Physicochemical parameters associated with the methods of application of salt baths and their field assessment of blood parameters of Atlantic salmon in water pre-smolt stage

Parámetros físico-químicos del agua asociados a los métodos de aplicación de baños de sal y su evaluación en terreno sobre parámetros sanguíneos de salmón del atlántico en etapa pre-smolt

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ABSTRACT. Salt baths in the production system of salmonids in fresh water, are important for the prevention and control of different diseases such as saprolegnia fungus. This standard procedure generates a rapid depletion of O2 in the water, leading to a significant increase of CO2, affecting blood parameters associated with animal welfare, such as Na+, Cl-, glucose and protracted hypercarbia. The aim of this study was to evaluate the response field of blood parameters related to fish welfare and the physical-chemical parameters of water to which they are subject during this period, comparing two sets of bath salt. One group of farmed Atlantic salmon (Salmo salar) in the pre-smolt stage was exposed in a salt bath system with the usual conditions and a second to a recirculation system with a waterfall for degasification, with two different exposure periods of one and two hours. The results of the concentration of CO2 in the water show significant variations between the two systems without recirculation compared to the control condition and salt. Significant differences were observed between the recirculating system and the control in blood levels of pCO2 and HCO3, but not pH. Our field data also show that salt baths that are performed regularly at the fish farms generate significant effects on blood parameters associated with animal welfare specifically fish pCO2 and HCO3 causing minor hypercarbia without generating blood acidosis. The addition of water recirculation with a waterfall does not affect blood parameters.

Key words: salt bath, water chemistry, saprolegnia, animal welfare.

RESUMEN. Los baños de sal, dentro del sistema productivo de salmonidos en agua dulce, son un paso clave en la prevención y el control de diferentes enfermedades como la saprolegnia. Este procedimiento habitual genera un agotamiento rápido del O2 en el agua, afectando parámetros sanguíneos asociados a bienestar animal, como Na+, Cl-, glucosa y en situaciones prolongadas hipercapnia. Este trabajo tiene como objetivo evaluar en terreno la respuesta de parámetros sanguíneos asociados a bienestar de los peces y parámetros físico-químicos del agua a la que están sometidos en este periodo, comparando dos sistemas de baño de sal. Para ello se sometió a un grupo de Salmo salar en etapa pre-smolt a un baño de sal habitual, otro grupo a un sistema de recirculación con cascada de agua para su desgasificación y un grupo control sin sal, exponiéndolos a diferentes períodos de una y dos horas. Los resultados de la concentración de CO2 en el agua muestran variaciones significativas entre ambos sistemas con y sin recirculación para la condición control y con sal. Los niveles sanguíneos evaluados en terreno de PCO2 y HCO3 presentaron diferencias significativas respecto del sistema con recirculación y al control, no así los niveles sanguíneos de pH. Nuestros datos de terreno demuestran que los baños de sal que se realizan con regularidad en los centros de cultivo generan efectos significativos sobre parámetros sanguíneos asociados a bienestar animal de los peces, específicamente PCO2 y HCO3, provocando hipercapnia leve sin generar acidosis sanguínea, al añadir recirculación del agua con una cascada a los baños de sal, no se ven afectados los parámetros sanguíneos.

Palabras clave: baño de sal, química del agua, saprolegnia, bienestar animal.

INTRODUCTION

In recent years, animal welfare has become an increasingly relevant subject for consumers and regulatory authorities in various countries, and the importance of animal welfare in the aquaculture industry has consequently grown (Hastein 2004). Animal welfare is particularly applicable to the aquaculture industry given the existing relationship between stressful situations and poor immune system responses in cultivated fish, which provokes increased rates of pathogenic infection (Dhabhar 2009, Tort 2011, Vargas-Chacoff et al 2014). There are a wide variety of stressful stimuli in salmonid farming, among which, animal management and confinement are the most significant (Kestin 1994). Confinement stress resulting from high stock density is produced due to a reduced water volume where fish are held, causing a social response and increased physiological activity (Thomas et al 1999). In turn, management procedures, such as vaccination, sanitary baths, transport, and taking biometric measurements,
activate biochemical processes associated with the stress response (Gesto et al. 2013).

In salmon farming, there are two types of stress responses. The first is an acute response to short-term events, such as fish capture and handling, biometric analyses, and transport. The second is a chronic response to persistent, long-term conditions such as high stock density, variations in water quality, exposure to new environments, social dominance, and exposure to certain diseases (Wendelaar Bonga 1997, Polakoff et al. 2006, Tort 2011, Vargas-Chacoff et al. 2014).

Salt baths are one of the management procedures used by the aquaculture industry. These baths are generally administered in non-circulating tanks, generating rapid O2 depletion in the water, a significant increase in CO2 concentration, and a consequent pH decrease (Auro de Ocampo and Ocampo-Camberos 1999). In goldfish (Carassius auratus), salt baths can significantly affect blood parameters, such as Na, Cl, and glucose, depending on the salt concentration and exposure time (Burgdorf-Moisuk et al. 2011).

In the culture of freshwater fish, salt baths are commonly used to treat pathogens such as Flavobacterium and Saprolegnia sp (Rasowo et al. 2007, Mifsud and Rowland 2008, Wangen 2012). Saprolegnia sp are oomycete fungi more closely related to chromophyte algae than other fungi, meaning that the fungicides used for the control of this genus are ineffective (Avendaño 2012). Saprolegnia sp is both primary and secondary, or opportunistic, pathogens that can infect completely healthy salmon or dead tissue. Saprolegniosis was for a long time kept under control using green malachite. However, the use of this carcinogenic chemical compound is now prohibited (Mifsud and Rowland 2008, Avendaño 2012). Currently, salt is still used as an effective control method against saprolegniosis. Salt baths typically last one hour and employ concentrations between 1.5-3.0%, with oomycetes of Saprolegnia sp unable to proliferate at NaCl concentrations greater than 1.75% (Avendaño 2012, Wangen 2012). On the other hand, low concentration salt baths counterbalance the osmotic stress produced by cutaneous lesions by preventing electrolyte loss (Van West 2006, Wangen 2012).

The objectives of the present study were to evaluate the effects of salt baths in the field on the physicochemical parameters of the water and how these would impact blood parameters associated with the welfare of pre-smolt Atlantic salmon (Salmo salar). To achieve these aims, two salt bath systems for the control of Saprolegnia sp. were evaluated in the field using the i-STAT equipment to determine variations in the physicochemical properties of the water and in blood parameters (i.e. glucose, Na+, K+, Cl-, pH, PCO2, HCO3).
RESULTS

Regarding the physicochemical properties of the water, no significant differences were observed in the O₂ levels for any of the treatments as compared to the controls (P > 0.05, table 1). In the case of the CS treatment group, 20 minutes after the start of the trial, O₂ was injected into the tank, but levels never surpassed 9.5-10.0 mg/L, maintaining the same conditions as the remaining groups. Significant differences (P < 0.05) were observed between the CO₂ levels of the control groups and treatment salt bath groups at both one and two hours. Specifically the traditional CS baths (figure 1A) evidenced a significant increase in CO₂ levels, surpassing the critical levels (10 mg/L) for salmonids (Tang et al 2009) in the two hour treatment group. In contrast, the CO₂ levels of the OS treatment groups (figure 1B) were slightly increased but remained well below critical levels for all treatment times.

The pH levels of the water did not show significant differences against the control (P > 0.05), with values between 6.6 and 6.1 in the CS treatment groups (table 1). Furthermore, no significant differences (P > 0.05) in water salinity were found between the CS and OS treatment groups (15.2 g/L; table 1). The control group, with no salt, presented a salinity of 0.06 g/L.

The plasmatic parameters of Na⁺ did not present significant differences between the treatment and control groups (figure 2A). A similar result was found for the blood levels of Cl⁻, which also showed no significant differences between groups (figure 2B). Additionally, the average levels of K⁺ in the blood were 3.42 ± 1.36 mmol/L, with no significant differences between the treatment and control groups (figure 3). Blood pH did not significantly vary in relation to the control groups, presented average values of 7.16 ± 0.036. Glucose levels also did not significantly vary between the treatments and controls (figure 3).

Table 1. Average concentration of oxygen, pH and salinity in the water treatment ponds (± SD).

<table>
<thead>
<tr>
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<th>Closed recirculation</th>
<th>Open recirculation</th>
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<tr>
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<td>Control</td>
<td>Treatment</td>
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<td>Time (minutes)</td>
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<tr>
<td>0</td>
<td>60</td>
<td>120</td>
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<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>8.2±0.8</td>
<td>8.3±1.0</td>
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<tr>
<td>pH</td>
<td>6.4±0.1</td>
<td>6.3±0.1</td>
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<tr>
<td>Salinity (g/L)</td>
<td>0.06±0.0</td>
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Figure 1. Water concentrations of CO₂ over the duration of the salt baths. A) Closed water recirculation system, CS. B) Open water recirculation system, OS. Data represent the mean ± S.E. * indicates significant differences (P < 0.05) compared to the control.
Regarding the blood levels of gases, PCO₂ presented significant differences between OS and CS fish, where the PCO₂ levels of the two-hour CS treatment group were significantly greater (P < 0.05) than the one-hour CS control group (figure 4). Furthermore, both the treatment and control OS groups had lower PCO₂ levels at two hours than the CS groups, but these differences were not significant. Fish exposed to a CS salt bath for two hours showed the highest blood levels of HCO₃⁻, reaching levels significantly different than the control (figure 5).
Figure 4. Plasma concentration of PCO₂ fish in both systems; CS without recirculation with recirculation OS. The data represent the mean ± SE of 6-8 fish. * Indicates significant differences (P < 0.05) compared to control.

Concentración plasmática de PCO₂ de peces *Salmo salar* en ambos sistemas; Sin recirculación CS, con recirculación OS. Los datos representan la media ± EE de 6 a 8 peces. * Indica diferencias significativas (P < 0.05) respecto del control.

Figure 5. Plasmatic HCO₃ variations n fish from closed recirculation (CS) and open recirculation (OS) systems. Data are represented as the mean ± S.E. of 6-8 fish. * indicates significant differences (P < 0.05) compared to the control; # indicates significant differences (P < 0.05) between systems.

Variación de los niveles plasmáticos de HCO₃ de peces *Salmo salar* en ambos sistemas; Sin recirculación CS, con recirculación OS. Los datos representan la media ± E.E.M. de 6 a 8 peces. * significa diferencias significativas (p < 0.05) respecto del control, # significa diferencias significativas (P < 0.05) entre sistemas.
DISCUSSION

Salt baths alter physiological functions, such as metabolism and osmoregulation, in fish (Burgdorf-Moisuk et al. 2011). These alterations are associated with increased respiration, as observed in the present study through the rapid uptake of O₂ and significant increase in CO₂ in the water of salt bath-treated groups (figure 1, table 1). Specifically, the CO₂ levels recorded for the water of the two-hour CS treatment group reached 12 mg/L, and CO₂ levels between 12 and 30 mg/L have been found to affect the growth, conversion rate, and immune status of Salmo salar (Smart et al. 1979, Wedemeyer 1996, Fivelstad et al. 1998). Wedemeyer (1996) suggested that salmonids can tolerate CO₂ levels up to 40 mg/L during transport or over short periods (Tang et al. 2009). In contrast, the control OS and CS groups did not surpass CO₂ levels of 4 mg/L, which were nearly basal as compared to the CS treatment groups. Importantly, as individual factors, salt and a CS were able to significantly increase the CO₂ levels in water, as evidenced by the lower CO₂ levels in both the CS and OS control groups (figure 1). The increased CO₂ levels in the treatment groups likely represent a stress response due to increased salinity. This would be in agreement with that reported by Morgan and Iwama (1991), who found that a sudden increase in salinity is related to increased metabolic costs and, consequently, greater oxygen uptake.

The oxygen levels in water were variable for both the control and treatment groups. In the case of the CS groups, the oxygen levels decreased to 7.5 mg/L after 20 minutes, requiring the injection of oxygen to maintain levels near 10 mg/L. The OS treatment group permitted a greater exchange of gases between the water and air, thus effectively reducing CO₂ excess while naturally increasing the availability of oxygen. Ultimately, this would result in better respiration for the fish, thereby reducing physical stress and facilitating better adaptation to salt baths.

The levels of Na⁺ and Cl⁻ in the plasma are relevant indicators of ion transfer through the gills, and changes in these levels represent the first response of a fish when the respiratory regulation of the acid-base balance becomes limited (Perry and Gilmour 2006). For example, rainbow trout (Oncorhynchus mykiss) evidence significantly increased plasma Na⁺ levels when exposed to conditions of hypercapnia, or high levels of CO₂ (Larsen and Jensen 1997). The results of the present study did not reflect changes in plasmatic Na⁺, which might be due to the short exposure period to hypercapnia conditions. Regarding Cl⁻, plasmatic Cl⁻ levels are reduced as a result of an electro-neutral exchange of ions with HCO₃⁻, resulting in a relationship between plasmatic Cl⁻ levels and HCO₃⁻ synthesis (Lloyd and White 1967, Fivelstad et al. 2003, Hosfeld et al. 2008, Fivelstad et al. 2015). Contrasting these prior studies, the present results did not evidence this positive relationship, which could, once again, be due to the short experimental period evaluated.

Likewise, blood glucose levels did not significantly vary between the currently assessed treatment and control groups. However, glucose levels in the CS treatment groups were slightly elevated as compared to the control groups for both evaluated time periods. In goldfish, one-hour salt baths at a concentration of 20 g/L modify plasmatic glucose levels (Burgdorf-Moisuk et al. 2011). The present study was performed under field conditions and in water with controlled oxygen levels, which is noteworthy considering the possible relationship between blood glucose levels and plasmatic oxygen levels (Perry et al. 1989).

It is very important to highlight that blood pH did not present significant variations between the assessed groups, suggesting an internal adaptation of fish to an acute period of stress. If plasmatic pH falls and respiratory acidosis occurs, rainbow trout are able to compensate for a period of 48-72 hours, while Atlantic salmon can compensate for 24-96 hours. One way in which salmonids adapt to pH variation is by increasing blood levels of HCO₃⁻, which consequently promotes CO₂ transport in the blood (Foss et al. 2007).

The recorded blood concentrations of HCO₃⁻ and PCO₂ reflect the physiochemical parameters of the water, where a relationship was found between plasmatic HCO₃⁻ and water CO₂ levels in both the OS and CS treatment conditions. This relationship is due to the high permeability of the gills to CO₂, and in conditions of hypercapnia, rapid internal equilibration occurs by elevating the levels of PCO₂ in the blood and tissues (Tang et al. 2009). Once internalised, CO₂ is rapidly transformed by the enzyme carbonic anhydrase into carbonic acid (H₂CO₃), resulting in blood acidosis. Furthermore, studies have found that prolonged hypercapnia leads to increased blood levels of PCO₂, activating the primary stress response in fish (Perry et al. 1989) and leading to changed pH levels (Eddy et al. 1977, Thomas and Le Ruz 1982), increased respiratory volume and rate (Janssen and Randall, 1975, Thomas et al. 1983, Fivelstad et al. 1999) PCO₂ = 5 Torr PWO₂ = 400 u2013450 Torr Salmo gairdneri R.9934Wuc0\u8211{}36, reduced O₂ levels, and variations in transport ability through the Bohr and Root effects (Eddy and Morgan 1969, Eddy et al. 1977, Wedemeyer 1996).

Blood pH was not significantly affected, indicating that the assessed fish did not suffer prolonged hypercapnia. However, HCO₃⁻ and PCO₂ levels were significantly affected, evidencing moderate hypercapnia, the effects of which were offset by the fish. Among the adaptation strategies of fish to hypercapnia exposure are increased ventilation and the use of chemosensors on the periphery of the gills that monitor environmental CO₂ levels (Burleson and Smatresk 2000, Reid et al. 2000, Gilmour 2001, Perry and McKendry 2001). These chemosensors are only stimulated by changes in the water concentration of CO₂ and not by changes in pH (Perry and McKendry 2001).

Salt baths administered with an open recirculation system did not significantly affect the physiochemical parameters
of the water as compared to the untreated control group. In contrast, salt baths administered with a closed recirculation system resulted in increased water CO₂ concentrations. Likewise, the two-hour, closed recirculation treatment group showed significantly affected blood parameters (i.e. PCO₂ and HCO₃⁻) as compared to the respective control. These changes resulted in moderate hypercapnia, directly affecting animal welfare in culture conditions.

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