4.

Triploid fish had a higher feed intake until 6 °C and were similar to diploids at 9 °C (Figure 5). The highest feed intake was measured at 12 and 15 °C in triploids and diploids, respectively. Hypoxia had a negative effect on feed intake at 6 °C in triploids, while significant decreased feed intake was observed in both ploidies during hypoxia at 18 °C.

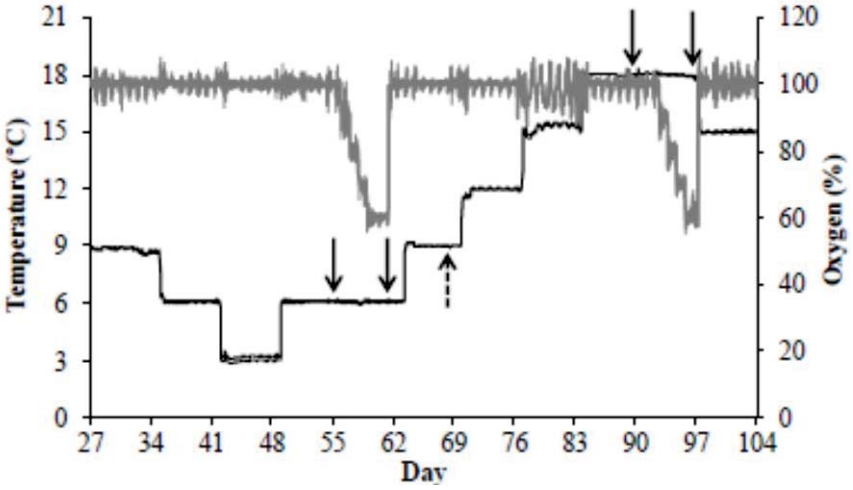

Figure 4. Experimental design in the small fish experiment. The water temperature was kept stable during an acclimation period at 9 °C for 34 days (data not shown). Thereafter the temperature was decreased to 6 and 3 °C for one week before increased in 3 °C steps per week until 18 °C. One additional week at the second 6 °C period and at 18 °C was included where DO was reduced from 100 % by 10 % day^{-1} until 60 % and kept stable until sampling. After sampling, DO was again raised to 100 %. Downwards pointing solid arrows indicate the four sampling points; the upwards pointing broken arrow indicates the length and weight sampling of 80 fish tank^{-1}.

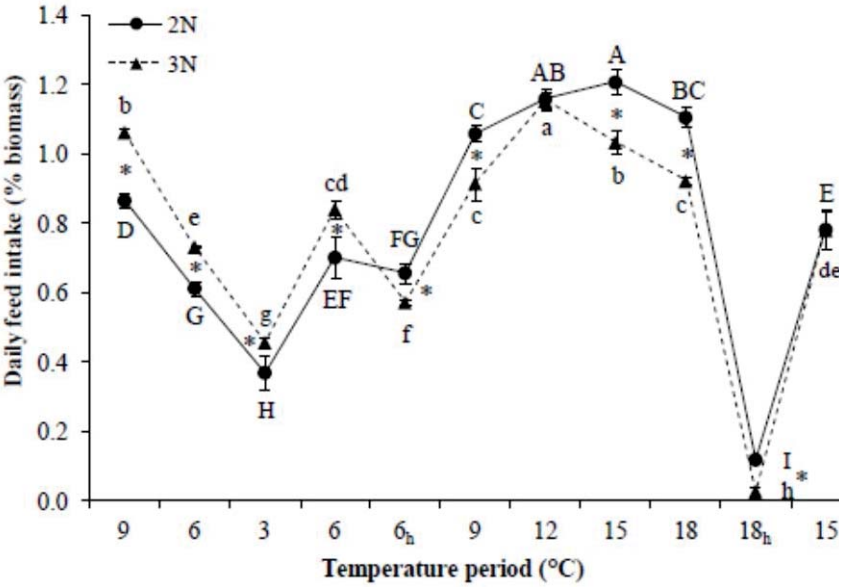

Figure 5. Mean ± SEM feed intake (% biomass) in triplicate groups of diploid and triploid Atlantic salmon post-smolts in seawater that were reared at a temperature regime with increasing temperature from 3 to 18 °C, and then down to 15 °C, with reduced DO from 100 to 60 % at 6 (6h) and 18 °C (18h). DO was otherwise 100 %. Unlike upper case letters indicate significant differences between periods in diploids. Unlike lower case letters indicate significant differences between periods in triploids. Asterisks indicate significant differences between ploidy (SNK, $p<0.05$) at the specific time point.

Overall growth, feed conversion and mortality were not statistically different between the two groups, but mortalities were higher in triploids at 18 °C and hypoxia (Table 1). There was no difference in the energy phosphate metabolism between ploidies, however, triploids had higher plasma glucose levels (data not shown) and lower monovalent plasma ion concentrations (Na+, K+, Cl-) during hypoxia at 18 °C (Figure 6). These results suggest that triploid Atlantic salmon post-smolts have a similar energy metabolism during varying environments, but differ homeostatically in their physiological response to suboptimal conditions. The cause of these altered responses remains to be studied. Nonetheless similar or even better performance at lower water temperatures could favour the use of triploid Atlantic

salmon not only in open net pen aquaculture in geographical areas with moderate maximum water temperatures, but also in closed flow-through systems.

Table 1. Mean ± SEM lengths (cm), weights (g) and condition factors in triplicate groups of diploid and triploid Atlantic salmon measured on day 0 (tank stocking, 9 °C), 68 (9 °C) and 104 (termination, 15 °C). Also length growth (mm day^{-1}), specific growth rate (SGR, % of biomass d^{-1}), and feed conversion ratio (FCR) during the first and second period and during the entire experiment. Results until day 68 refer to measurement of 80 fish $tank^{-1}$ (240 fish $ploidy^{-1}$). Mortality until day 83 represents the mortality from tank stocking until the end of the 15 °C period; day 84 – 97 represents 18 °C and 18_h °C.

Day	Parameter	Diploid	Triploid	p-value
0	Length	29.1 ± 0.1	30.4 ± 0.1	**<0.001**
	Weight	303 ± 1	339 ± 3	**<0.001**
	CF	1.22 ± 0.00	1.20 ± 0.00	**<0.001**
68	Length	34.7 ± 0.1	37.3 ± 0.1	**<0.001**
	Weight	580 ± 9	667 ± 5	**<0.001**
	CF	1.37 ± 0.01	1.28 ± 0.02	**<0.001**
104	Length	39.3 ± 0.1	41.6 ± 0.1	**<0.001**
	Weight	811 ± 14	939 ± 5	**<0.001**
	CF	1.33 ± 0.02	1.30 ± 0.01	**<0.001**
0 – 68	mm d^{-1}	0.82 ± 0.02	1.01 ± 0.01	**0.001**
	SGR	0.96 ± 0.03	1.00 ± 0.03	0.342
	FCR	0.71 ± 0.02	0.81 ± 0.02	**0.016**
69 - 104	mm d^{-1}	1.27 ± 0.04	1.20 ± 0.05	0.335
	SGR	0.93 ± 0.05	0.96 ± 0.03	0.730
	FCR	1.02 ± 0.04	0.87 ± 0.04	0.057
0 - 104	mm d^{-1}	0.98 ± 0.01	1.09 ± 0.01	**<0.001**
	SGR	0.96 ± 0.02	0.99 ± 0.01	0.189
	FCR	0.82 ± 0.01	0.83 ± 0.01	0.489
0 - 83	Mortality	0.66 ± 0.33	1.15 ± 0.59	0.121
84 - 97	Mortality	0.50 ± 0.29	3.41 ± 1.25	**0.048**
0 - 104	Mortality	0.67 ± 0.33	2.68 ± 0.94	0.091

p-values in bold indicate significant differences between ploidy (t-test, $p<0.05$)

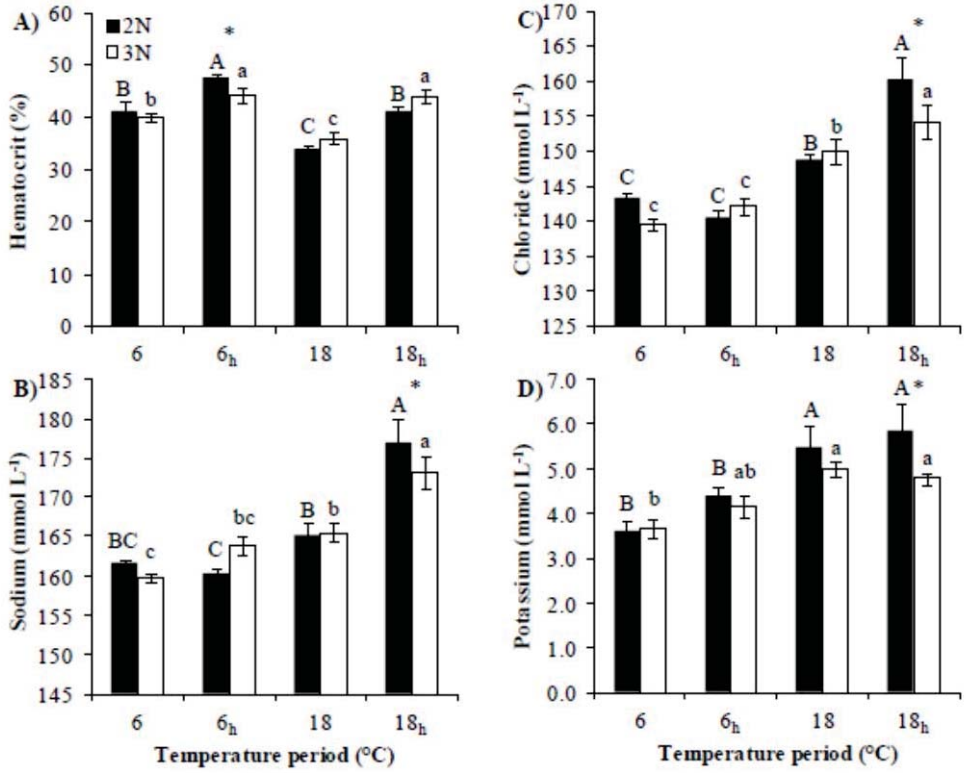

Figure 6. Mean ± SEM hematocrit (A), plasma sodium (B), chloride (C), and potassium (D) (μmol L^{-1}) in diploid and triploid Atlantic salmon post-smolts sampled at 6 and 18 °C, at 100 (6, 18) and 60 % DO (6h, 18h). The fish had been reared in seawater at a temperature regime with increasing temperature from 3 to 18 °C and reduced DO from 100 to 60 % at 6 and 18 °C. DO was otherwise 100%. Different upper case letters indicate significant differences between temperature periods in diploids. Different lower case letters indicate significant differences between temperature periods in triploids. Asterisks indicate significant differences between ploidy (SNK, $p<0.05$).

Sambraus, F. Remen, M., Olsen, RE., Waagbø, R., Hansen, TJ., Torgersen, T., Lock, EJ., Imsland, A., Fjelldal, PG. Water temperature and oxygen: The effect of triploidy on performance and metabolism in adult farmed Atlantic salmon (*Salmo salar*). Production performance and physiological status in adult diploid and triploid Atlantic salmon (*Salmo salar* L.) subjected to increasing temperature and hypoxic periods.

In this study, adult diploid and triploid Atlantic salmon were acclimated to 9 °C and then given a weekly temperature changing regime from 9 to 3 °C and 3 to 18 °C in 3 °C steps. Furthermore, the fish were exposed to lowered oxygen saturation (70 %) at low (6_h °C) and high (18_h °C) temperature (Figure 7.).

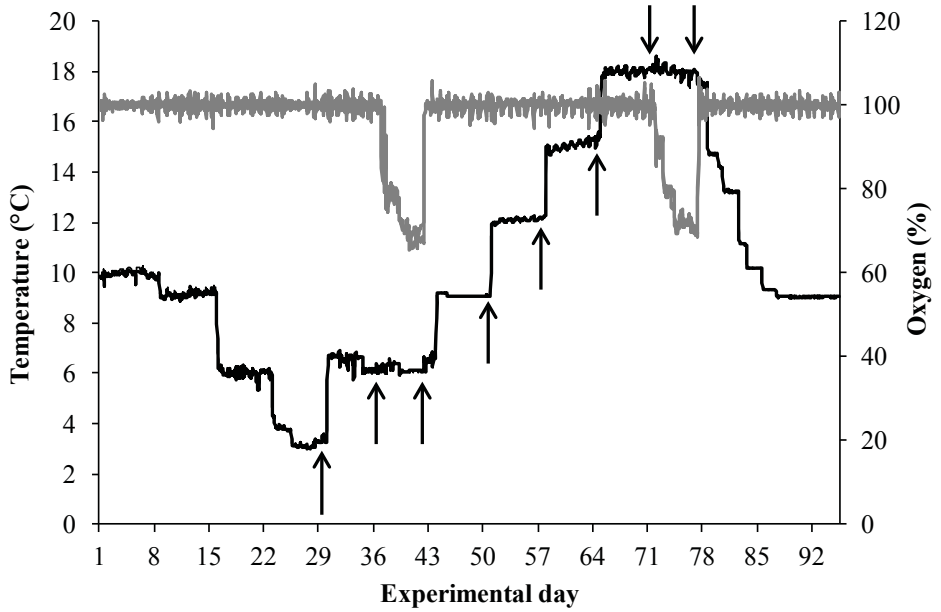

Figure 7. Experimental design to study feed intake, performance and physiology of adult diploid and triploid Atlantic salmon. The water temperature was kept stable during an acclimation period at 9 °C for 21 days (data not shown). Thereafter the temperature was kept at 9±1 °C for two weeks and then reduced to 6 and 3 °C for one week, following an increase in 3°C-steps per week until 18 °C. One additional week at the 6 °C and at 18 °C was included where the oxygen concentration was reduced from 100 % DO by 10 % day^{-1} until 70 % and maintained for three days until sampling. Arrows indicate the sampling points.

Commercial production parameters (feed intake, growth, feed conversion, survival) were recorded from tank stocking to termination. At the end of each week of exposure starting at 3 °C a range of physiological (including energy related parameters from white muscle) haematological and stress related parameters from blood and plasma were taken. Additionally, ocular cataracts, which are generally more prevalent in triploids, were examined in both ploidies at different temperature periods.

Triploids had higher feed intake between 3 and 9 °C and were similar to diploids at 12 °C (Figure 8). At 15 °C the feed intake significantly dropped in both ploidies, but considerably more in triploids. During hypoxia, feed intake was higher in triploids at 6 and equal to diploids at 18 °C.

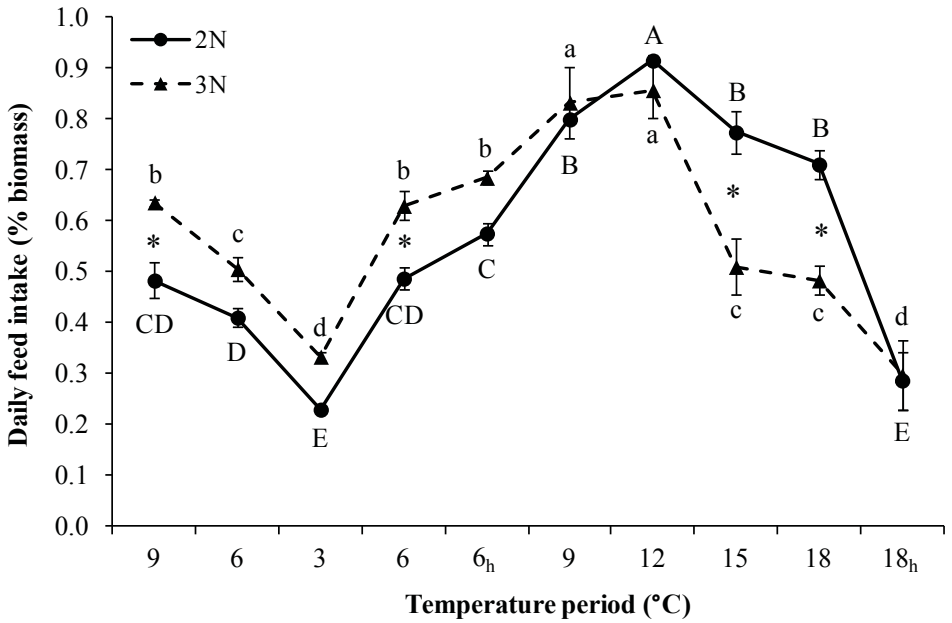

Figure 8. Daily feed intake (% biomass; mean ± SEM) in triplicate groups of adult diploid and triploid Atlantic salmon in seawater, reared under a temperature regime with decreasing water temperature from 9 to 3 °C, then increasing until 18 °C and decreasing to 12 °C in 3 °C steps until 18 °C. The fish were subjected to reduced DO from 100 to 70 % during an additional period at 6 (6_h) and 18 °C (18_h). Different upper case letters indicate significant differences between periods in diploids, different lower case letters indicate significant different differences between periods in triploids (2-way factorial ANOVA, $p<0.05$; SNK, $p<0.05$)

Triploids grew better at colder temperatures and the feed conversion ratio was similar between ploidy. Mortality was low (<1%) and similar in both ploidies. Muscle energy phosphates (CrP, ATP) were consistently higher in diploids (Figure 9 A,B) and blood haemoglobin and hematocrit were higher in diploids with increasing temperatures. Plasma lactate levels were higher in triploids and increased with increasing temperatures and at hypoxia in both ploidies, respectively (Figure 10C). Cortisol increased in both ploidies at high temperatures and was higher in diploids at 18 °C and in triploids at 18_h °C, respectively. The present findings show that adult diploid and triploid Atlantic salmon differ in their energy and physiological metabolism. Triploids perform better at colder temperatures and could substitute for diploid salmon under commercial practices with moderate water temperatures and sufficient oxygen levels throughout the year.

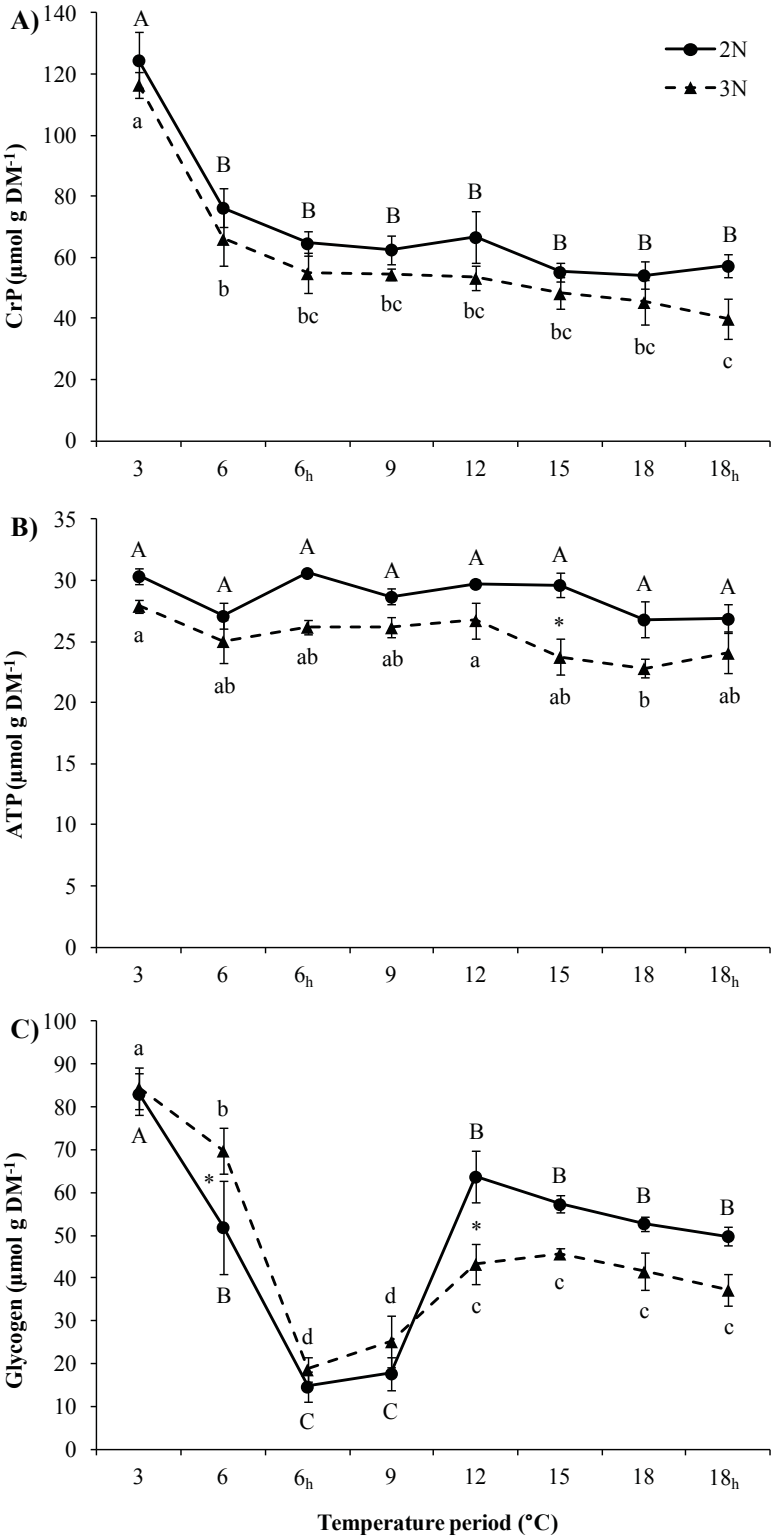

Figure 9. Mean ± SEM white muscle CrP (A), ATP (B) and glycogen (C) levels (µmol g DM^{-1}) in adult diploid and triploid Atlantic salmon in seawater. The fish had been reared under a regime with increasing temperatures from 3 to 18 °C and sampled at 6 different periods (3, 6, 9, 12, 15 and 18 °C) at 100 % DO and additionally at 6 (6_h) and 18 °C (18_h) at 70 % DO. Unlike upper case letters indicate significant differences between periods in diploids, unlike lower case letters indicate significant different differences between periods in triploids (2-way factorial ANOVA, $p<0.05$; SNK, $p<0.05$).

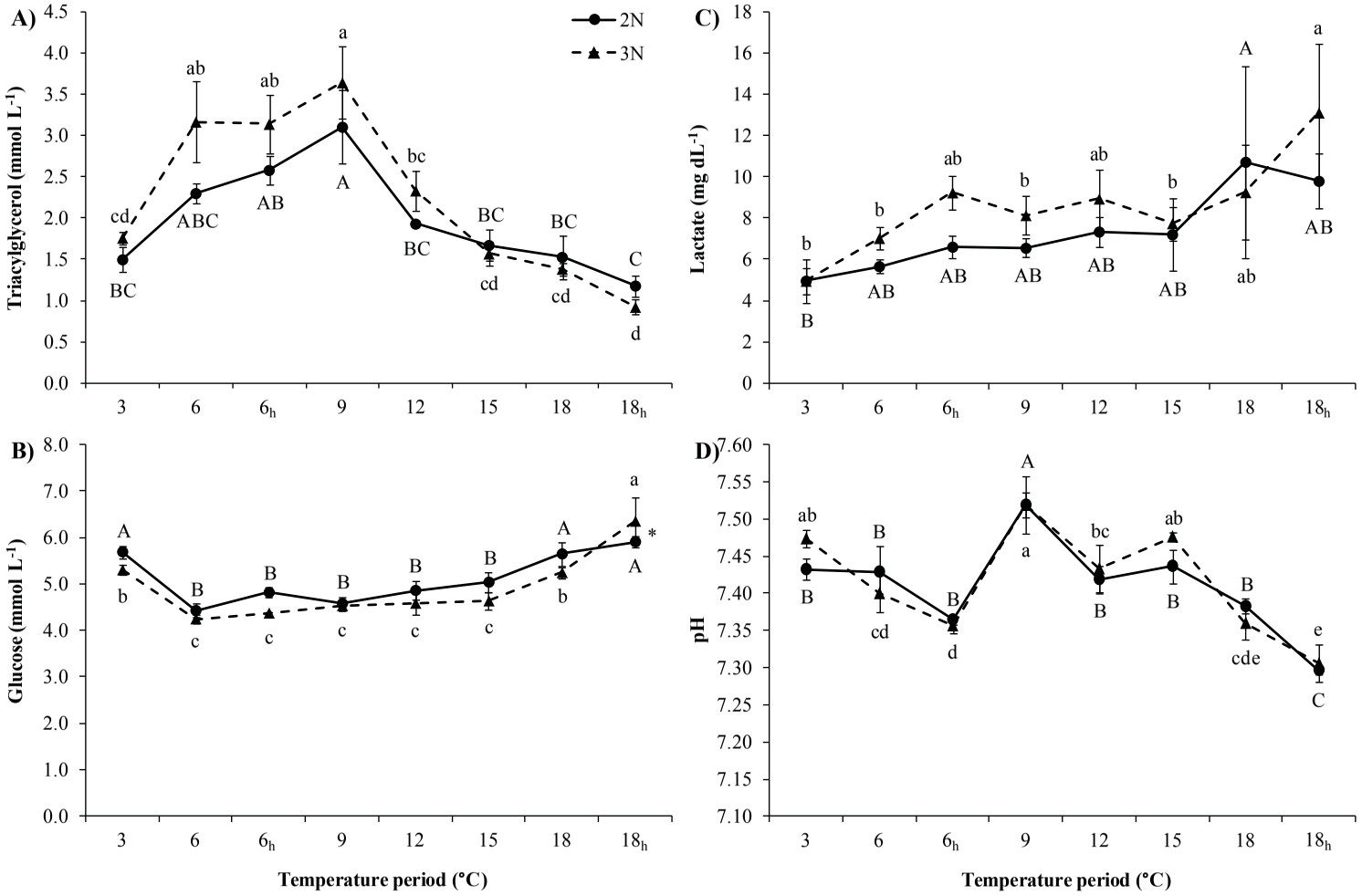

Figure 10. Mean ± SEM plasma (A) triacylglycerol (mmol L^{-1}), (B) glucose (mmol L^{-1}), (C) lactate (mg dL^{-1}) and (D) pH levels in adult diploid and triploid Atlantic salmon in seawater. The fish had been reared under a regime with increasing temperatures from 3 to 18 °C and sampled at 6 different periods (3, 6, 9, 12, 15 and 18 °C) at 100 % DO and additionally at 6 (6_h) and 18 °C (18_h) at 70 % DO. Unlike upper case letters indicate significant differences between periods in diploids, unlike lower case letters indicate significant different differences between periods in triploids (2-way factorial ANOVA, $p<0.05$; SNK, $p<0.05$)

WP3: Dietary demands of histidine and phosphorous

Histidine

Ocular cataracts are a widely reported problem in the North Atlantic salmon farming industry and a higher occurrence of cataracts in triploid Atlantic salmon has been observed. Generally a strong correlation between rapid growth and cataract has been documented (Breck and Sveier, 2001) and cataract outbreaks typically occur during periods of high or fluctuating water temperatures and rapid growth (Bjerkås et al., 2003). Interestingly, cataractogenesis can be reduced by feeding diets properly balanced in with anti-oxidants (Waagbø et al., 2003) or supplemented with histidine (His) in form of blood meal, high His fish meals or crystalline His (Breck et al., 2003). The mechanism underlying the higher occurrence of ocular cataracts in triploids than diploids has not been identified. This project investigated whether development of cataracts in triploids are associated with temperature and His nutrition.

Sambraus, F., Fjelldal, PG., Remø, SC., Hevrøy, EM., Nilsen, TO., Thorsen, A., Hansen, TJ., Waagbø, R. Water temperature and dietary histidine affect cataract formation in large yearling Atlantic salmon (Salmo salar) diploids and triploids undergoing parr-smolt-transformation.

The aim of the present study was to demonstrate the effect of between diploid (2N) and triploid (3N) Atlantic salmon smolts and post-smolts at two water temperatures (10 and 16 °C) and low (LH, 10.4g kg^{-1}) or high (HH, 13.1g kg^{-1}) dietary histidine levels on smolting physiology and the development of cataracts 7 weeks before until 13 weeks after change from freshwater (FW) to seawater (SW). A full crossing of the treatments resulted in eight experimental groups with quadruple replicates. At the experimental start, all groups were kept at ambient water temperature (5.5 °C) and gradually increased (1°C day^{-1}) to their target temperatures thereafter.

At that point, the prevalence and severity of cataracts was minor in both ploidies. In FW, a grave cataract outbreak was recorded in groups at 16 °C, although with a reduced severity in both ploidies fed the HH diet. Growth rates in FW were higher in 2N at 16 °C, compared to the remaining groups. After change to SW, groups at 16 °C displayed osmoregulatory stress in form of insufficient gill Na^+, K^+-ATPase activity, elevated plasma electrolyte concentrations and high mortalities among both ploidies. The respective 10 °C groups performed similarly to each other with respect to growth and mortality, however both, ploidy and diet, affected cataract formation in SW. Lens histidine and N-acetyl histidine concentrations reflected the dietary histidine level, although absolute values decreased in all groups during the experiment, particularly in FW. At the end of the FW period, white muscle free imidazoles were lower in groups fed the LH diet, but higher in groups fed the HH diet compared to levels at the experimental start. Groups fed the HH diet displayed elevated histidine and anserine levels in the white muscle compared to elevations in non-essential amino acids among groups fed the LH diet. The findings of this study demonstrate the importance of environmental conditions in the husbandry of triploid Atlantic salmon with

regards to smoltification and the need for adjusted diets to counteract production issues such as lens opacities.

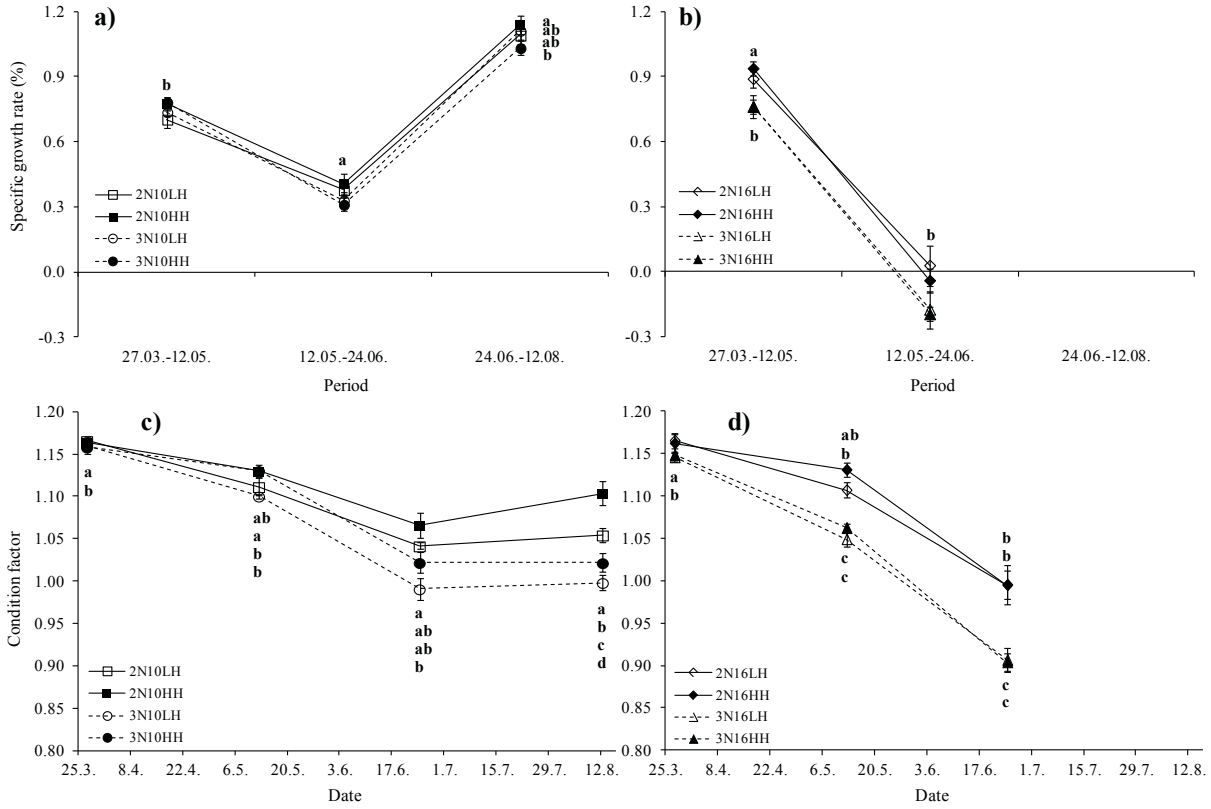

Figure 11. Specific growth rates (a, b) and change in condition factor (c, d) (mean ± SEM) of diploid (2N) and triploid (3N) Atlantic salmon at 10 (a, c) and 16 °C (b, d), fed two experimental diets differing in the level of histidine (LH: 10.4; HH: 13.1 g kg^{-1}). The experimental diets replaced a commercial diet on 30.03. and the water temperature was then elevated from ambient (5.5 °C) to either 10 or 16 °C (1 °C day-1). The water inflow was changed from freshwater to seawater on 14.05.. Groups at 16 °C were terminated on 24.06., while 10 °C groups were terminated on 12.08.. Superscripts denote significant differences (SNK, $P<0.05$) between the groups, but within the parameters (a, b; c, d), at each sampling point and period, respectively.

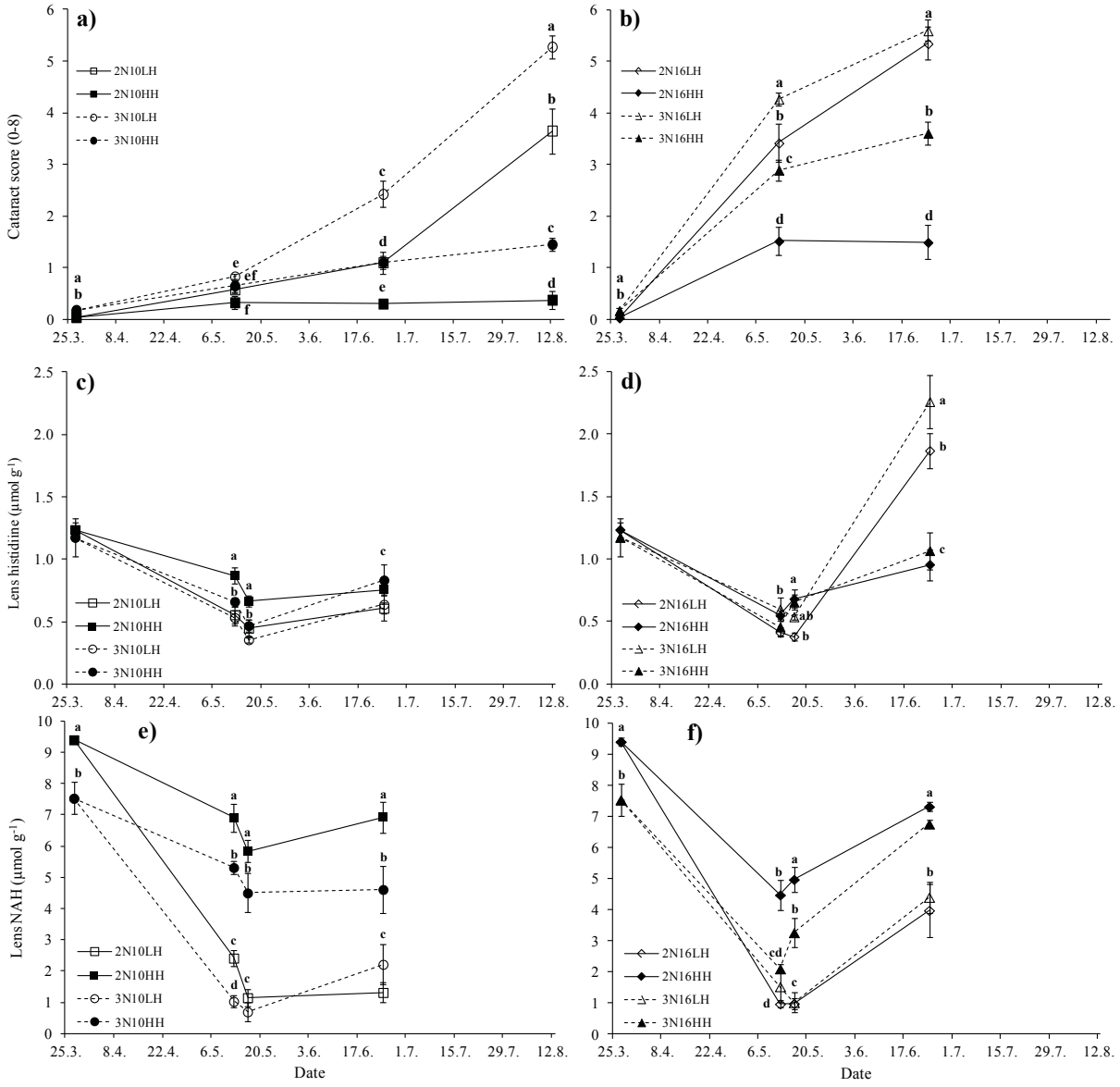

Figure 12. Development of cataract (a, b) (sum score 0-8; mean ± SEM), lens histidine (c, d) and lens N-acetyl-histidine (e, f) (mean ± SEM; n = 4 pooled samples) concentrations in diploid (2N) and triploid (3N) Atlantic salmon smolts and post smolts (seawater change 14.05.) at 10 and 16 °C, fed two 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 (5.5°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

Phosphorous

An increased prevalence of morphological deformities in triploids has been readily reported, but the mechanisms by which such deformities are affected by growth, nutrition and environmental variables have not been much studied in triploid salmon. Several causal mechanisms of deformities in triploids have been proposed (Benfey, 1999; Sadler et al., 2001) and one of them are probably a sub-optimal P nutrition caused by a higher P demand in triploids during early life stages (Fjelldal et al., 2011).

An extra experiment was conducted prior to the study that was originally planned for the project. In this experiment, diploid and triploid siblings were feed diet with 0.4, 0.6 and 1.2% available P from first feeding until transfer to seawater. The ash content in the bone was equal between ploidy at sea transfer for the 0.4 and 1.2% P groups, but higher in diploids than triploids for the 0.6% P diet group (Table 2).

Table 2: Mean values (±SE) for ash content (% defatted dry weight) in vertebral bone and ALP and TRACP enzyme activity (µg pNP mg $^{-1}$ h^{-1}) in scales at transfer to seawater. Triplicate groups of diploid and triploid Atlantic salmon were fed experimental diets with a low (LP), medium (MP) or high (HP) P content for 203 days from first-feeding (11 March 2010) to seawater transfer (30 September 2010). N = 15 per ploidy diet group (5 per tank) for ash content, and ALP/TRACP analysis, N = 3 per ploidy diet group (pooled samples of 5 individuals per tank) for the relative gene expression analysis. Different lower case letters indicates significant differences.

Parameter	LP		MP		HP		P-values*		
	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid	Diet	Ploidy	Interaction
Ash (%)	26±0.9c	26±0.8c	47±0.3a	45±0.4b	49±0.3a	49±0.3a	***<0.001***	0.224	0.0964

*Values in bold and italic indicate a significant effect (2-way ANOVA, P<0.05) of diet, ploidy or interaction between diet and ploidy.

The occurrence of fish with vertebra deformities at transfer to seawater, and 426 days later is shown in Table 2. All groups were feed a commercial diet from transfer to seawater to termination 426 days after transfer to seawater.

Radiological examination of random fish at transfer to seawater and at termination showed a significant higher occurrence of fish with deformed vertebra in triploids than diploids when fed the 0.6% P diet, but no ploidy difference when fed the 1.2% diet. Both results on vertebra bone ash content (Table 2) and occurrence of vertebra deformity (Table 3) points to that triploids have a higher P demand then diploids in seawater.

The last experiment was conducted to explore if the P demand was higher in triploids only during certain periods in freshwater. The fish used in this study was fed a commercial first feeding diet from first feeding until 3 g body size. Then, diploid and triploid salmon were fed a 1.2% available P diet from 3 g body weight until 10 g, 30 g, and then fed a 0.6% available P diet until transfer to seawater (75 g). I addition diploids and triploids were feed either 0.6 or 1.2% available P diets from 3 g until transfer to seawater. At transfer to seawater, all groups were pittaged and transferred to a common 12x12 m seacage for on-growth on a commercial diet until harvest size to measure the treatment effects on the animals long-term performance. The period with different feeds (until seawater transfer) was performed according to the plan, but unfortunately, there was a calibration failure in the central feeding system in the sea-cage facility, causing a underfeeding of the experimental groups during the first period in seawater. The experiment was terminated because of this error.

Table 3: Occurence (mean ± S.E.) of fish with externally detectable vertebra deformities (Vertebra), and with one or more deformed vertebrae on radiographs (Def V x-ray). Triplicate groups of diploid and triploid Atlantic salmon were fed experimental diets with a low (LP), medium (MP) or high (MP) phosphorus level for 203 days from start-feeding (11.03.10) to seawater (SW) transfer (30.09.10), and then followed up for 426 days on one common commercial diet in seawater until harvest size (Termination, 30.11.11). Different lower case letters indicates significant differences.

Sampling	Param.	LP		MP		HP		P-values[1]		
		Dip	Trip	Dip	Trip	Dip	Trip	Diet	Ploi	Inter
Seawater transfer	Def V x-ray (%)[2]	35±6b	56±5a	17±7c	45±3ab	8±2c	10±3c	***<0.0001***	***0.0007***	***0.0353***
Termination	Vertebra external (%)[2]	90±2b	94±1a	2±1c	4±1c	0.6±0.4c	2±1c	***<0.0001***	***0.0153***	0.7092
	Def V x-ray (%)[2]	97±3a	100±0a	31±8c	58±4b	31±11c	25±7c	***<0.0001***	0.1471	0.0684

[1]Values in bold and italic indicate a significant effect (2-way ANOVA, $P < 0.05$) of diet, ploidy or interaction between diet and ploidy. 'N def V x-ray' was tested by Kruskal-Wallis test due to the large difference in number of deformed individuals between groups (SW transfer: $P = 0.0120$, Termination: $P < 0.0001$). [2]N = 3 (occurrence per tank/sea-cage) for LJD, Spinal external and Def V x-ray. For external deformities (LJD and Spinal), 200 fish per tank were evaluated at SW transfer (totally 3600 fish), while 42 to 172 fish per cage were evaluated at termination (all fish evaluated; N varied according to group differences in mortality rate; totally 2438 fish). For radiological detectable vertebra deformities 20 fish per tank were radiographed at SW transfer (totally 360 fish), while 10 per cage were radiographed at termination (totally 160 fish).

Data on vertebra bone mineralization up to seawater transfer (75 g) showed that the first period – up to 10 g – was most critical regarding diet P for triploids (Figure 13). When fed 0.6% P from 3 g, triploids had lower vertebra ash content than diploids at 10 and 30 g, but had reach the level of diploids at 75 g size. This indicates that the P demand is higher in triploids than diploids, especially up to 30 g. The parallel increase in vertebra ash between diploids and triploids between 10 and 30 g, when feed 0.6% P throughout, points to that the period between 3 and 10 g is most critical for triploids. Since, triploids in this feeding group recovered to the same ash content as in diploids between 30 and 75 g, it seems like triploids have a good capacity for bone mineralization in this size range. There was a tendency for triploids having lower vertebra ash content than diploids at 10 g, also when fed the 1.2% P diet, further pointing to that the period 3 to 10 g are critical for triploids with regard to bone mineralization. When switched to 0.6% P at 10 and 30 g, diploids and triploids responded in a similar manner, with reduced vertebra ash. Diploids and triploids fed 1.2% P throughout had equally high vertebra ash content at 30 and 75 g. The study shows that the period between 3 and 10 g seems critical for triploids with regard to dietary P.

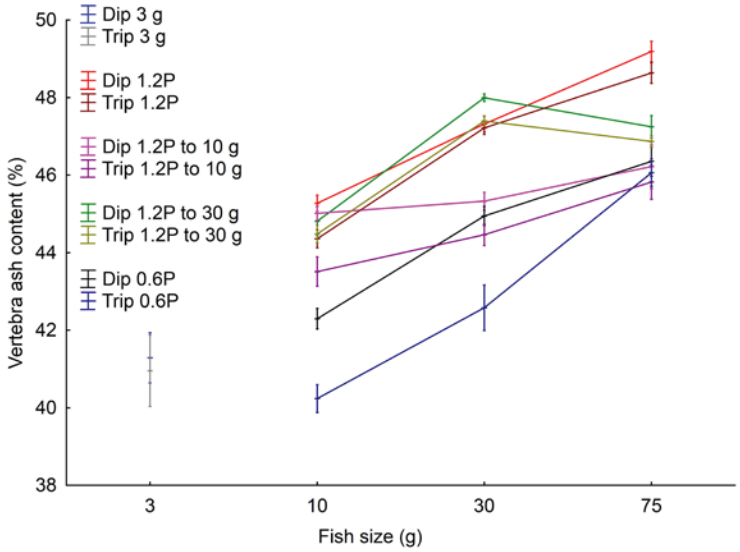

Figure 13. Vertebra ash content (% of defatted dry weight, mean ± S.E.) in diploid and triploid juvenile salmon fed: (*i*) 0.6% available P diet from 3 g until 75 g (Dip 0.6P, Trip 0.6P); (*ii*) 1.2% available P diet from 3 g until 10 g followed by 0.6% available P diet until 75 g (Dip 1.2P to 10g, Trip 1.2P to 10 g); (*iii*) 1.2% available P diet from 3 g until 30 g followed by 0.6% available P diet until 75 g (Dip 1.2P to 30g, Trip 1.2P to 30 g); (*vi*) 1.2% available P diet from 3 g until 75 g (Dip1.2P, Trip 1.2P). The diploid and triploid salmon used in the study were fed a commercial first feeding diet up to 3 g body weight.

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