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The vertebral column and exercise in Atlantic salmon — Regional effects

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ABSTRACT

This study investigated the effect of swimming speed on the mineralization (mineral rate, Ca, P, Mg, Zn, K, Mn, Cu) and morphology of vertebra (V) in different anatomical regions of the vertebral column in Atlantic salmon. Triplicate groups of Atlantic salmon post-smolt were forced to swim at slow ($0.2 \pm$ SEM 0.02 BL s^{-1}), moderate ($0.8 \pm$ SEM 0.01 BL s^{-1}) and fast ($1.5 \pm$ SEM 0.02 BL s^{-1}) water current velocities for six weeks in raceways. The specific growth rate (SGR) was lower at fast (1.41 ± 0.04 SEM) compared to moderate (1.49 ± 0.02 SEM) and slow velocities (1.51 ± 0.03 SEM). In the post-cranial region (V1 → 8) fish at: (*i*) moderate and fast velocities developed higher relative V lengths, and mineral rate (% ash weight of dry weight), compared to fish at slow velocity; (*ii*) moderate velocity developed higher ratio between V length and dorso-ventral diameter (l/d-ratio) – more elongated V – and lower potassium ash content, compared to fish at slow, while those at fast velocity displayed intermediate values for both parameters. In the posterior-truncal region (V9 → 30), fish at moderate and fast velocity developed lower potassium ash content than those at slow. In the anterior-caudal region (V31 → 49), fish at: (*i*) fast velocity developed higher mineral rate than those at slow, with those at moderate displaying intermediate values; (*ii*) moderate velocity developed lower phosphorus and magnesium ash contents than those at slow and fast. In the ural region (V50 → 58), fish at: (*i*) slow velocity developed higher relative V lengths than those at moderate and fast; (*ii*) slow velocity developed higher l/d-ratio than those at fast, with fish at moderate displaying intermediate values. Also, ontological shifts in mineral rate, and Ca and K ash contents along the spine were observed; with time, Ca increased in all regions except the ural, while K decreased in all regions except the ural, where it increased, accompanied with a decrease in mineral rate.

The present results show that the positive effects of exercise on vertebrae in farmed Atlantic salmon are region specific.

Statement of relevance

Exercise promotes vertebra mineralization in Atlantic salmon.

1. Introduction

The vertebral column of Atlantic salmon (*Salmo salar*) consists of on average 58 amphicoelous vertebrae. These can be sub-divided into 4 distinct anatomical regions: (*i*) post-cranial region (R1) with vertebra nos. 1 to 8; (*ii*) posterior-truncal region (R2) with vertebra nos. 9 to 30; (*iii*) anterior-caudal region (R3) with vertebra nos. 31 to 49; (*iv*) ural region (R4) with vertebra nos. 50 to 58 (Kacem et al., 1998, Fig. 1). The vertebrae in R3 have the largest cranial to caudal length, dorso-ventral diameter, lateral diameter, and dry weight, but there is also an increase in the three latter parameters towards the head within R1 (Kacem et al., 1998). The shape of the vertebra, measured as the ratio between the cranial to caudal length and the dorso-ventral diameter (l/d ratio), also changes along the vertebral column, and there is a general increase with age i.e. the vertebrae gets more elongated in post-smolts (Fjelldal et al., 2005). Vertebrae in the anterior-caudal region have the highest l/d ratio, mineral rate, and mechanical strength throughout life (Fjelldal et al., 2005; Fjelldal et al., 2006; Fjelldal et al., 2009). Ontologically, the vertebrae in the anterior-caudal and ural regions increases in relative size during parr-smolt transformation (Fjelldal et al., 2006), probably as an adaption to increased swimming activity in post-smolts compared to parr. The teleost vertebral column is not weight bearing, and mechanical loading is restricted to muscular contractions imposed by the large lateral muscle during swimming and locomotion. Swimming in salmonids is classified as sub-carangiform swimming where the posterior part of the body is undulating to generate a forward force (Sfakiotakis et al., 1999). Considering this swimming mode, the mechanical load is likely greatest in the anterior-caudal and ural regions of the vertebral column.

Exercise has a positive effect on vertebra in salmonids, shown by enhanced mineralization of anterior-caudal vertebrae with increasing swimming speed (Deschamps et al., 2009; Totland et al., 2011). The

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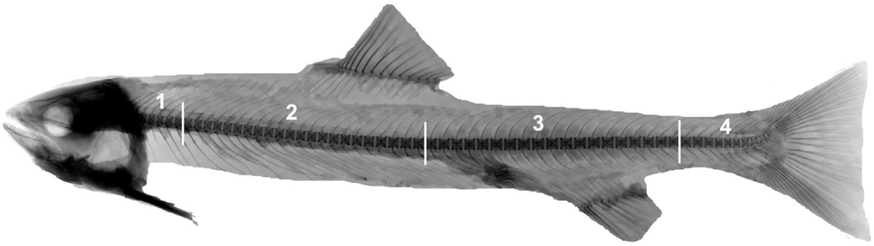

Fig. 1. Lateral radiograph of the Atlantic salmon. The vertebral (V) regions are shown: (*i*) post-cranial region (R1) with vertebra nos. 1 to 8; (*ii*) posterior-truncal region (R2) with vertebra nos. 9 to 30; (*iii*) anterior-caudal region (R3) with vertebra nos. 31 to 49; (*iv*) ural region (R4) with vertebra nos. 50 to 58.

two major bone minerals are Ca and P, but also other elements like Zn, Mn, K, Mg and Cu are present in lower amounts (Roy and Lall, 2003). There are no teleost studies on how exercise affects vertebra mineralization and morphology in different regions of the vertebral column, or how mineral composition varies accordingly.

Swimming speed in the marine phase of wild salmonids is on average 1 body length (BL) s^{-1} (Drenner et al., 2012), which correspond well with what has been documented for farmed salmon (Blyth et al., 1993; Juell and Westerberg, 1993; Kadri et al., 1991; Sutterlin et al., 1979). Swimming speeds of 0.8 to 1.0 BL s^{-1} has also been reported as the preferred swimming speed in brook charr (*Salvelinus fontinalis*) when the fish was free to choose (Tudorache et al., 2011). However, following the trend of more farms being located in exposed areas with fast water current velocities fish may have to swim faster than what is considered optimal. In some areas there are reports of water current velocity up to 80 cm s^{-1} and areas with current velocity of 150 cm s^{-1} are being considered for salmon farming (Pers. Comm. Øystein Patursson Ph.D. Fiskaaling, Aquaculture Research Station at the Faroes). The effect that these fast water currents have on fish is scarcely investigated. In sheltered areas with slow water current velocity the fish have the possibility to choose their swimming speed. But, in exposed areas the fish must swim at the prevailing velocity (Johansson et al., 2014). Increased swimming speeds are often conceived as positive for farmed fish with increases in growth, protein deposition, cardiac output, energy stores, metabolic rate, swimming performance etc. (Castro et al., 2011; Christiansen and Jobling, 1990; Davison, 1997; East and Magnan, 1987; Farrell et al., 1991; Farrell et al., 1990; Houlihan and Laurent, 1987; Jørgensen and Jobling, 1993; McFarlane and McDonald, 2002; McKenzie et al., 2012; Pearson et al., 1990; Totland et al., 1987).

In this study we examined the effects of slow (0.2 BL s^{-1}), moderate (0.8 BL s^{-1}), and fast (1.5 BL s^{-1}) swimming speeds on vertebra in different regions of the vertebral column in Atlantic salmon post-smolts, with emphasize on vertebrae size and morphology, mineralization and mineral composition.

2. Materials and methods

2.1. Experimental design and animals

Postsmolt Atlantic salmon, 98.6 ± 20 g, 22.3 ± 1.3 cm, mean ± SD, (AquaGen strain, hatched March 2011) were forced to swim at different water current velocities during a six week trial from April till May 2012, in the Tank Environmental Laboratory at the Institute of Marine Research, Matre (Norway). Fish were kept at three different velocities in triplicate raceways ($n = 80$ per raceway) submerged in tanks (∅ 3 m, 5.3 m^3). The raceways were designed as an open cylinder creating homogenous water current (Solstorm et al., 2015). The trans-sectional area of the cylinder was 0.10 m^2 (∅ 0.36 m) and the length available for the fish was 2.0 m, giving a volume of 0.20 m^3. Slow, moderate and fast (0.2 ± 0.02, 0.8 ± 0.01 and 1.5 ± 0.02 BL s^{-1} (mean ± SEM)) water currents were generated with an electric engine (Minn Kota RT80/EM, Johnson Outdoors Marine Electronics, Inc., Racine, WI, USA) with adjustable speed. A honeycomb (5.0 mm opening and 101.6 mm long, PC 5.0 G4, Plascore GmbH & Co KG, Waldlaubersheim, Germany) assured a laminar flow. A plastic coated wire netting (10 mm × 10 mm) was placed at the outlet and on the top to keep the fish inside. Fish were transferred to raceways at a stocking density of 38.7 ± SEM 0.28 kg m^{-3} ($n = 80$ per raceway). Five fish from each replicate were removed every second week during the trial to minimize increases in density. During the trial, constant temperature (10 °C), water exchange (120 l min^{-1}), salinity (33 psu) and continuous light were maintained. Dissolved oxygen (DO) levels were always above 80%. Fish were fed commercial feed (Skretting Spirit 75) in excess throughout the day (24 h) to ensure sufficient food supply.

All fish were anesthetized (100 mg/l Finquel®), weighed (±1 g) and measured (±0.5 cm) before and after the trial.

2.2. Bone analysis

Fish were rapidly killed and frozen prior to and after the trial for vertebra analysis. 30 fish were collected before the trial and 10 from each of the 9 replicate after the trial. All the frozen fish were thawed and carefully filleted to remove surrounding flesh around the vertebral column. This was done in order to increase the quality of the radiograph images. Subsequently, the vertebral columns were radiographed (Porta 100 HF; Eickemeyer Medizintechnik für Tierärzte KG, Tuttlingen, Germany) onto a 35 × 43 cm image plate in a rigid cassette (Dürr Medical, Bietigheim-Bissingen, Germany) with 40 kV and 10 mAs with a distance of 70 cm. The image plate was scanned (CR 35 VET; Dürr Medical) and the resulting image converted into a TIFF file (Vet-Exam Plus Software, version 4.14.0). The software program Adobe Photoshop CS2 was used for the evaluation of vertebral deformities, and Image J was used to measure vertebrae cranial-caudal lengths and dorso-ventral diameters. Five random fish from each replicate was selected for further analysis of mineral rate (% ash weight of dry weight) and mineral composition. Analyses were performed on three vertebrae in each of the four anatomical regions; post-cranial (R1) – vertebra numbers (V nos) 3–5, posterior-truncal (R2) – V nos 18–20, anterior-caudal (R3) – V nos 40–42 and ural (R4) – V nos 53–55. Mineral rate was measured according to Grini et al. (2011). For analysis of mineral composition, ash samples were pooled per replicate and region and sent to ALS Laboratory Group Norway AS (Oslo, Norway) for analysis of calcium (Ca), phosphorus (P), zinc (Zn), magnesium (Mg), potassium (K), manganese (Mn) and cupper (Cu) content in mg kg^{-1} ash.

2.3. Statistics

Statistical analysis were performed in STATISTICA (StatSoft, Inc. 2012. data analysis software system, version 11. www.statsoft.com). Data were analysed with nested ANOVA to check for possible tank effects. Significant nested ANOVAs ($p < 0.05$) were followed up by one-way ANOVAs, followed by Fisher LSD-test. All values are presented per region and are shown as mean ± standard error of mean (SEM).