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

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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 ± 87g) than 2N SP or 3N SP (3007± 64g; 2965 ± 88g), while there was no significant difference in weight between ploidy in SP diet. Higher visible vertebral (9.6 ± 0.4%) and jaw deformities (10.6 ± 1.2%) were observed in 3N compared to 2N (0.9 ± 0.1%; 1.3 ± 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**

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 ± 87g) than 2N SP or 3N SP (3007± 64g; 2965 ± 88g), while there was no significant difference in weight between ploidy in SP diet. Higher visible vertebral (9.6 ± 0.4%) and jaw deformities (10.6 ± 1.2%) were observed in 3N compared to 2N (0.9 ± 0.1%; 1.3 ± 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.

## Introduction

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

In particular, definition of nutritionally complete aquafeeds will be essential in triploid salmon culture in order to fully meet the nutritional requirements of the animals for somatic growth and metabolic function. However, to date, virtually all studies exploring production traits of triploids have used standard commercial diets formulated for diploids, and specific experiments on triploid nutritional requirements are limited (Burke et al., 2010; 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

## 2. Methods and Materials

### *2.1 Fish Stock and Husbandry*

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

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: $(W_f^{1/3}-W_i^{1/3}) \times (\sum D^o)^{-1}$, where $W_f$
133 is the final body weight, $W_i$ is the initial body weight and $D^o$ 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

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

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

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

*2.4 Parameters analysed*

*2.4.1 Fillet Quality*

Of terminal samples collected at harvest per pen (2N = 10 / pen; 3N = 20 / pen), the left hand side fillet was carefully removed for flesh quality analysis carried out with the assistance of Biomar (Grangemouth, UK). Fillets were assessed for pigmentation inside a light box using Roche SalmoFan Lineal Card (Hoffman-La Roche, Basel, Switzerland) scoring by two independent observers (Roche SOP). Fillets were then assessed for gaping and 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 x Heart Weight (HW)) / 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

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

*2.4.5 Vertebra Mechanical Properties*

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

*2.5 Statistics*

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

## 3. Results

*3.1 Growth and Mortality*

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

concomitant decrease in $O_2$ saturation and higher water temperature (Fig. 1). Cumulative mortality levels were comparable (2N SP: 3.83 ± 0.68; 3N SP: 3.64 ± 0.34; 3N BP: 3.75 ± 1.21) during this period, and overall mortality for the duration of the trial did not differ significantly between any treatment and was 6.7 ± 1.0, 7.4 ± 0.1 and 6.8 ± 1.8% for 2N SP, 3N SP and 3N BP respectively. From November onwards, water temperature cooled and fish showed signs of recovery whereby 3N BP achieved a significantly greater final harvest weight than 3N SP or 2N SP dietary groups. Irrespective of dietary treatment, triploids maintained a significantly lower K than diploids from September until harvest (Fig. 2c).

*3.2 Deformity*

*3.2.1 Cataract and Externally Visible Deformity*

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

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

*3.2.2 Radiological Deformity*

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

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

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

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

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

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

*3.4 Heart morphometrics*

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

*3.5 Harvest Weight & Fillet Quality*

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

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

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

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

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

## 4. Discussion

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

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

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

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

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

From late July to November, triploid growth rate (TGC) in both dietary groups dropped significantly compared to diploids. Peak water temperatures were achieved during this period with a concomitant reduction in oxygen saturation. In a previous study at the same site, Taylor et al. (2013) reported a similar drop in growth performance during the same period with comparable oxygen and temperature profiles. These observations are consistent with recent findings that showed triploid Atlantic salmon have reduced heart rate (Atkins & Benfey, 2008), and lower aerobic metabolic scope at high temperature (19˚C) and moderate hypoxia (70% $O_2$ saturation) (Hansen et al., 2015). Thus the inability of triploid salmon to withstand extended periods of high temperature and moderate hypoxia could set limits to the geographical distribution of triploid salmon farming (Hansen et al., 2015). In the case of our study the reduced growth performance is highly likely a result of a metabolic compromise under the environmental conditions, further exacerbated by a combined outbreak of AGD and HSMI during this period. However, of significant importance is that during this "challenge" period, mortality rates did not differ between ploidy. Following a return to normal environmental conditions, both triploid groups recovered to pre-challenge growth rates, albeit recovery time was longer than diploids. Environmental and disease challenge pressures may place further strain on the cardiac system. In this respect it was evident that triploids under the standard diet had a more acute angle of the bulbus arterious at slaughter consistent with other studies in Atlantic salmon (Leclercq et al., 2011; Fraser et al., 2013b), suggestive that triploids could experience more cardiac workload to diploids. Although triploid heart morphology has been shown to be influenced by egg incubation temperature (Fraser et al., 2013a) and vaccination (Fraser et al., 2014b), it was also evident in our study that diet equally

406 affected angle of the bulbus arterious, 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. ≥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.

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

Fish bone strength is highly impacted by mechanical stress, and mechanical stimuli induce extra strength and vertebral support in the form of mineralisation (Lall & Lewis-McCrea, 2007; Ytteborg et al., 2013). In particular, the tail region (R3) undergoes the greatest mechanical strain due to lateral muscular activity and is the region most associated with spinal pathology in seawater (Fjeldall, et al., 2009; Totland et al., 2011). In our study this region not only displayed the highest occurrence of vertebral deformity, but also the greatest 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 ($Ca_5(PO_4)_3(OH)$) 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 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

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

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**Figure Legends**

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

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 ± 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 ± 0.1$^{a}$ | 81.0 ± 1.3$^{b}$ | 79.7 ± 2.9$^{b}$ |
| Jaw (%) | n/a | n/a | 0.9 ± 0.0$^{b}$ | 9.7 ± 2.3$^{a}$ | 11.4 ± 2.6$^{a}$ |
| Vertebral (%) | n/a | n/a | 1.3 ± 0.5$^{b}$ | 9.3 ± 0.5$^{a}$ | 9.9 ± 0.1$^{a}$ |
| **B.) Radiological Vertebral Deformity** | | | | | |
| Ave. V No. | 59.4$^{a}$ | 58.4$^{b}$ | 59.2 ± 0.2$^{a}$ | 58.4 ± 0.1$^{b}$ | 58.4 ± 0.1$^{b}$ |
| Ave. no. dV | 1.9$^{b}$ | 5.8$^{a}$ | 3.3 ± 0.0$^{c}$ | 11.5 ± 1.6$^{a}$ | 6.0 ± 1.6$^{b}$ |
| 0dV (%) | 63.0 | 23.6 | 60.0 ± 0.0$^{a}$ | 15.0 ± 5.0$^{b}$ | 15.0 ± 5.0$^{b}$ |
| 1-5dV (%) | 37.0 | 43.1 | 25.0 ± 5.0$^{ab}$ | 10.0 ± 10.0$^{b}$ | 45.0 ± 5.0$^{a}$ |
| 6-9dV (%) | 0.0 | 19.4 | 10.0 ± 0.0$^{b}$ | 30.0 ± 0.0$^{a}$ | 25.0 ± 5.0$^{a}$ |
| ≥10dV (%) | 0.0 | 13.9 | 5.0 ± 5.0$^{b}$ | 45.0 ± 5.0$^{a}$ | 15.0 ± 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** | $13{\cdot}10 \pm 0{\cdot}03^{a}$ | $12{\cdot}70 \pm 0{\cdot}39^{ab}$ | $12{\cdot}18 \pm 0{\cdot}0^{b}$ |
| **P** | $6{\cdot}76 \pm 0{\cdot}10^{a}$ | $6{\cdot}58 \pm 0{\cdot}1^{b}$ | $6{\cdot}25 \pm 0{\cdot}0^{b}$ |
| **Ca:P** | $1{\cdot}94 \pm 0{\cdot}02$ | $1.93 \pm 0.03$ | $1{\cdot}95 \pm 0{\cdot}01$ |
| **Mg** | $0{\cdot}172^{a} \pm 0{\cdot}001$ | $0{\cdot}169^{ab} \pm 0{\cdot}003$ | $0{\cdot}160^{b} \pm 0{\cdot}001$ |
| **V** | $3{\cdot}26*10^{-3}\ 0{\cdot}14^{b}$ | $3{\cdot}13*10^{-3} \pm 0{\cdot}20^{b}$ | $4{\cdot}21*10^{-3} \pm 0{\cdot}15^{a}$ |
| **Zn** | $0{\cdot}0119 \pm 0{\cdot}001^{a}$ | $0{\cdot}0118 \pm 0{\cdot}0001^{a}$ | $0{\cdot}0105 \pm 0{\cdot}0001^{b}$ |

**Table 4.** Breakdown of harvest weight and condition factor (mean ± 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**

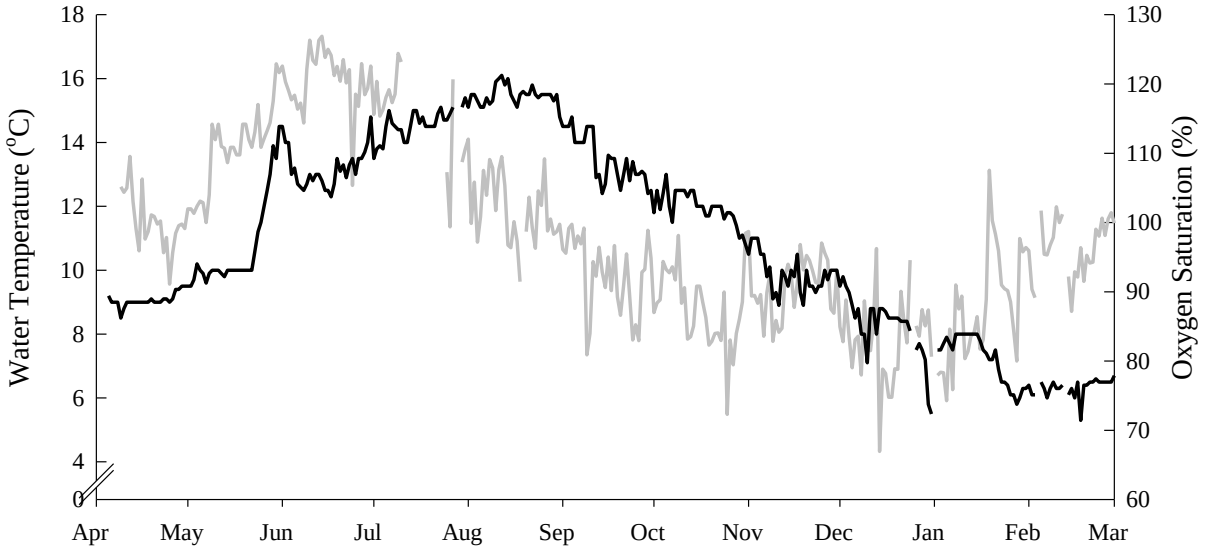


Figure 1.

**Figure 2**

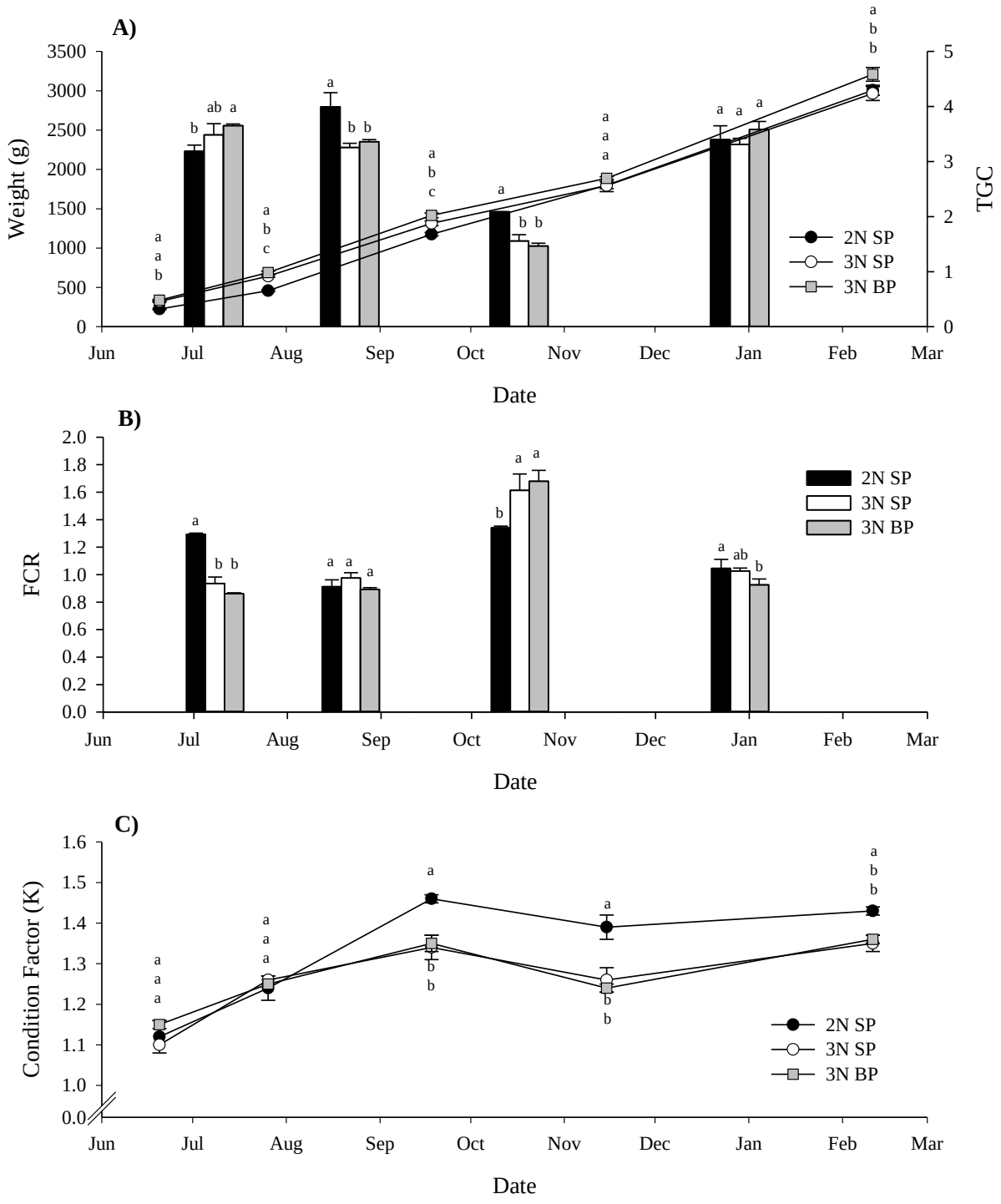


Figure 2.

**Figure 3**

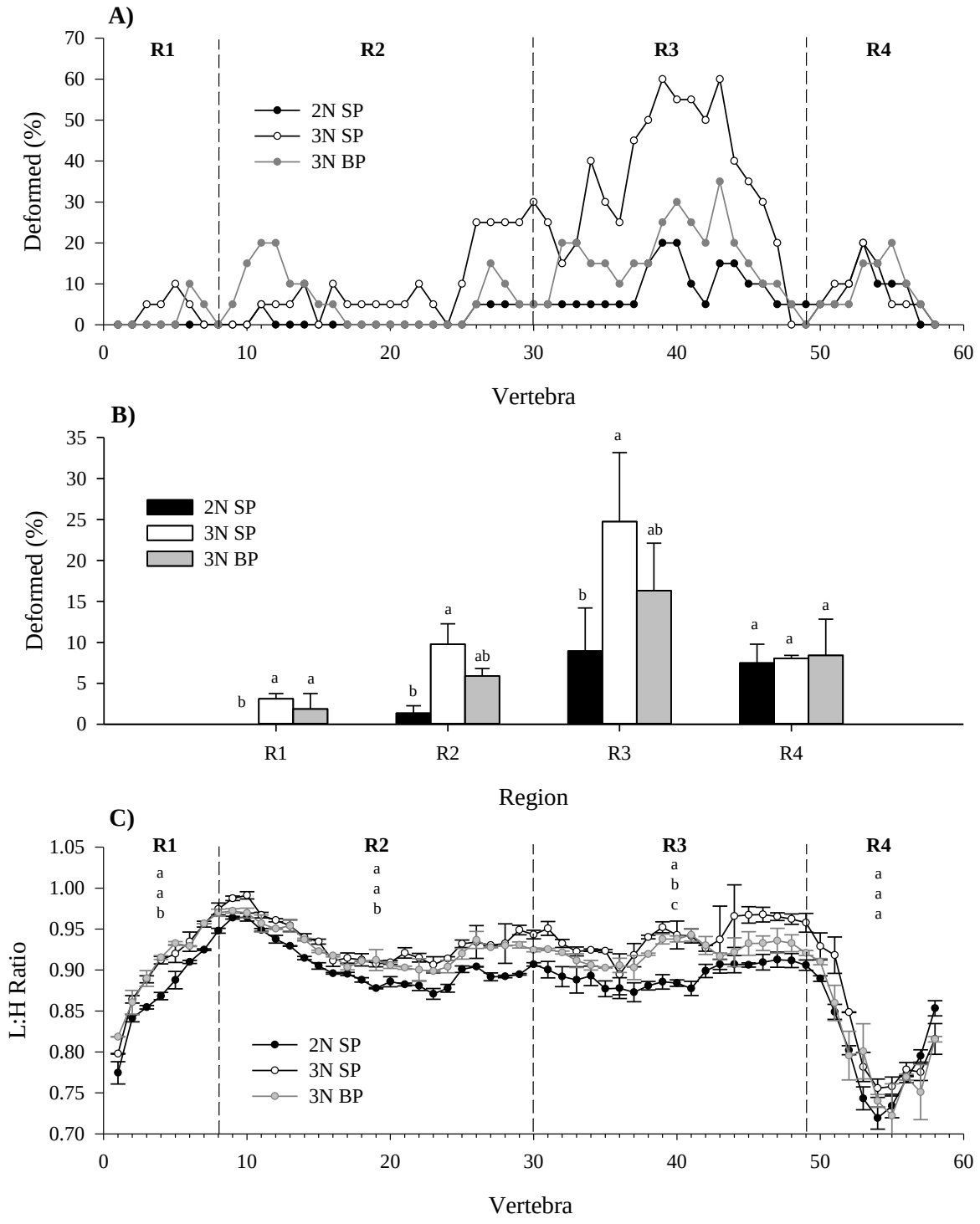


Figure 3.

**Figure 4**

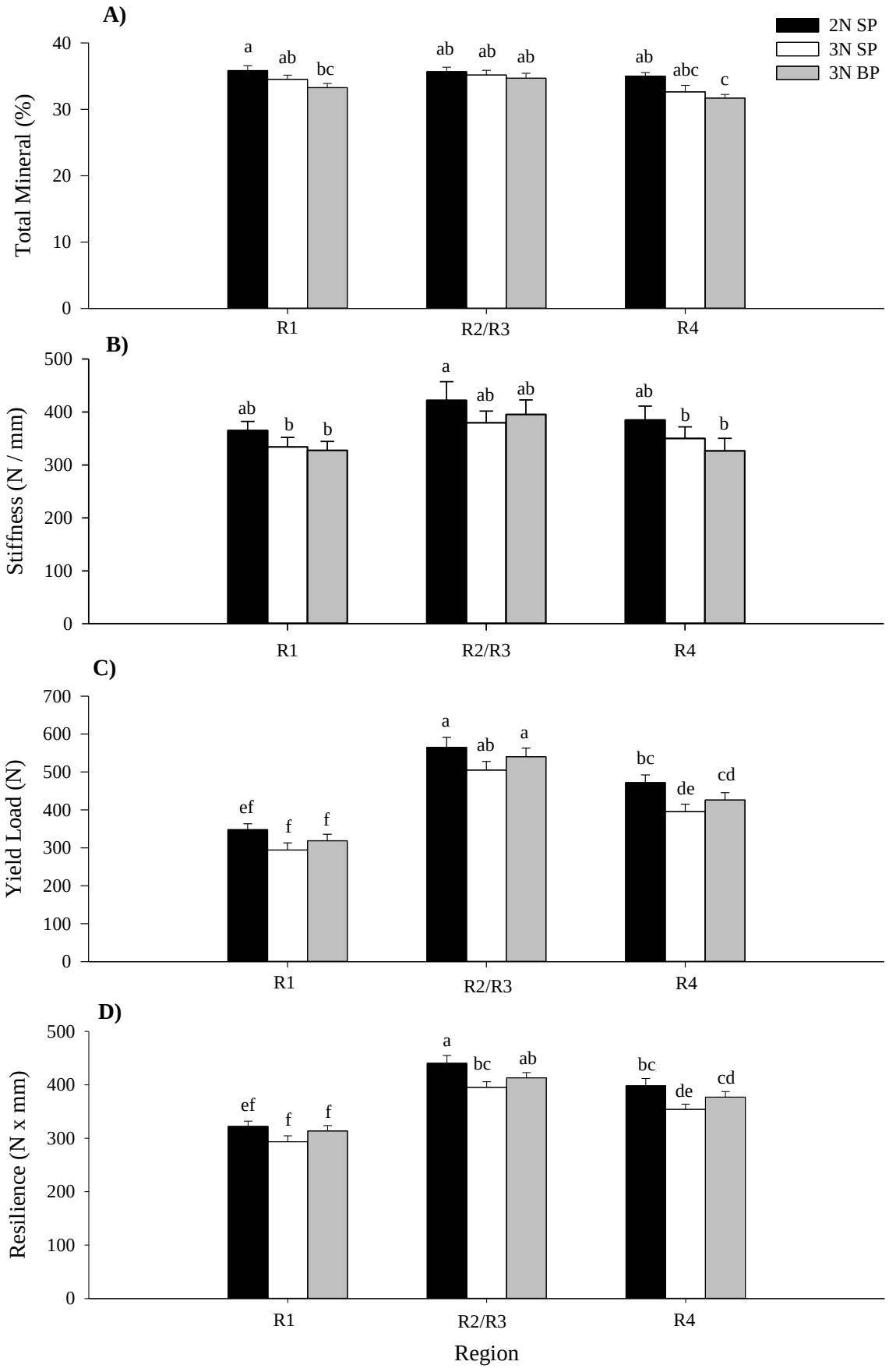


Figure 4.

**Figure 5**

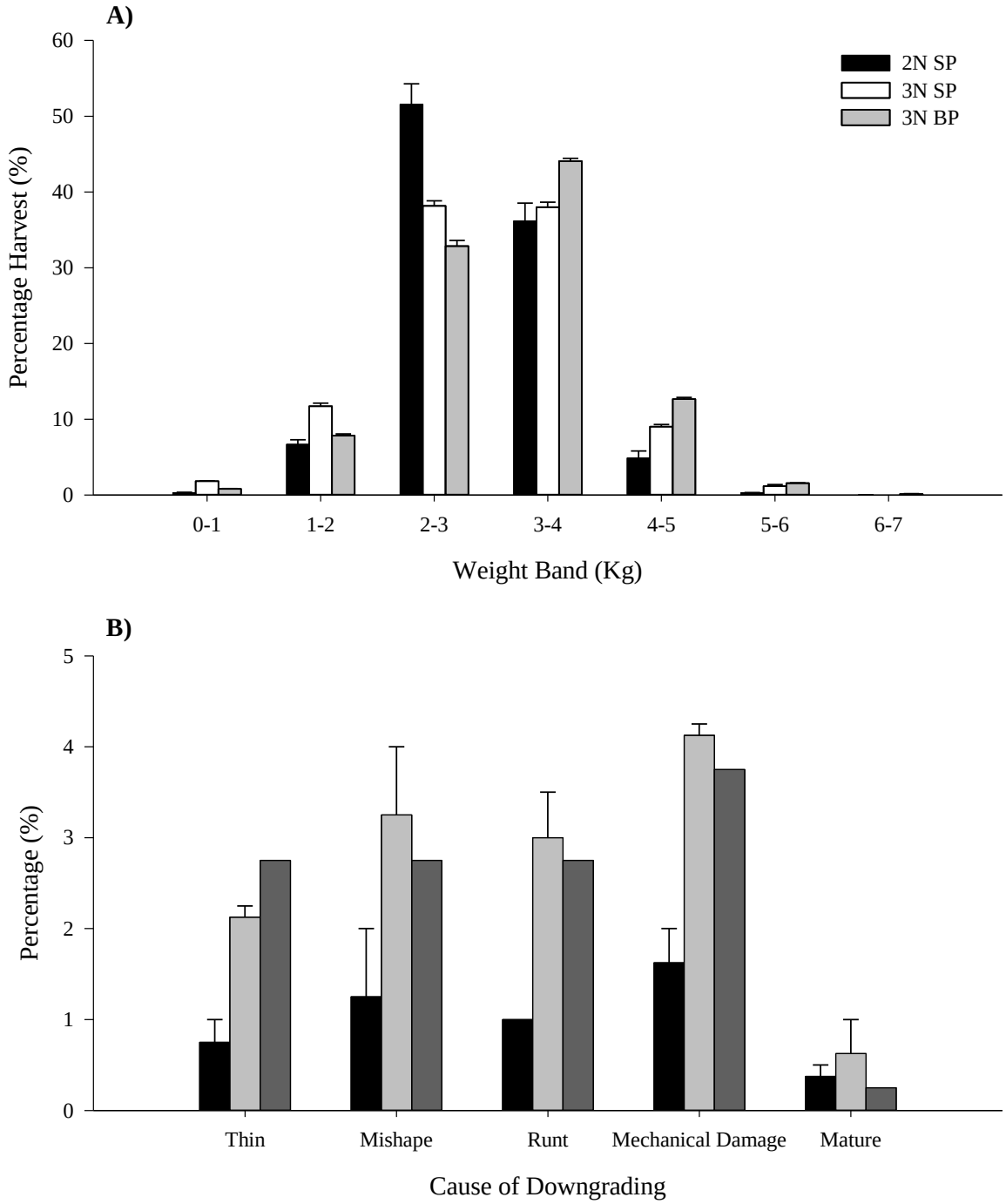


Figure 5.