Effect of chronic confinement on sperm quality and male reproductive physiology in Eurasian perch, *Perca fluviatilis*.

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## INTRODUCTION

Stress plays a key role in the ability of fish to perform reproduction (Milla et al., 2010; Schreck et al., 2010). Given the huge species differences in fish reproductive strategies, large variability in the effects of stress on reproductive efficiency could be expected. Eurasian perch (*Perca fluviatilis*) is mainly represented in Europe and reared under intensive rearing conditions (e.g. high confinement), which are known to impair with many physiological functions (Douxfils et al., 2011). In fish, stress induces an increase of cortisol, but little is known regarding the effect of stress on male reproductive capacity in percid fish. The aim of the present study was to examine the impact of chronic confinement applied during the final maturational period on sperm quality (concentration and motility) and reproductive physiology of perch.

## MATERIALS AND METHOS

Perch breeders were reared in an outdoor recirculating system in 3 confinement conditions  $(0.07 \text{ m}^3, 0.5 \text{ m}^3 \text{ and } 2 \text{ m}^3)$  in duplicate (except for 0.07 m<sup>3</sup>) at the same density (12 ind/m<sup>3</sup>) from September to April at a 50:50 sex ratio (to allow a chilling process and maturational process). Early and late March, 3 males from each tank were randomly handled and anesthetized for sperm and blood collection.

## Sperm quality assessment

For each male, the genital papilla was dried, sperm was collected by stripping and 50-fold dissolved in extender (a Bicine solution, Moore, 1996). 50  $\mu$ L of this sperm solution was a second time 40-fold dissolved in extender and then 10-fold dissolved in formol (10%). Sperm concentration was assessed by counting spermatozoa using a Burker's cell.

Sperm motility assessed with CASA system (Rougeot et al., 2004) by studying the main relevant velocity parameters for perch : percentage (%) of progressive and motile spermatozoa, average pathway velocity-VAP, curvilinear velocity - VCL and straight line velocity – VSL.

## Steroid assays

Blood was sampled in the caudal vein and 4000g centrifuged for 20 min at  $4^{\circ}$ C to collect plasma. Sex steroid hormones (17 $\beta$ -estradiol, 11-keto-testosterone and testosterone) were assayed by RIA.

#### RESULTS

Sperm concentration decreased with the increase of confinement from  $70.5\pm17.2 \times 10^9$  spermatozoa.ml<sup>-1</sup> in 2m<sup>3</sup> to  $61.5\pm9.9 \times 10^9$  in 0.07m<sup>3</sup>. On the other hand, sperm motility in terms of VAP (from 32.6 to 40.2 µm/sec), VSL (from 23.5 to 30.0 µm/sec), VCL (from 81.6 to 89.5 µm/sec), MOT (from 56.0 to 85.0 %) and PROG (from 21.5 to 28.7) were not significantly influenced by the confinement level. Plasma sex steroids levels increased with the advancement of the reproductive season, but were not significantly affected by confinement.

#### DISCUSSION AND CONCLUSIONS

Sperm density ranged within the values obtained in previous studies on perch (Piironen and Hyvärinen, 1983; Rougeot et al., 2004) and were significantly decreased by the confinement level. This negative effect of stress on sperm density was previously reported in rainbow trout (Campbell et al., 1992) and reviewed by Milla et al. (2009). Sperm velocity parameters, as well as plasma sex steroids hormones, were closed to those obtained by Rougeot et al. (2004). In perch, unlike other fish species (Milla et al. 2009), these parameters were not significantly changed by confinement during the final maturational period

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