Aquaculture 436 (2015) 151–159

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Welfare assessment of rainbow trout reared in a Recirculating Aquaculture System: Comparison with a Flow-Through System

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ARTICLE INFO

Article history:
Received 18 April 2014
Received in revised form 29 September 2014
Accepted 29 October 2014
Available online 12 November 2014

Keywords:
Rainbow trout
Recirculating Aquaculture System
Stress
Swimming behavior
Welfare

ABSTRACT

A multi-parameter analysis was applied in order to acquire an integrated and objective assessment of the effects of recirculating aquaculture systems in rainbow trout farms on a range of welfare indicators. We compared growth, cortisol and behavior in trout previously held in a Recirculating System (RS) developed in the PEIMA (Brittany, France) with optimized water quality, with trout held in a Flow-Through System (FTS) 100% supplied with neighboring lake water. After acclimation to experimental tanks using a flow-through system, the fish were filmed 30 minutes per day, two days per week for two weeks. At the end of this observation period, we performed an Emotional Reactivity (ER) test created by the social isolation of the trout transferred into a novel environment. Trout were individually video-taped in the novel tank for 20 minutes and stress-related behaviors were automatically analyzed. Plasma cortisol was measured in undisturbed fish and in tested fish 30 minutes after the start of the ER. Learning ability was measured by group food-anticipatory activity. Mean weight was higher in the RS group, probably resulting from warmer water temperature in that group. Basal cortisol and cortisol levels measured after the acute stress did not differ between the RS and FTS fish. Group swimming activity and between-individuals dispersion registered during the observation periods were similar in both groups. Learning performance was comparable between the two groups. The ER test showed no major behavioral differences between RS and FTS trout, except for the maximum velocity recorded during the first 5 minutes which was higher for trout previously held in a FTS. The integration of performance and stress-related parameters suggests that, when water quality is kept optimum, a recirculating aquaculture system does not induce welfare impairment for rainbow trout. This study highlights the importance of integrating multi-parameter approaches for an objective and accurate trout welfare assessment.

1. Introduction

Current societal concerns about environmental alterations justify the need for developing less polluting breeding systems. In aquaculture, recirculating systems (RS) have been developed in order to reduce natural water use and to improve waste management, making fish production more compatible with environmental sustainability. An increasing number of European countries are now applying recirculating systems technology and the production of RS-cultured fish has strongly increased in France between 2005 and 2009 (Martins et al., 2010). The consequences of these systems on fish production have been explored and some studies have demonstrated performance uniformity comparing recirculating aquaculture systems and flow-through systems in rainbow trout (d'Orbcastel et al., 2009a,b). However little has been reported about fish welfare in RS. Some studies were carried out on rainbow trout and have shown caudal fin damage (d'Orbcastel et al., 2009b) or impaired swimming activity, probably due to deformities (Davidson et al., 2011b) but the authors of this latter article suspected the role played by poor water quality due to low water exchange in these recirculating systems. Indeed they later observed more side-swimming fish when the recirculating system contained high NO3-N concentrations (Davidson et al., 2014). Despite the relevance of these parameters, they should be extended to complementary tests for a more accurate fish welfare evaluation in a recirculating system ensuring optimal water quality.

The Pisciculture Expérimentale INRA des Monts d'Arrée (PEIMA, Brittany, France) has developed a salmonid rearing system based on recirculating water with water quality always close to the recommended values for salmonid farms (d'Orbcastel, 2008; Labbé et al., 2014). This optimized system required only 7.2 m^3 of fresh water per kilogram of fish produced instead of the 100 m^3 conventionally needed for a flow-through system. An initial study of this system has demonstrated the absence of negative consequences on fish reproduction, growth performance and meat quality, despite higher temperature fluctuations compared to the flow-through system (Labbé et al., 2014). However, direct

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http://dx.doi.org/10.1016/j.aquaculture.2014.10.047
0044-8486/

consequences on fish welfare parameters were never accurately assessed for such an optimized water recirculating system.

Several methods are commonly used to evaluate fish welfare. In fish farms, it is generally admitted that disruptions of swimming behavior or of fish shoal cohesion within the tank is a relevant indicator of stress. The swimming behavior of a shoal can be modified by various environmental stressors such as poor water quality (Baganz et al., 2005; Davidson et al., 2011a; Santos et al., 2010) or suboptimal temperature (Hasler et al., 2009; Quigley and Hinch, 2006). Although group behavior is a sensitive and simple welfare indicator for fish farmers, it is more difficult to evaluate the mental state of each individual, though this could provide crucial information for describing individual's well-being. A proper welfare analysis needs to investigate group behavior with a close connection with individual stress indicators such as physiological and behavioral responses during an emotional reactivity test.

Emotional reactivity of an individual is its propensity to express fear when alarming stimuli occur. Emotional responses are measured by specific adaptive behavior and physiological parameters. Like in other species, exposure to potentially threatening situations provokes stress responses in fish. The open-field test commonly used in mammals (Boissy and Bouissou, 1995; Pare, 1994; Sarkisova and Kulikov, 1994; Sudakov et al., 2013; Van Reenen et al., 2013) has its equivalent in fish with the novel-tank diving test (Champagne et al., 2010; Rouger et al., 1998; Steele, 1983). For example, isolated zebrafish exhibit freezing behavior, reduced exploration and erratic movements which are considered as anxiety-related patterns (Egan et al., 2009). Salmonids subjected to a novel-tank test avoid the central area and display altered swimming activity (Drangsholt et al., 2013). In addition, plasma cortisol is a common stress-marker in fish that can be measured after a novel-tank test, since the relationship between cortisol and specific behavioral responses to stress is now well-established (Kittilsen et al., 2009; Overli et al., 2005, 2007; Winberg et al., 2007). The emotional physiological and behavioral responses are known to be modified after chronic stress in mammals (Boissy et al., 2001; Destrez et al., 2013; Mineur et al., 2006; Willner, 2005; Wood et al., 2008) and birds (Calandreau et al., 2011; Laurence et al., 2012). In agreement with these authors, we considered that measuring altered fearfulness in the novel-tank test can be an indicator of chronic stress in fish.

Learning abilities of fish are now widely demonstrated. Like mammals, fish are able to associate neutral stimuli to food-rewards (Bratland et al., 2010; Nilsson et al., 2008, 2010; Yue et al., 2008) or to punishment (Moreira et al., 2004; Yue et al., 2008). Fish are also capable of spatial learning (Braithwaite and de Perera, 2006; Holbrook and de Perera, 2013; Odling-Smee et al., 2008) and show for example increased swimming activity around feeders prior to feeding. This food-anticipatory activity is commonly considered as an indicator of good welfare (Folkedal et al., 2012; Kristiansen and Ferno, 2007; Spruijt et al., 2001) since foraging and feeding motivation may translate a better well-being than prostration.

The aim of this study was to describe welfare in fish that were reared in a flow-through system (as a control) versus a recirculating aquaculture system, supplied with acceptable water quality in regards to the recommended thresholds. If detrimental effects were observed on rainbow trout reared in RS in particular after the fish had returned to a flow-through system, it would indicate that the recirculating system was perceived by the animals as a stressor. To further provide an objective assessment of fish welfare status, we performed a multi-parameter analysis based on group behavior on the one hand, and on individual endocrine and behavioral responses registered in an emotional reactivity test on the other hand.

2. Materials and methods

The experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), under the official license of V. Colson (35-114). At the end of the experiment, animals were reared under classical conditions up to 250 g, weight at which they are commercialized to fishing corporations for recreational purposes or up to 2500 g, weight at which they commercialized for human food production.

2.1. Husbandry and aquaculture systems

2.1.1. Recirculating Aquaculture System (RAS)

Ten 6.5 m^3 (3 m diameter) circular polyester resin tanks were used outdoors. In the RAS, 90% of the water was re-injected into the breeding tanks after water treatments. Under these conditions, only 10% of water was withdrawn from the natural environment (Drennec lake). Mean feed loadings of 0.125 kg/day per m^3/day make-up flow were maintained. A drum filter (mesh 60 μm) treated the waste water which contained macros suspended solids collected by an external fecal-trap positioned against each tank. The overflow of the system was evacuated in a vertical elevating pump for the biological filtration. Just before the water was pumping, sodium bicarbonate was added in the water at 1 kg for 5 kg of feed. The bio-filtration transforming ammonia into nitrate was achieved in a fluidized bed biofilter. A blower sent air (150 m^3/h) to prevent filter clogging and to strip CO_2. The re-oxygenation was achieved using pure O_2 gas from a pressurized liquid O_2 tank. Water quality analyses indicated that total suspended solid (TSS) concentrations, N-NO_2, N-NO_3, and N-NH_4 were always conformed to recommended values for salmonid farms (d'Orbcastel, 2008). Water measurements over the rearing period (between May 19th and November 4th 2011) were as follows: N-NH_4^+: 0.66 ± 0.13 $mg \cdot l^{-1}$, N-NO_2^-: 0.014 ± 0.07 $mg \cdot l^{-1}$, and N-NO_3^-: 5.46 ± 0.38 $mg \cdot l^{-1}$. The TSS average concentration was 2.9 ± 0.32 $mg \cdot l^{-1}$. The mean O_2 and CO_2 values were 8 $mg \cdot l^{-1}$ (min: 5.5, max: 14 $mg \cdot l^{-1}$) and 12.6 $mg \cdot l^{-1}$ (min: 10.2, max: 18 $mg \cdot l^{-1}$), respectively. The TSS average concentration was 2.9 ± 0.32 $mg \cdot l^{-1}$. Mean temperature over the period was 16.4 °C with a minimum of 14.2 °C and a maximum of 19.2 °C. Mean number of degrees per day was 2765° during the rearing period (Table 1). The mean daily temperature range was 1.2 ± 0.7 °C.

2.1.2. Flow-Through System (FTS)

Salmonid farming in recirculating system in France use circular tanks because of their capacity for self-cleaning which prevents fecal settling areas. In order to make the two systems more comparable, we also used circular tanks in the flow-through system.

Six covered circular flow-through tanks with a volume of 2 m^3 (2 m diameter), maintained outdoors and supplied with the Drennec lake water were used. Water physicochemical quality is not stable over time. It varies according to the season. Each tank was supplied in "new water" by two different pipes ("new water" and "hyper oxygenate water").

New water measurements were as follows: N-NH_4^+: 0.127 ± 0.1 $mg \cdot l^{-1}$, N-NO_2^-: 0.013 ± 0.007 $mg \cdot l^{-1}$, and N-NO_3^-: 1.46 ± 0.66 $mg \cdot l^{-1}$. The TSS value was 0.8 ± 0.3 $mg \cdot l^{-1}$. The O_2 values in the outfeed water were higher than 5.5 $mg \cdot l^{-1}$ (like in the RS) and CO_2 was systematically below 3 $mg \cdot l^{-1}$. The mean temperature during

Table 1
Sum of degrees per day, mean initial and final weights, specific growth rate (SGR; in % of body weight per day), feed conversion ratio (FCR) and thermal growth coefficient (TGC) of fish reared in Recirculating System (RS) and Flow-Through System (FTS) between May and November 2011.

	Degrees/day	Mean initial weight (g)	Mean final weight (g)	SGR (%BW/day)	FCR	TGC (%)
RS	2537	3.1	116.1 ± 5.72	2.16	0.84	1.36
FTS	2394	3.1	97.67 ± 3.05	2.05	0.97	1.24

Values are means and their standard deviation associated (SD) ($n = 3$).