Aquaculture 464 (2016) 469–479

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Atlantic salmon (*Salmo salar*) and Coho salmon (*Oncorhynchus kisutch*) display differential metabolic changes in response to infestation by the ectoparasite *Caligus rogercresseyi*

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ARTICLE INFO

Article history:
Received 24 April 2016
Received in revised form 25 July 2016
Accepted 26 July 2016
Available online 27 July 2016

Keywords:
Amino acids
Atlantic salmon
Carbohydrates
Coho salmon
Lipids
Plasma

ABSTRACT

Caligus rogercresseyi sea lice negatively impact Chilean salmonid farming, but no complete characterization for the metabolic effects of *Caligus* infestation currently exists. Therefore, the aim of this study was to analyze the effects of *C. rogercresseyi* infestation on the metabolic responses of *Salmo salar* and *Oncorhynchus kisutch*. Energy metabolism responses to *C. rogercresseyi* were examined over a time-course infestation of both salmonid species. Plasma metabolite levels and enzymatic activities related to intermediate metabolisms of carbohydrate, amino acid, and lipid were evaluated in the liver and muscle. Plasma glucose levels changed in both salmonid species, increasing at 1–3 days post-infestation. In turn, triglyceride levels increased at days 3 and 7 in *O. kisutch* and *S. salar*, respectively, while protein and total α-amino acids increased in *O. kisutch* but decreased in *S. salar* during infestation. Amino acid intermediate catabolic metabolism in the liver and muscle of *O. kisutch* increased during infestation, indicating a higher use of the gluconeogenic pathway than *S. salar*. Lipid intermediate anabolic metabolism increased in *O. kisutch* liver, remained unchanged in *S. salar* liver, and increased 1 day post-infestation in the muscle of both salmonids. Liver and muscle carbohydrate intermediate anabolic metabolism in *O. kisutch* increased during infestation, suggesting that this species preferentially uses the glycogenolytic-glucolytic pathway, in contrast with *S. salar*. In conclusion, amino acid and carbohydrate catabolism enzymes in *O. kisutch* activated soon after initial sea lice infestation, which would allow this species to dispose of energy substrates earlier than *S. salar*. This physiological data contributes towards the ability of *O. kisutch* to more adeptly cope with the increased energy demand imposed by *C. rogercresseyi* infestation.

1. Introduction

Aquaculture is the most consistently expanding industry in the world. In marine farming, changes in abiotic conditions (e.g. temperature, salinity, photoperiod), biotic factors (e.g. fish nutritional status, parasitism), management procedures (e.g. manipulation, transport, stocking density), and interactions with other species can activate endogenous stress systems in fish (Barton, 2002; Vargas-Chacoff et al., 2009a, 2014a; Wendelaar-Bonga, 1997). In turn, the endogenous stress system induces a variety of physiological responses, categorized as primary (catecholamine and cortisol release), secondary (changes in metabolism, respiration rates, hydromineral balance, immune function, and cellular responses), and tertiary responses (alterations in reproduction and growth, inhibition of disease resistance, and modified survival) (Barton, 2002; Wendelaar-Bonga, 1997).

Metabolic stress responses in teleost fish are characterized by changes in the levels of plasma glucose and hepatic glycogen, which are mobilized to peripheral tissues to cope with energy-demanding restoration processes (Barton, 2002; Polakof et al., 2006; Wendelaar-Bonga, 1997). Stress may cause a high consumption of energy reserves, triggering the reallocation of existing metabolic energy to other physiological processes that could negatively affect immunological capacity. In turn, this would impact the infection resistance of fish that is normally achieved through

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http://dx.doi.org/10.1016/j.aquaculture.2016.07.029
0044-8486/

the coordination and modulation of distinct immune system components (Barton and Iwama, 1991; Iwama and Nakanishi, 1997; Mommsen et al., 1999; Pickering, 1998; Wendelaar-Bonga, 1997). Teleost fish exposed to stressful conditions can produce an energetic mobilization that could potentially interfere with immunological responses to pathogens, including bacteria, viruses, and parasites (Barton et al., 1986; Mommsen et al., 1999; Wendelaar-Bonga, 1997).

Ectoparasites can substantially affect hosts by impacting physiological, behavioral, and morphological traits, as well as by damaging the integument of the host (Bunkley-Williams and Williams, 1998; Lehmann, 1993; Vargas-Chacoff et al., 2014a; Wagner et al., 2003; Wendelaar-Bonga, 1997). One such integument-damaging ectoparasite is the Southern Hemisphere sea louse (*Caligus rogercresseyi*), one of the major problems affecting the salmonid industry worldwide (Hamilton-West et al., 2012; Jaramillo et al., 2015, 2016). In recent years, Chilean salmonid production has been negatively affected by *C. rogercresseyi*, the costs of which (e.g. through chemical control treatments and associated fish mortality) have made sea lice the most economically detrimental parasite affecting the industry (González and Carvajal, 2003). The host fish of this parasite are both endemic to Chile and introduced. Infestation begins with attachment of the copepodid, the free-swimming planktonic stage of *C. rogercresseyi*, to the scales and fin rays of hosts. Once attached, sea lice mature into adults (González and Carvajal, 2003), feeding on host mucus and skin as a sole energy source.

Recently, González et al. (2015) studied the primary physiological responses to *C. rogercresseyi* infestation in Atlantic salmon (*Salmo salar*), also described changes in blood parameters in field (González et al., 2016). Parasite infestation leads to osmotic stress and generally reduces the condition of fish, resulting in susceptibility to secondary infections (Dawson et al., 1999; Wells et al., 2006). Fast et al. (2002) described coho salmon (*Oncorhynchus kisutch*) to be less susceptible to sea lice, but the metabolic consequences of infestation in this host fish are still unknown.

The present work is the first study to experimentally assess the effects of *C. rogercresseyi* infestation over time on the metabolic responses of Atlantic and coho salmon, the primary salmonid species farmed in Chile. This investigation contributes towards furthering current understandings in aquaculture by comparing the effects of sea lice infestation in salmonid species previously characterized as either infestation-susceptible (Atlantic salmon) or -resistant (coho salmon).

2. Materials and methods

The experiments were performed under the guidelines for the use of laboratory animals established by the Comisión Nacional de Ciencias y Tecnología de Chile (CONICYT) and the Universidad Austral de Chile.

2.1. Fish and experimental design

A group of juvenile post-smolt Atlantic salmon (166.4 ± 17.5 g body weight, n = 250) and coho salmon (161.2 ± 15.8 g body weight, n = 250) were purchased from the Puerto Phillipi and Chaparano fish farms, respectively. All fish were verified free from pathogens by accredited laboratories. The salmonids were then transported to the Lenca Laboratory of Fundación Chile in Quillaipe, Chile and distributed into tanks (500 L) maintained with a continuous flow-through system, a 12:12 light:dark-hour photoperiod cycle, and 12 ± 2 °C. The fish were completely acclimated for two weeks and then maintained under the same conditions for a further three weeks. During these acclimation and maintenance stages, fish were fed ad libitum using EWOS Transfer 100, without boost, pellet feed.

2.2. Experimental conditions

The metabolic responses of Atlantic and coho salmon to *C. rogercresseyi* infestation were evaluated. For this, triplicate experimental (i.e. infested) and duplicate control tanks were established for each salmonid species. Each experimental tank was infested with 35 *C. rogercresseyi* copepodids per fish. Sea lice were obtained from previously collected specimens maintained at the Fundación Chile Laboratory in Puerto Montt, Chile according to the protocols defined by González et al. (2015). The control tanks were not subjected to parasite infestation. Samples were taken at time 0 (prior to infestation), and at 1, 3, 7, and 14 days post-infestation (dpi). Ten fish were sampled from each tank at each sampling time-point, including from the non-infested control tank.

2.3. Sampling procedure

Fish were netted, submitted to a lethal dose of clove oil AQUI-S™ (Bayer Company, Germany) (50 mg L^{-1}), and euthanized by spinal sectioning before tissue removal. The fish, water, and collection trays were inspected for detached parasites, which were counted and classified by developmental stage (González and Carvajal, 2003). Fish were weighed, and blood was collected from the caudal peduncle in 1 mL heparinized syringes (25,000 units ammonium heparin, 3 mL of 0.6% NaCl saline solution). Plasma was separated from cells by centrifuging whole blood for 5 min at 2000 × *g* and 4 °C. The collected plasma was snap frozen in liquid N_2 and stored at −80 °C until analyses. Muscle portions and the complete liver were taken from each fish, frozen in liquid N_2, and stored at −80 °C.

2.4. Plasma parameters

Plasma glucose and triglycerides were measured using the Glucose-HK Ref. 1001200 and Triglyceride Ref. 1001311 commercial kits (Spinreact, Girona, Spain) adapted to 96-well microplates. Plasma protein was determined using the Pierce BCA Protein Assay Kit #23225 (Thermo Fischer Scientific, Waltham, MA, USA). Total α-amino acid levels were assayed colorimetrically using the ninhydrin method described by Moore (1968) and adapted to a microplate assay. All assays were performed with a Multiskan GO Microplate Reader (Thermo Fischer Scientific, Waltham, MA, USA) using SkanIt v.3.2.

2.5. Tissue metabolites and enzymatic activities

Frozen liver and muscle tissues were finely minced in an ice-cooled petri dish and divided into two aliquots to assess enzymatic activities and metabolite levels. Tissues used for metabolite concentration assessments were homogenized by ultrasonic disruption with 7.5 volumes of ice-cooled 0.6 N perchloric acid, neutralized using 1 M potassium bicarbonate, and centrifuged for 30 min at 13,000 × *g*) in an Eppendorf 5415R (Sigma-Aldrich, St. Louis, MO, USA). The supernatant was then used to assay tissue metabolite levels. Tissue triglyceride levels were spectrophotometrically determined using the Triglyceride Ref. 1001311 commercial kit (Spinreact, Girona, Spain). Tissue glycogen concentrations were assessed using the Keppler and Decker (1974) method. Glucose obtained after glycogen breakdown (i.e. after subtracting free glucose levels) was determined with the Glucose-HK Ref. 1001200 commercial kit (Spinreact, Girona, Spain). Total α-amino acid levels were colorimetrically assessed using the ninhydrin method described by Moore (1968) and adapted to a microplate assay.

The tissue aliquots used for assessing enzymatic activities were homogenized by ultrasonic disruption with 10 volumes of ice-cold stopping buffer (pH 7.5) containing 50 mM HCl, 1 mM 2-mercaptoethanol, 50 mM NaF, 4 mM 165 EDTA, 250 mM sucrose, and 0.5 mM p-methylsulphonyl fluoride (Sigma Chemical Co., St. Louis, MO, USA), which were added as dry crystals immediately before homogenization. The homogenate was centrifuged for 30 min at 13,000 × *g*), and the resulting supernatant was used in enzyme assays of fructose 1,6-bisphosphatase (FBP, EC 3.1.3.11), glutamate dehydrogenase (GDH, EC 1.4.1.2), alanine aminotransferase (GPT-AT, EC 2.6.1.2), aspartate aminotransferase

Table 1
Plasma metabolite values for glucose, triglycerides, total proteins, and total α-amino acids from untreated *O. kisutch* (day 0) and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). Values represent the means ± SEM of 10 fish per group. Different letters indicate significant differences between days at the same condition. The symbol (+) indicates significant differences between control and infested fish groups at the same experimental time (two way ANOVA, post hoc Tukey-test, $P < 0.05$).

Plasma *O. kisutch*	Condition	Time (days)				
		0	1	3	7	14
Glucose (mM)	Control	4.45 ± 0.12	4.41 ± 0.07	4.36 ± 0.21	4.36 ± 0.43	4.56 ± 0.31
	Infested	4.58 ± 0.15b	5.31 ± 0.14a +	5.21 ± 0.21a +	4.72 ± 0.19ab	4.71 ± 0.16ab
Triglycerides (mM)	Control	0.71 ± 0.14	0.81 ± 0.14	0.74 ± 0.07	0.67 ± 0.05	0.87 ± 0.22
	Infested	0.83 ± 0.13b	0.89 ± 0.14b	1.36 ± 0.18a +	0.81 ± 0.08b	0.94 ± 0.13b
Total proteins (mg/mL)	Control	41.15 ± 3.21	37.21 ± 3.99	36.01 ± 3.02	36.29 ± 4.39	38.25 ± 1.33
	Infested	42.86 ± 2.19b	41.42 ± 1.91b	44.21 ± 1.18a +	38.98 ± 1.29b	42.06 ± 2.47b
Total α-aminoacids(mM)	Control	34.58 ± 2.61a	32.71 ± 2.24ab	27.19 ± 0.59ab	21.41 ± 3.12b	28.64 ± 1.88ab
	Infested	41.86 ± 3.22a	35.41 ± 2.07a	39.06 ± 1.75a +	26.41 ± 1.95b	29.25 ± 1.79b

Table 2
Plasma metabolite values for glucose, triglycerides, total proteins, and total α-amino acids from untreated *S. salar* (day 0) and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). Values represent the means ± SEM of 10 fish per group. Different letters indicate significant differences between days at the same condition. The symbol (+) indicates significant differences between control and infested fish groups at the same experimental time (two wayANOVA, post hoc Tukey-test, $P < 0.05$).

Plasma *S. salar*	Condition	Time (days)				
		0	1	3	7	14
Glucose (mM)	Control	4.85 ± 0.12	5.02 ± 0.23	4.88 ± 0.12	5.06 ± 0.05	4.93 ± 0.15
	Infested	4.75 ± 0.11b	5.37 ± 0.19a	5.41 ± 0.28a	4.83 ± 0.13ab	4.38 ± 0.08ab
Triglycerides (mM)	Control	0.37 ± 0.02	0.35 ± 0.09	0.31 ± 0.08	0.36 ± 0.06	0.31 ± 0.11
	Infested	0.37 ± 0.06b	0.37 ± 0.02b	0.51 ± 0.06a	0.81 ± 0.11a +	0.26 ± 0.02b
Total proteins (mg/mL)	Control	36.02 ± 2.81	43.05 ± 0.91	40.91 ± 1.57	41.26 ± 1.79	39.28 ± 1.09
	Infested	36.31 ± 1.31	36.06 ± 1.58	38.46 ± 1.83	39.15 ± 0.87	34.02 ± 1.65
Total α-amino acids (mM)	Control	40.35 ± 2.24	41.24 ± 0.75	40.75 ± 1.53	39.46 ± 1.52	41.14 ± 2.66
	Infested	39.61 ± 2.23b	35.17 ± 1.91a +	36.18 ± 0.94a +	35.92 ± 0.77a +	35.65 ± 1.02a +

Table 3
Hepatic metabolite values for glycogen, glucose, triglycerides, soluble proteins, and total α-amino acids values from untreated *O. kisutch* (day 0) and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). Values represent the means ± S.E.M of 10 fish per group. Further details: Table 1 legend.

Liver *O. kisutch* (μmoles/g wet weight)	Condition	Time (days)				
		0	1	3	7	14
Glycogen	Control	83.72 ± 3.24	86.66 ± 12.01	85.23 ± 11.92	87.23 ± 12.25	79.98 ± 15.18
	Infested	80.95 ± 9.61a	38.62 ± 7.91b +	64.94 ± 4.34a	80.53 ± 10.91a	77.89 ± 7.43a
Glucose	Control	20.37 ± 1.87	19.81 ± 2.51	18.92 ± 1.87	21.34 ± 0.99	20.74 ± 1.58
	Infested	21.14 ± 1.92a	12.81 ± 1.19b +	11.03 ± 1.41b +	9.91 ± 1.01b +	16.41 ± 1.98a
Triglycerides	Control	1.32 ± 0.25	1.21 ± 0.17	1.49 ± 0.21	1.54 ± 0.13	1.31 ± 0.12
	Infested	1.55 ± 0.19b	1.61 ± 0.25b	2.69 ± 0.12a +	1.75 ± 0.11b	1.98 ± 0.12b +
Soluble proteins	Control	113.42 ± 4.09	114.41 ± 2.94	113.66 ± 3.39	115.38 ± 1.82	114.41 ± 2.05
	Infested	114.01 ± 2.91b	124.85 ± 1.81a +	116.14 ± 1.51b	112.15 ± 4.49b	112.15 ± 2.24b
Total α-amino acids	Control	85.53 ± 7.91	80.55 ± 13.7	75.85 ± 5.44	84.78 ± 9.45	86.19 ± 9.19
	Infested	91.01 ± 12.37b	71.61 ± 3.57b	127.21 ± 13.66a +	82.87 ± 9.72b	77.09 ± 9.21b

Table 4
Hepatic metabolite values for glycogen, glucose, triglycerides, soluble proteins, and total α-amino acids from untreated *S. salar* (day 0) and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). Values represent the means ± S.E.M of 10 fish per group. Further details: Table 1 legend.

Liver *S. salar* (μmoles/g wet weight)	Condition	Time (days)				
		0	1	3	7	14
Glycogen	Control	17.76 ± 1.98	16.36 ± 5.27	19.05 ± 5.85	15.51 ± 4.76	15.57 ± 9.23
	Infested	19.96 ± 4.43a	3.07 ± 2.64b +	34.57 ± 6.99a	29.31 ± 12.68a	12.29 ± 9.29a
Glucose	Control	12.21 ± 1.93	12.64 ± 1.49	12.36 ± 1.25	13.53 ± 0.98	13.51 ± 2.21
	Infested	13.58 ± 0.45	16.12 ± 1.18 +	16.64 ± 1.01 +	16.11 ± 0.88	16.81 ± 0.84
Triglycerides	Control	3.17 ± 0.01	2.98 ± 0.33	3.32 ± 0.25	2.93 ± 0.29	3.15 ± 0.27
	Infested	3.21 ± 0.24	2.61 ± 0.14	3.73 ± 0.19	3.49 ± 0.23	3.17 ± 0.18
Soluble proteins	Control	129.95 ± 2.64	130.54 ± 2.81	131.15 ± 4.81	129.57 ± 1.35	128.74 ± 1.46
	Infested	133.16 ± 4.32	130.91 ± 2.73	113.33 ± 1.34a +	125.19 ± 4.04	130.68 ± 2.48
Total α-amino acids	Control	78.22 ± 10.46	83.63 ± 11.89	79.11 ± 10.99	78.61 ± 8.38	80.68 ± 8.86
	Infested	83.48 ± 13.32	76.54 ± 6.51	79.29 ± 4.63	84.95 ± 7.44	97.42 ± 6.16

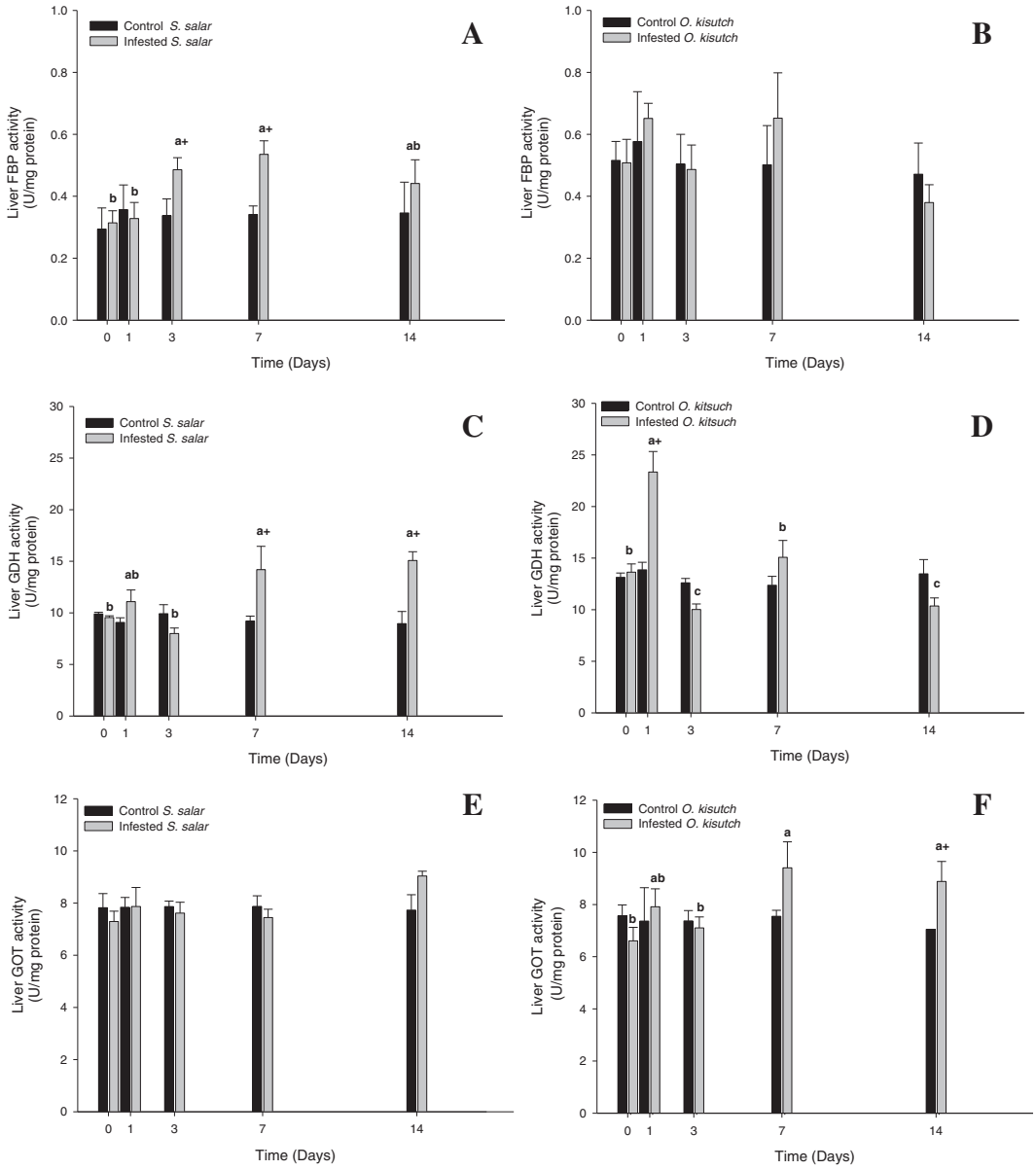

Fig. 1. Hepatic enzyme activities from untreated *S. salar* and *O. kisutch* (day 0), and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). A–B) Fructose 1,6-bisphosphatase [FBP], C–D) Glutamate dehydrogenase [GDH], E–F) Aspartate aminotransferase [GOT].Values are mean ± SEM of 10 fish per group. Different letters indicate significant differences among days of experiment. Symbol (+) indicates significant differences between control and infested fish group (two way ANOVA, post hoc Tukey-Test, $P < 0.05$).

(GOT-AT, EC 2.6.1.1), glycerol-3-phosphate dehydrogenase (G3PDH, EC 1.1.1.8), glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), pyruvate kinase (PK, EC 2.7.1.40), and glycogen phosphorylase (GP, EC. 2.4.1.1). Enzymatic activities were determined using a Multiskan GO microplate reader (Thermo Fischer Scientific, Waltham, MA, USA) and SkanIt v.3.2software 3.2. Enzyme reaction rates were determined by changes in absorbance of NAD(P)H at 340 nm. The reactions were started by adding homogenates (15 μL) at a pre-established protein concentration, omitting the substrate in control wells (final volume 275–295 μL), and allowing the reactions to proceed at 37 °C for pre-established times (5–15 min). Protein levels were assayed in triplicate using the Pierce BCA Protein Assay Kit #23225 (Thermo Fischer Scientific, Waltham, MA, USA). Enzyme assays were carried out at initial velocity conditions.

The conditions for enzyme assays, described in detail elsewhere (Sangiao-Alvarellos et al., 2003, 2005a, 2005b; Vargas-Chacoff et al., 2009b, 2014a,b,c, 2016a,b), were as follows: FBP in the liver was determined in 85 mM imidazole-HCl (pH 7.7), 0.5 mM NADP, and 5 mM $MgCl_2$ 6 H_2O, with excess phosphoglucose isomerase and G6PDH (Sigma Chemical Co., St. Louis, MO, USA), and 0.1 mM FBP as the substrate. GDH activities in the liver and muscle were assessed in 50 mM imidazole-HCl (pH 7.8), 250 mM ammonium acetate, 0.1 mM NADH, and 1 mM ADP, with 80 mM α-ketoglutaric acid as the substrate. Hepatic and muscle GPT-AT was assessed in 50 mM imidazole-HCl (pH 7.8), 0.025 mM pyridoxal 5′-phosphate, 10 mM α-ketoglutaric acid, and 0.2 mM NADH, with excess LDH (Sigma Chemical Co., St. Louis, MO, USA) and 2.5 mM L-alanine as the substrate. Hepatic and muscle GOT-AT was assessed in 50 mM imidazole-HCl (pH 7.8), 10 mM α-ketoglutaric, and 0.32 mM NADH, with 10.5 mM L-aspartate as the substrate and MDH as the enzymatic solution. G3PDH activities in the liver and muscle were assessed in 50 mM imidazole-HCl (pH 7.8) and 0.15 mM NADH, with 0.2 mM DHAP as the substrate. Hepatic and

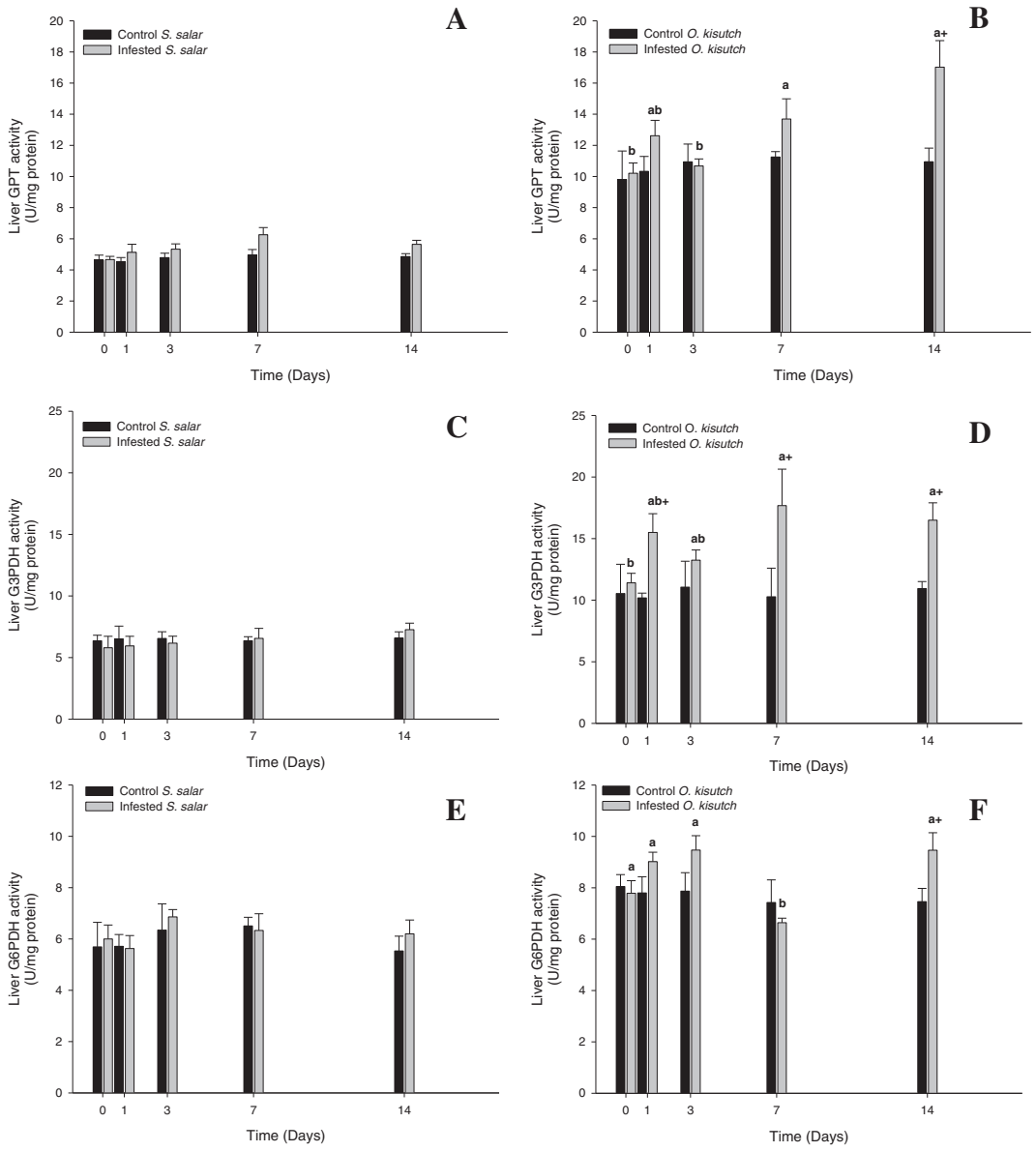

Fig. 2. Hepatic enzyme activities from untreated *S. salar* and *O. kisutch* (day 0), and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). A–B) Alanine aminotransferase [GPT], C–D) Glycerol-3-phosphate dehydrogenase [G3PDH], E–F) Glucose-6-phosphate dehydrogenase [G6PDH]. Values are mean ± SEM of 10 fish per group. Further details in the legend of Fig. 1.

muscle G6PDH was assayed in 78 mM imidazole-HCl (pH 7.7), 5 mM $MgCl_2$, and 0.5 mM NADP, with 1 mM glucose 6-phosphate as the substrate. Hepatic and muscle PK activity was assessed using 50 mM imidazole-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM KCl, 0.15 mM NADH, 1 mM ADP, and 21 U mL^{-1} LDH, with 0.5 mM PEP as the substrate. Only in the liver was 25 μM of FBP added as a co-factor. Finally, GP activity in the liver and muscle was assessed using 50 mM phosphate buffer (pH 7.0), 27 mM $MgSO_4$, 19.5 mM EDTA, 0.5 mM NADP, 2.5 AMP, 0.527 U $well^{-1}$ phosphoglucomutase, 2.2 U $well^{-1}$ G6PDH, and 100 μM glucose 1,6-bisphosphate as co-factor, with 5 mg ml-1 glycogen as the substrate. Active GP activity was measured with 10 mM caffeine, and total GP activity was estimated without caffeine.

2.6. Statistical analyses

Assumptions of normality and homogeneity for the variances were tested. For each metabolic variable, two-way analysis of variance was performed. The factors of variance were the infested fish and time. A post hoc Tukey-test was used to identify significantly different groups. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Fish survival

No fish mortalities were observed in any group throughout the acclimatization, maintenance, or experimental periods.

3.2. Plasma parameters

Both coho and Atlantic salmon displayed increased glucose levels 1 and 3 dpi, with coho showing statistically different results when compared to the control group on both sampling days.

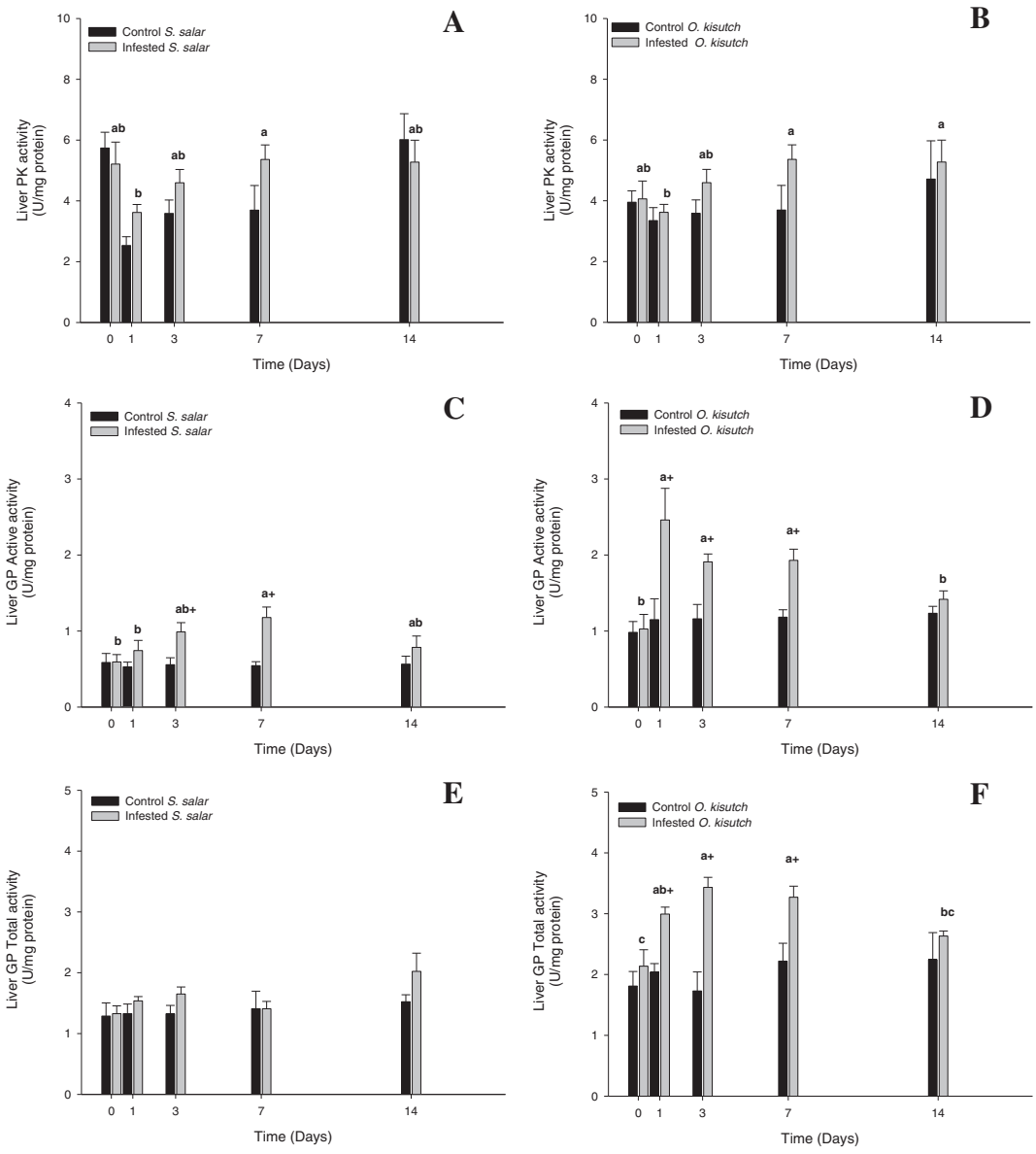

Fig. 3. Hepatic enzyme activities from untreated *S. salar* and *O. kisutch* (day 0), and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). A–B) Pyruvate kinase [PK], C–D) Glycogen phosphorylase active [GP active), E–F) Glycogen phosphorylase total [GP total]. Values are mean ± SEM of 10 fish per group. Further details in the legend of Fig. 1.

Triglyceride levels increased at 3 dpi in coho salmon, and at 3 and 7 dpi in Atlantic salmon. Proteins levels were high only at 3 dpi in coho salmon. Total α-amino acids level increased at 3 dpi in coho salmon, but decreased in Atlantic salmon during the experimental period (Tables 1 and 2).

3.3. Metabolic parameters

3.3.1. Liver metabolism

Glycogen levels decreased in both species at 1 dpi. While glucose levels in coho salmon continued to increase from 1 and 3 dpi to

Table 5
Muscle metabolite values for glycogen, glucose, triglycerides, soluble proteins, and total α-amino acids from untreated *O. kisutch* (day 0) and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). Values represent the means ± S.E.M of 10 fish per group. Further details: Table 1 legend.

Muscle *O. kisutch* (μmoles/g wet weight)	Condition	Time (days)				
		0	1	3	7	14
Glycogen	Control	2.24 ± 0.67	1.92 ± 0.51	2.31 ± 1.06	2.21 ± 0.81	2.44 ± 1.05
	Infested	2.32 ± 0.47	1.95 ± 1.21	3.07 ± 2.64	3.73 ± 3.47	2.23 ± 0.86
Glucose	Control	5.74 ± 0.53	5.29 ± 0.53	5.06 ± 0.37	5.15 ± 0.62	5.01 ± 0.21
	Infested	5.27 ± 0.39b	6.29 ± 0.22ab	7.29 ± 0.88a+	7.81 ± 1.25a	6.71 ± 0.61ab
Triglycerides	Control	1.02 ± 0.09	1.08 ± 0.06	1.06 ± 0.05	1.07 ± 0.06	1.01 ± 0.09
	Infested	1.14 ± 0.06	1.28 ± 0.06	1.33 ± 0.15	0.94 ± 0.05	1.16 ± 0.11
Soluble proteins	Control	39.43 ± 1.38	39.81 ± 1.31	40.67 ± 0.51	40.05 ± 1.06	38.57 ± 1.59
	Infested	38.89 ± 1.16a	35.61 ± 0.63b+	34.29 ± 1.28b+	36.09 ± 1.07b+	39.19 ± 0.71a
Total α-aminoacids	Control	37.79 ± 5.81	38.19 ± 3.01	38.61 ± 6.34	43.36 ± 3.58	36.83 ± 2.76
	Infested	39.09 ± 5.88b	62.11 ± 5.94a+	61.68 ± 4.47a+	45.96 ± 7.53b	41.16 ± 6.53b

Table 6
Muscle metabolite values for glycogen, glucose, triglycerides, soluble proteins, and total α-amino acids from untreated *S. salar* (day 0) and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation. Values represent the means ± S.E.M of 10 fish per group. Further details: Table 1 legend.

Muscle *S. salar* (μmoles/g wet weight)	Condition	Time (days)				
		0	1	3	7	14
Glycogen	Control	8.35 ± 1.25	6.94 ± 1.55	7.32 ± 2.43	6.31 ± 1.09	6.46 ± 2.26
	Infested	6.32 ± 0.73	6.01 ± 0.64	5.89 ± 0.67	4.62 ± 0.67	3.51 ± 0.91
Glucose	Control	31.13 ± 8.35	29.39 ± 6.79	26.42 ± 3.17	33.58 ± 3.44	33.95 ± 6.97
	Infested	36.46 ± 6.45b	43.72 ± 5.49ab	50.75 ± 3.43ab+	49.67 ± 5.81ab+	59.11 ± 2.66a+
Triglycerides	Control	1.29 ± 0.13	1.34 ± 0.07	1.22 ± 0.07	1.36 ± 0.04	1.29 ± 0.05
	Infested	1.41 ± 0.05b	1.45 ± 0.15ab	1.62 ± 0.06a+	1.47 ± 0.04ab	1.51 ± 0.02ab
Soluble Proteins	Control	62.46 ± 1.81	63.19 ± 2.42	61.45 ± 2.78	61.71 ± 2.95	64.83 ± 3.72
	Infested	61.21 ± 0.51	63.61 ± 2.47	62.79 ± 1.42	63.51 ± 1.46	67.91 ± 0.54
Total α-aminoacids	Control	34.96 ± 6.76	35.63 ± 4.19	38.61 ± 0.93	36.79 ± 6.73	34.56 ± 5.29
	Infested	37.59 ± 6.41	36.05 ± 5.21	30.48 ± 5.42	39.91 ± 3.35	40.02 ± 3.58

respect at control group, Atlantic salmon showed no further statistical differences as compared to the control group, although increased on 1 and 3 after infestation to respect at time cero. Triglyceride levels increased at 3 and 14 dpi only in coho salmon. Similarly, soluble protein and amino acid levels increased at 1 and 3 dpi, respectively, in coho salmon, while Atlantic salmon evidenced no differences

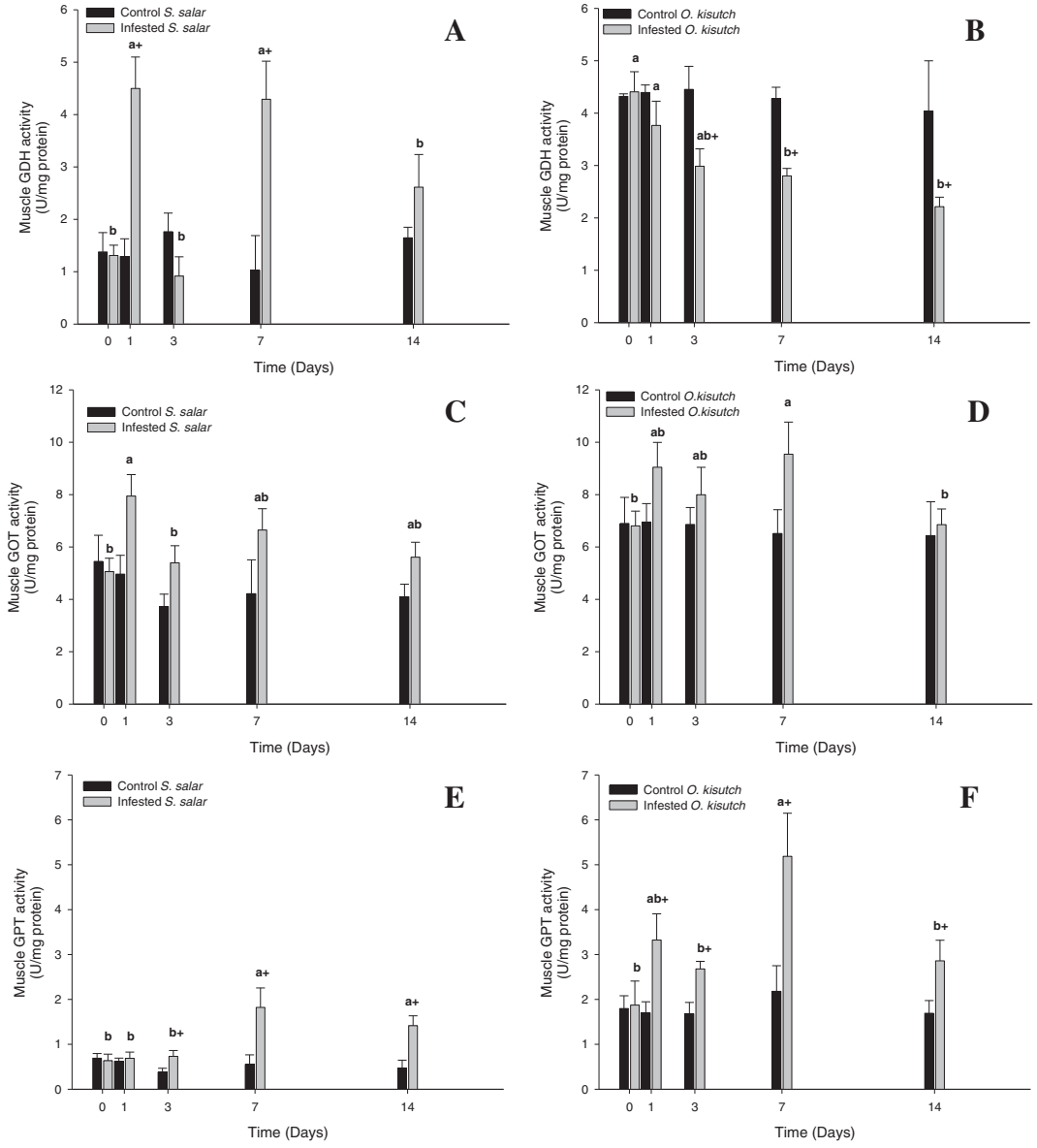

Fig. 4. Muscle enzyme activities from untreated *S. salar* and *O. kisutch* (day 0), and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). A–B) Glutamate dehydrogenase [GDH], C–D) Aspartate aminotransferase [GOT], E–F) Alanine aminotransferase [GPT]. Values are mean ± SEM of 10 fish per group. Different letters indicate significant differences among days of experiment. Symbol (+) indicates significant differences between the control and infested fish group (two way ANOVA, post hoc Tukey-Test, $P < 0.05$).

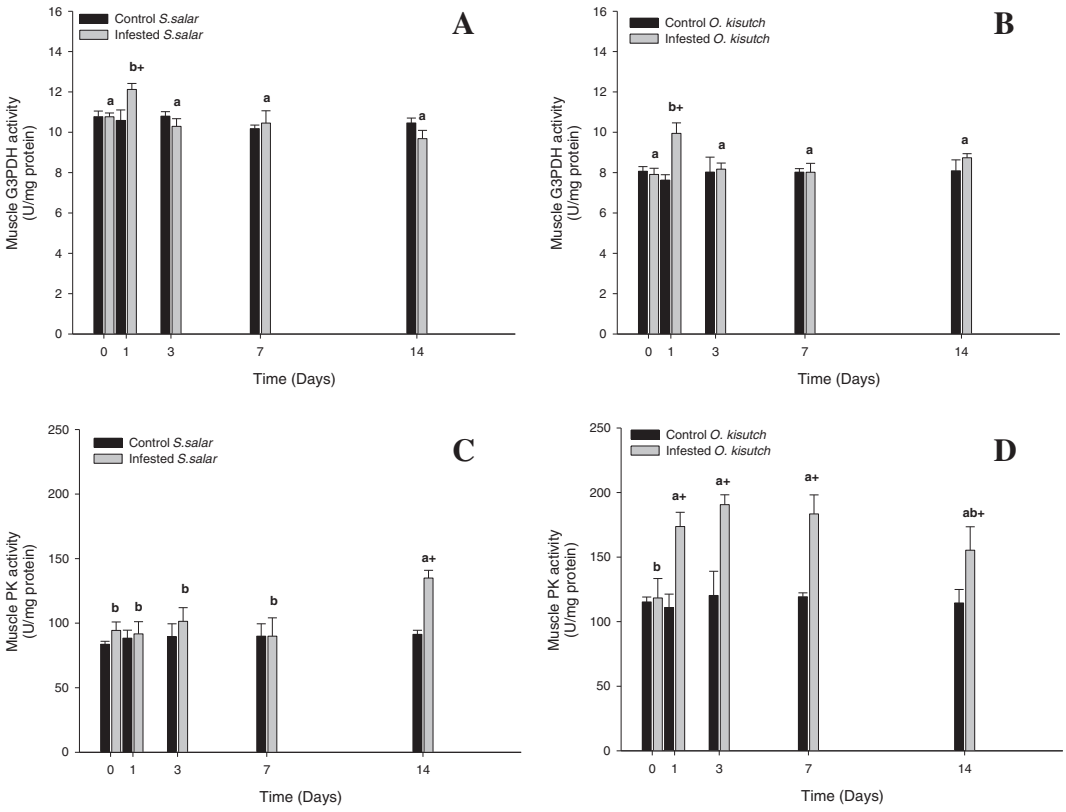

Fig. 5. Muscle enzyme activities from untreated *S. salar* and *O. kisutch* (day 0), and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). A–B) Glycerol-3-phosphate dehydrogenase [G3PDH], C–D) Pyruvate kinase [PK]. Values are mean ± SEM of 10 fish per group. Further details in the legend of Fig. 4.

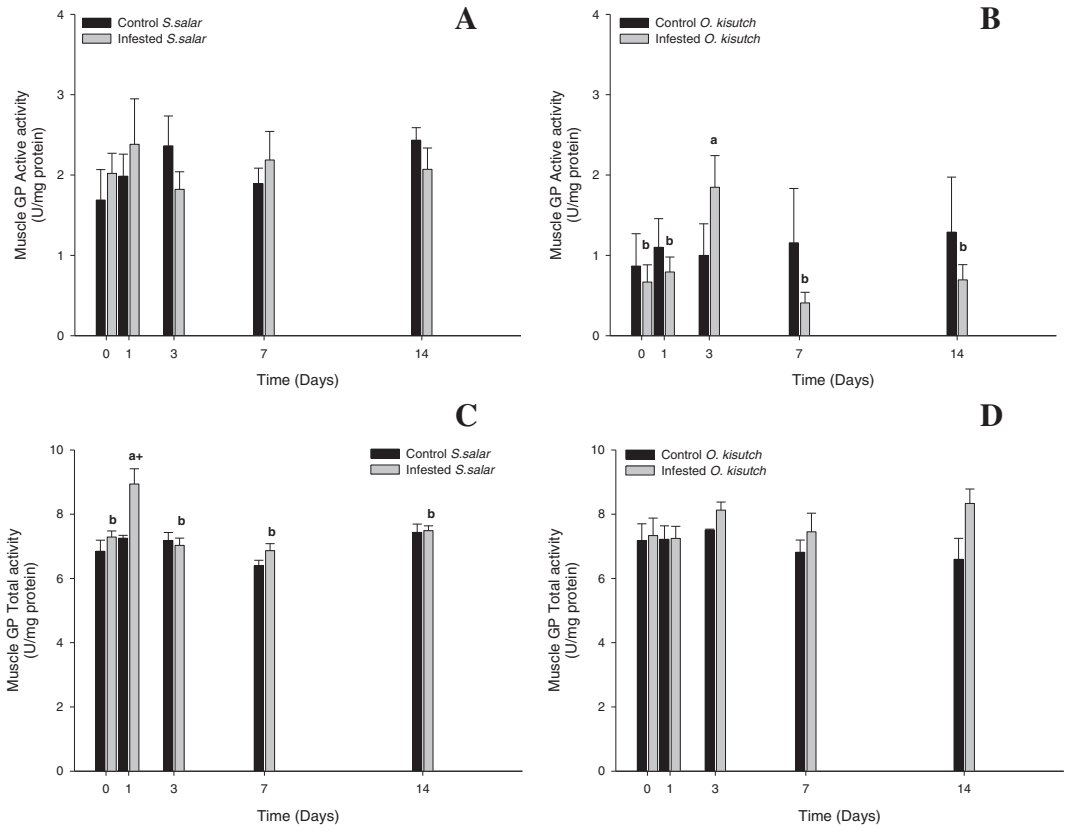

Fig. 6. Muscle enzyme activities from untreated *S. salar* and *O. kisutch* (day 0), and from fish infested with *C. rogercresseyi*. Animals were sampled at 1, 3, 7, and 14 days post-infestation (dpi). A–B) Glycogen phosphorylase active [GP active), C–D) Glycogen phosphorylase total [GP total]. Values are mean ± SEM of 10 fish per group. Further details in the legend of Fig. 4.

between the infested and control groups for either of these variables (Tables 3 and 4).

Various intermediate metabolism enzymes (amino acid and carbohydrate) were assessed, including FBP, a carbohydrate metabolism enzyme implicated in gluconeogenesis; GDH, GOT and GPT as amino acid metabolism enzymes. FBP activity increased in Atlantic salmon from 3 dpi until 14 dpi, while coho salmon displayed no changes at any sampling time (Fig. 1A, B). GDH activity increased at 7 and 14 dpi in Atlantic salmon, while the highest GDH activity in coho salmon occurred at 1 dpi (Fig. 1C, D). GOT and GPT activities were highest at 14 dpi in coho salmon, but levels were insignificantly changed in Atlantic salmon, although GPT levels were lower than in coho salmon (Figs. 1E, F and 2A, B). G3PDH, part of lipid metabolism, activity did not change in Atlantic salmon, but coho salmon displayed increased activity of G3PDH at 7 and 14 dpi (Fig. 2C, D). Carbohydrate metabolism, including G6PDH, PK, active GP, and total GP, exhibited different responses according to salmon species. G6PDH activity remained unchanged in Atlantic salmon, while in coho salmon, G6PDH activity decreased at 7 dpi before increasing at 14 dpi (Fig. 2E, F). PK activity in both salmonids presented the same pattern, with a drop in activity at 1 dpi and a significant recovery at 7 dpi (Fig. 3A, B). Active GP activity in Atlantic salmon increased at both 3 and 7 dpi, while in coho salmon, activity increased at 1, 3, and 7 dpi, but presented similar values to respect at control group over the remaining experimental period (Fig. 3C, D). Total GP activity did not present changes in Atlantic salmon, while coho salmon displayed a pattern similar to that of active GP activity (Fig. 3E, F).

3.3.2. Muscle metabolism

The glycogen levels of both species presented no significant differences between sampling days or fish groups, but glucose levels in Atlantic salmon increased over the experiment. Coho salmon evidenced statistically increased glucose levels only at 3 dpi. Triglyceride levels significantly increased at 3 dpi in Atlantic salmon, with no significant changes in coho salmon. The soluble protein and amino acid levels in Atlantic salmon exhibited no changes over the course of the study. In contrast, coho salmon protein levels decreased on days 1 to 7 after infestation compared to the control group, while amino acids increased on days 1 and 3 dpi (Tables 5 and 6).

In muscle, enzyme activities related to amino acid metabolism, such as GDH, GOT, and GPT, presented differentiated responses. GDH activity increased at 1 and 7 dpi in Atlantic salmon, whereas coho salmon GDH activity decreased, displaying the lowest values at 7 and 14 dpi (Fig. 4A, B). GOT activity in both species increased at 1 dpi (Fig. 4C, D). GPT activity increased at 7 and 14 dpi in Atlantic salmon and in coho salmon from 1 until 14 dpi (Fig. 4E, F). G3PDH activity presented a similar pattern in both fish species, increasing only at 1 dpi (Fig. 5A, B). The enzymatic activity of PK, which is related to carbohydrate metabolism, showed a differential responses, with increased activity at 14 dpi in Atlantic salmon and increased activity from 1 until 14 dpi in coho salmon (Fig. 5C, D).Active GP activity in Atlantic salmon exhibited no significant changes, but coho salmon increased activity at 3 dpi (Fig. 6A, B). Total GP activity in Atlantic salmon increased at 1 dpi, while coho salmon presented no changes (Fig. 6C, D).

4. Discussion

In Atlantic and coho salmon, the ectoparasite *C. rogercresseyi* modified the main pathways related to energy metabolism in two metabolically important tissues, the liver and muscle. This is the first study to report the effects of sea lice on energetic metabolism responses in Atlantic and coho salmon. The obtained results suggest that *C. rogercresseyi* induces hyperglycemia and enhances triglyceride plasma levels in both salmonid species at the beginning of infestation. This could stimulate intermediate catabolic metabolism to increase the plasma levels of energy metabolites. Differences between the responses of the salmon species during the course of the infestation were also observed. These results concur with studies conducted in the Northern hemisphere with other salmonids and sea lice species (Dawson et al., 1999; Wells et al., 2006).

For clarity, the different effects of sea lice on metabolite pathways (amino acid, lipid and carbohydrates), are separately discussed for every salmon species.

4.1. Amino acid metabolism

Salmonid fish being able to preferentially use amino acids to accomplish different metabolic functions (Halver and Hardy, 2002) as well as to the fact that amino acids can act as metabolic signals (Li et al., 2009). Amino acids have a central role in the defense mechanisms since they are involved in the synthesis of an array of proteins such as antibodies and in the control of key immune regulatory pathways. In vertebrates, several amino acids are involved in sustaining immune competence and disease resistance. Amino acid imbalances as well as their antagonisms could affect nutrient utilization and can have a direct consequence on immune organs and responses (Li et al., 2007).

Plasma concentrations for total amino acids decreased from 1 dpi in specimens of Atlantic salmon, while in coho salmon, levels increased at 3 dpi, suggesting the mobilization of this metabolite, a potential energy substrate in peripheral tissues, via gluconeogenesis.

GDH activity is a biomarker for amino acid catabolism in fish (Peh et al., 2010), with Saha et al. (2002) describing GDH assimilates ammonia and produces glutamate, “or vice versa”. In this study, both salmon species presented different responses. Atlantic salmon displayed increased GDH activity in the liver at 7 dpi and in the muscle at 1 and 7 dpi. In turn, amino acid content decreased in the plasma, but no changes were found in the liver or muscle (Figs. 1C, 5A), indicating low protein catabolism rates in these tissues. In coho salmon, liver GDH activity increased at 1 dpi. Also in coho salmon, amino acid levels increased at 3 dpi in plasma and the liver and at 1 dpi in muscle (Figs. 1D, 5B). The amino acids resulting from protein breakdown would likely enter gluconeogenic pathways and the Krebs cycle to cope with the energetic demands caused by salmon lice infestation.

The FBP enzyme is involved in anabolic pathways such as gluconeogenesis, which in vertebrates mainly occurs in the liver. In turn, the liver utilizes non-carbohydrate substrates, such as amino acids, glycerol, or lactate, to generate glucose. The presently obtained FBP results showed that hepatic activity increased only in Atlantic salmon from 3 to 7 dpi, indicating the use of amino acids. This was corroborated by the decreased plasma amino acid levels to produce glucose. In coho salmon, FBP increased, although insignificantly, from 1 to 7 dpi (Fig. 1A–B), coinciding with increased plasma glucose levels (Table 1). This hyperglycemia may be associated with the stress of sea lice infestation, concurring with other researchers (Bowers et al., 2000; González et al., 2015; Wells et al., 2006) and studies on different stressors, such as on the effects of high density and changes in temperature and salinity (Arjona et al., 2009; Mancera et al., 2008; Polakof et al., 2006; Vargas-Chacoff et al., 2009a, 2014b, 2014c).

Certain enzymes related to protein metabolism, such as the transaminases GOT and GPT, play important roles in the utilization of amino acids for oxidation and/or gluconeogenesis (Kumar et al., 2012). There was increased activity of these enzymes in the liver and muscle of both salmon species, although levels were higher in coho salmon (Figs. 1E–F, 2A–B). These increased GOT and GPT activities could explain the enhanced amino acid and glucose levels observed in the liver and muscle, tissues in which amino acids and glucose serve as substrates for the first steps of gluconeogenesis, thus feeding the Krebs cycle without triggering FBP activity (Soengas et al., 2007). The energy produced through this pathway could then be used to fuel the immune system extruding the excess ammonia produced by protein catabolism (Vargas-Chacoff et al., 2014b). In general term the coho salmon increased protein catabolism at compare to Atlantic salmon, exporting amino acid to the blood stream. At coho salmon those amino acids

will be used as primary metabolites to produce glucose in the liver, as the GOT/GPT and G3PDH activities suggested. In Atlantic salmon liver proteins are catabolically degraded (GDH activity), producing amino acids, with those increased its gluconeogenic pathways (FBP) to produce glucose.

4.2. Lipid metabolism

Lipid metabolism, as assessed by G3PDH activity and triglyceride levels, varied significantly between sampling days in the liver of coho salmon, whereas increased activity was observed only at 1 dpi in muscle (Figs. 2D, 5B). This suggests that lipid mobilization from storage tissues to the bloodstream, possibly due to increased triglyceride levels in the liver and plasma at 3 dpi, could be a mechanism to cope with an increased metabolic rate and the subsequent energy demand triggered by sea lice infestation. The G3PDH enzyme serves as a major link between lipid and carbohydrate metabolism as its role in lipid biosynthesis is through the reduction of glycolytic substrates (Robinson et al., 2011). In Atlantic salmon, lipid oxidation as an energy source occurred 1 dpi in muscle, while hepatic G3PDH activity was unaffected by parasite infestation (Fig. 2C). These observations indicate that neither lipid biosynthesis nor gluconeogenesis through glycerol pathways were affected in the liver.

G6PDH is involved in the oxidative phase of the pentose phosphate shunt, supplying NADH for the reductive biosynthesis of fatty acids, which is related to G3PDH (Polakof et al., 2006; Vargas-Chacoff et al., 2016a, b). Only the G6PDH activity in coho salmon liver increased (Fig. 2E–F), further supporting the idea that the effects of sea lice infestation on metabolism vary according to the tissue and fish species.

4.3. Carbohydrate metabolism

The liver is the main site of glycogen/glucose turnover, ammoniagenesis, fatty acid synthesis, and gluconeogenesis in teleosts (Mommsen et al., 1999; Peragón et al., 1999). In this study, Atlantic and coho salmon liver glycogen levels diminished at 1 dpi with *C. rogercresseyi* (Fig. 3A–D), suggesting that parasite exposure increases energy demands that are subsequently met through increased glycogenolysis. Indeed, activation of the glycogenolytic pathway was reflected by increased hepatic activity of total GP in both salmonid species. When considered together, the increased total GP and PK activities indicate enhanced glycogenolytic and glucolytic potentials.

Glycogenolysis produces glucose-1-phosphate that is converted to glucose-6-phosphate by phosphoglucomutase and used in glycolysis and/or exportation to peripheral tissues via dephosphorylation. In muscle, PK activity indicated enhanced glucolytic potentials, utilizing the Krebs cycle as an energy source, which was observed more in coho than Atlantic salmon, while GP activity displayed no changes in glucolytic potentials (Figs. 5C–D, 6A–D). Similarly, other studies have not found clear or significant changes in glucolytic dietary carbohydrate metabolism s in fish white muscle (Hemre et al., 2002; Kirchner et al., 2005; Panserat, 2009), supporting low glycolytic capacity in fish muscle despite elevated muscle glycogen contents (Polakof et al., 2012).

5. Conclusions

In conclusion, amino acid and carbohydrate catabolism enzymes in coho salmon activated soon after initial sea lice infestation, which would allow this species to dispose of energy substrates earlier than Atlantic salmon. The main difference between both species is that coho salmon evidenced a higher protein catabolism in the muscle that Atlantic salmon. This response highlighted the mobilization of amino acid to the blood, serving as substrates for the gluconeogenesis pathway and for the formation of other necessary compounds to combat the sea lice infestation. This pattern contributes towards the ability of coho salmon to more adeptly cope with the increased energy demand imposed by *C. rogercresseyi* infestation, a finding further corroborated by the observed plasmatic and liver protein levels, with higher levels observed via muscle proteins during early infestation. According to Johnson and Albright (1992), coho salmon are capable of mounting a localized immune response in the skin, inducing hyperplasia of epithelial cells and a more dispersed inflammatory response, which may contribute to parasite rejection. Furthermore, the early availability of energy resources in coho salmon could help satisfy energy demands but could also induce a rapid inflammatory response, facilitating the ability of coho salmon to reject parasite infestation.

Conflict of interest

The authors declare that there are no conflicts of interest.

Ethical approval

All experimental procedures complied with the guidelines regarding the use of laboratory animals of the Comisión Nacional de Ciencias y Tecnología de Chile (CONICYT) and the Universidad Austral de Chile.

Acknowledgements

This study was carried out within the framework of INNOVA CORFO 13IDL2 23565, Fondecyt 11130308, 11121498, 1160877, and PAI 7912010009, Fondap-Ideal Grant 15150003. The authors also acknowledge the Dirección de Investigación, Universidad Austral de Chile for their support.

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