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Short communication

Effects of the ectoparasite *Caligus rogercresseyi* on *Salmo salar* blood parameters under farm conditions

Margarita P. González [a,b,1], José L.P. Muñoz [c,*,1], Víctor Valerio [d], Luis Vargas-Chacoff [e]

[a] Programa de Doctorado en Ciencias de la Acuicultura, Universidad Austral de Chile, Los Pinos s/n Balneario Pelluco, Puerto Montt, Chile
[b] Departamento de Salud Hidrobiológica, Instituto de Fomento Pesquero, Balmaceda 252, Puerto Montt, Chile
[c] Centro de Investigación y Desarrollo i~mar, Universidad de los Lagos, Casilla 557, Puerto Montt, Chile
[d] South Smart Solutions for engineering, Puerto Montt, Chile
[e] Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Independencia, 641, Valdivia, Chile

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ABSTRACT

Between 2006 and 2009, the Chilean salmon industry suffered a major sanitary crisis caused by outbreaks of infectious salmon anemia virus and *Caligus rogercresseyi*. Additionally, *Piscirickettsia salmonis*, the causative agent of piscirickettsiosis, and the parasite *C. rogercresseyi* continue to impact the salmon industry. This study evaluates the effects of both *C. rogercresseyi* infestation and *P. salmonis* infection concurrently on the blood parameters of *Salmo salar* (Atlantic salmon) under field conditions, providing an estimated parasite threshold at which the least impact occurs on host physiology. The presence of *P. salmonis* and *C. rogercresseyi* increased *S. salar* hematocrit, plasma glucose, and pCO_2 levels but decreased hemoglobin and pO_2 levels. Significant threshold values of parasite abundances were estimated for glucose, hematocrit, hemoglobin, and lymphocytes. This is the first study to evaluate the effects of *C. rogercresseyi* on *S. salar* physiology and to provide estimated abundance threshold values under farm conditions. Although the threshold estimations will likely be of great value for surveillance and control programs, they are an initial approximation that requires further confirmation. The prevalence of *C. rogercresseyi* and *P. salmonis* in Chilean salmon farms necessitates further research on the development and establishment of on-farm pathogen indicators.

Statement of relevance: The manuscript is the first study of *C. rogercresseyi* effects on *S. salar* blood parameters on farm conditions, with an estimation of abundances thresholds values. This information is of great relevance in an integrated pest management program and *S. salar* welfare.

1. Introduction

Parasitic sea lice are a major problem for the salmon and trout industries worldwide. In Chile, the sea louse *Caligus rogercresseyi* (Boxshall and Bravo, 2000) is a primary concern for salmon farms located in the south of the country. Sea lice impose a number of economic burdens on salmonid producers due to the costs of antiparasitic treatments and the monetary losses resulting from parasite-induced skin lesions and weight loss in fish (Carvajal et al., 1998; Costello, 2009; Helgesen et al., 2014; Jaramillo et al., 2015). Moreover, Oelckers et al. (2014) indicated that *C. rogercresseyi* is a potential mechanical vector of the infectious salmon anemia virus that affects Atlantic salmon (*Salmo salar*). This ectoparasite could also play an important role in the transmission of the bacterium *Piscirickettsia salmonis*, another major pathogen affecting Chilean salmonid farms (Rozas and Enríquez, 2014), but further studies are needed to confirm this point.

Due to the high loads of sea lice observed during the Chilean salmon crisis of 2006–2007, the Chilean National Fishing and Aquaculture Service (SERNAPESCA) implemented a control and surveillance program in farms. This program included the establishment of sea lice thresholds for the application of pharmacological treatments (González et al., 2015a; González et al., 2015b; Hamilton-West et al., 2012; Rozas and Asencio, 2007; Valdes-Donoso et al., 2013). Threshold values are an important component of an integrated pest management program (Ehi-Eromosele et al., 2013) and are central to the current Chilean management program.

González et al. (2015b) were the first to determine *C. rogercresseyi* threshold values for physiological responses in *S. salar*. Specifically, loads of 6–7 adult sea lice per fish induced increased plasma cortisol and glucose levels. However, the study of González et al. (2015b) was performed under controlled laboratory conditions. Therefore, the aims

* Corresponding author.
E-mail addresses: margaritapgg@hotmail.com, margarita.gonzalez@ifop.cl (M.P. González), joseluis.munoz@ulagos.cl (J.L.P. Muñoz), vvalerio@3se.cl (V. Valerio), luis.vargas@uach.cl (L. Vargas-Chacoff).
[1] These authors contributed equally to this work.

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of the present study were to evaluate the effects of *C. rogercresseyi* and *P. salmonis* infections on the blood parameters of *S. salar* under field conditions. These analyses were performed to estimate the physiologically negligible parasite load threshold for *S. salar*.

2. Materials and methods

2.1. Animals and sample collection

Twenty-five adult *S. salar* (3.9 ± 0.92 kg) were available for sampling, a condition imposed by the salmon producer who provided the *S. salar* samples. Fish were taken from a marine cage farm located in the Aysén Region (45°34′12″S–72°03′58″W) of southern Chile. Mean water temperature and oxygen concentration at the sampling site were 8.5 °C and 8.0 mg/l, respectively. Eight laboratory-reared *S. salar* (≈1.5 kg) with no history of *C. rogercresseyi* infestation or *P. salmonis* infection were used as the control group, in tanks at 10.5 °C water temperature and 10.0 mg/l oxygen concentration. Despite that the latter group was under controlled conditions, its inclusion responds to the need to obtain a parasite abundances gradient starting from zero, and also to make comparisons among groups with and without pathogens. Before tissue sampling, fish were exposed to a lethal 50 mg l^{-1} dose of Aqui-S™ clove oil and euthanized by spinal transection following the guidelines established for the use of laboratory animals by the National Commission for Scientific and Technological Research of Chile and the Universidad Austral de Chile. The fish, water tray, and holding tray were inspected for detached parasites, which were counted and classified by developmental stage according to González and Carvajal (2003). These counts were recorded as the number of parasites per fish. Fish blood samples were collected from the caudal peduncle using 3 ml syringes and added to 1.5 ml heparinized tubes (1000 units of porcine heparin per 1 ml of 0.9% NaCl).

2.2. Blood analysis

Aliquots (250 μl) were taken from the experimental and control groups to evaluate pH, glucose, pCO_2, and pO_2 parameters using a CG8 + cartridge and the i-STAT equipment. Temperature correction ($\Delta T = 37\ ^\circ C$ − mean water temperature of 8.5 °C, as fish temperature) was applied to pH, pCO_2, and pO_2 parameters using the following formulas described in Hosfeld et al. (2008), Gallagher et al. (2010), and Merkin et al. (2010): pH correction = pH i-STAT + 0.013 * (ΔT); pCO_2 correction = pCO_2 i-STAT * (10 − 0.019 * (ΔT)); and pO_2 correction = pO_2 i-STAT * (10 − 0.0058 * (ΔT)). Blood smears (50 μl aliquots) were prepared from whole blood on glass microscope slides, air dried, and stained with the commercial Quick Diff™ Stain Set (Medion Diagnostics, Switzerland). A hemogram was performed on each blood sample by counting cells using a light microscope, thus obtaining erythrocyte, hemoglobin (Hb), and lymphocyte levels. A hematocrit (Hct) test was performed using i-STAT equipment.

2.3. Pathogen detection

Pathogens were detected in sampled fish following methodologies for aquaculture samples established by the Chilean National Fishing and Aquaculture Service. Detection tests were performed for the infectious salmon anemia virus, *Renibacterium salmoninarum*, and *P. salmonis*. For this, head and posterior kidney samples were analyzed by reverse transcription polymerase chain reaction. Additionally, the presence of *P. salmonis* was assessed using an immunofluorescence antibody test IFAT. Control fish were also analyzed for pathogens, but no clinical signs of disease were observed.

2.4. Sampling design

Pathogen analyses were used to detect the incidence of both *P. salmonis* and *C. rogercresseyi* in farmed *S. salar*. Although the main

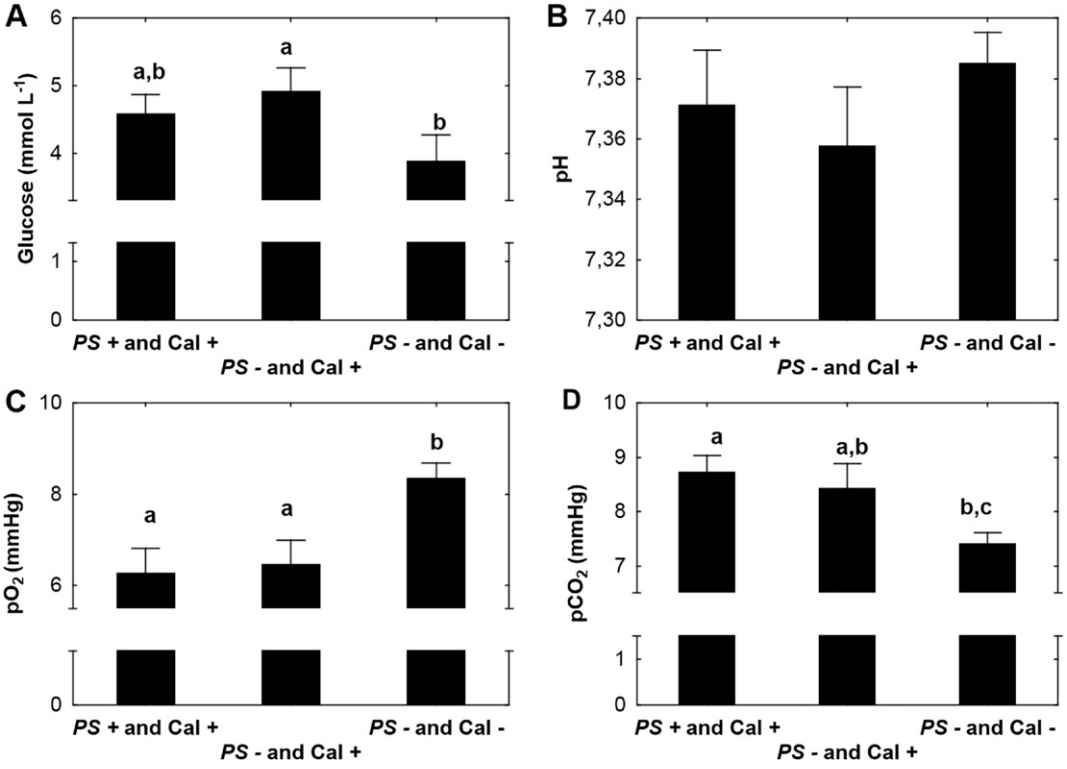

Fig. 1. Plasma levels of A) glucose, B) pH, C) pO_2, and D) pCO_2 for *Salmo salar* in relation to *Caligus rogercresseyi* abundances and *Piscirickettsia salmonis* presence in farm conditions. PS + and Cal + indicates *P. salmonis* positive and *C. rogercresseyi* infested; PS − and Cal + indicates *P. salmonis* negative and *C. rogercresseyi* infested; and PS − and Cal − indicates the control group without pathogens. Different letters indicate significant ($p < 0.05$) differences among treatments.