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Steroids accumulate in the rearing water of commercial recirculating aquaculture systems

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ABSTRACT

Little information is available on steroid concentrations in the rearing water of aquaculture systems and whether they accumulate in recirculating aquaculture systems (RAS). Therefore this study aimed at determining (1) the concentrations and variation of cortisol and sex steroids in RAS, (2) the contribution of fish rearing conditions to steroid concentrations in seven commercial RAS. Each RAS was sampled twice at three different points: (1) make-up water; (2) influent and (3) effluent of the rearing unit. The results showed significant higher steroid concentrations in the influent and effluent when compared with the make-up water. On average cortisol concentration was 15.7% higher in the effluent when compared with the influent. Mean steroid concentrations in the rearing unit effluent varied between: 3.8–217.0 ng/L for cortisol, 3–12.5 ng/L for testosterone, 0.9–7.1 ng/L for 11-ketoteststerone and 1.8–12.8 ng/L for 17,20β-dihydroxypregn-4-en-3-one. Stocking density, Total Ammonia-Nitrogen concentration and orthophosphate-P concentration (a measure of make-up water usage) showed a positive correlation with sex steroids in the water. The steroid concentrations from the present study were orders of magnitude lower than initial estimations indicating a water treatment efficiency of >99%. The results suggest that an intensification of fish production through decrease of make-up water use and increase of stocking density will lead to a build-up of steroids in the water. Although intensification is critical for the economical success of RAS, this ultimately could affect fish performance as steroids accumulates in the water of RAS at levels that can potentially be detected by some fish species.

1. Introduction

Recirculating aquaculture systems (RAS) are among the most environmentally sustainable systems to culture fish due to their reduced make-up water usage (new water supply) and release of nutrients to the environment (Martins et al., 2010). In response to the increasing demand for aquaculture products, production methods in RAS have been intensified. Intensification of fish production is achieved by increasing stocking densities, which increases the amount of metabolites released into the water (Fanouraki et al., 2008). However, intensification of fish production may not always result in optimal rearing conditions.

It is known that situations eliciting the production of fish steroids, will increase their release into the water and ultimately bioaccumulation in RAS (Scott et al., 2008). Studies with rainbow trout *Oncorhynchus mykiss* (Ellis et al., 2004) and Atlantic salmon *Salmo salar* (Ellis et al., 2007) showed that the stress hormone cortisol is released at higher quantities into the water after exposure to acute handling stress. European sea bass *Dicentrarchus labrax* kept at stocking densities of 50 kg/m^3 increased both blood plasma concentrations and cortisol release rates into water when compared to fish kept at 20 kg/m^3 (Fanouraki et al., 2008). Other steroid hormones potentially accumulating in RAS are sex steroids such as testosterone, 11-ketotestoterone and the maturation-inducing steroid 17,20β-dihydroxypregn-4-en-3-one (17,20β-*P*). Sex steroids can be transferred from one group of fish to another group of fish (Budworth and Senger, 1993) as reported

Abbreviations: RAS, recirculating aquaculture systems; 17,20β-*P*, 17,20β-dihydroxypregn-4-en-3-one; TA-N, Total Ammonia-Nitrogen; TFA, trifluoroacetic acid; RIA, radioimmunoassay; TLC, thin-layer chromatography; CV, coefficient of variation.

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Table 1
Overview of the rearing unit, water treatment unit and water quality parameters of the seven sampled RAS. Values are mean ($N = 2$, except RAS 1 where $N = 1$).

	RAS 1	RAS 2	RAS 3	RAS 4	RAS 5	RAS 6	RAS 7
Rearing unit[a]							
Species	*Solea solea*	*Anguilla anguilla*	*Psetta maxima*	*Stizostedion lucioperca*	*Clarias gariepinus*	*Oreochromis niloticus*	*Seriola lalandi*
Common name	*Dover sole*	*European eel*	*Turbot*	*Pike-perch*	*African Catfish*	*Nile tilapia*	*Yellowtail amberjack*
Fish tanks	Raceways	Circular	Raceways	Circular	Rectangular	Rectangular	Circular
Standing stock (kg)	20,000		65,000	7500	6000	4750	19,000
Stocking density (kg/m^3)	104	175	59	43	162	68	50
Feed load (kg/d)	60	300	331	48	100	48	180
Weight range (g)	16–555	5–1500	8–2500	370–1800	100–1500	100–800	400–1800
Sex ratio (M:F)	1:3	9:1	Unknown	1:1	1:1	1:1	Unknown
Volume (m^3)	193	200	1100	175	37	70	378
Water treatment unit[a]							
Mechanical filtration	Drum	Drum	Drum	Drum	Settling	Sieves/settling	Drum
Bio-reactor	Trickling	Trickling	Moving bed	Trickling	Trickling	Trickling	Trickling
Ozone	Present	–	Present	–	–	–	–
UV	–	–	–	–	Present	–	Present
Make-up water (L/kg feed/d)	1000	480	920	450	100	74	1000
Volume (m^3)	7	30	2500	25	13	10	122
Water quality							
Temperature (°C)[a]	19	24	17	25	26	24	21
pH[a]	5.9	5.5	7.7	6.2	6.6	7.5	7.7
Conductivity (μs/cm)[a]	40,600	2500	40,000	1900	4000	4200	36,300
TA-N (mg/L)[b]	5.7	63.5	0.3	1.4	48.8	5.9	1.0
Nitrite-N (mg/L)[b]	0.14	0.15	0.05	0.22	4.6	1.3	0.31
Nitrate-N (mg/L)[b]	64.5	92.3	27.0	91.1	53.5	72.3	73.6
Orthophosphate-P (mg/L)[b]	4.9	21.6	2.1	7.1	13.1	6.5	5.0

Fish sexual maturation: immature in RAS 1, 2, 3, 4, 5 and 7 and 90% of the standing stock mature in RAS 6, according to information provided by the facility managers.
[a] Value or information provided by the facility manager.
[b] Value measured.

for rainbow trout (Vermeirssen and Scott, 1996) and tench *Tinca tinca* (Scott et al., 2005).

Besides acting as endogenous signals, steroids are also used by fish as exogenous signals, e.g. pheromones that synchronize gamete maturation or spawning interactions (Stacey, 2003). For instance, three steroids (androstenedione, 17,20β-*P* and 17,20β-*P* sulphate) that are released by female goldfish *Carassius auratus* can elicit behavioural and physiological changes in males at very low concentrations (nM threshold) (Stacey and Sorensen, 2006). In addition, testosterone is reported to be a potent odorant in precocious male Atlantic Salmon parr (Moore and Scott, 1991).

Whether steroids in RAS occur at concentrations that can be sensed or taken up by fish remains to be investigated. Therefore the present study aimed at determining (1) the concentrations and variation of cortisol and sex steroids in RAS (2) the contribution of fish rearing conditions to the concentration of steroids in RAS.

2. Materials and methods

2.1. Sampling sites and sample collection

Water samples were collected from seven commercial recirculating aquaculture systems (RAS) in full operation located in The Netherlands. None of these RAS were in the start up phase. Details of the systems (rearing unit, water treatment unit and water quality) provided by the facilities managers are presented in Table 1. Five different commercial RAS were sampled twice with an interval of ±15 months, one sampled twice (RAS 7) with and interval of ±2 months and one RAS (1) was sampled once (the farm closed down during the second sampling period). Three different points were sampled in each RAS: (1) make-up water; (2) influent of rearing unit and; (3) effluent of rearing unit (Fig. 1).

Water samples for steroid analysis were collected in 1 L containers and immediately placed on ice water, transported to the laboratory and stored at −20 °C. Additional water samples (10 mL) were taken (Fig. 1), placed on ice water and transported to the laboratory for Total Ammonia-Nitrogen (TA-N), Nitrite-N, Nitrate-N and Orthophosphate-P analysis using a SAN autoanalyzer (Skalar, The Netherlands). Temperature, pH, and conductivity were measured at the sampling sites using portable meters or provided by the facilities managers. Nitrile gloves were used during all water sampling and processing activities to prevent contamination with steroids.

2.2. Steroid analysis

Steroid hormones were measured by radioimmunoassay of their free forms. For this it required extraction of steroids from the water and hydrolysis of conjugates to release the free forms as previously reported (Canario and Scott, 1989; Scott and Sorensen, 1994). Briefly, water samples for steroid analysis were first paper filtered (2 μm; VWR, France) followed by a membrane filter (0.45 μm; Millipore, Ireland). The sample volume (±500 mL) was determined gravimetrically and pumped (±12 mL/min) through an Oasis HLB Plus solid phase extraction cartridge (Oasis®, Waters, Milford, U.S.A.) previously activated with methanol (5 mL) and washed with distilled water (5 mL). Cartridges were eluted (3 mL 100% ethanol) and the eluate evaporated in a dry bath at 45 °C under a gentle flow of nitrogen. The dried residue was re-dissolved in 0.1 mL distilled water, 3× 3 mL of diethyl ether was added, the tubes vigorously shaken, and centrifuged at low speed to separate the organic and water phases. The water phase was frozen in liquid nitrogen. The diethyl ether was transferred to another tube and evaporated under nitrogen. To the residue was re-constituted in radioimmunoassay (RIA) buffer (sodium phosphate 0.05 M, pH 7.6, containing 1% gelatine) and stored (−20 °C) until assay.

The remaining aqueous fraction containing the conjugated steroids was evaporated at 40 °C and 1 mL of trifluoroacetic acid (TFA)/ethyl acetate (1/100, v/v) was added to the dried residue and incubated in a water bath at 40 °C overnight for the chemical hydrolysis of the sulphate steroids. The TFA/ethyl acetate was subsequently evaporated in a dry bath at 45 °C under a gentle nitrogen flow and the dried residue was re-dissolved in 0.5 mL sodium