Low temperature stress in a cultured fish (*Piaractus mesopotamicus*) fed with *Pyropia columbina* red seaweed-supplemented diet

Analía Ale · Carla Bacchetta · Andrea S. Rossi · Pablo A. Scarabotti · Jimena Cazenave

Abstract This study aimed to analyze the cold stress effects (in terms of hematology, energy reserves, and oxidative stress) in *Piaractus mesopotamicus* (pacú) and their mitigation by a *Pyropia columbina* red seaweed-supplemented diet. For this purpose, juvenile fish were fed with a control (CD) or a red seaweed-supplemented diet (RD) for 60 days, and then, the animals were exposed to a low temperature (14 °C) and a control temperature (24 °C) for 24 h. The cold shock generated an increase of hemoglobin levels in fish fed with both diets. In CD-fed fish, plasmatic triglycerides, cholesterol, and hepatic glycogen decreased after the thermal shock; meanwhile, the animals fed with RD showed decreased hepatic proteins, but increased cholesterol and hepatic glycogen. Regarding oxidative stress, antioxidant enzymes augmented their activity in the liver, intestine, and gills; meanwhile, lipid oxidative damage was observed in the liver and intestine of fish exposed to 14 °C and fed with both diets. Pacú was sensitive to cold shock, but no mitigation effects were observed in fish fed with the supplemented diet. Further research should target higher concentrations of *P. columbina* in supple-mented diets to take advantage of this valuable resource.

Keywords Aquaculture · Cold shock · Nutrition · Hematology · Energy metabolism · Oxidative stress

Introduction

Temperature is an important stress factor in fish aquaculture since its changes could disrupt biochemical reactions and physiological functions (Wen et al. 2017). However, most of the studies took into account biological aspects of fish after long periods of exposure to constant temperatures, and there is a lack of available information considering short-term variations and stress biomarker responses like metabolic parameters (Pinto et al. 2019). Particularly, cold shock (natural or anthropogenic) in fish is a common stress situation which occurs when fish have been acclimated to a specific water temperature or range of temperatures and are subsequently exposed to a rapid decrease in temperature, resulting in a cascade of physiological and behavioral responses and, in some cases, death (Donaldson et al. 2008).

Temperature under the optimum limits of a species could negatively influence the health status and increase susceptibility to diseases (Ahmad et al. 2011). Changes in hematological parameters have been proposed as easy detectors and excellent biomarkers of the health status of fish to represent early diagnosis of pathological disorders generated by variable temperatures (Pinto et al.

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2019). Moreover, thermal stress causes homeostasis modifications which can be evaluated via fish energy reserves (Wang et al. 2012; Nobrega et al. 2019). Lastly, it is widely reported that low water temperature increases endogenous reactive oxygen species (ROS) and trigger DNA, protein, and lipid damage (Cheng et al. 2018).

In order to cope with the prevalence and appearance of new diseases and overcome obstacles to sustainable aquaculture, fish nutrition is recognized to be one of the most important topics in management of fish farming. Thus, increasing attention has been given to the development of nutritional strategies that could mitigate the deleterious effects of stressors associated with aquaculture practices (Gasco et al. 2018). Functional diets have become a suitable alternative for the inclusion of natural ingredients which tend to be more biodegradable than synthetic ones and are less likely to generate resistance (Muñoz et al. 2018).

There are several reports which tested different kinds of supplemented diets. In a recent study, mitigation effects by β-carotene-supplemented diet on physiological and antioxidant biomarkers were analyzed in *Piaractus mesopotamicus* exposed to 14 °C (Bacchetta et al. 2020). In *Cyprinus carpio* fish, a diet supplemented with myrcene and menthol reduced the adverse effects of ammonia in terms of tissue damage and anemia (Hoseini et al. 2019). Another study showed that a diet supplemented with cottonseed meal and exogenous protease improves growth, nutrient assimilation, and hematology parameters in Nile tilapia (Hassaan et al. 2019). Other authors found that synthetic astaxanthin supplementation improved antioxidant activity and resistance to thermal stress in fish (Goda et al. 2018; Cheng et al. 2018).

Dietary algae as feed additive for fish has proved to have many benefits like acceleration of ascorbic acid and improvement of physiological conditions in relation to vitamin C nutrition and lipid metabolism (Nakagawa 1997). Besides, seaweeds are considered a valuable food source that contains high levels of proteins, dietary fibers, well-balanced amino acid profile, and significant amounts of vitamins, omega-3 fatty acids, pigments, and minerals (Burtin 2003; Ngo et al. 2011). In this way, there have been growing commercial interests regarding seaweed diet supplementations in most aquatic species and in many land-farmed animals (Sotoudeh and Mardani 2017). However, up to date, there is still lack of studies that have focused on the evaluation of micro-

or macroalgae as dietary supplements for fish despite their abundance and easy availability in nature (Teimouri et al. 2019).

Pyropia columbina is a red seaweed with high economic interest, usually found on hard substrates in Patagonia Argentina coasts. It was demonstrated that *P. columbina* has bioactive compounds with antioxidant properties (Cian et al. 2014, 2016). Moreover, Cian et al. (2019) found that a supplemented diet with this seaweed improved antioxidant status, promoted higher iron bioavailability, and had a lipid-lowering effect in juvenile fish (*P. mesopotamicus*). Another study showed that Atlantic salmon (*Salmo salar*) fed with a *P. columbina*-based feed improved their immune system based on the expression of immune-relevant genes and white blood cell lysozyme expression (Muñoz et al. 2018).

P. mesopotamicus (commonly named pacú) represents a worldwide important resource for fisheries and aquaculture because of its potential of rustic management, good growth rates, and acceptance in the consumer market. Besides, its herbivorous/omnivorous habits represent an excellent attribute to deal with feed restrictions (Barbieri and Vigliar Bondioli 2013; Claudiano et al. 2019). Optimal range for pacú culture is 20–28 °C, as such, it has been a challenge for farmers to deal with cold temperatures in winter like poor growth performance and low survival rates (Bacchetta et al. 2020). As aquaculture systems are commonly challenged to deal with cold stress, this study aimed to evaluate hematological, energetic, and oxidative stress parameters in *P. mesopotamicus* after a cold-shock exposure and the mitigation effects in fish fed with a *Pyropia columbina* red seaweed-supplemented diet.

Materials and methods

Diets

The formulation and chemical composition of both control (CD) and red seaweed (*Pyropia columbina*)-supplemented (RD) diets are detailed in Table 1. The CD ingredients consisted of commercial cornmeal (613 g kg^{-1}), soybean meal (200 g kg^{-1}), bovine plasma protein concentrate (130 g kg^{-1}), cornstarch (20 g kg^{-1}), vitamin-mineral mix (7 g kg^{-1}), and canola oil (30 g kg^{-1}). In the case of RD, the ingredients were the same as CD but 35 g kg^{-1} of cornmeal was replaced with

P. columbina. The level of red seaweed inclusion was selected according to a previous study of Cian et al. (2019), where the production of the RD-based diet is also described. From the raw *P. columbina* material, the samples were washed in distilled water, dried at 100 ± 4 °C, and ground up into a particle size lower than 1 mm using a laboratory hammer mill (Retsch, Haan, Germany). The powder obtained was passed through a 20-mesh sieve (0.85 mm) and stored at 4 °C in plastic bags until analysis or diet formulation.

Fish and feeding assays

All assays were conducted in the Aquaculture Laboratory at the Instituto Nacional de Limnología (CONICET-UNL, Argentina). Juvenile *Piaractus mesopotamicus* fish were purchased from a local fish farm (Pez Campero, Argentina) ($N = 210$; 7.0 ± 0.5-cm standard length; 12.2 ± 2.5 g). Firstly, fish were acclimated to laboratory conditions for 2 weeks at controlled temperature (24 ± 1 °C). Then, the animals were divided in 35 individual groups per 300-L tanks and fed with CD or RD twice a day at a rate consisted of 5% biomass weight per day. The feeding assay lasted 60 days. All treatments were replicated three times with the consent of the national and institutional guidelines for the protection animal welfare (CONICET 2005).

Low temperature exposure

The thermal-stress exposure was defined at 14 °C, as 7–7.5 °C is considered the lethal temperature for *P. mesopotamicus* according to Milstein et al. (2000).

Table 1 Composition of the control (CD) and red seaweed-supplemented (RD) diets

Components (g kg^{-1}) *	CD	RD
Dry matter	895.3 ± 5.3	892.7 ± 0.9
Crude protein	276.8 ± 4.7	268.2 ± 4.3
Crude lipid	34.4 ± 2.6	31.5 ± 1.6
Total starch	444.2 ± 4.3	443.5 ± 1.2
Ash	29.5 ± 1.4	30.9 ± 1.0
Calcium	1.6 ± 0.1	1.7 ± 0.1
Phosphorus	2.2 ± 0.2	2.4 ± 0.2
Zinc	0.1 ± 0.0	0.1 ± 0.0
Iron	0.2 ± 0.0	0.2 ± 0.0

*Chemical composition expressed as mean $\pm$ SD ($n = 3$)

Artificial climate chambers were employed for exposing fish fed with CD or RD ($n = 18$ per diet) to control (24 ± 0.1 °C) or cold temperature (14 ± 0.1 °C) in 10-L aquaria ($n = 3$ per aquarium, in triplicate). After 24 h, animals were anesthetized with 100 mg L^{-1} of benzocaine (Parma de Croux 1990). Then, blood was taken from the caudal peduncle according to Reichenbach-Klinke (1980) to measure hematological parameters. Lastly, the fish dissection was performed, and the liver, intestine, gills, and muscle were extracted and stored at −80 °C.

Hematological parameters

The following parameters were measured from blood samples of fish ($n = 9$): red blood cell count (RBC), hematocrit (Ht) through the micro-method, and hemoglobin concentration (Hb) employing the cyanmethemoglobin method (Houston 1990). From those values, the hematimetric indexes were calculated as proposed by Cazenave et al. (2005): mean cell volume (MCV = Ht × 10/RBC), mean cell hemoglobin (MCH = Hb × 10/RBC), and mean cell hemoglobin concentration (MCHC = Hb × 100/Ht).

Blood metabolites and energy reserves in the liver and muscle

Plasmatic total protein, triglycerides, cholesterol, and glucose levels ($n = 9$) were measured employing colorimetric commercial kits tested in dish (Wiener Lab®). Total plasma protein concentration was measured by a kit reagent containing EDTA/Cu complex in an alkaline medium that reacts with peptide bonds to yield a purple-blue complex. Plasma levels of total cholesterol and triglycerides were analyzed by using standard enzymatic-colorimetric test, and finally, plasma glucose was assayed by a colorimetric test based on the glucose oxidase method (Rossi et al. 2017).

In the liver and muscle, glycogen, total proteins, and lipid contents ($n = 6$) were quantified. Glycogen was measured according to Seifter et al. (1950). Briefly, 20 mg of hepatic and 60 mg of muscle tissues were treated with 1 ml KOH 30% and 0.5 ml KOH 60% at 10 °C. After alkaline tissue disruption, glycogen was precipitated by ethanol, and glucose was determined using the anthrone reagent method. Lipid content was extracted using chloroform: methanol (2:1) by the method described by Folch et al. (1957), and total protein

concentration was determined in tissue homogenates according to Lowry et al. (1951) using bovine serum albumin as standard. All biochemical analyses were measured in triplicate.

Oxidative stress

Enzyme extracts from the liver, intestine, gills, and muscle of fish ($n = 6$) were made as proposed by Bacchetta et al. (2014). Briefly, tissues were homogenized using 0.1 M sodium phosphate buffer, pH 6.5 containing 20% (v/v) glycerol, 1 mM EDTA, and 1.4 mM dithioerythritol (DTE). The homogenate was centrifuged at 20,000g at 4 °C for 30 min, and the supernatant was collected and stored at −80 °C for enzyme measurement.

The activity of the enzyme superoxide dismutase (SOD, EC 1.15.1.1) was determined by its ability to inhibit the epinephrine autoxidation (Misra and Fridovich 1972). Catalase activity (CAT, EC 1.11.1.6) was measured according to the method of Beutler (1982) following the decomposition of H_2O_2. The assay mixture consisted of 1 M Tris-HCl, 5 mM EDTA (pH 8.0), 10 mM H_2O_2, and enzyme extract. The activity of glutathione-S-transferase (GST, EC 2.5.1.18) was determined following the conjugation of reduced glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) that produces a dinitrophenyl thioether as described by Habig et al. (1974). Glutathione reductase activity (GR, EC 1.6.4.2) was determined as described by Tanaka et al. (1994) by measuring the oxidation of NADPH. The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.5), 20 mM oxidized glutathione, 2 mM NADPH, and enzyme extract. Lastly, lipid peroxidation levels ($n = 6$) were analyzed by measuring the formation of thiobarbituric reactive substances (TBARS) (Yagi 1976). Enzymatic activities and TBARS levels were calculated in terms of the protein content according to Bradford (1976) using serum bovine albumin as standard.

Statistical analyses

All results were reported as mean ± standard error (SE). To corroborate normality and homogeneity of variance, Shapiro-Wilk's and Levene's tests were carried out, respectively. When normal distribution was not accomplished, variables were transformed to $\log_{10}$. A two-way ANOVA was used to analyze the effects of temperature and diet and the interaction between them for the parametric variables. After two-way ANOVA, we performed simple main effects analysis to test the effects of temperature separately for each diet treatment. This test was based in the lineally independent pair-wise comparisons between marginal estimated means using the error terms and the degree of freedom of the whole design (Logan 2010). We did not perform post hoc tests because there are only two temperatures for each diet treatment. Significant differences were considered when $p < 0.05$. Variables that remained non-parametric after transformation were analyzed using Mann-Whitney tests. For these variables, we performed one test to evaluate the effects of temperature and another test to evaluate the effects of the diet. Then, we performed another test to evaluate the effects of temperature stress separately for each diet. All tests were carried out using the SPSS software (SPSS Inc., Chicago, USA).

Results

No mortality was evidenced during the feeding or low temperature-exposure assays. Hematological measurements are shown in Table 2. After the thermal stress, both hemoglobin levels and MCHC increased significantly. There were no differences in hematological measurements between the diets. No interactions were evidenced through the two-way ANOVA.

Blood metabolites and energy reserves in the liver and muscle are summarized in Table 3. After the low temperature stress, fish fed with CD showed diminished plasmatic triglycerides and cholesterol levels. In the case of RD, cholesterol increased in low temperature-exposed fish. Two-way ANOVA showed a significant interaction between diet and temperature for glycogen in the liver. Hepatic glycogen content decreased after the low temperature exposure in CD-fed fish but increased in RD-fed fish. The hepatic protein levels decreased in fish fed with RD exposed to thermal stress.

Activities of antioxidant enzymes and TBARS levels are presented in Table 4. The two-way ANOVA showed significant interactions for SOD in the liver and GST in the intestine. Activities of SOD and GR in the liver and GST in the intestine augmented in fish fed with CD and exposed to 14 °C. In the case of RD-fed fish, the SOD activity increased in gills of fish exposed to 14 °C. TBARS levels significantly increased in the liver and

Table 2 Hematological parameters of *Piaractus mesopotamicus* fed with control (CD) and red seaweed-supplemented (RD) diets and exposed to low temperature stress. Values are expressed as means ± SE. In the rows with parameter names, asterisks in the cell to the right in the columns of diet and temperature represent the significance of the effect of each factor in two-way ANOVA for parametrical variables and difference between levels of each factor in Mann-Whitney tests non-parametric variables. For non-parametric variables, the significance of interaction was not assessed (na), and asterisks indicate the significance of Mann-Whitney's tests

Parameters	Diet		Temperature		Temperature × diet interaction
	CD	RD	24 °C	14 °C	
RBC (10^6 μL^{-1})	2.25 ± 0.07	2.17 ± 0.07	2.1 ± 0.08	2.34 ± 0.07	na
Hematocrit (%)	30.41 ± 0.71	29.41 ± 0.78	29.96 ± 0.79	30.07 ± 0.71	ns
Hemoglobin (g dL^{-1})	8.52 ± 0.2	8.61 ± 0.22	7.93 ± 0.14	9.12 ± 0.18***	ns
MCH (pg)	38.72 ± 1.84	40.42 ± 1.36	38.78 ± 1.56	40.2 ± 1.91	ns
MCV (μm^3)	13.96 ± 0.64	13.94 ± 0.85	14.68 ± 0.85	13.19 ± 0.64	ns
MCHC (%)	277.1 ± 10.54	295.27 ± 12.23	265.59 ± 9.76	301.99 ± 12.23*	ns

RBC red blood cells count, *MCH* mean corpuscular hemoglobin, *MCV* mean corpuscular volume, *MCHC* mean corpuscular hemoglobin concentration, *ns* not significant, *na* not assessed

Data with asterisk in a row are significantly different (*$p<0.05$; **$p<0.01$; ***$p<0.001$)

Discussion

Cold-suboptimal temperatures have been widely reported to cause negative impacts in aquaculture production and generate economic loss (Nobrega et al. 2019). This problem is particularly important for fish since, as ectothermic animals, they are challenged to adapt their physiological functions and overcome thermal stress (Turchini et al. 2010). Moreover, when temperature radically changes, the effects on organisms are quite different from those exposed to low but constant temperature (Barbieri 2009a, b). As such, research regarding how fish nutrition could improve the health status of animals and mitigate the negative effects of environmental stressors has gained importance in order to achieve a sustainable aquaculture and an optimal management of fish farming (Gasco et al. 2018).

In the present study, a 24-h exposure to low temperature (or cold shock) increased the hemoglobin level in juvenile *Piaractus mesopotamicus* fed with CD and RD, which could be a compensatory response and an adaptation to temperature-imposed increases in oxygen requirements. A study carried out by Panase et al. (2018), who found an increase in hemoglobin content (among other hematological parameters) in Nile tilapia exposed

intestine of fish exposed to thermal stress but did not show differences between diets in any tissue.

to 13 °C during 24–72 h, reinforces this statement. The authors explained that fish exposure to low temperatures increases the oxygen demand to enhance the ATP synthesis for maintaining the body temperature.

Stress responses and preservation of homeostasis increase energetic costs (Jager et al. 2014). Cortisol is the principal glucocorticoid secreted under stress condition by the interrenal tissue located in the head-kidney of teleost fish (Geslin and Benoit Auperin 2004; Castillo et al. 2008). It activates different processes to produce energy according to the increased demand during the stressful event (Donaldson et al. 2008). Rotllant et al. (2001) reported that a drop in water temperature affects the pituitary-interrenal axis in gilthead seabream, triggering cortisol release. As cholesterol is the precursor of cortisol, these two parameters are expected to show a similar trend (Miller 1988; Castillo et al. 2008). However, Panase et al. (2018) observed decreased cholesterol and increased cortisol levels in plasma of *Oreochromis niloticus* exposed to cold shock. In the present study, fish fed with CD showed decreased plasmatic levels of cholesterol after the cold stress event, while fish fed with RD showed an increment of this biochemical parameter. This difference in fish response could be attributed to the presence of the seaweed in the diet, as Wang et al. (2019) observed that the concentrations of serum cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol all gradually increased with increasing levels of

Table 3 Plasma metabolites and tissue energetic reserves of *Piaractus mesopotamicus* fed with control (CD) and red seaweed-supplemented (RD) diets and exposed to low temperature stress. Values are expressed as means ± SE. In the rows with parameter names, asterisks in the cell to the right in the columns of diet and temperature represent the significance of the effect of each factor in two-way ANOVA for parametrical variables and difference between levels of each factor in Mann-Whitney tests non-parametric variables. In the row "Simple main effects", the asterisks in the cell to the right in the columns of temperature correspond to the significance of the differences between the temperatures for each diet (RD or CD) tested with this analysis. In the row "Mann-Whitney," the asterisks in the cell to the right in the columns of temperature correspond to the significance of the differences between the temperatures for each diet (RD or CD) tested with this analysis. In the column "Temperature × diet interaction," asterisks mean significant effect of interaction in two-way ANOVA

Parameters	Diet			Temperature		Temperature × diet interaction
	CD	RD		24 °C	14 °C	
Plasma						
Glucose (g L^{-1})	0.68 ± 0.03	0.75 ± 0.02		0.69 ± 0.02	0.74 ± 0.03	ns
Triglycerides (g L^{-1})	3.7 ± 0.44	2.22 ± 0.14*		3.67 ± 0.46	2.45 ± 0.25*	na
Mann-Whitney			CD	5.06 ± 0.63	2.69 ± 0.43*	
			RD	2.29 ± 0.24	2.15 ± 0.17	
Cholesterol (g L^{-1})	1.65 ± 0.16	1.15 ± 0.16		1.76 ± 0.16	1.09 ± 0.07*	na
Mann-Whitney			CD	2.34 ± 0.13	0.97 ± 0.07***	
			RD	1.01 ± 0.04	1.40 ± 0.13**	
Total protein (g L^{-1})	2.62 ± 0.05	2.77 ± 0.11		2.78 ± 0.1	2.55 ± 0.04	ns
Liver						
Glycogen (μmol g wt^{-1})	460.7 ± 30.3	472.19 ± 41.35		442.04 ± 36.45	494.2 ± 34.98	***
Simple main effects			CD	514.38 ± 39.42	393.63 ± 14.80*	
			RD	369.72 ± 45.56	574.66 ± 25.16**	
Lipid (μmol g wt^{-1})	57.24 ± 4.52	62.99 ± 4.69		60.89 ± 5.21	59.33 ± 4.13	na
Protein (mg g wt^{-1})	93.58 ± 6.79	141.1 ± 8.01		116.48 ± 12.82	118.2 ± 10.33	
Simple main effects			CD	142.90 ± 10.22	151.39 ± 14.45	
			RD	145.39 ± 6.29	122.27 ± 7.62*	
Muscle						
Glycogen (μmol g wt^{-1})	1.88 ± 0.08	2.36 ± 0.1		2.04 ± 0.11	2.2 ± 0.11	na
Lipid (μmol g wt^{-1})	7.77 ± 1.14	11.47 ± 1.75		9.88 ± 1.51	9.53 ± 1.78	
Protein (mg g wt^{-1})	93.58 ± 6.79	141.1 ± 8.01**		116.48 ± 12.82	118.2 ± 10.33	

wt wet tissue, *ns* not significant, *na* not assessed

Data with asterisk in a row are significantly different (*$p<0.05$; **$p<0.01$; ***$p<0.001$)

dietary *Sargassum horneri* algae to juvenile turbot (*Scophthalmus maximus*).

Plasmatic triglycerides were diminished in juvenile fish fed with CD and exposed to 14 °C. Decreased triglyceride levels were also found by Lermen et al. (2004) in *Rhamdia quelen* exposed to 15 °C for 21 days. In fasted fish, plasma triglyceride levels represent the result between the rate of hepatic secretion of very-low-density lipoproteins and the rate of their clearance by peripheral tissues (Greene and Selivonchick 1987). In addition, low water temperature could modify the rate of lipolysis or fatty acid reesterification. Then, the decrease in plasmatic triglyceride levels observed in *P. mesopotamicus* was probably due to the utilization of fat stores to face thermal stress.

Hepatic glycogen levels decreased in CD-fed fish. This result was also observed in *H. littorale* and *R. quelen* exposed to 10–15 °C after acute or chronic conditions (Lermen et al. 2004; Rossi et al. 2017). Additionally, Ibarz et al. (2010) analyzed the liver proteome of gilthead sea bream (*Sparus aurata*) exposed to a cold stress challenge and found a rise in glycogen-

Table 4 Antioxidant enzyme activities and TBARS levels in the liver, intestine, gills, and muscle of *Piaractus mesopotamicus* fed with control (CD) and red seaweed-supplemented (RD) diets and exposed to low temperature stress. In the rows with parameter names, asterisks in the cell to the right in the columns of diet and temperature represent the significance of the effect of each factor in two-way ANOVA for parametrical variables and difference between levels of each factor in Mann-Whitney tests non-parametric variables. In the row "Simple main effects", the asterisks in the cell to the right in the columns of temperature correspond to the significance of the differences between the temperatures for each diet (RD or CD) tested with this analysis. In the row "Mann-Whitney", the asterisks in the cell to the right in the columns of temperature correspond to the significance of the differences between the temperatures for each diet (RD or CD) tested with this analysis. In the column "Temperature × diet interaction," asterisks mean significant effect of interaction in two-way ANOVA

Parameters	Diet			Temperature		Temperature × diet interaction
	CD	RD		24 °C	14 °C	
Liver						
SOD (U SOD mg prot⁻¹)	31.15 ± 6.71	45.02 ± 3.7**		28.72 ± 6.18	47.45 ± 3.05***	**
Simple main effects			CD	14.81 ± 1.96	44.57 ± 6.37***	
			RD	42.64 ± 6.75	47.41 ± 3.84	
CAT (U mg prot⁻¹)	39.57 ± 4.08	42.71 ± 3.25		42.26 ± 3.94	40.01 ± 3.47	ns
GST (mU mg prot⁻¹)	165.61 ± 14.45	175.53 ± 25		178.5 ± 24.72	162.3 ± 14.67	ns
GR (mU mg prot⁻¹)	50.66 ± 8.48	63.32 ± 5.95		41.44 ± 5.74	69.81 ± 5.55*	*
Simple main effects			CD	33.98 ± 5.08	67.36 ± 11.14*	
			RD	51.40 ± 9.61	78.28 ± 3.96	
TBARS (nmol mg prot⁻¹)	0.26 ± 0.02	0.2 ± 0.01		0.18 ± 0.01	0.27 ± 0.01***	ns
Intestine						
SOD (U SOD mg prot⁻¹)	49.29 ± 2.27	37.01 ± 3.43*		41.3 ± 4.19	44.47 ± 2.88	ns
CAT (U mg prot⁻¹)	5.27 ± 0.42	3.61 ± 0.13**		4.56 ± 0.38	4.41 ± 0.51	ns
GST (mU mg prot⁻¹)	116.75 ± 6.31	111.14 ± 3.87		104.39 ± 3.66	122.83 ± 4.98**	*
Simple main effects			CD	101.31 ± 5.54	129.39 ± 5.95**	
			RD	108.25 ± 4.51	113.46 ± 6.23	
GR (mU mg prot⁻¹)	24.27 ± 0.9	25.22 ± 1.07		24.58 ± 1.12	24.9 ± 0.87	ns
TBARS (nmol mg prot⁻¹)	1.09 ± 0.2	0.99 ± 0.19		0.55 ± 0.03	1.58 ± 0.08***	ns
Gills						
SOD (U SOD mg prot⁻¹)	13.24 ± 2.38	7.56 ± 0.44		8.05 ± 0.97	13.41 ± 2.56*	na
Mann-Whitney			CD	101.31 ± 5.54	129.39 ± 5.95**	
			RD	108.25 ± 4.51	113.46 ± 6.23	
CAT (U mg prot⁻¹)	1.19 ± 0.11	1.27 ± 0.14		1.03 ± 0.04	1.39 ± 0.14	ns
GST (mU mg prot⁻¹)	70 ± 2.22	78.41 ± 4.32		70.41 ± 1.3	78 ± 4.77	ns
GR (mU mg prot⁻¹)	11.89 ± 0.67	11.7 ± 0.51		10.95 ± 0.29	12.84 ± 0.71	ns
TBARS (nmol mg prot⁻¹)	1.08 ± 0.11	0.88 ± 0.03**		0.94 ± 0.09	1.03 ± 0.09	ns
Muscle						
SOD (U SOD mg prot⁻¹)	12.8 ± 1.15	14.59 ± 1.86		14.52 ± 1.9	12.88 ± 1.09	ns
CAT (U mg prot⁻¹)	1 ± 0.12	0.81 ± 0.07		0.94 ± 0.12	0.85 ± 0.06	ns
GST (mU mg prot⁻¹)	11.51 ± 0.79	13.24 ± 0.92		11.51 ± 0.82	13.06 ± 0.89	ns
GR (mU mg prot⁻¹)	5.79 ± 0.6	4.75 ± 0.52		5.67 ± 0.72	4.89 ± 0.3	ns
TBARS (nmol mg prot⁻¹)	0.07 ± 0.003	0.1 ± 0.005		0.085 ± 0.007	0.085 ± 0.006	ns

SOD superoxide dismutase, *CAT* catalase, *GST* glutathione S-transferase, *GR* glutathione reductase, *ns* not significant, *na* not assessed

Data with asterisk in a row are significantly different (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

phosphorylase levels in agreement with their observation of liver glycogen depletion. These results indicate that energy substrates, such as glycogen, are broken down into glucose to provide the extra energy needed (Viant et al. 2003; Lermen et al. 2004; Chatzifotis et al. 2010; Sun et al. 2019). On the other hand, rats fed with the seaweed *Undaria pinnatifida* showed that gluconeogenesis was upregulated and, by contrast, glycolysis-related genes were downregulated (Yoshinaga et al. 2018). These mechanisms could be operating in fish as increased liver glycogen reserves were observed in RD-fed fish exposed to 14 °C. Additionally, this group of fish showed lower hepatic protein levels. This issue requires further research to elucidate the mechanism involved in seaweed effects on this macromolecule.

Temperature oscillations lead to changes in fish metabolic rate and, consequently, in the generation of reactive oxygen species (ROS) (Pavlović et al. 2010). Then, farmers are challenged to overcome this situation and keep the animals under optimal welfare and developmental conditions; thus, early detection of damage in macromolecules like proteins and lipids has gained importance (Pinto et al. 2019). Bioactive compounds have been identified in *P. columbina* red seaweed which are good electron donors and could act as antioxidants (Cian et al. 2014); however, no consistent differences were found in comparison with the CD. In the present study, *P. mesopotamicus* fed with both diets and exposed to a 24-h thermal shock showed lipid oxidative damage in the liver and intestine although augmented antioxidant activities were observed in these tissues. Particularly, SOD and GR were increased in fish liver of CD-fed fish exposed to 14 °C, meanwhile GST augmented in the intestine. In fish fed with RD, only SOD activity was increased in gills. Joy et al. (2017) found increased activities of SOD and CAT in several tissues (liver, gills, brain, and muscle) of *Etroplus suratensis* exposed to cold water for 24–48 h. SOD and CAT enzymes are considered as the first line of antioxidant defense, as SOD catalyzes the dismutation of superoxide anion, and CAT breaks down the hydrogen peroxide in water and oxygen (Halliwell and Gutteridge 1999). Since GST enzyme conjugates aldehyde products of lipid peroxidation process (Schlenk et al. 2008), its activation seems important to prevent oxidative damage. However, in the intestine of *P. mesopotamicus* exposed to 14 °C and CD, it was not enough probably due to the lack of activation of other antioxidant enzymes.

The high activity of antioxidant enzymes did not prevent oxidative damage generated by cold stress, since increased levels of TBARS were observed in the liver and intestine of fish fed with both diets. Increased TBARS levels were also found in the liver of *Solea senegalensis* and *Danio rerio* exposed to low temperatures for 24 and 1–12 h, respectively (Castro et al. 2012; Wu et al. 2015). Joy et al. (2017) explained that low temperatures could enhance the formation of ROS and proton leakage, and then, favor peroxidation of lipids due to increased polyunsaturation in the mitochondrial membranes and the respiration rates. Oxidative stress seems increased because low temperature enhances oxygen solubility; meanwhile, the transfer of electrons could be disrupted, and the ROS production enhanced because the mitochondrial membrane may diminish its fluidity (Weiss 1970; Hazel 1995).

Conclusions

The cultured fish *Piaractus mesopotamicus* was found to be sensitive to cold shock based on several biomarkers. Our results contribute to the current knowledge regarding cold shock in fish, a situation which could occur either in aquaculture or during animal transport. We hypothesized that the thermal stress effects would be mitigated in fish fed with the *Pyropia columbina* seaweed based-diet, but no significant differences were observed between fish fed with the algae-based diet and the ones fed with the control diet. Although it is widely known that seaweeds contain several bioactive substances with potential health properties (Sørensen et al. 2019), fish responses to dietary seaweed inclusion are dependent on seaweed species and dose, besides being species dependent. We suggest that further research needs to consider different powder algae concentrations in supplemented diets, to take advantage of this valuable and abundant natural resource. Last, we consider this report as relevant to complement the baseline information regarding nutritional requirements of this important farmed species *P. mesopotamicus*.

Declarations

Conflict of interest   The authors declare no competing interest.

Ethics approval   All experiments were carried out following the national and institutional guidelines for the protection of animal welfare (CONICET 2005) and approved by the Committee of Ethics and Safety in Experimental Work (Scientific-Technological Center, CONICET Santa Fe, Argentina).

Consent to participate   It is not applicable.

Consent for publication   It is not applicable.

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