Aquaculture 438 (2015) 47–54

Contents lists available at ScienceDirect

Aquaculture

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

Effects of *Caligus rogercresseyi* (Boxshall and Bravo, 2000) infestation on physiological response of host *Salmo salar* (Linnaeus 1758): Establishing physiological thresholds

Margarita P. González [a,*], Sandra L. Marín [b], Luis Vargas-Chacoff [c]

[a] Programa de Doctorado en Ciencias de la Acuicultura, Universidad Austral de Chile, Los Pinos s/n, Balneario Pelluco, Puerto Montt, Chile
[b] Instituto de Acuicultura, Universidad Austral de Chile, Los Pinos s/n, Balneario Pelluco, Puerto Montt, Chile
[c] Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Independencia 641, Valdivia, Chile

ARTICLE INFO

Article history:
Received 18 October 2014
Received in revised form 23 December 2014
Accepted 27 December 2014
Available online 6 January 2015

Keywords:
Physiological thresholds
Caligus rogercresseyi
Experimental infestation
Salmo salar

ABSTRACT

After the sanitary crisis suffered by the Chilean salmon industry (infectious salmon anemia virus and *Caligus rogercresseyi*), the Chilean National Fisheries Service implemented an official surveillance and control program, including a threshold at which control measures of lice must be taken. However, this threshold was set as a way to prevent outbreaks of the parasite population and did not consider salmon welfare. This study evaluated how different abundances of attached and mobile stages of *C. rogercresseyi* affected the physiological response of *Salmo salar* and identified the threshold at which parasite populations caused negligible physiological effects on the host. Three groups of *S. salar* juveniles, with two replicates each, were randomly assigned to six tanks. Two experimental groups were subjected to infestation pressures of 50 and 100 copepodids per fish, and the control group was not exposed to any copepodids. Sampling was done at 1 day pre-infestation and 1, 8, 16, and 22 days postinfestation. During sampling, *C. rogercresseyi* specimens were classified as either copepodid, chalimus I–II, chalimus III–IV and adults (female and male). The physiological variables measured in the sampled fish were cortisol (as the primary stress response) and plasma proteins, amino acids, triglycerides, lactate, osmolality, and number and diameter of skin mucous cells (as secondary stress responses). All variables were analyzed using a piecewise linear approach. Significant effects were observed for all variables and along the development of the parasite. The lowest threshold of which negligible impact was observed was six adult *C. rogercresseyi* per fish: above this level, the fish physiology was altered. Advanced stages of the parasite were the most detrimental to fish physiology, as suggested by both high cortisol levels and chronic energy demand. This is the first study to evaluate the effects of *C. rogercresseyi* on *S. salar* physiology, information that constitutes a valuable, objectively derived tool to aid in the development of effective, integrated pest management programs.

1. Introduction

Caligus rogercresseyi (Boxshall and Bravo, 2000) is the dominant sea lice parasite affecting the salmon and trout industry in southern Chile. The parasitic life cycle includes eight developmental stages separated by a molt (González and Carvajal, 2003). Three stages are planktonic, including two naupliar stages and one copepodid stage, the latter being the infective stage. The parasite continues to develop, going through four attached parasitic stages (chalimus I–IV) and one mobile, adult stage (Marín et al., 2014).

Between 2004 and 2007, both the number of *C. rogercresseyi* positive cages and parasite counts on Chilean salmon farms increased (Hamilton-West et al., 2012). In June 2007, the infectious salmon anemia virus (ISAv) was first isolated from farmed *Salmo salar* on a farm with high mortalities in southern Chile (Godoy et al., 2008; Mardones et al., 2009; Valdes-Donoso et al., 2013). Within two years, the disease had spread throughout southern Chile (Valdes-Donoso et al., 2013).

Oelckers et al. (2014) demonstrated that *C. rogercresseyi* is capable of being a mechanical vector for the ISA virus, as Nylund et al. (1994) had suggested for *Lepeophtheirus salmonis*. The sanitary and economic impacts of ISA and *C. rogercresseyi* on salmon production (Valdes-Donoso et al., 2013) prompted the Chilean National Fisheries Service to implement official surveillance and control programs for these two diseases (Hamilton-West et al., 2012; Valdes-Donoso et al., 2013).

The *Caligus* control program set out to monitor parasite loads and implement control measures if necessary (Sernapesca, 2012). Although fish welfare has become of great concern all over the world (Huntingford and Kadri, 2009), this program did not consider salmon

* Corresponding author at: Instituto de Acuicultura, Universidad Austral de Chile, Los Pinos s/n, Balneario Pelluco, Puerto Montt, Chile. Tel.: +56 65 2277119.
E-mail addresses: margaritapgg@hotmail.com (M.P. González), smarin@spm.uach.cl (S.L. Marín), luis.vargas@uach.cl (L. Vargas-Chacoff).

http://dx.doi.org/10.1016/j.aquaculture.2014.12.039
0044-8486/

welfare. Broom (1988) defined individual welfare as the state of an animal as regards its attempts to cope with its environment, and Segner et al. (2012) mentioned that good welfare can be viewed upon as the ability to maintain homeostasis and normal biological functions.

A stress response is defined as a state of threatened homeostasis which is re-established by a complex repertoire of physiological and behavioral adaptive responses of the organism (Chrousos, 1998; Ellis et al., 2012) and can be observed at three levels: primary (catecholamines and cortisol release), secondary (changes in metabolism, hydromineral balance, immune function, and cellular responses), and tertiary (alteration in reproduction and growth, inhibition of resistance to disease, and, ultimately, survival) (Barton, 2002; Wendelaar Bonga, 1997).

As a primary response, Sutherland et al. (2014) found elevated plasma cortisol in chum salmon infected with *L. salmonis*. A short-term (5–10-day) infection with as few as ten pre-adult or adult parasites per fish was enough to cause a stress response in *S. salar* (Nolan et al., 1999; Wagner et al., 2008). Information about secondary responses indicates an increase in fish energy demand at 14 and 21 days postinfestation (dpi) with *L. salmonis* (Wells et al., 2006). The advanced stages of *L. salmonis* were the most detrimental for the fish (Bowers et al., 2000).

Most physiological studies have focused on the effects of *L. salmonis* on different hosts, and studies regarding the effects of *C. rogercresseyi* are scarce. If one of the main goals of a control program is to establish thresholds for controlling the parasite and protecting the host species (Brooks, 2009), then threshold definitions should include maintaining both low parasite abundances and good host conditions. The aims of this study was to evaluate the effects of different abundances of attached and mobile stages of *C. rogercresseyi* on the physiological response of *S. salar* not previously infected with this parasite and to detect an objective parasite threshold at which negligible physiological effects occur in the host.

2. Materials and methods

2.1. Animals and maintenance conditions

S. salar juveniles were randomly distributed in six circular tanks (300 L) and acclimated for 30 days. Mean fish weight among tanks was not significantly different therefore the mean fish weight grouping of all fish was 81.4 g (n = 156, weight range: 77.05–85.74 g, confidence interval (CI): 95%). Final mean weight was 124.94 g in a range of 116.9–133.0 g, (CI: 95%), with no significant differences among tanks. Fish were maintained under a flow-through (7.5 L min^{-1}) seawater system with seawater filtered at 2 μm and a photoperiod of 8:16 h light:dark (Marín et al., 2014). The food given to the fish was Biomar CPK 100 diet (Proteins: 41–49%; Lipids: 17–25%). Fish were fed "ad libitum" daily using commercial dry pellets at a ratio of 1.5% body weight and fish satiety was used as an indicator to end fish feeding. Lack of appetite was not observed in fish at any time. Salinity (mean: 31 psu; 95% CI: 30.86–31.18), temperature (mean: 11.3 °C; 95% CI: 11.27–11.37), and oxygen saturation (mean: 86.8%; 95% CI: 86.42–87.25) were registered daily using a YSI 85 multifunction meter (Yellow Springs Instruments Inc.).

2.2. Production of infective stages and fish infection

Copepodids for fish infection were obtained from *C. rogercresseyi* females collected from fish maintained at the Ecological Interactions Laboratory of Universidad Austral de Chile in Puerto Montt, Chile. Females were transported to the laboratory and egg-strings were carefully removed and classified as mature (dark) and immature (white). Only mature eggs were incubated in 5-L tanks containing seawater at 32 psu (filtered at 5 μm and UV-disinfected). The culture chamber was kept at 12 °C, with a 12:12 photoperiod and constant aeration. Infection trials began when 90% of the free-living lice reached the copepodid stage. The infection trials were performed according to Marín et al. (2014), except that the detention time of the water flow was 6 h. The control group was exposed to the same conditions as the infection groups, but with no copepodids added to the tank.

2.3. Experimental design

The fish groups (two experimental and one control) were randomly assigned to six tanks with two replicates each. In order to obtain a gradient of parasite loads on fish, one group was exposed to an infestation pressure of 50 copepodids per fish (50 IP), the second group was exposed to 100 copepodids per fish (100 IP), and the control group was not exposed to any copepodids (NE). This design was used because incremental levels of infestation by *C. rogercresseyi* copepodids do not produce a linear response in terms of settlement on *S. salar* (Araya et al., 2012) and because there is a high variability in the infestation success (Marín et al., 2014). Sampling times were carried out at 1 day pre-infestation and 1, 8, 16, and 22 dpi to find the stages of copepodid, chalimus I–II (Ch I–II), chalimus III–IV (Ch III–IV) and adult (female and male).

2.4. Sampling procedure

On each sampling day, five fish were randomly removed from each tank and euthanized to obtain the samples for estimating welfare condition parameters. Each fish was individually netted and subjected to lethal doses of benzocaine chlorhydrate (>250 mg L^{-1}) and completely euthanized by spinal cord separation before tissues were removed. Fish blood was collected from the caudal peduncle using 1-mL syringes and added to 1.5-mL heparinized tubes (1000 units of porcine heparin per 1 mL of 0.9% NaCl). Plasma was separated by centrifuging the whole blood (5 min, 11.000 g, 4 °C) and then stored at −20 °C. Fish, tray water, and trays were inspected for detached parasites, which were counted and classified by developmental stage according to González and Carvajal (2003) and named as no. of parasite * $fish^{-1}$. Fish were weighed and skin samples were taken from the left side of the fish. Skin samples (approximately 10 cm) were triangular in shape, simulating the shape of the fish. They were preserved in 100 mL Davidson AFA solution.

2.5. Plasma parameters

Plasma glucose, triglyceride, and lactate levels were measured using commercial kits from Spinreact (Glucose-HK Ref. 1001200; Lactate Ref. 1001330) adapted for 96-well microplates (Vargas-Chacoff et al., 2009). Plasma cortisol was measured by ELISA using a commercial kit from DIA Source Immuno Assays S.A. (Cortisol Ref. KAPDB270). The inter-assay coefficient of variation at 50% binding was 5.6% (n = 4), whereas the mean intra-assay coefficient of variation (calculated from the sample duplicates) was 5.6%. The mean percentage of recovery was 95% (n = 4). Main cross-reactivity of 100% given by cortisol was detected with prednisolone (13.6%), deoxycorticosterone (7.2%), cortisone (6.2%), and corticosterone (7.6%) (Vargas-Chacoff et al., 2014). Plasma protein and amino acid levels were determined using a bicinchoninic acid BCA Protein Assay Kit (Pierce #23225) (Vargas-Chacoff et al., 2009). All assays were performed with a Microplate Reader (Multiscango, Thermo Scientific), using Software Scan 3.2 of Multiscango. Plasma osmolality was measured with a vapor pressure osmometer (Advanced Instruments, INC, model 3320) and expressed as mOsm kg^{-1}.

2.6. Skin histology procedures

Skin samples (2 × 2 cm) were dissected from the mid-dorso-lateral body on the left side (where a line drawn from approximately the start of the dorsal fin would intersect with the lateral line). The site was selected according to the description of *C. rogercresseyi* distribution on