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# Gut morphology of diploid and triploid Atlantic salmon (*Salmo salar* L.)

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## Abstract

Triploid fish may differ from diploids in a number of morphological and physiological characteristics. The present study was undertaken to examine the effects of ploidy on the gut morphology of Atlantic salmon post-smolts (*Salmo salar* L.). For this purpose, we compared the intestinal length (Relative Gut Length, RGL), pyloric caeca number and mass of commercially produced diploid and triploid siblings. The mean body weights of diploid and triploid salmon (n=30 fish/ploidy) were 135±1g and 132±1g, respectively. Diploid fish had significantly higher RGL (0.47±0.05 vs. 0.40±0.04), pyloric caeca number (56±4 vs. 45±3) and mass (6.41±0.61g vs. 4.80±0.59g) than triploids. Morphological differences in the guts of diploids and triploids could influence digestive efficiency and play a role in determining subsequent growth or welfare of the fish.

## Introduction

Incentives for farming sterile fish relate to potential improvements in post-pubertal somatic growth, survival and carcass quality. Sterility may also minimize the risk of genetic interactions between farmed and wild stocks. As such, the production of sterile, triploid fish for commercial farming may be an option for reproductive control and genetic containment that meets both industrial and environmental criteria (Maxime 2008; Piferrer et al. 2009). The production of triploid Atlantic salmon (*Salmo salar* L.) is common in Australia (Lijalad and Powell 2009), and commercial evaluation is presently under way in Norway.

Triploid fish may differ from diploids in a number of morphological and physiological characteristics, including skeletal and gill anatomy, hematology, respiration and cardiovascular physiology (Maxime 2008; Piferrer et al. 2009), and in Atlantic salmon diploid and triploid fish differ in incidence of ocular cataracts and vertebral malformations (Fraser et al. 2013). Although the reasons for this are not clear, differences in egg quality (Taylor et al. 2011) and nutritional requirements (Burke et al. 2010; Taylor et al. 2014) might be key factors.

There are significant differences in gut morphology between diploid and triploid Atlantic cod (*Gadus morhua* L.) raised under similar conditions (Peruzzi et al. 2013). With regard to Atlantic salmon, gut morphology of wild, diploid fish has been described (Løkka et al. 2013), but information about the digestive system of farmed triploids is lacking.

In this work, we focus on the effects of ploidy on the gut morphology of Atlantic salmon post-smolts. For this purpose, we compared the intestinal length, pyloric caeca number and mass of diploid and triploid siblings produced commercially in Northern Norway.

## Materials and methods

Sibling diploid and triploid Atlantic salmon post-smolts (n=30 fish/ploidy) were obtained from a commercial producer in Northern Norway (Cermaq Norway AS, Hopen, Nordland). Prior to sampling the diploid and triploid fish had been held separately in circular tanks supplied with a mixture of flowing fresh and sea water.

The fish had completed the parr-smolt transformation and were ready for transfer to sea cages. The fish were sampled at random, sacrificed and transported on ice to the University of Nordland (Bodø, Norway). Immediately upon arrival, a small gill clip was taken from each individual, placed in a 1.5ml Eppendorf tube and deep-frozen (-80°C) for flow cytometric analysis. Fish were measured for body weight, standard length, gut length, pyloric caeca mass and number as described in Peruzzi et al. (2013). Briefly, the digestive system was excised and intestinal length was measured to the nearest 0.1cm from the end of the pyloric caeca region to the anus. The intestine was placed in relaxed position but not stretched. The pyloric caeca region was then cut at its junctions with the stomach and upper intestine, weighed to the nearest 0.01g, and stored at -20°C. The pyloric caeca samples were part-thawed and then fixed in 10% neutral buffered formalin for at least 48 hours prior to counting of pyloric caeca. After overnight rinsing in running tap water, the total numbers of pyloric caeca were counted using a dissecting microscope. Relative Gut Length (RGL) was calculated as: RGL = Intestine Length (cm)/Standard Length (cm). The ploidy status of all fish was verified by flow cytometry using Propidium Iodide (PI) as fluorescent stain following Peruzzi et al. (2013). Flow cytometry analyses confirmed the ploidy status of the fish, with triploids having ca. 1.5 (mean±SD, 1.52±0.06) more nuclear DNA than their diploid siblings. Results relating to morphological measures are expressed as mean±95% Confidence Interval (CI). Morphological data were compared using a two-tailed Student’s *t*-Test (SYSTAT v 13.1, San Jose, CA, USA) and differences were considered statistically significant at $P<0.05$.

## Results

The diploid and triploid salmon did not differ in body weight or standard length (Table 1). The diploid fish had relatively longer guts ($P<0.001$) and higher pyloric caeca numbers and mass ($P<0.001$) than the triploids (Table 1, Fig. 1).

**Table 1.** Mean (±CI) body weight, standard length, relative gut length (RGL), pyloric caeca number and mass of diploid and triploid salmon post-smolts (n=30 fish/ploidy). The *P*-values of differences between the two ploidy groups identified by *t*-Test are indicated.

| Trait | Diploid | Triploid | *P*-value |
|---|---|---|---|
| Body weight (g) | 135.1±14.4 | 132.3±11.1 | 0.77 |
| Standard length (cm) | 23.3±0.7 | 23.8±0.6 | 0.32 |
| Relative gut length (RGL) | 0.47±0.02 | 0.40±0.01 | <0.001 |
| Pyloric caeca number | 55.6±1.3 | 45.5±1.3 | <0.001 |
| Pyloric caeca mass (g) | 6.41±0.61 | 4.80±0.59 | <0.001 |

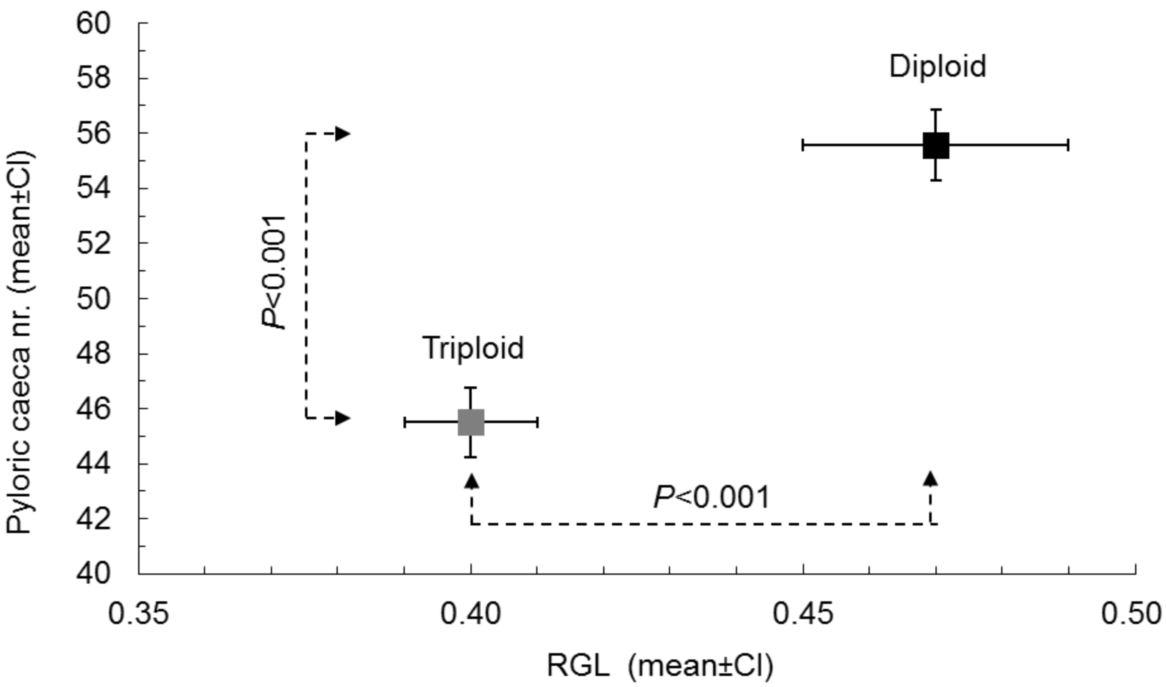


**Figure 1.** Relative gut length (RGL) and pyloric caeca numbers (mean±CI) of diploid and triploid salmon (n = 30 fish/ploidy). The *P*-values of differences between the two ploidy groups identified by *t*-Test are shown.

## Discussion

Ploidy had a marked influence on several aspects of the gut morphology of the sibling salmon post-smolts. The pyloric caeca mass of triploids was reduced by nearly 25% compared to their diploid siblings. The triploids had approximately 20% fewer pyloric caeca and 15% shorter guts (RGL) than the diploid fish. This is in agreement with findings for Atlantic cod (Peruzzi et al. 2013). The numbers of pyloric caeca of farmed diploid salmon (range 50-62) found in our study overlap those reported for wild fish (range 55-75) (Løkka et al. 2013).

Pyloric caeca are blind extensions of the intestine whose functions include secretion of digestive enzymes and the absorption of nutrients (Rust 2002). Our results indicate that there are morphological differences between diploid and triploid Atlantic salmon that could lead to lower digestive capacity and absorptive surface area in triploids in comparison with diploids. Numbers of pyloric caeca have been shown to vary among strains and families of salmonids (e.g. Bergot et al. 1976; Townsend, 1944) and this could also be the case for Atlantic salmon. However, our comparison reveals that there are also differences between full sibling diploid and triploid salmon as has previously been reported for Atlantic cod (Peruzzi et al. 2013).

There is some evidence that diploid and triploid salmon may differ in their ability to absorb, metabolize and retain nutrients. For example, triploid salmon may require more dietary phosphorus than diploids (Burke et al. 2010), and require dietary supplements of histidine to reduce the incidence of ocular cataracts (Taylor et al. 2014). In addition, diploid and triploid Atlantic salmon may differ in gut microbiota, with potential consequences for culture performance and health (Cantas et al. 2011). As such, ploidy status may alter not only the gut morphology but also the bacterial flora in triploids. Various nutritional and environmental factors may play additional roles to influence the level and type of acquired bacteria with resulting differences in microbiota colonization and composition between ploidies.

Overall, morphological and physiological differences in the guts of diploid and triploid salmon could be hypothesized to play a role in determining the digestive efficiency and subsequent growth or welfare of fish that differ in ploidy status.

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