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Title: NUTRIENT SUPPLEMENTATION ENHANCES SEAWATER GROWTH AND REDUCES SEVERITY OF VERTEBRAL MALFORMATION IN TRIPLOID ATLANTIC SALMON (*Salmo salar* L.)

Article Type: Research Paper

Keywords: Triploid; Salmon; Phosphorous; Nutrition; Deformity

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Abstract: Diploid (2N) and triploid (3N) sibling post-smolts were divided between six sea pens and fed: a standard nutrient package diet (2 x 2N SP, 2 x 3N SP), or an iso-energetic nutrient boosted package (2 x 3N BP) until market size. 3N groups initially grew significantly faster than 2N, and by harvest, 3N BP weighed significantly more (3210 ± 87 g) than 2N SP or 3N SP (3007 ± 64 g; 2965 ± 88 g), while there was no significant difference in weight between ploidy in SP diet. Higher visible vertebral ($9.6 \pm 0.4\%$) and jaw deformities ($10.6 \pm 1.2\%$) were observed in 3N compared to 2N ($0.9 \pm 0.1\%$; $1.3 \pm 0.5\%$). However, x-ray radiography revealed that 3N BP and 2N SP had comparable levels of severely affected individuals to that at time of sea transfer, while 3N SP showed a 3 fold increase in the severity of malformed individuals. The tail region (R3) in 3N SP fish had both the lowest vertebral strength and stiffness and the highest number of deformed vertebrae. Fillet quality attributes were comparable between diet and ploidy. These findings showed that triploid growth rate can be sustained until harvest throughout the seawater phase by using a nutrient boosted diet, and furthermore, the progression of spinal deformity beyond that at sea transfer can be stabilised by increasing dietary P during the marine phase.

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8th June 2015

Dear Prof. Gatlin,

Please find a research article entitled “Nutrient supplementation enhances seawater growth and reduces severity of vertebral malformation in triploid Atlantic salmon (*Salmo salar*)” for consideration for publication in Aquaculture. The study generates new data on dietary requirements of triploid Atlantic salmon that provides a significant improvement in farmed triploid welfare, allowing exploitation of faster growth rates and highlights the need to develop “triploid specific” aquafeeds rather than the use of conventional diploid diets.

We look forward to receiving your feedback.

Yours sincerely,

Dr. John Taylor
Research Fellow, Institute of Aquaculture

Highlights (for review)

- Triploid Atlantic salmon growth rate can be sustained during marine rearing using nutrient boosted diets
- Progression of skeletal malformation development can be prevented during marine rearing of triploid Atlantic salmon by increasing dietary phosphorous
- The occurrence of skeletal malformation in triploid Atlantic salmon must be addressed during freshwater rearing in the first instance

*Statement of Relevance

This study demonstrates that triploid Atlantic salmon have higher dietary requirements than their diploid siblings and that supplementing dietary phosphorous can prevent further progression of deformity during marine rearing. Tailored triploid specific aquafeeds must be formulated to support growth and prevent deformity in order to minimise welfare implications and allow exploitation of faster growth potential of triploid salmon within industry.

NUTRIENT SUPPLEMENTATION ENHANCES SEAWATER GROWTH AND REDUCES SEVERITY OF VERTEBRAL MALFORMATION IN TRIPLOID ATLANTIC SALMON (*Salmo salar* L.)

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Running Title: Nutrient supplementation supports triploid salmon development

Keywords: Triploid; Salmon; Phosphorous; Nutrition; Deformity.

Abstract

1 Diploid (2N) and triploid (3N) sibling post-smolts were divided between six sea pens
2 and fed: a standard nutrient package diet (2 x 2N SP, 2 x 3N SP), or an iso-energetic nutrient
3 boosted package (2 x 3N BP) until market size. 3N groups initially grew significantly faster
4 than 2N, and by harvest, 3N BP weighed significantly more ($3210 \pm 87\text{g}$) than 2N SP or 3N
5 SP ($3007 \pm 64\text{g}$; $2965 \pm 88\text{g}$), while there was no significant difference in weight between
6 ploidy in SP diet. Higher visible vertebral ($9.6 \pm 0.4\%$) and jaw deformities ($10.6 \pm 1.2\%$)
7 were observed in 3N compared to 2N ($0.9 \pm 0.1\%$; $1.3 \pm 0.5\%$). However, x-ray radiography
8 revealed that 3N BP and 2N SP had comparable levels of severely affected individuals to that
9 at time of sea transfer, while 3N SP showed a 3 fold increase in the severity of malformed
10 individuals. The tail region (R3) in 3N SP fish had both the lowest vertebral strength and
11 stiffness and the highest number of deformed vertebrae. Fillet quality attributes were
12 comparable between diet and ploidy. These findings showed that triploid growth rate can be
13 sustained until harvest throughout the seawater phase by using a nutrient boosted diet, and
14 furthermore, the progression of spinal deformity beyond that at sea transfer can be stabilised
15 by increasing dietary P during the marine phase.

16 **Introduction**

17 Commercial adoption of triploid Atlantic salmon (*Salmo salar*) is being considered in
18 Europe due to their potential for faster growth compared to diploids (Taylor et al., 2012;
19 Fraser et al., 2013b) and to remove the risk of interbreeding between escapees and wild
20 populations (McGinnty et al., 2003). However, although growth in freshwater is generally
21 superior than diploids (Fjellidal & Hansen, 2010; Taylor et al., 2012), it is the loss of growth
22 at sea (Fraser et al., 2013b; Taylor et al., 2013) and increase in skeletal deformity (Fjellidal &
23 Hansen, 2010; Leclercq et al., 2011; Taylor et al., 2011) and cataract (Taylor et al., 2015) that
24 have hindered full scale uptake as these traits reduce harvest weight (Hansen et al., 2010),
25 increase production time and downgrading (Michie et al., 2001), and raise welfare concerns
26 (Hansen et al., 2010). Aetiologies of skeletal malformations in diploid Atlantic salmon are
27 well documented and include high egg incubation temperatures (Wargelius et al., 2005),
28 genetic factors (Gjerde et al., 2005), vaccination (Berg et al., 2006), S0+ smolt regimes
29 (Fjellidal et al., 2006) and nutritional deficiencies (Lall & Lewis-McCrea, 2007) in particular
30 dietary phosphorous (P) (Baeverfjord et al., 1998; Fjellidal et al., 2009; Fjellidal et al., 2012).
31 It is now recognised that triploids should be treated as a ‘new species’ and environmental
32 optima, disease resistance, behavioural and nutritional requirements must be defined in order
33 that stock performance be at least comparable, if not better than diploids (Fraser et al.,
34 2012a).

35 In particular, definition of nutritionally complete aquafeeds will be essential in
36 triploid salmon culture in order to fully meet the nutritional requirements of the animals for
37 somatic growth and metabolic function. However, to date, virtually all studies exploring
38 production traits of triploids have used standard commercial diets formulated for diploids,
39 and specific experiments on triploid nutritional requirements are limited (Burke et al., 2010;
40 Fjeldal et al., 2015; Taylor et al., 2015). Triploids appear to have similar overall conversion,

41 utilisation and behavioural feeding characteristics (Olivia-Teles & Kaushik, 1990; Carter et
42 al., 1994; Preston et al., 2014). However, recent research suggests triploid fish may have a
43 higher nutritional requirement for growth than diploids in part related to altered metabolic
44 function and differential gene regulatory pathways. In rainbow trout (*Oncorhynchus mykiss*) a
45 series of studies have shown that triploids have increased fatty acid turnover due to increased
46 β -oxidation in the liver (Manor et al., 2015); increased potential for muscle protein gain
47 compared to diploids (Cleveland et al., 2012); lower expression of autophagy-related genes
48 (*atg4b* and *lc3b*), indicative of lower rates of protein catabolism (Cleveland & Weber, 2013);
49 improved anabolic signalling in peripheral tissues by increased free IGF-I in the plasma, and
50 altered expression of muscle regulatory factors, leading to improved myogenesis and muscle
51 growth (Cleveland & Weber, 2014). Given that the rate of protein accumulation in skeletal
52 muscle largely determines growth rate (Bureau et al., 2006) and that a positive correlation
53 exists between amino acid consumption and rate of protein synthesis (Houlihan et al., 1995),
54 it is possible that triploids have higher protein and amino acid requirements for growth if feed
55 intake cannot be increased to meet demand or dietary formulations are not sufficient to meet
56 requirements. To date no commercial feed charts exist for recommended feeding rates of
57 triploid Atlantic salmon. In addition specific dietary essential amino acid (EAA) and protein
58 requirement studies have yet to be conducted in triploid Atlantic salmon. However, evidence
59 exists to show a higher dietary histidine (His) requirement to prevent cataracts in triploids (17
60 vs. 12g kg⁻¹) (Taylor et al., 2015) while there is a known differential His requirement for
61 growth or cataract prevention in diploid salmon (Remo et al., 2014). Thus other protein and
62 essential amino acids requirement studies are essential as it is well established in diploid
63 salmon that EAA deficiencies such as methionine can lead to growth depression and
64 increased protein catabolism (Belghit et al., 2014).

65 Nutritional supplementation is also known to mitigate skeletal malformation in
66 diploid salmonids (Lall & Lewis-McCrea, 2007) and may have potential for improvement of
67 triploid skeletal health (Fraser et al., 2012a) particularly dietary phosphorous
68 supplementation. In diploid post-smolts, less skeletal deformity, higher mineral retention and
69 increased vertebral strength was observed in fish fed high dietary P (9.3g available P kg⁻¹)
70 than those without supplementation (6.3g available P kg⁻¹) when fed for 17 weeks
71 immediately following sea transfer (Fjelldal et al., 2009). By contrast a similar study using
72 comparable dietary P levels but at later stage (>200g) post-sea transfer found no beneficial
73 effect on malformation suggesting a stage specific requirement (Gil-Martens et al., 2012).
74 More recently, Fjelldal et al. (2015) demonstrated that feeding 9.4g total P kg⁻¹ to triploid
75 Atlantic salmon from first feeding throughout freshwater rearing minimised skeletal
76 malformations at the end of saltwater ongrowing and improved final weight when
77 subsequently fed a standard seawater diet relative to those previously fed a lower P diet (7.1g
78 total P kg⁻¹). However, feeding high P diets during hatchery rearing raises environmental
79 sustainability concerns due to the potential for eutrophication of freshwater bodies by
80 increased P discharge. As yet triploid dietary P requirements for optimal skeletal
81 development in saltwater are yet to be defined and may provide a means to stabilising
82 skeletal malformation while minimising environmental impacts.

83 Thus the aim of the present study was to investigate whether a diet supplemented with
84 increased dietary phosphorous and protein during seawater grow-out of triploid Atlantic
85 salmon could reduce vertebral malformations whilst sustaining growth in comparison to
86 triploids fed a standard commercial diploid diet.

87

88 2. Methods and Materials

89 2.1 Fish Stock and Husbandry

90 On 26th November 2010, fish eggs (20,000 / ploidy) from the Aquagen strain were
91 induced for triploidy at the Aquagen Broodstock Site, Hemne, Norway. Triploidy was
92 induced using a hydrostatic pressure shock of 655 bar applied 37 minutes post fertilisation for
93 6.25 minutes at 8°C. Eyed ova (~380 °days) were transferred to Marine Harvest Inchmore
94 Hatchery, Glenmorrison, Scotland (57°N, 5°W) on 13th of January 2011 and on-grown under
95 commercial protocols (Thermal regime: eye-hatch, $4.4 \pm 0.8^\circ\text{C}$; hatch-1st feed, $5.9 \pm 1.6^\circ\text{C}$).
96 First feeding fry were reared under constant light (LL) and ambient water temperature ($12.0 \pm$
97 2.2°C). On the 9th of August 2011, fry (~5g) were transferred to the Glenfinnan cage site and
98 raised in two separate pens 10 x 10 x 5m (1 / ploidy) under ambient photoperiod and water
99 temperature ($9.9 \pm 3.1^\circ\text{C}$) and fed a standard diploid salmon feed (Skretting, UK) according
100 to manufacturer's guidelines until sea transfer. Fish were vaccinated on the 16th November
101 2011 with Birnagen Forte. Completion of smoltification was verified in house by gill Na^+, K^+
102 ATPase activity (McCormick 1993) and skin silvering (Sigholt et al., 1995). Diploid control
103 groups had significantly smaller nuclear lengths than pressure shock triploid groups (2 N 6.9–
104 7.8 μm ; 3 N 9.1–10.2 μm) confirming that all fish that were subjected to hydrostatic pressure
105 shock were likely to be triploids. All experimental procedures and husbandry practices used
106 in the present study were conducted in compliance with the Animals Scientific Procedures
107 Act 1986 (Home Office Code of Practice) in accordance with EU regulation (EC Directive
108 86/609/EEC) and approved by the Animal Ethics and Welfare Committee of the University
109 of Stirling.

110

111 *2.2 Experimental Design*

112 On 5th April 2012, triploid smolts (mean weight 79.0 ± 17.4g) were transferred to
113 seawater (SW) at Marine Harvest Ardnish Farm Trial Unit, Lochailort, Scotland (57°N, 6°W)
114 and divided into four 10 x 10 x 15m pens, (n = 6625 / pen). Diploids (mean weight 88.0g ±
115 20.8g) smolted later and were transferred on 28th of April to two pens (n = 6625 / pen). All
116 fish up until the 20th of June were fed a standard commercial feed (Biomar, CPK) after which
117 duplicate pens of diploid and triploid smolts were fed a standard nutrient package (SP), while
118 a further two pens of triploids were fed a boosted nutrient package (BP). Feed formulations
119 for the experimental period are provided in Table 1. Fish were handfed three times daily in
120 accordance with manufacturer feeding table recommendations and feed recorded daily. Due
121 to the scale of the study no feed collection devices were used and satiation was observed
122 visually. Mortality, environmental data including water temperature, salinity, dissolved
123 oxygen and clarity was recorded on a daily basis (Fig. 1).

124

125 *2.3 Sampling Protocol*

126 In June, July, September and November 2012 a total of 100 fish / pen were
127 anaesthetised (50ppm MS222, Pharmaq, UK) and individual body weight (BW ± 10g) and
128 fork length (FL ± 0.5cm) recorded. Each fish was assessed for cataracts using a handheld
129 ophthalmoscope according to [Wall & Richards \(1992\)](#) and externally assessed for vertebral
130 and jaw deformities in accordance with [Taylor et al. \(2014\)](#). Weight data was used to
131 calculate thermal growth coefficient (TGC) and feed conversion rate (FCR) for each
132 sampling period until harvest where TGC was calculated as: $(W_f^{1/3} - W_i^{1/3}) \times (\sum D^0)^{-1}$, where W_f
133 is the final body weight, W_i is the initial body weight and D^0 is the cumulative sum of water
134 temperature in degrees per day. FCR was calculated as: $F / (B_f - B_i + B_m)^{-1}$ where F is the food

135 fed (kg) B_f is the final biomass (kg), B_i is the initial biomass (kg) and B_m is the mortality
136 biomass for the period (kg).

137 On the 7th February 2013 a final sampling was carried out prior to harvest. From the
138 100 fish anaesthetised / pen, terminal samples were collected (10 and 20/pen for 2N and 3N
139 respectively) using a percussive blow to the head and severing of the gill aorta in accordance
140 with schedule 1 UK Home Office procedure. Triploid fish were subjectively selected
141 according to normal/no visible deformity (n = 10 / pen) or the appearance of externally
142 observable lower jaw deformity (n = 5 / pen) or vertebral deformity (n = 5 / pen). The heart
143 was dissected out from each fish and preserved in 10% neutral buffered formalin. Fish were
144 number tagged using a cable tie, placed in polystyrene boxes, packed flat with ice and left for
145 72 hours to achieve rigor prior to fillet quality analysis.

146 From Feb 25th one pen per day was harvested according to commercial protocol. 500
147 fish per pen were individually assessed for externally visible deformities on each harvest day
148 to determine overall deformity prevalence within each cage population. All harvested fish
149 were classified as superior, ordinary or rebate according to Marine Harvest Quality standards.

150

151 *2.4 Parameters analysed*

152 *2.4.1 Fillet Quality*

153 Of terminal samples collected at harvest per pen (2N = 10 / pen; 3N = 20 / pen), the left
154 hand side fillet was carefully removed for flesh quality analysis carried out with the
155 assistance of Biomar (Grangemouth, UK). Fillets were assessed for pigmentation inside a
156 light box using Roche SalmoFan Lineal Card (Hoffman-La Roche, Basel, Switzerland)
157 scoring by two independent observers (Roche SOP). Fillets were then assessed for gaping and
158 texture (Biomar SOP). A Norwegian quality cut (NQC) was removed from each fillet and

159 frozen for later fatty acid composition analysis using near-infrared NIR analysis and
160 additional pigment analysis (Marine Harvest SOP).

161

162 *2.4.2 Texture Analysis*

163 Texture analysis was carried out according to [Johnston et al. \(2004\)](#). Briefly, two
164 cuboid sections of flesh were removed from the side fillet below the dorsal fin measuring
165 20mm x 40 mm x 40 mm and chilled to 4°C before analysis was carried out using a texture
166 analyser (TA-HDi Texture Analyser, Stable Micro Systems, Haslemere, UK) with a steadily
167 advancing Warner-Bratzler blade set to travel at 1 mm second⁻¹. The cutting load was
168 continuously recorded and used to calculate the maximum force (N) required and the total
169 work done (WD).

170

171 *2.4.3 Heart Morphology*

172 Sample hearts were pinned and photographed with the cranio-ventral surface facing
173 uppermost before being turned and photographed from a side view according to the method
174 of [Poppe et al. \(2003\)](#). Image analysis was carried out on each using Fiji (version 1.47b, NIH,
175 USA). Heart width and height was measured along with the angle of the bulbous arteriosis.
176 The heart was squeezed to remove excess fixative and weighed to calculate the cardio-
177 somatic index (CSI) such that $CSI = (100 \times \text{Heart Weight (HW)}) / \text{Body Weight (BW)}$.

178

179 *2.4.4 Vertebra Radiological Assessment*

180 After careful removal of the side fillet, two radiographs (anterior and posterior) were
181 taken of each fish using a portable x-ray unit (Celtic SMR PX40 HF) with an extremity plate
182 measuring 24 X 30 cm, and each plate exposed for 32 mAs at 40kV. Images were then
183 digitized (AGFA CR-35X) and radiographs examined using Adobe Photoshop CS 6 (version

184 13.0.1, Adobe system Incorporated, California, USA). The spine was divided into four
185 regions (R1, 2, 3, and 4) as per [Kacem et al. \(1998\)](#), and deformities classified based on
186 [Witten et al. \(2009\)](#), with the total number of vertebra recorded for each fish.

187

188 *2.4.5 Vertebra Mechanical Properties*

189 Vertebra number 6, 7, and 8 from the anterior region (R1), v28, 29 and 30 from the
190 middle region (R2/3) and v52, 53 and 54 from the caudal region (R4) were carefully
191 dissected out post radiography. Each vertebra was crushed individually (n = 3 vertebra /
192 region / pen) using a texture analyser fitted with a 10cm compression plate (TA-HDi Texture
193 Analyser, Stable Micro Systems, Haslemere, UK) to a distance of 4mm at a speed of
194 0.1mm/s. Yield Load (N), Stiffness (N / mm) and resilience (N x mm) were calculated for
195 each vertebra according to modified protocols of [Fjelldal et al. \(2004\)](#). After mechanical
196 crushing, the three vertebrae from each region were pooled and mechanically stripped of any
197 remaining flesh, defatted in baths of iso-hexane for 24 hours, oven dried at 105°C for 24
198 hours and incinerated at 600°C for 16 hours. Weights (1×10^{-3} mg) of dried and ashed
199 vertebrae were used to calculate the Bone Mineral content (BM%) of each region according
200 to [Fjelldal et al. \(2006\)](#) as Mineral content = (ashed weight / dry weight) x 100. Samples were
201 then digested in nitric acid using a Mars Microwave digestion system (10 min. heating phase
202 to 160°C, 20 min. at 160°C, 30 min. cooling phase) and analysed for inorganic elements by
203 Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a Thermo X Series II ICP-
204 MS (collision cell model). Percentage concentrations were calculated for Phosphorous,
205 calcium, magnesium, zinc and vanadium.

206

207 2.5 Statistics

208 Results are reported as mean \pm standard error of the mean (SEM). Statistical analysis
209 was carried out using Minitab (Version 16.2.3, Minitab Inc, Pennsylvania, USA). Differences
210 between weight, K, and flesh quality parameters were assessed using a general linear model
211 (GLM) and one-way ANOVA with replicates nested within treatment. Statistically significant
212 differences were considered as $p < 0.05$. Post hoc tests were carried out using Tukey's multiple
213 comparisons. Two-way ANOVA manipulated through GLM was used to analyse heart
214 morphology, bone mineral and bone strength attributes. All proportions were transformed
215 using arcsine and all data were checked for normality using a Kolmogorov-Smirnov test and
216 homogeneity of variance using Levene's test and observations of residual plots. X-rays of
217 deformed vertebrae were ranked according to severity and analysed for differences using a
218 PERMANOVA (Version 1.6, University of Auckland, New Zealand).

219

220 3. Results

221 3.1 Growth and Mortality

222 From June to September, both triploid groups maintained a significantly higher weight
223 than diploids (Fig. 2a). Furthermore, from July to September, 3N BP attained a significantly
224 higher weight than 3N SP. Greater weight was reflected in a higher TGC and more efficient
225 FCR (Fig. 2a,b) of both triploid diets than diploids during period 1. However, both 3N dietary
226 groups TGC significantly decreased and FCR was less efficient during period 2 and 3, as
227 such there was no significant difference in weight between any treatment by mid-November.
228 Diploids also showed a marked reduction in TGC and FCR efficiency between September
229 and November. This period of reduced growth and feeding efficiency (July-November)
230 coincided with a combined outbreak of amoebic gill disease (*Neoparamoeba perurans*) and
231 heart and skeletal muscle inflammation (HSMI). During this period there was also a

232 concomitant decrease in O₂ saturation and higher water temperature (Fig. 1). Cumulative
233 mortality levels were comparable (2N SP: 3.83 ± 0.68; 3N SP: 3.64 ± 0.34; 3N BP: 3.75 ±
234 1.21) during this period, and overall mortality for the duration of the trial did not differ
235 significantly between any treatment and was 6.7 ± 1.0, 7.4 ± 0.1 and 6.8 ± 1.8% for 2N SP,
236 3N SP and 3N BP respectively. From November onwards, water temperature cooled and fish
237 showed signs of recovery whereby 3N BP achieved a significantly greater final harvest
238 weight than 3N SP or 2N SP dietary groups. Irrespective of dietary treatment, triploids
239 maintained a significantly lower K than diploids from September until harvest (Fig. 2c).

240

241 *3.2 Deformity*

242 *3.2.1 Cataract and Externally Visible Deformity*

243 Cataract prevalence at harvest was very low in this study (incidence of 2.0 ± 1.0%) with
244 a mean score of 2.9 ± 1.6 for affected individuals and did not differ between ploidy (data not
245 shown).

246 At harvest both triploid dietary groups exhibited similar levels of external deformity
247 (19-21%) in comparison to diploids (~2.2%) with jaw and vertebral pathologies accounting
248 for approximately equal proportions of deformity (9.3-11.4%) within triploid treatments
249 (Table 2a).

250

251 *3.2.2 Radiological Deformity*

252 X-ray assessment showed that triploids had on average one less vertebra than diploids
253 (Table 2b). At smolt, 37% of diploids and 76.4% of triploids were classified as radiologically
254 deformed, having at least 1 or more deformed vertebra (dV). Triploids also had a
255 significantly higher number of dV per deformed fish than diploids at smolt, and only triploids
256 showed individuals with 6-9dV or ≥10dV (Table 2b).

257 At harvest, diploids showed a slight increase (+3%) in radiologically deformed
258 individuals (40%) compared to that at smolt, while triploids showed a greater increase
259 (+8.6%), with 85% of fish classified as radiologically deformed (Table 2b). 3N SP had a
260 significantly higher average no. dV per deformed fish than 3N BP, with 2N SP having
261 significantly lower average no. dV than either triploid dietary group. Finally, comparing fish
262 with ≥ 10 dV (i.e. likely to compromise welfare, Hansen et al., 2010) at harvest and smolt
263 showed a small increase in 2N SP (+5%) and 3N BP (+1.1%), but a notable increase
264 (+31.1%) in 3N SP (Table 2b). Furthermore, a greater proportion of triploids were classified
265 as having mild deformities (range 1-5dV) in the BP than SP diet (~45 % vs. 10%).

266 Deformed vertebrae were observed in all four spinal regions in triploids, but not in the
267 cranial trunk (R1) in diploids, with the predominate locality of all deformed vertebrae in the
268 tail region (R3), principally v39-v43, irrespective of ploidy (Fig 3a). Triploid dietary groups
269 did not differ significantly in total deformed vertebrae in R1 (Fig. 3b), while 3N SP had
270 significantly higher deformed vertebrae in R2 than 2N SP, with 3N BP intermediate to both,
271 and not differing significantly from either ploidy on the SP diet. A similar pattern was
272 reflected in the tail region (R3), with 3N BP showing a reduced prevalence to 3N SP, and
273 statistically comparable to 2N SP (Fig. 3b). Finally, no significant differences between
274 treatments were observed in the tail fin (R4). Of deformity types observed compression type
275 pathologies (type 2 predominant in diploids and type 5 in triploids) were most common
276 accounting for 43-63% of all deformed vertebra recorded, and symmetry deviations
277 accounting for 22-29% of all pathologies irrespective of ploidy (data not shown).

278 Ploidy and diet had a significant effect on vertebral L:H ratio (Fig. 3c). In R1 and R2
279 triploids had a significantly higher L:H ratio than diploids irrespective of diet. In R3, 3N SP
280 had a significantly higher L:H ratio than 3N BP (mean: 0.94 ± 0.00 vs. 0.92 ± 0.01),
281 predominantly evident in v45-v49, which were significantly higher than 2N SP (mean $0.89 \pm$

282 0.01). Finally, no significant difference in L:H ratio was observed between ploidy or diet in
283 R4.

284

285 *3.3 Vertebral Composition and Strength*

286 Total mineral content did not differ between spinal region in 2N SP or 3N SP groups
287 (Fig. 4a). There was no significant difference between R1 and R2/R3 within 3N BP, however,
288 R4 had a significantly lower mineral content than R2. Comparing all three treatments, R2/R3
289 had comparable total mineral content, while 2N SP had a significantly higher mineral content
290 than 3N BP in both R1 and R4.

291 Vertebral mineral analysis revealed no significant differences between regions for
292 specific minerals, as such all vertebra data were pooled per treatment (Table 3). Calcium
293 content was significantly higher in 2N SP than 3N BP, but not 3N SP, while phosphorous
294 content was significantly higher in 2N SP than either of the triploid groups. By contrast, Ca:P
295 ratio did not differ between any treatment. Magnesium content was significantly higher in 2N
296 SP than 3N BP, but not 3N SP, although no difference between triploids was observed.
297 Vanadium content was significant higher in 3N BP than either ploidy in the SP diet. Both 2N
298 SP and 3N SP had significantly higher vertebral zinc content than 3N BP.

299 Vertebral stiffness did not differ significantly within region between treatments (Fig.
300 4b). Lowest stiffness was generally observed in R1 and highest in R2/R3. Mechanical testing
301 showed significant differences in the yield load (N) between the three regions with R1
302 demonstrating the lowest yield load, R4 then R2/R3 (Fig. 4c). No significant difference
303 between the three dietary groups was found within R1 and R2/3. In R4, 3N SP showed a
304 significantly lower value than 2N SP. No significant differences were observed between 3N
305 BP and 2N SP. R2/R3 showed a significantly higher resilience (N x mm) than any other
306 region with the lowest resilience observed in R1 (Fig. 4d). No significant differences were

307 found between treatments within R1. In R2/R3 and R4, 2N SP resilience was significantly
308 higher than 3N SP but there was no statistical difference between 2N SP and 3N BP groups.

309

310 *3.4 Heart morphometrics*

311 No significant differences between ploidy or diet were found for CSI (0.17 - 0.18) and
312 H:W ratio (0.09 - 1.12). A significant difference was however found between the angle of the
313 bulbous arteriosis between the 2N SP (35.9 ± 1.6) and 3N SP (30.9 ± 1.4) but not the BP diet
314 (34.7 ± 1.1) (data not shown).

315

316 *3.5 Harvest Weight & Fillet Quality*

317 Size classification at harvest varied between the dietary groups and there was an
318 overall trend towards larger fish in triploids than diploids with triploid BP showing a greater
319 proportion of fish in the 3-4 and 4-5kg grades (Fig. 5a). By contrast, $51.6 \pm 3.78\%$ of fish
320 harvested in 2N SP weighed in the smaller weight class of 2-3kg compared to $32.9\% \pm 1.3\%$
321 in 3N BP and $38.2 \pm 2.8\%$ 3N SP.

322 In both diploids and triploids, fish with jaw malformation showed a lower harvest
323 weight than those without (Table 4). In diploids, vertebral deformity did not affect harvest
324 weight, by contrast, harvest weight was significantly higher in triploids with visible vertebral
325 deformity than those without. Condition factor was also significantly higher in the fish with
326 vertebral deformities in all ploidy groups, while those with jaw malformation showed a
327 tendency towards a lower condition factor (Table 4).

328 Final harvest saw a greater proportion of fish classed as superior in 2N SP than 3N SP
329 or 3N BP (Table 5a). Consequently, the proportion of fish classed as ordinary was higher for
330 the triploid dietary groups. Rebate for 3N SP was significantly higher than 3N BP. 2N SP had
331 the lowest level of rebate at harvest. The major cause of downgrading were mainly

332 attributable to thin, misshapen, runts and mechanical damage, with triploids showing a higher
333 relative proportion than diploids (Fig. 5b).

334 Total percentage fillet fat, DHA content, and ratio of n-3:n-6 fatty acids did not differ
335 significantly between ploidy or diet (Table 5b). EPA was significantly higher in 3N SP than
336 3N BP ($P = 0.01$). Fillet pigment content was significantly lower in both triploid groups
337 relative to diploids, but did not differ between triploid dietary groups (Table 5c). Although
338 Roche scores did not show significant difference between treatments and ploidy, scores did
339 correlate with reduced total pigment. Fillet texture, gaping, or mechanical strength showed no
340 significant difference between diet and ploidy (Table 5d).

341

342 **4. Discussion**

343 This study successfully demonstrated that triploid Atlantic salmon growth rate can be
344 superior to diploids, and more importantly, sustained until harvest when fed a nutrient
345 enriched diet rather than a conventional diploid diet. Furthermore, by supplementing dietary
346 P in the marine phase, progression of skeletal malformation was successfully stabilised and is
347 a major step forward towards improving triploid welfare.

348 Triploids fed the boosted nutrient diet showed significantly greater growth during the
349 trial, achieving a +7% greater mean body weight at harvest than diploids or triploids reared
350 on a standard commercial diploid diet. This is one of only two studies to show triploid growth
351 can actually be sustained at a higher rate over the entire marine phase (Oppedal et al., 2003).
352 By comparison, triploids fed the standard diet also showed a significantly higher growth rate
353 than diploids but only in the first 5 months before weight advantage was lost and this is
354 consistent with previous studies to date (O'Flynn et al., 1997; Friars et al., 2001; Cotter et al.,
355 2002; Leclercq et al., 2011; Fraser et al., 2013b; Taylor et al., 2013; Tibbets et al., 2013).

356 Collectively, these differences in growth potential of triploids between the two diets in the
357 current study clearly demonstrate that triploids do indeed appear to have a higher nutritional
358 requirement to support growth.

359 Given that the rate of protein accumulation in skeletal muscle largely determines
360 growth rate (Bureau et al., 2006) and that a positive correlation exists between amino acid
361 consumption and rate of protein synthesis (Houlihan et al., 1995) it is likely that triploids fed
362 the nutrient boosted package, benefited from increased inclusion of dietary protein, +7%,
363 which then facilitated sustained muscle growth. This would agree with observations in
364 triploid rainbow trout which have been shown to have higher protein synthesis rates
365 (Cleveland et al., 2012), reduced protein catabolism (Cleveland & Weber, 2013) and
366 improved myogenesis (Cleveland & Weber, 2014) which are all suggestive of different
367 metabolic rates between ploidy. In this respect, increasing dietary protein may also be having
368 an energy sparing effect (Tibbets et al., 2013) as non-protein energy source (oil) were
369 comparable between the standard and nutrient boosted diets, and given that triploids have
370 different fatty acid turnover rates (Manor et al., 2015) this sparing effect may be conserving
371 amino acids for protein biosynthesis. Finally, during the high growth periods of this study,
372 triploid FCR was also more efficient than diploids. Thus, in theory, per kilogram of feed
373 consumed triploids on the standard diet would be consuming less nutrients per kilogram of
374 muscle growth than would be theoretically available through the nutrient boosted package.
375 This potential nutrient shortfall may also reflect why triploids have been reported to have
376 higher feed intakes to compensate for resource deficiency (Cleveland & Weber, 2013) and
377 are more prone to nutritional deformities. Furthermore, feeding rates are traditionally
378 assessed in diploids through confirmation of satiation by surface observations. However,
379 observations in this study suggested a deeper feeding behaviour as previously reported in
380 brown trout, *Salmo trutta*, (Preston et al., 2014). If confirmed, this could mean satiation in

381 older studies may not have been met in triploids. Successful production of triploids will thus
382 rely on developing triploid specific feeding tables in addition to specific aquafeeds in order to
383 provide optimum nutrition.

384 From late July to November, triploid growth rate (TGC) in both dietary groups
385 dropped significantly compared to diploids. Peak water temperatures were achieved during
386 this period with a concomitant reduction in oxygen saturation. In a previous study at the same
387 site, [Taylor et al. \(2013\)](#) reported a similar drop in growth performance during the same
388 period with comparable oxygen and temperature profiles. These observations are consistent
389 with recent findings that showed triploid Atlantic salmon have reduced heart rate ([Atkins &
390 Benfey, 2008](#)), and lower aerobic metabolic scope at high temperature (19°C) and moderate
391 hypoxia (70% O₂ saturation) ([Hansen et al., 2015](#)). Thus the inability of triploid salmon to
392 withstand extended periods of high temperature and moderate hypoxia could set limits to the
393 geographical distribution of triploid salmon farming ([Hansen et al., 2015](#)). In the case of our
394 study the reduced growth performance is highly likely a result of a metabolic compromise
395 under the environmental conditions, further exacerbated by a combined outbreak of AGD and
396 HSMI during this period. However, of significant importance is that during this “challenge”
397 period, mortality rates did not differ between ploidy. Following a return to normal
398 environmental conditions, both triploid groups recovered to pre-challenge growth rates, albeit
399 recovery time was longer than diploids. Environmental and disease challenge pressures may
400 place further strain on the cardiac system. In this respect it was evident that triploids under
401 the standard diet had a more acute angle of the bulbus arteriosus at slaughter consistent with
402 other studies in Atlantic salmon ([Leclercq et al., 2011](#); [Fraser et al., 2013b](#)), suggestive that
403 triploids could experience more cardiac workload to diploids. Although triploid heart
404 morphology has been shown to be influenced by egg incubation temperature ([Fraser et al.,
405 2013a](#)) and vaccination ([Fraser et al., 2014b](#)), it was also evident in our study that diet equally

406 affected angle of the bulbus arteriosus, in that the nutrient boosted diet had a significantly
407 higher angle than those on the standard diet, and comparable to that in diploids. However,
408 what effect the angle of the bulbus arteriosus has on salmon heart function is currently
409 unknown, and future studies on cardiac performance in triploids are suggested.

410 Finally, both triploid groups had lower condition factors than diploids from early
411 autumn, suggestive of increased skeletal growth relative to muscle gain, and is again
412 consistent with previous studies. [Fjelldal et al. \(2015\)](#) suggested that this diverging pattern
413 between skeletal and somatic growth in triploids may have an effect on dietary P demand, as
414 an animal with a rapidly growing skeleton will need a higher mineral input to support normal
415 bone mineralization. However, examination of externally visible deformity at harvest did not
416 reveal a difference in occurrence between triploids on the nutrient boosted or standard dietary
417 packages. Nonetheless, and of fundamental importance was that x-ray radiography revealed
418 that triploids fed the supplemented diet (+30% dietary P) had three fold less fish (15 vs 45%)
419 with severe spinal deformities (i.e. $\geq 10dV$, that would be expected to affect welfare,
420 according to [Hansen et al., 2010](#)) than their triploid siblings fed a standard commercial diet.
421 Furthermore, the average number of deformed vertebrae per deformed fish remained more or
422 less the same at harvest as the point of sea transfer (smolt) in the supplemented group (5.8 to
423 6.0), but had doubled in the standard dietary triploid group (from 5.8 to 11.5). Thus the
424 progression of deformity during seawater in triploids was largely arrested by dietary
425 supplementation suggesting that deformity in triploids may indeed be tackled by diet during
426 the early seawater phase. Previously, mineral supplementation for 17 weeks following sea
427 transfer has been shown to reduce spinal malformation in diploid post-smolts ([Fjelldal et al.,](#)
428 [2009](#)). However, given the high prevalence of pre-existing malformation observed at smolt, a
429 greater emphasis must be placed on egg incubation regimes and first feeding diets of triploids
430 to minimise the occurrence of deformity in the first instance.

431 Malformation affected both weight and body morphology at harvest. Condition factor
432 and body weight was highest in those with compressive spinal deformities in accordance with
433 [Hansen et al. \(2010\)](#), which may be indicative of pathology associated with fast growth rates
434 under nutrient deficient conditions, while fish exhibiting jaw deformities were generally of
435 lower weight (-20%) than non-deformed fish, which may reflect impaired feeding or
436 respiratory ability ([Roberts et al., 2001](#); [Venegas et al., 2003](#); [Lijald & Powell, 2009](#); [Taylor
437 et al., 2013](#)). However, unlike spinal deformity, no positive effect of diet on reducing jaw
438 malformation was evident in our study, although dietary vitamin C and P supplementation
439 during seawater rearing have previously been suggested as preventive nutritional factors
440 ([Roberts et al., 2001](#)). Jaw malformation may also be caused by mechanical stress and
441 weakening of the lower jaw bones through excessive buccal-opercular pumping associated
442 with high temperatures and reduced oxygen availability. It has recently been shown that
443 incubating triploid eggs at lower temperature (6°C) to the point of eyeing significantly
444 reduced occurrence of jaw malformation ([Fraser et al., 2014a](#)), and that supplementing
445 dietary P from first feeding in combination with low temperature incubation ([Fjellidal et al.,
446 2015](#)) further reduces the occurrence of jaw deformity. This further supports the concept that
447 these skeletal weaknesses may be inbuilt from early life stages during freshwater and should
448 be tackled during the hatchery phase.

449 Fish bone strength is highly impacted by mechanical stress, and mechanical stimuli
450 induce extra strength and vertebral support in the form of mineralisation ([Lall & Lewis-
451 McCrea, 2007](#); [Ytteborg et al., 2013](#)). In particular, the tail region (R3) undergoes the greatest
452 mechanical strain due to lateral muscular activity and is the region most associated with
453 spinal pathology in seawater ([Fjeldall, et al., 2009](#); [Totland et al., 2011](#)). In our study this
454 region not only displayed the highest occurrence of vertebral deformity, but also the greatest
455 dietary effect on vertebral strength and morphology (L:H ratio). In general, diploids had the

456 highest bone strength properties in each respective region, while triploids fed the standard
457 diet generally the weakest properties, and those on the boosted diet were intermediary to
458 both, although differences were generally non-statistically significant. Decreased bone
459 mineralisation and increased vertebral deformities in fast growing fish are considered features
460 of a sub-optimal diet (Fjelldal & Hansen, 2010) that manifest as reduced vertebral strength
461 (Ytteborg et al., 2010) which were clearly evident in triploids fed the standard nutrient
462 package diet. Furthermore, triploids also had higher L:H ratios than diploids in R1-R3
463 indicative of more elongated vertebral bodies within these regions as observed in triploid
464 yearling smolts (Fraser et al., 2014a). In addition, L:H ratio was also affected by diet, being
465 significantly higher in triploids fed the standard diet compared to those fed the supplemented
466 diet in R3. Such changes in vertebral morphology could be attributed to the need for
467 elongation of individual vertebral bodies to compensate for compressive pathologies (the
468 most common pathology observed in this study) elsewhere within the spinal column, thus
469 providing increased strength within the spine while under mechanical strain, particularly
470 under conditions of mineral deficiency.

471 Hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) is the key mineral structure in bone and its
472 formation is limited through dietary P and Ca absorption directly from the aquatic
473 environment (Lall & Lewis-McCrea, 2007). NRC (2011) recommendations for dietary P for
474 diploid Atlantic salmon are estimated at 8 g Kg^{-1} available P. In seawater, Gil-Martens et al.
475 (2012) failed to observe a reduction in vertebral deformity in diploid post-smolts using 6 g or
476 9 g kg^{-1} available P, whereas Fjelldal et al. (2012) found a reduction in vertebral
477 malformation in diploid smolts when previously fed 11.7g kg^{-1} available P as opposed to 6.3g
478 and 8.9g kg^{-1} . In a more recent study on triploid smolts, vertebral malformation was
479 prevented when previously fed 12 g kg^{-1} available P rather than 4 g or 6 g kg^{-1} (Fjelldal, et al.,
480 2015). These observations are in agreement with Helland et al. (2005) who previously

481 suggested that commercial levels ($<10 \text{ g Kg}^{-1}$ total P) may be too low for fast growing
482 salmon to maintain skeletal integrity. In the current study we observed clear beneficial effects
483 on improved spinal health in triploid post-smolts by increasing total dietary P by +20% (9.9
484 vs. 12 g P kg^{-1}).

485 Phosphorus is not only important for bone growth but also plays an essential role in
486 many anabolic, catabolic and metabolic processes such as energy and DNA synthesis ([Burke
487 et al., 2010](#)). Maintenance processes taking precedence over bone mineralisation offer a
488 possible explanation of the high level of deformities in the triploid standard diet at the end of
489 the trial. Although severity of deformity was improved in the nutrient boosted package,
490 interestingly vertebral mineral content, P and Ca levels were in general lower than diploid
491 and triploids fed the standard diet, thus suggesting that improvement of vertebral integrity
492 through P supplementation is not simply through accumulation. Minerals such as P may be
493 used preferentially to facilitate higher growth rates in the supplemented diet without
494 compromising bone strength or stiffness. Higher levels of vanadium, a known biometal
495 suppressor of ECM mineralisation ([Tiago et al., 2008](#)) were also observed in triploids fed the
496 supplemented diet compared to the standard package diet, and may reflect suppressed
497 hydroxyapatite formation in the presence of sufficient mineral resources for skeletal
498 development, but as yet remains unclear. Certainly in the case of triploids fed the standard
499 diet, deformed fish had significantly higher weights at harvest suggestive of spinal deformity
500 being a function of fast growth under nutrient deficient conditions in the standard diet.
501 Collectively these results indicate that the nutrient boosted package appears to facilitate better
502 spinal mineralisation during development which would otherwise be compromised at the
503 expense of accelerated growth under a standard diet.

504 Irrespective of dietary treatment virtually all triploid flesh quality attributes were
505 comparable to the diploid control and concur with other studies in triploid Atlantic salmon

506 (Taylor et al., 2013). However, in this study we did observe a significant reduction in total
507 pigment in triploids relative to diploids. Differences in pigment and other flesh quality
508 attributes at harvest may also be highly influenced by season. Improved pigment retention
509 through reproductive arrestment has often been cited as a potential benefit for producers of
510 triploid salmon, although this has so far only been shown in rainbow trout (Choubert &
511 Blanc, 1989; Choubert et al., 1997). However, fish in our study were harvested in February
512 and would not be expected to entering into an active gonadal development at this stage,
513 therefore we cannot relate these pigment differences to differing maturation rates between
514 ploidy. A positive relationship between visual colour score and muscle fibre density
515 independent of chemical pigment content has been reported in Atlantic salmon (Johnston et
516 al., 2000), however, Bjørnevik et al. (2004) concluded that differences in muscle fibre
517 structure between ploidy are not a major factor influencing flesh redness. It was however
518 noted that texture may affect fillet redness, and similarly in our study we also found a
519 decrease in pigment content with increased texture score, albeit non-significant. Reduced
520 pigment deposition may also stem from the decreased surface area to volume ratio and/or
521 binding affinity of triploid cells and requires further study to elucidate differences between
522 ploidy.

523 In conclusion, this trial demonstrated that increased dietary supplementation of
524 protein and phosphorous can achieve a higher growth rate in triploids compared to diploids or
525 triploids fed a standard diploid seawater diet. Furthermore, of significant importance was that
526 the development of vertebral malformation beyond that present at time of seawater transfer
527 (i.e. smolt) could be stabilised and prevented from progressing further by increasing dietary P
528 supplementation. However, the incidence of malformation observed at time of sea transfer
529 still remains above ethically acceptable levels and supports reports in other studies whereby
530 the initial formation of deformities should be addressed in freshwater through triploid specific

531 diets and egg incubation temperatures. Finally, this study also provided anecdotal evidence
532 to suggest that triploid fish are not necessarily more susceptible to disease challenge, but they
533 are more sensitive to sub-optimal environmental conditions, particularly elevated temperature
534 and reduced oxygen saturation. Collectively, this study makes significant contributions
535 towards improving triploid welfare standards and achieving viable commercial
536 implementation.

537

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549

550 **Figure Legends**

551 **Figure 1.** Water temperature (°C; black line) and oxygen saturation (%; grey line line) for the
552 cage site during the trial period. Oxygen saturation has been corrected for salinity and
553 temperature.

554

555 **Figure 2.** Change in **A)** weight (symbols) and thermal growth coefficient (TGC, vertical
556 bars) for each growth period; **B)** feed conversion ratio (FCR) for each growth period; and **C)**
557 condition factor (K) of diploid and triploid Atlantic salmon fed a standard (SP) or nutrient
558 boosted package (BP) diets during seawater grow out. Lower case superscripts denote
559 significant differences between ploidy and diet.

560

561 **Figure 3.** **A)** Percentage of deformed vertebra along the vertebral column; **B)** Mean
562 percentage of total deformed vertebra within each spinal region; and **C)** Vertebral length-
563 height ratio (L:H) along the vertebral column in diploid and triploid Atlantic salmon fed a
564 standard (SP) or nutrient boosted package (BP) diet during seawater grow out. Lower case
565 superscripts denote significant differences between dietary treatments within region. The
566 vertebral column has been divided into four regions as defined by [Kacem et al., \(1998\)](#).

567

568 **Figure 4.** **A)** Total mineral content (% bone dry weight); **B)** Stiffness (N / mm), **C)** Yield
569 load (N) required to crush an individual vertebra; and **D)** resilience (N x mm) as a measure of
570 total energy required to crush a single vertebra for each of the three regions examined (R1 v6-
571 8; R2/3 v28-30 and R4 v52-54) at the end of seawater grow out in diploid and triploid
572 Atlantic salmon previously fed a standard (SP) or nutrient boosted package (BP) diet. Results
573 are the pool of three vertebra from each region per fish analysed. Lower case superscripts
574 denote significant differences within regions between dietary groups.

575

576 **Figure 5.** Final harvest data showing **A)** distribution of harvested fish weight classification
577 (2N SP: n=13,452; 3n SP: n= 11,075; 3N BP: n= 11854); and **B)** cause of downgrading at
578 final processing according to Marine Harvest Quality Standards.

579

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Table 1. Composition (%) of the standard nutrient diet (SP) and boosted nutrient diet (BP) fed during the experimental period.

	SP	BP
Diets as formulated (%)		
Fish/Crustacean meal	25.1	28.3
Pea protein	1.2	1.2
Soy Protein Concentrate	3.8	4.6
Corn gluten	8.3	8.2
Sunflower expeller	10.0	5.7
Wheat	7.0	5.4
Wheat gluten	7.6	6.6
Dehulled beans	5.8	7.1
Fish oil	13.4	13.9
Rape oil	14.6	14.9
Additives*	3.1	4.2
Nutritional content		
Oil (%)†	32.8	32.8
Protein (%)‡	37.6	40.1
Energy (KJ/g) ‡	24.8	24.8
Total Phosphorus (%)§	0.99	1.20

* BioFish premix (not commercially available) with additional Essential Amino Acids

† Nutritional Analytical Service, University of Stirling, UK

‡ BioMar, Grangemouth, UK

§ Eurofins, Denmark

Table 2. A) Total visible external deformity (% , mean \pm SEM) observed at smolt (n = 72-92 ploidy) and at harvest (n = 500 / pen) for diploid (2N) and triploid (3N) fed the standard nutrient diet (SP) or boosted nutrient diet (BP). **B)** Radiological deformed vertebra (dV) and severity of affected vertebra per deformed fish at smolt (n = 72-92 ploidy) and at harvest (n = 20 / ploidy / diet) for fish exhibiting no externally visible signs of deformity. Lower case superscripts denote significant differences between ploidy at smolt, or between dietary treatments and ploidy at harvest.

	Smolt		Harvest		
	2N	3N	2N SP	3N SP	3N BP
A.) External Visible Deformity					
None (%)	n/a	n/a	97.8 \pm 0.1 ^a	81.0 \pm 1.3 ^b	79.7 \pm 2.9 ^b
Jaw (%)	n/a	n/a	0.9 \pm 0.0 ^b	9.7 \pm 2.3 ^a	11.4 \pm 2.6 ^a
Vertebral (%)	n/a	n/a	1.3 \pm 0.5 ^b	9.3 \pm 0.5 ^a	9.9 \pm 0.1 ^a
B.) Radiological Vertebral Deformity					
Ave. V No.	59.4 ^a	58.4 ^b	59.2 \pm 0.2 ^a	58.4 \pm 0.1 ^b	58.4 \pm 0.1 ^b
Ave. no. dV	1.9 ^b	5.8 ^a	3.3 \pm 0.0 ^c	11.5 \pm 1.6 ^a	6.0 \pm 1.6 ^b
0dV (%)	63.0	23.6	60.0 \pm 0.0 ^a	15.0 \pm 5.0 ^b	15.0 \pm 5.0 ^b
1-5dV (%)	37.0	43.1	25.0 \pm 5.0 ^{ab}	10.0 \pm 10.0 ^b	45.0 \pm 5.0 ^a
6-9dV (%)	0.0	19.4	10.0 \pm 0.0 ^b	30.0 \pm 0.0 ^a	25.0 \pm 5.0 ^a
\geq 10dV (%)	0.0	13.9	5.0 \pm 5.0 ^b	45.0 \pm 5.0 ^a	15.0 \pm 10.0 ^b

n/a: not assessed

Table 3. Mineral content (%) of the vertebrae for diploid (2N) and triploid (3N) fed a standard nutrient (SP) or a boosted nutrient (BP) diet. Significant differences between treatments are denoted using lower case superscripts.

	2N SP	3N SP	3N BP
Ca	13.10 ± 0.03^a	12.70 ± 0.39^{ab}	12.18 ± 0.0^b
P	6.76 ± 0.10^a	6.58 ± 0.1^b	6.25 ± 0.0^b
Ca:P	1.94 ± 0.02	1.93 ± 0.03	1.95 ± 0.01
Mg	$0.172^a \pm 0.001$	$0.169^{ab} \pm 0.003$	$0.160^b \pm 0.001$
V	$3.26 * 10^{-3} 0.14^b$	$3.13 * 10^{-3} \pm 0.20^b$	$4.21 * 10^{-3} \pm 0.15^a$
Zn	0.0119 ± 0.001^a	0.0118 ± 0.0001^a	0.0105 ± 0.0001^b

Table 4. Breakdown of harvest weight and condition factor (mean \pm SEM) into fish exhibiting no externally visible signs of deformity (2N SP n=195; 3N SP n=160; 3N BP n=147); Jaw; those exhibiting jaw deformity (2N SP n=2; 3N SP n=21; 3N BP n=38) and Vertebral; individuals with externally visible vertebral deformity (2N SP n=3; 3N SP n=19; 3N BP n = 12). Upper case superscripts denote significant differences between the three categories ('no visible deformity', 'jaw' and 'vertebral') within a given treatment, while lower case superscripts denote significant differences between treatments within each category.

	2N SP	3N SP	3N BP
Harvest Weight (g)			
No visible deformity	3010 \pm 40 ^{bA}	2900 \pm 50 ^{bB}	3270 \pm 0 ^{aB}
Jaw	2430 \pm 90 ^{aB}	2830 \pm 270 ^{aB}	2730 \pm 0 ^{aC}
Vertebra	2960 \pm 0 ^{bA}	3680 \pm 130 ^{aA}	3480 \pm 30 ^{aA}
Condition Factor (K)			
No visible deformity	1.42 \pm 0.01 ^{aAB}	1.32 \pm 0.02 ^{bB}	1.34 \pm 0.01 ^{bB}
Jaw	1.38 \pm 0.05 ^{aB}	1.37 \pm 0.07 ^{aB}	1.28 \pm 0.01 ^{aC}
Vertebra	1.51 \pm 0.00 ^{aA}	1.58 \pm 0.05 ^{aA}	1.51 \pm 0.02 ^{aA}

Table 5. Harvest summary of (A) percentage grading of harvested fish classified as superior, ordinary or rebate (Fish scored according to Marine Harvest quality standards); (B) fillet fat content; (C) fillet colour and total pigment; and (D) Mechanical and textural properties. Significant differences between treatments are denoted using lower case superscripts. **NB:** all data presented (B-C) is taken from the fish classed as showing no signs of external deformity (n=20 / ploidy / diet).

	2N SP	3N SP	3N BP
A) Harvest Grade (% Total Harvest)			
Superior	95.0 ± 1.2 ^a	80.0 ± 1.7 ^b	83.1 ± 1.3 ^b
Ordinary	3.7 ± 0.9 ^b	13.3 ± 2.0 ^a	13.6 ± 1.3 ^a
Rebate	1.4 ± 0.3 ^c	6.7 ± 0.3 ^a	3.3 ± 0.0 ^b
B) Fat Analysis (%)			
NQC Fat	11.61 ± 0.43	11.72 ± 0.06	11.84 ± 0.77
Calculated SQC Fat	19.84 ± 0.68	20.15 ± 0.73	19.35 ± 0.20
DHA	0.93 ± 0.02	0.90 ± 0.01	1.04 ± 0.06
EPA	0.63 ± 0.01 ^{ab}	0.68 ± 0.02 ^a	0.60 ± 0.01 ^b
Ratio n-3:n-6	1.93 ± 0.02	2.00 ± 0.03	2.03 ± 0.17
C) Fillet Colour			
Pigment (mg/kg)	5.87 ± 0.24 ^a	5.11 ± 0.07 ^b	4.81 ± 0.09 ^b
Roche Average	26.60 ± 0.25	26.25 ± 0.38	25.92 ± 0.01
D) Fillet Texture and Mechanical Properties			
Texture	2.95 ± 0.15	3.05 ± 0.10	3.13 ± 0.18
Gaping	1.30 ± 0.10	1.05 ± 0.10	1.28 ± 0.08
Cutting Force (N)	17.63 ± 0.76	17.98 ± 0.82	17.08 ± 0.23
Total Work (mJ)	160.5 ± 5.6	166.6 ± 8.0	159.6 ± 1.6

Figure 1

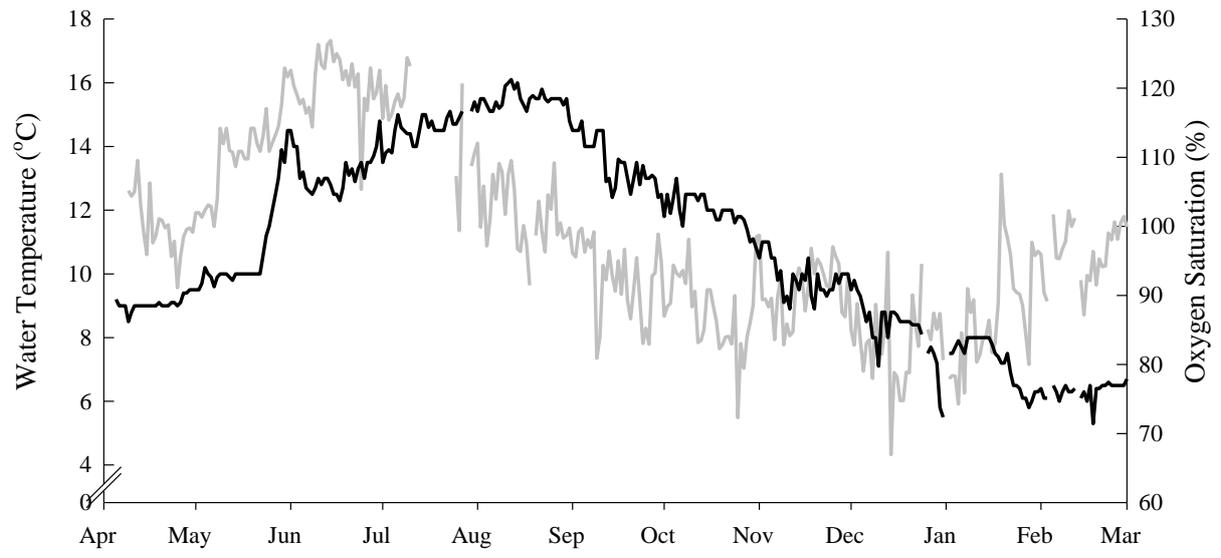


Figure 1.

Figure 2

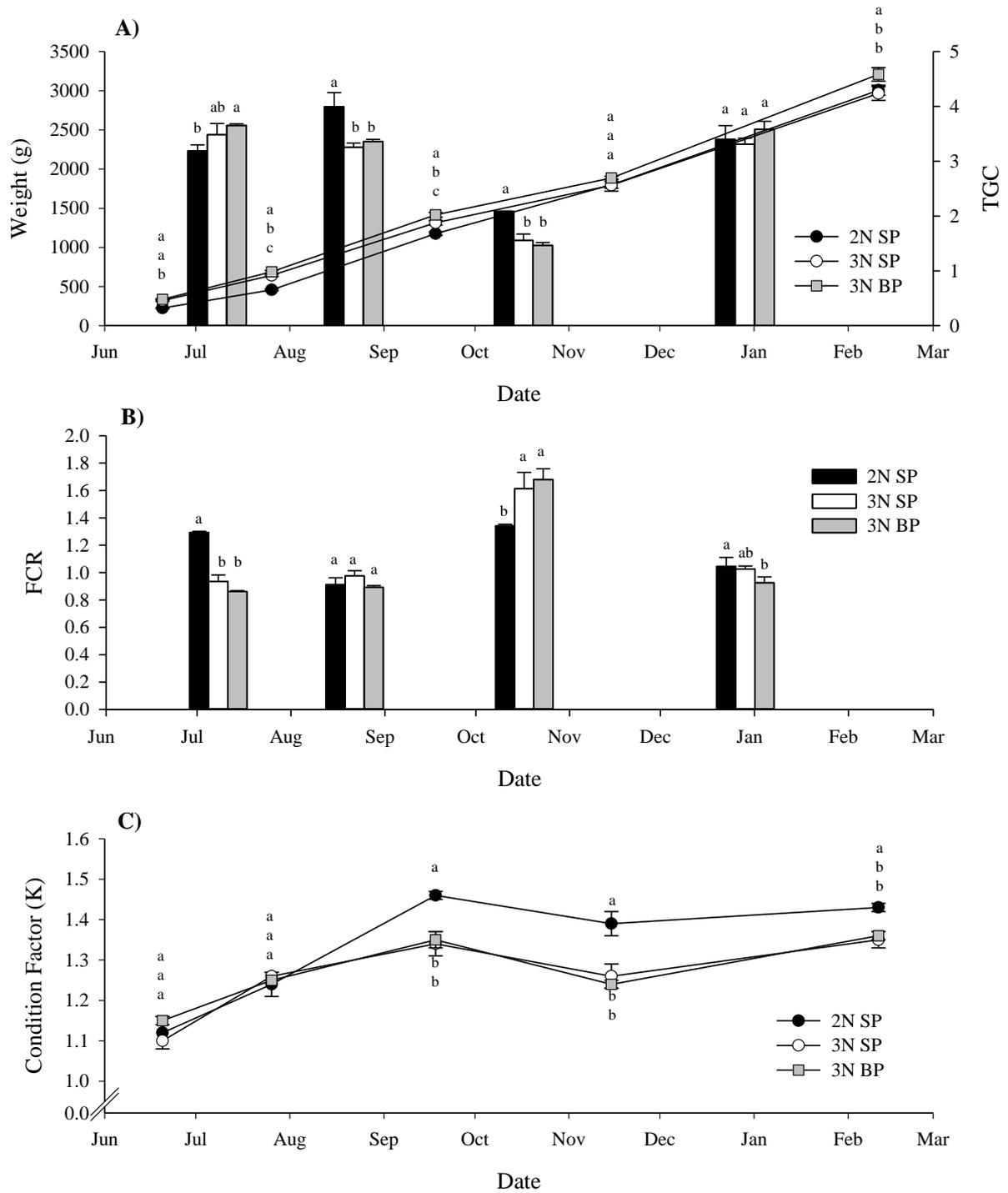


Figure 2.

Figure 3

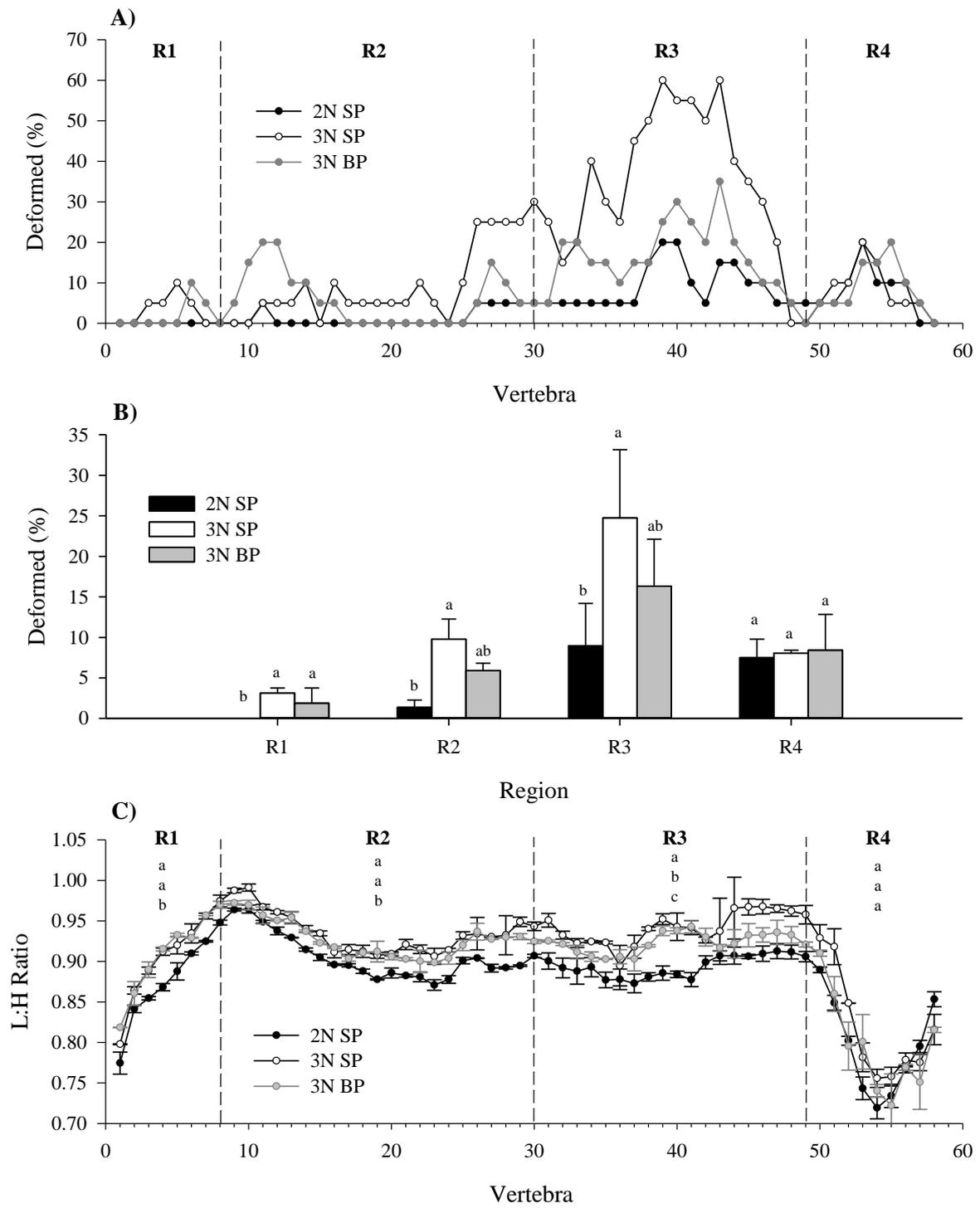


Figure 3.

Figure 4

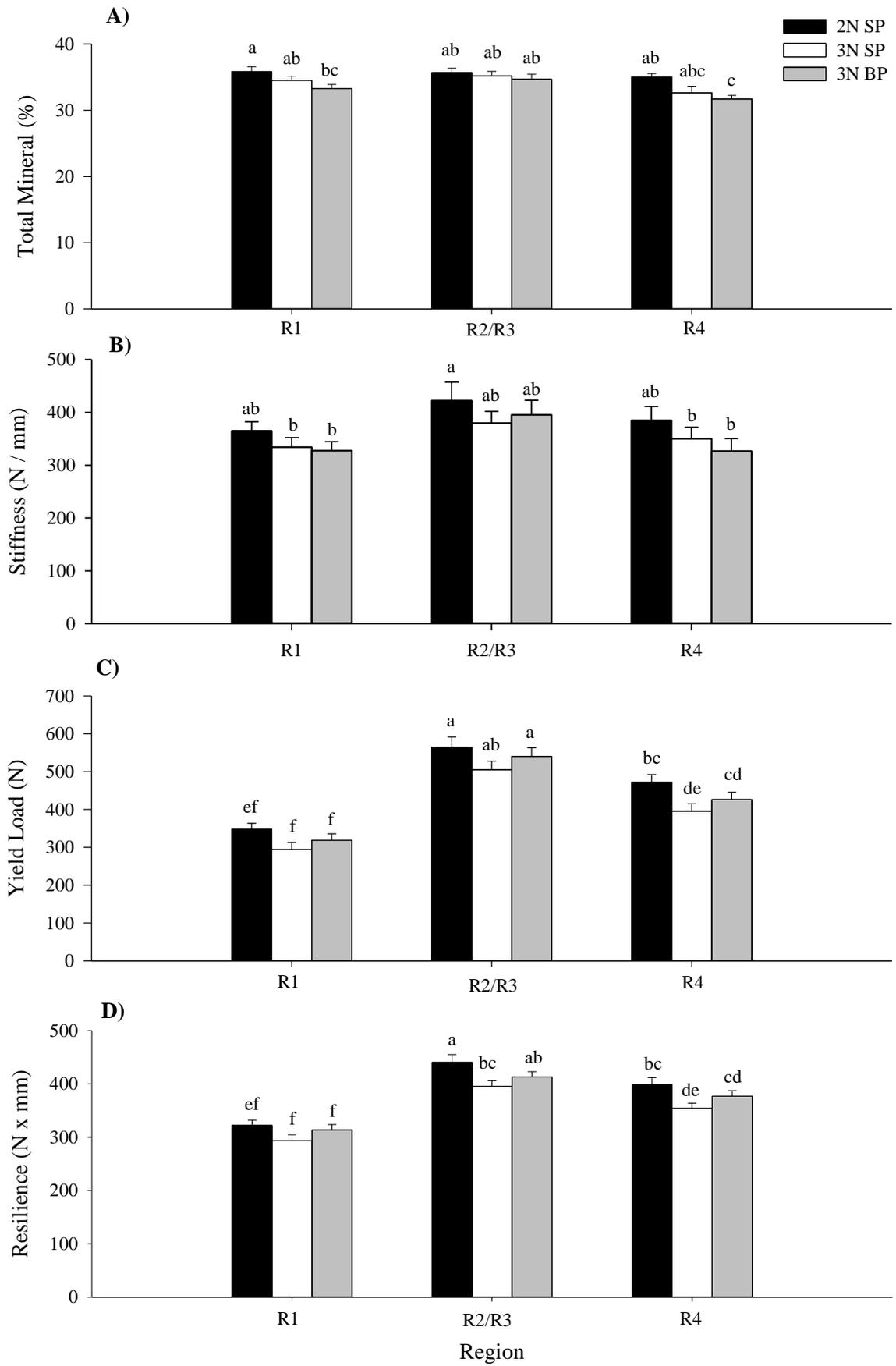


Figure 4.

Figure 5

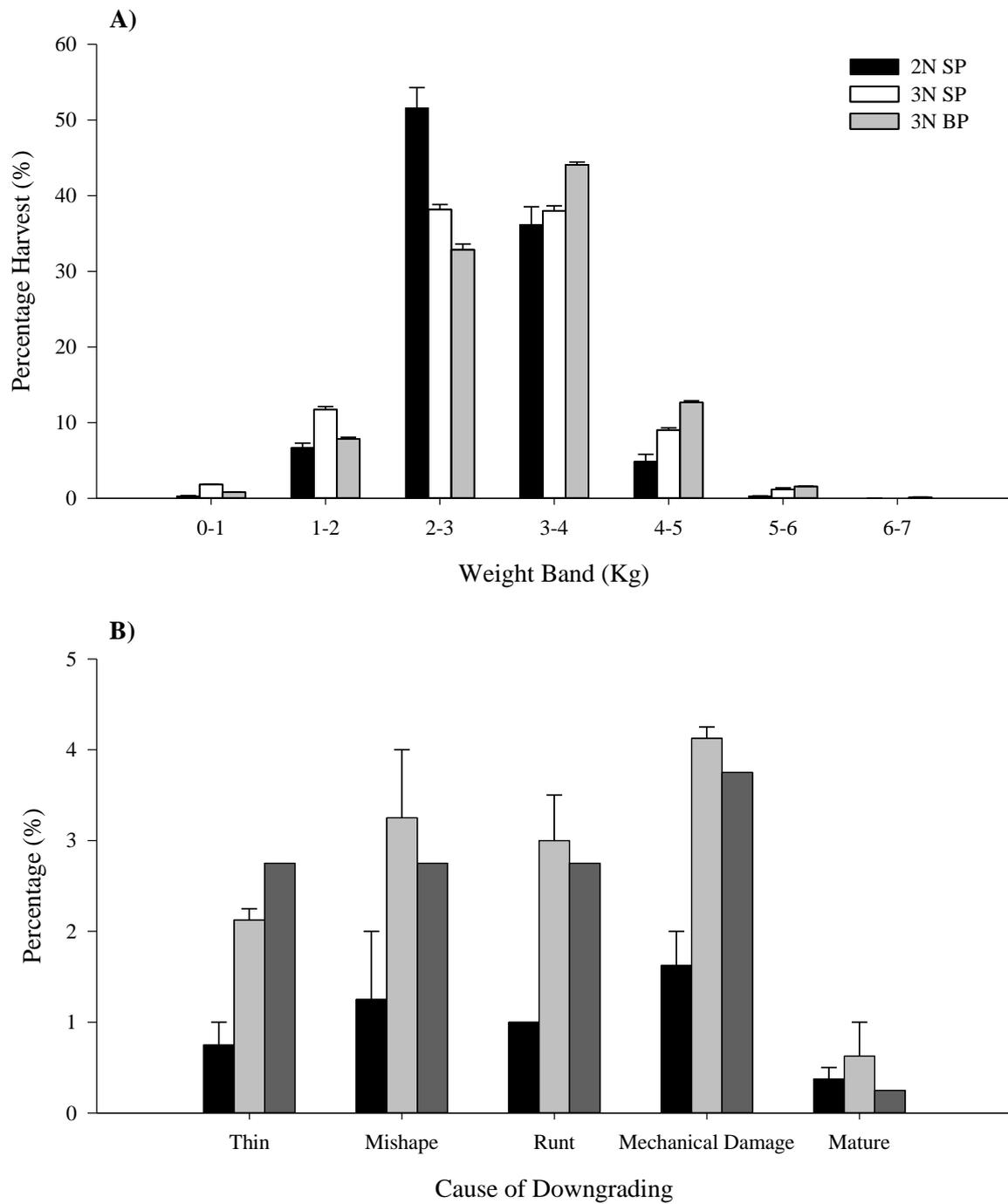


Figure 5.