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ORIGINAL PAPER

Effects of acclimation to high environmental temperatures on intermediary metabolism and osmoregulation in the sub-Antarctic notothenioid *Eleginops maclovinus*

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Abstract

Eleginops maclovinus is a sub-Antarctic fish endemic to the South American coast and is a monotypic species of the Eleginopidae family. A scarce amount of scientific information exists regarding the effects of temperature on *E. maclovinus* physiology. In this study, *E. maclovinus* specimens were acclimated for 2 weeks at 10 °C (control), 14, and 18 °C. Posterior assessments were performed for the intermediary metabolism of carbohydrates, lipids, and amino acids (in the plasma, liver, gills, and kidney) and on the osmoregulatory capacity [plasma osmolality and gill, kidney, and foregut Na^+, K^+-ATPase (NKA) activities]. In the plasma, only lactate and total amino acid levels were affected by the temperature. NKA activity presented a linear relationship with the increased temperatures. The liver exhibited no change in the carbohydrates metabolism, but the glycogen/glucose metabolite levels did differ. Amino acid metabolism showed a direct relationship between temperature and GDH and Asp-AT activities, and an inverse relationship between temperature and Ala-AT activity. Lipid metabolism was similar to Ala-AT. In the gills, carbohydrate metabolism (G6PDH and HK activities) presented a direct relationship with the increased temperatures. In the kidney, carbohydrate metabolism (only G6PDH activity) was greater at higher temperatures, and amino acid metabolism (GDH activity) showed a direct relationship with temperature. Lipid metabolism (G3PDH activity) was greatest at 14 °C, presenting significant differences compared to 10 °C. The obtained results suggest that *E. maclovinus* can acclimate to the projected thermal increase of climate change, with a reorganization of the intermediary metabolism components being tissue-dependent and osmoregulatory effects being affected by NKA activity and greater rates of nitrogenous waste extrusion.

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Introduction

The physiological processes of teleosts, i.e., poikilothermic fish, are profoundly affected when the environmental temperatures fluctuate outside of the species-specific optimum (Pörtner et al. 2006; Farrell et al. 2011; Sandblom et al. 2014; Somero 2010; Schulte 2015). Particularly, two of them affected physiological processes, they are intermediary metabolism and osmoregulation. Metabolic pathways can be monitored through metabolite concentrations and enzymatic activities in metabolically important tissues, such as the liver. Regarding carbohydrate metabolism, certain metabolic pathways are indicated by activities of unidirectional enzymes. For example, the glycolytic pathway, gluconeogenic route, and pentose phosphate pathway can be, respectively, monitored by the hexokinase (HK), fructose 1,6-bisphosphatase (FBPase), and glucose-6-phosphate dehydrogenase (G6PDH) enzymes (Mommsen 1986; Hemre et al. 2002). Carbohydrate metabolism can also be deduced

by quantifying metabolites such as glucose and glycogen. Regarding lipid metabolism, monitoring is possible through G3PDH activity and triglycerides levels. Finally, amino acid metabolism can be monitored by measuring the enzymatic activities of transaminases, such as aspartate aminotransferase (Asp-AT), alanine aminotransferase (Ala-AT), and deaminase glutamate dehydrogenase (GDH), as well as by quantifying protein and amino acid concentrations (Johnston and Dunn 1987; Vargas-Chacoff et al. 2009).

In turn, temperature affects osmoregulation, since ion balance and, consequently, osmotic homeostasis is controlled by a series of membrane proteins, homeoviscous adaptations, and enzymes with specific, temperature-influenced kinetic traits (Metz et al. 2003; Arjona et al. 2010; Costas et al. 2012). Osmotic balance in teleost fish can be monitored by determining the percentage of water in the muscle (Freire et al. 2008), cortisol levels (Mommsen et al. 1999), plasma osmolality (Imsland et al. 2003), and the enzymatic activity of Na^+, K^+, ATPase (NKA) in the gills, kidney, and intestine, all of which are tissues with osmoregulatory functions (Gonzalez 2012). The primary function of NKA, which is ubiquitous across tissues, is to generate and maintain tissue-specific secondary electrochemical gradients. This regulatory role is achieved by actively transporting three Na^+ ions to the extracellular medium and two K^+ to the intracellular medium, as fueled by ATP hydrolysis (Hwang et al. 2011; Kreiss et al. 2015).

Teleosts adapt to the new environmental temperatures through a number of physiological mechanisms, such as modifying certain metabolic enzymes. This well-studied mechanism is achieved through (1) changes in the primary structure that cause long-term adaptive variations in kinetic properties and protein stability, (2) changes in protein concentrations, as mediated by modified gene expressions and protein turnover, and (3) changes in the enzymatic environment that aid in lowering the molecular mass of constituents, or osmolytes. Worth noting, primary structure and protein concentration modifications can adjust seasonally (Somero 2004).

One teleost subject to fluctuations in environmental temperatures is *Eleginops maclovinus,* the monotypic Eleginopsidae species of the notothenioidei suborder. This sub-Antarctic fish is endemic to the Atlantic and Pacific Coasts and is one of the few notothenioids inhabiting the constantly cold waters near Antarctica that exhibits euryhaline and eurythmic tolerances (Pequeño et al. 2010; Vargas-Chacoff et al. 2014a). In contrast to *E. maclovinus,* a number of reports exist on the projected physiological responses of the other Antarctic members of the notothenioid group to the expected temperature increase of climate change (Beers and Sidell 2011; Sandersfeld et al. 2015; Klein et al. 2017; Sandersfeld et al. 2017). Enzor et al. (2017) indicated that some notothenioids may require physiological trade-offs to fully offset the energetic costs of long-term acclimation for climate change-related stressors. One available study for *E. maclovinus* describes the effects of two cold environmental temperatures (4 and 10 °C) on the growth, food intake, and swimming energy of juveniles (Vanella et al. 2012). A slightly more recent investigation measured the effect of four environmental temperatures (1, 6, 11, and 21 °C) on *E. maclovinus* carbohydrate metabolism via enzyme activities and metabolite levels (Magnoni et al. 2013). Nonetheless, data remains scarce regarding the effects of increased temperature on the intermediary metabolism and osmoregulatory capacity of *E. maclovinus*.

Depending on the modeled scenario (e.g., high versus low emissions), air temperature is projected to increase between 1 and 7 °C within the next 100 years (Ficke et al. 2007; Stitt et al. 2014; IPCC 2014). Consequent increases in the ocean temperatures could affect the physiological processes of *E. maclovinus*, a fish that naturally inhabits shallow coastal areas (Pequeño 1989). Shallow coasts are the marine habitat most affected by air and water temperature fluctuations. Negative physiological impacts arising from increased environmental temperatures have already been reported for other teleosts inhabiting coastal waters (Hevroy et al. 2013; Madeira et al. 2012; Paaijmans et al. 2013; Bozinovic and Pörtner 2015).

Since, *E. maclovinus* is a eurythermal species, we hypothesized that this species would exert metabolic and osmoregulatory responses to maintain internal homeostasis over a wide range of environmental temperatures. This hypothesis was assessed by determining enzymatic activities and metabolite levels specific to intermediary metabolism and osmoregulation in *E. maclovinus* specimens subjected to single-point experiments at environmental temperatures of 10 °C (control), 14, and 18 °C.

Materials and methods

Ethics statement

All the handling and experimental procedures complied with ethical guidelines regulating the use of laboratory animals established by the National Commission for Scientific and Technological Research (CONICYT, Spanish acronym) and the Universidad Austral de Chile.

Fish

Immature *E. maclovinus* specimens (136.7 ± 21.1 g mean body mass ± standard deviation) were captured in the Valdivia River Estuary during winter and transferred to the Calfuco Coastal Laboratory (Faculty of Science, Universidad Austral de Chile, Valdivia, Chile). Fish were acclimated

for 30 days in flow-through system tanks (500 L) maintained under the following conditions: seawater (32 psu, 1085 mOsm/kg); natural photoperiod; and temperature of 10 ± 1 °C. Fish were fed daily in proportion to 1% average body weight with commercial dry pellet (Skretting 100, containing 48% protein, 22% fat, 13% carbohydrates, 8% moisture, and 8.5% ash).

After acclimation, fish were randomly distributed among six tanks (500 L) maintained at three differing temperatures (control group, 10 °C; experimental groups, 14, and 18 °C). Each group was comprised of 14 individuals ($n = 7$ per tank, in duplicate). For the experimental groups, water temperature was gradually increased by 1 °C per day to allow the animals to acclimate. Following an additional acclimation period (7 days), the fish were kept at the established experimental temperatures for 14 days. This experimental period was chosen based on prior results showing 14 days to be sufficient for reaching complete acclimation and a new steady-state in eurythermal teleosts (Goldspink 1995; Arjona et al. 2010; Vargas-Chacoff et al. 2009, 2014a, b).

Sampling procedure

Fish were fasted 24 h before sampling. Fish were netted, exposed to lethal doses of 2-phenoxyethanol (1 mL/L), and euthanized by spinal sectioning before tissue removal. Blood was immediately collected from the caudal peduncle using 1 mL heparinized syringes (25,000 units of ammonium heparin in 0.9% NaCl, Sigma H6279). Plasma, obtained by centrifuging whole blood (5 min, 2000×*g*), was snap-frozen in liquid nitrogen and stored at − 80 °C until analysis.

The second gill arch was removed from each fish and blotted dry with absorbent paper. Fine-point scissors were then used to obtain 3–5 branchial filaments (McCormick 1993). Small sections were also collected from the posterior kidney and foregut. The intestinal epithelium was obtained by scraping with a scalpel blade. All the collected tissue samples were placed in ice-cold SEI buffer (100 µL; containing 150 mM sucrose, 10 mM EDTA, and 50 mM imidazole, pH 7.3) and frozen at − 80 °C (McCormick 1993). Additional gill arch, kidney, muscle and liver portions were collected, frozen in liquid nitrogen, and stored at − 80 °C until posterior use in metabolism assays.

Plasma measurements

Plasma osmolality (mOsm/Kg H_2O) was measured with a freezing point depression micro-osmometer (Model 3320; Advanced Instruments, Inc.). Plasma glucose, triglycerides, and lactate levels were measured on 96-well microplates using commercial kits from Spinreact (Glucose Ref. 1001200; Triglycerides Ref. 1001310; and Lactate Ref. 1001330) following the manufacturer's recommendations. Total plasma protein levels were determined with the bicinchoninic acid method using the BCA Protein Assay Kit (Kit #23225; Pierce) and albumin as the standard. All the assays were performed on a MultiscanGo Microplate Reader (Thermo Scientific) using the ScanIT v3.2 software. Plasma cortisol levels were measured through an enzyme-linked immunosorbent assay using a commercial kit (Cortisol Ref. KAPDB270; DIA Source Immuno Assays S.A.), following the manufacturer's recommendations.

NKA enzymatic activity

NKA activity in the gills, posterior kidney, and foregut was determined using the micro-assay method, described by McCormick (1993), with author-recommended modifications for non-salmonid fish. Modifications made for *E. maclovinus* were for ionic K^+ concentration (84 mM) and temperature measurements. Ouabain-sensitive ATPase activity (µmol ADP/mg protein/h) was detected through the enzymatic coupling of ATP dephosphorization to NADH oxidation, with measurements taken at the respective water temperature of each group (10, 14, and 18 °C). Total protein contents were measured in undiluted samples and in triplicate (BCA Protein Kit #23225; Pierce). Both the assays were run on a MultiscanGo Microplate Reader (Thermo Scientific) using the ScanIT v3.2 software.

Intermediate metabolism

The methodology for assessing intermediate metabolism was similar to that described by Vargas-Chacoff et al. (2014a, b, 2015, 2016). Briefly, frozen liver, gill, and kidney samples were finely minced in an ice-cold Petri dish, vigorously mixed, and divided into two portions for the measurement of metabolites and enzymes from intermediary metabolism.

For the quantification of metabolites, tissue samples were homogenized by ultrasonic disruption in cold 0.6 N perchloric acid in a volume equivalent to 7.5 times the tissue weight. The same volume of 1 M potassium bicarbonate was then used to neutralize the sample. Homogenates were centrifuged at 4500×*g* for 30 min at 4 °C, and supernatants were frozen in liquid nitrogen and stored at − 80 °C until metabolite measurements. Triglycerides and lactate were determined on 96-well plates using adapted commercial kits from Spinreact (see above). Glycogen concentrations were determined using the amyloglucosidase method (Keppler and Decker 1974). This enzyme generates glycogen breakdown to release glucose units. These units were then measured as detailed above through the use of commercial kits from Spinreact. Total α-amino acids were determined using the ninhydrin method (Moore 1968), with adaptation to 96-well microplates.

For the quantification of intermediary metabolism enzymes, tissue samples were finely minced in an ice-cold Petri dish and homogenized by ultrasonic disruption with 10 volumes of ice-cold stopping buffer (50 mM Imidazole–HCl, pH 7.5; 1 mM 2-mercaptoethanol; 50 mM NaF; 4 mM EDTA; 250 mM sucrose; and 0.5 mM *p*-methylsulphonyl fluoride). The homogenate was centrifuged at 4500×*g* for 30 min at 4 °C. The resulting supernatants were used to measure the enzymes detailed in Table 1. Enzymatic activities were determined using a MultiscanGo Microplate Reader (Thermo Scientific) and the ScanIT v3.2 software. Enzyme reaction rates were determined by changes in the absorbance of NADH or NADPH at 340 nm. The reactions were started by adding homogenates (15 µL) at pre-established protein concentrations, thereby allowing reactions to occur at the distinct acclimation temperatures (i.e., 10, 14, and 18 °C). Protein levels were assayed in triplicate using the BCA Kit (Pierce), as with plasma samples (see above).

Muscle water content

The muscle water content was determined gravimetrically after drying at 100 °C for 48 h (Tang et al. 2009).

Statistical analyses

Differences in enzymatic activity among groups (i.e., 10, 14, and 18 °C) were tested by nested one-way analysis of variance (ANOVA) using temperature as the main factor and the tank as the nested variable. Differences among groups were then assessed by Tukey's test. Data were checked for normality and homoscedasticity before performing nested ANOVA. When necessary, data were logarithmically transformed to fulfill the required conditions for parametric ANOVA. However, data are presented as decimal values for clarity. Where ANOVA conditions were not met by logarithmic transformation, a nonparametric Newman–Keuls rank test was performed. Analyses of linear regression were performed to quantify the coefficient of determination (R^2) and to determine trends in enzymatic activity for intermediary metabolism and NKA in fish acclimated to 10, 14, and 18. A significance level of $P < 0.05$ was used in all the analyses (Quinn and Keough 2002).

Results

No mortalities or alterations in health status were detected in any of the fish during the experimental period.

Plasma parameters

No significant changes in plasma glucose or triglycerides levels were observed, although lactate levels increased at 14 °C. Protein levels were not significantly different, but amino acid levels were lower at both the experimental temperatures. No significant differences in cortisol levels, osmolality, or muscle water content were detected (Table 2).

NKA activity in the gills, kidney, and foregut

Enzymatic NKA activity increased in both the experimental groups. Furthermore, a direct relationship was established between enzymatic NKA activity and temperature ($R^2 = 0.9477$ in the gills; $R^2 = 0.9476$ in the kidney; and $R^2 = 0.8795$ in the foregut; Fig. 1a–c, respectively).

Metabolites and enzymes of intermediary metabolism

Liver

Hepatic changes in metabolite concentrations and enzyme activities are presented in Table 3 and Fig. 2, respectively. Glycogen and glucose levels differentially responded to higher temperatures, with glycogen decreasing and glucose increasing. Protein and triglyceride levels did not show significant differences among groups. In turn, α-amino acid levels were significantly lower in the 14 °C group, as compared to the control group. Regarding carbohydrate metabolism, G6PDH and FBP did not significantly vary between the tested temperatures, but both the enzymes did present relative increase in the activity at higher temperatures ($R^2 = 0.8145$ for G6PDH and $R^2 = 0.9914$ for FBP; Fig. 2a, b, respectively). Hepatic enzymatic HK activity was not measured since levels are undetectable in teleost fish (Hemre et al. 2002). Amino acid metabolism presented two kinds of responses. While GDH and Asp-AT activities showed a linear relationship with temperature ($R^2 = 0.9873$ for GDH and $R^2 = 0.9803$ for Asp-AT; Fig. 2c, d, respectively), Ala-AT activity presented an inverse relationship with temperature, with enzymatic activity levels being lowest at 14 and 18 °C ($R^2 = 0.835$; Fig. 2e). Regarding triglyceride metabolism, G3PDH activity decreased as temperature increased, with the lowest activity levels recorded at 18 °C ($R^2 = 0.9956$; Fig. 2f).

Gills

Changes in metabolite concentrations and enzyme activities for the gills are presented in Table 4 and Fig. 3, respectively. Glycogen values decreased as temperature increased, and glucose levels were higher at 14 and 18 °C, as compared to

Table 1 Enzymes and the respective specific conditions used

Enzyme	Abbreviation	Actions	Reagents (mM)	Metabolic pathway
Hexokinase	HK (EC 2.7.1.1)	Phosphorylates glucose to form glucose-6-phosphate	50 imidazole–HCl (pH 8), 5 $MgCl_2$, 0.15 NADP, 1 ATP; excess G6PDH and 135 glucose as substrate	Glycolysis
Fructose-1,6-bisphosphatase	FBP (EC 3.1.3.11)	Hydrolyses fructose-1,6-bisphosphate to fructose-6-phosphate and phosphorus inorganic	85 imidazole–HCl (pH 7.7), 0.5 NADP, 5 $MgCl_2$ $6H_2O$; excess phosphoglucose isomerase, G6PDH; 0.1 fructose 1,6-bisphosphate as substrate	Gluconeogenesis
Glucose-6-phosphate dehydrogenase	G6PDH (EC 1.1.1.49)	Generates NADPH and 6-phosphoglucono-δ-lactone from glucose-6-phosphate	78 imidazole–HCl (pH 7.7), 5 $MgCl_2$ and 0.5 NADP, 1 glucose-6-phosphate as substrate	Pentose phosphate
Glycerol-3-phosphate dehydrogenase	G3PDH (EC 1.1.1.8)	Generates Glycerol-3-phosphate from Dihydroxyacetone phosphate	50 imidazole–HCl (pH 7.8), 0.15 NADH, 0.2 DHAP as substrate	Triglyceride synthesis
Glutamate dehydrogenase	GDH (EC 1.4.1.2)	Generates Glutamate from α-ketoglutaric acid and ammonium acetate	50 imidazole–HCl (pH 7.8), 250 ammonium acetate, 0.1 NADH, 1 ADP, 80 ketoglutaric acid as substrate	Deamination
Aspartate aminotransferase	Asp-AT (EC 2.6.1.1)	Transfers the amino group of aspartate to α-ketoglutarate to produce glutamate and oxaloacetate	50 imidazole–HCl (pH 7.8), 10 α-ketoglutaric acid and 0.32 NADH, 10.5 L-aspartate as substrate and an excess of MDH	Transdeamination
Alanine aminotransferase	Ala-AT (EC 2.6.1.2)	Transfers the amino group of alanine to α-ketoglutarate with formation of glutamate and pyruvate	50 imidazole–HCl (pH 7.8), 0.025 pyridoxal 5′-phosphate, 10 α-ketoglutaric acid, 0.2 NADH; excess LDH2.5 L-alanine as substrate	Transdeamination
Lactate dehydrogenase-oxidase	LDH-o (EC 1.1.1.27)	Oxidizes lactate to pyruvate	50 imidazole–HCl (pH 8.5) and 2.5 NAD^+ and 6.25 lactic acid as substrate	Gluconeogenesis

Table 2 Plasma parameter levels and water muscle content (%) in *Eleginops maclovinus* juveniles acclimated to 10 °C (control), 14, and 18 °C for 14 days

Plasma	Temperature		
	10 °C	14 °C	18 °C
Glucose (mmol)	2.96 ± 0.18	3.44 ± 0.27	3.17 ± 0.17
Lactate (mmol)	0.29 ± 0.05B	0.51 ± 0.05A	0.42 ± 0.06A,B
Triglycerides (mmol)	3.26 ± 0.51	2.63 ± 0.43	2.41 ± 0.41
Proteins (mg mL^{-1})	43.51 ± 1.91	45.56 ± 2.28	45.47 ± 1.91
Total α-amino acids (mmol)	34.03 ± 2.58A	22.98 ± 2.25B	23.85 ± 2.51B
Cortisol (ng mL^{-1})	55.99 ± 20.77	59.11 ± 18.46	52.65 ± 14.21
Osmolality (mOsm kg^{-1})	322.07 ± 1.05	324.61 ± 1.32	322 ± 2.04
Muscle water (%)	80.73 ± 0.35	80.86 ± 0.42	80.29 ± 0.46

Data are shown as the mean ± SEM (n = 14 per group). Different letters indicate significant difference between groups ($P < 0.05$, one-way ANOVA followed by Tukey's test)

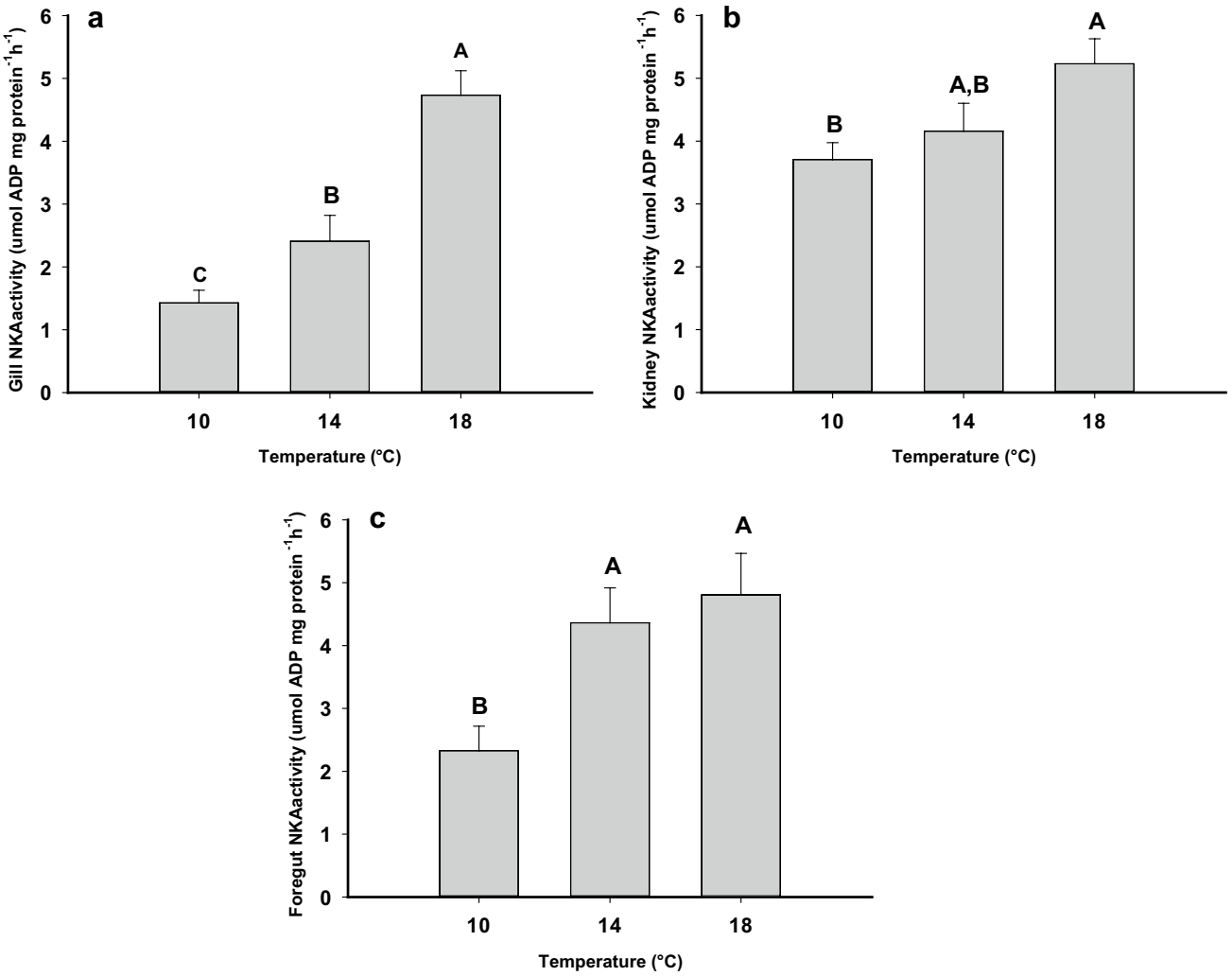

Fig. 1 Na^+, K^+-ATPase (NKA) activity in the **a** gills, **b** kidney, and **c** foregut of *Eleginops maclovinus* acclimated for 2 weeks to 10 °C (control), 14 and 18 °C. Data are shown as the mean ± SEM (n = 14 per group). Different letters indicate significant difference between the temperature groups ($P < 0.05$, one-way ANOVA followed by Tukey's test)

Table 3 Hepatic metabolites in *Eleginops maclovinus* juveniles acclimated to 10 °C (control), 14, and 18 °C for 14 days

Liver (μmoles g wet weight^{-1})	Temperature		
	10 °C	14 °C	18 °C
Glycogen	106.85 ± 17.07A	69.74 ± 17.44A,B	41.33 ± 9.53B
Glucose	13.99 ± 1.49B	17.92 ± 2.88A,B	25.18 ± 2.91A
Proteins	88.11 ± 2.95	92.04 ± 2.95	96.17 ± 3.47
Total α-amino acids	55.88 ± 2.03A	41.56 ± 3.07B	53.31 ± 2.81A
Lactate	Not detected	Not detected	Not detected
Triglycerides	2.91 ± 0.33	3.19 ± 0.31	2.94 ± 0.31

Different letters indicate significant difference between groups ($P < 0.05$, one-way ANOVA followed by Tukey's test)

the 10 °C control. Just as in the liver, protein and α-amino acid levels were lowest at 14 °C, whereas no significant changes were found at 18 °C, as compared with the control. Triglycerides levels were significantly higher at 18 °C, and no significant differences in lactate levels were detected.

Regarding carbohydrate metabolism, only G6PDH activity showed significant differences. A direct relationship to temperature ($R^2 = 0.9999$) was found, and the highest enzymatic G6PDH activity levels were recorded at 18 °C (Fig. 3a). HK enzymatic activity showed only a slight increase with temperature ($R^2 = 0.94$), but this change was not significant (Fig. 3b). No significant changes in FBP activity were detected (Fig. 3c). Both GDH and G3PDH showed the same activity pattern, increasing at 14 °C, but without significant differences at 18 °C, as compared to the control group (Fig. 3d, e, respectively). Finally, enzymatic LDH-O activity did not show significant differences between the tested temperatures ($R^2 = 0.6436$; Fig. 3f).

Kidney

Changes in metabolites concentrations and enzyme activities in the kidney are presented in Table 5 and Fig. 4, respectively. Significant differences were found in glycogen and α-amino acid levels. The lowest glycogen levels were recorded at 18 °C, while α-amino acid levels were lowest at 14 and 18 °C. In contrast, no statistical differences were found for glucose, protein, lactate, or triglycerides levels. Regarding carbohydrates metabolism, only enzymatic G6PDH activity was higher at 14 and 18 °C ($R^2 = 0.7566$; Fig. 4a). Enzymatic HK and FBP activities did not show significant differences among the tested temperatures ($R^2 = 0.5236$ for HK and $R^2 = 0.8924$ for FBP; Figs. 4b, c, respectively). Regarding amino acid metabolism, enzymatic GDH activity was directly related to temperature ($R^2 = 0.9991$), with the highest activity of this enzyme recorded at 18 °C (Fig. 4d). For triglyceride metabolism, enzymatic G3PDH activity evidenced no significant differences between groups, but the activity did show a relative increase with temperature ($R^2 = 0.9965$) (Fig. 4e). Enzymatic LDH-O activity could not be detected in the kidney with the methodologies used in the present study.

Discussion

Fish performance is optimum (i.e., greater growth, best metabolic enzyme functioning) within a certain temperature range. Outside of this range, a number of metabolic, molecular, and physiological changes aid in maintaining internal homeostasis. Nevertheless, these changes generate a new allostatic state, or stress response, that may impair normal physiological functions (McEwen and Wingfield 2003). Additionally, previous studies suggest that some notothenioid fish are particularly sensitive to temperature increases (Beers and Sidell 2011). Interestingly, some Antarctic fish appear to have retained the ability to compensate for chronic temperature change (Seebacher et al. 2005). The results obtained in the present study indicate that *Eleginops maclovinus* can acclimate to environmental temperatures of 14 and 18 °C without any signs of apparent stress, evidenced by no significant differences in plasma glucose and cortisol levels, or changes in behavior or mortality (Barton 2002; Martínez-Porchas et al. 2009). However, *E. maclovinus* evidenced a new allostatic state through a marked reorganization of intermediary metabolism components, the processes of which are tissue-dependent, likely due to the specific physiological functions of each tissue (Feidantsis et al. 2015). In the present study, all of the analyzed tissues presented metabolic modifications, especially in the gills, where marked metabolic and osmoregulatory adjustments were recorded.

Osmoregulatory performance

Eleginops maclovinus did not show differences in plasma osmolality or muscle water content, suggesting that this species can maintain osmotic homeostasis at 14 and 18 °C. In turn, enzymatic NKA activity increased with temperature. Similar results in NKA activity following maintenance at

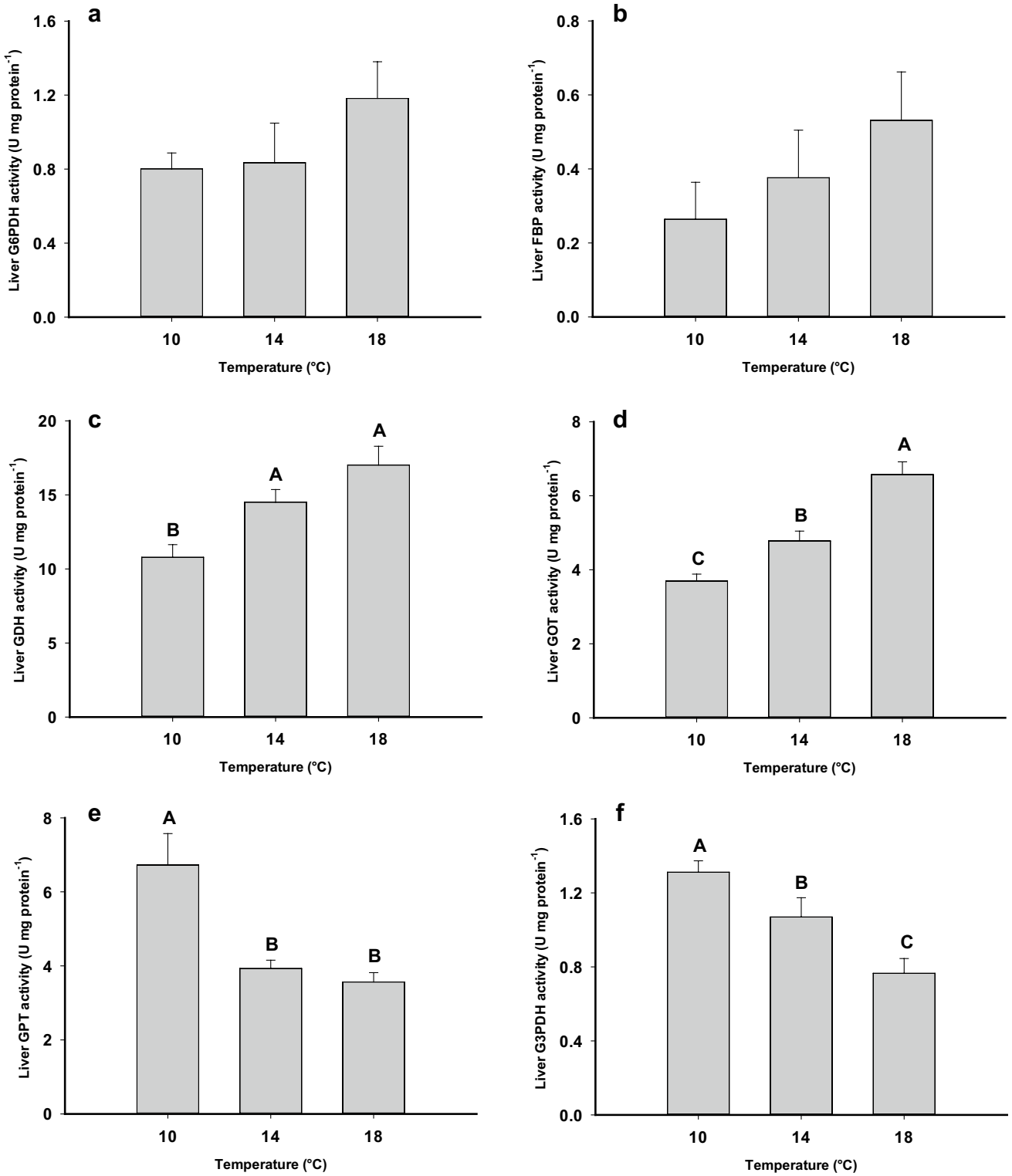

Fig. 2 Hepatic activities of the intermediate metabolism enzymes **a** G6PDH, **b** FBP, **c** GDH, **d** GOT, **e** GPT, and **f** G3PDH in *Eleginops maclovinus* acclimated for 2 weeks to 10 °C (control), 14 and 18 °C. Data are shown as the mean ± SEM ($n = 14$ per group). Different letters indicate significant difference between the temperature groups ($P < 0.05$, one-way ANOVA followed by Tukey's test)

Table 4 Gill metabolites in *Eleginops maclovinus* juveniles acclimated to 10 °C (control), 14, and 18 °C for 14 days

Gills (μmoles g wet weight^{-1})	Temperature		
	10 °C	14 °C	18 °C
Glycogen	4.72 ± 0.35A	3.16 ± 0.31B	1.32 ± 0.16C
Glucose	2.61 ± 0.22B	3.55 ± 0.31A	3.17 ± 0.13A,B
Proteins	61.18 ± 0.96A	53.98 ± 1.44B	61.54 ± 2.19A
Total α-amino acids	35.17 ± 1.66A	26.48 ± 1.51B	34.17 ± 2.02A
Lactate	0.15 ± 0.02	0.13 ± 0.01	0.13 ± 0.01
Triglycerides	0.88 ± 0.08B	1.02 ± 0.06A,B	1.28 ± 0.09A

Different letters indicate significant difference between groups ($P < 0.05$, one-way ANOVA followed by Tukey's test)

higher environmental temperatures were reported in the gills and other tissues of the common carp (*Cyprinus carpio*) (Metz et al. 2003), the Antarctic *Trematomus bernacchii* and *Trematomus newnesi* (Gonzalez-Cabrera et al. 1995), juvenile turbot (*Scophtalmus maximus*) (Imsland et al. 2003), juvenile yellowtail kingfish (*Seriola lalandi)* (Abbink et al. 2012), and tilapia hybrids *(Oreochromis mossambicus* × *O. urolepis hornorum*) (Sardella et al. 2004). This direct relationship between temperature and enzymatic NKA activity, as found in the present study and across phylogenetically distinct species, suggests the existence of a conserved mechanism among teleost fish for acclimating to increased environmental temperatures. Since NKA is an enzyme dependent on ATP, successful acclimation to higher environmental temperatures requires a trade-off between greater enzymatic NKA activity and a greater consumption of energy substrates from intermediary metabolism, such as hepatic glycogen or triglycerides, to maintain the ATP supply (Pörtner et al. 2006; Sandersfeld et al. 2015; Enzor et al. 2017).

Additionally, increased enzymatic activity of NKA in the gills at higher temperatures may be due to a higher catabolism of amino acids resulting in more nitrogenous wastes, such as NH_3 or NH_4^+ (Randall and Wright 1987). This waste is ultimately excreted by the gills, mainly through NKA (Wilkie 1997; Ip et al. 2012). In teleost fish, only the posterior region of the kidney has osmoregulatory functions. At higher temperatures (14 and 18 °C), this region presented the greatest levels of NKA activity. This observation could be due to greater water absorption and the excretion of divalent cations (Mg^{2+}, Ca^{2+}, and SO_4^{2-}), along with a greater absorption of some organic molecules, such as amino acids and glucose. These absorption processes recycle energy substrates through co-transportation in the proximal tubules of the nephron (Beyenbach 2000; McCormick et al. 2013). In the anterior intestine, adjacent to the pyloric caeca region, the recorded increase in enzymatic NKA activity is likely associated with the generation of electrochemical gradients necessary for a greater osmotic absorption of water and ions (Na^+ and Cl^-), with consequent energy expenditures (Grosell 2006; Whittamore 2012).

Carbohydrates metabolism

The liver is the main metabolic organ in charge of storing and processing glycogen. To maintain the higher metabolism rate caused by increased environmental temperatures, the liver of the teleost fish breaks down glycogen to glucose and sends this metabolite to different tissues that demand specific energy substrates (Polakof et al. 2012). In the present study, liver glycogen levels significantly decreased as environmental temperature increased, and glucose levels increased linearly with temperature. These results suggest greater glycogenolysis rates in the liver, which would generate glucose-1-phosphate and, through subsequent phosphoglucomutase, glucose-6-phosphate. This final metabolic intermediary can be used in hepatic glycolysis or be exported through the bloodstream to peripheral tissues requiring glucose as an energy substrate. Similar results have been reported in Antarctic fish (Windisch et al. 2011), suggesting that the hepatic glycolysis process is an acclimation strategy among teleost species when faced with high environmental temperatures.

Plasma glucose levels did not present significant differences among the distinct temperature. This lack of change might be due to glycemic turnover or various homeostatic mechanisms that maintain constant blood glucose levels in *E. maclovinus* (Polakof et al. 2012). Gill and kidney glycogen levels, by contrast, followed a pattern similar to that found in the liver, with lower glycogen levels recorded at higher temperatures. These observations reflect a greater use of endogenous energy reserves by each organ due to the higher metabolism rate imposed by increased environmental temperatures. However, differences in glucose levels were only significant in the gills and were highest in the 14 °C group. This result is likely due to increased glycogenolysis and the use of hepatic exogenous glucose. Indeed, teleost gill tissue is a very active organ in regards to glucose use (Hemre et al. 2002).

G6PDH activity was significantly greater at higher temperatures in the gills and kidney, but not the liver. This finding indicates that an increase in the oxidative phase of glucose to the pentose phosphate pathway occurred only

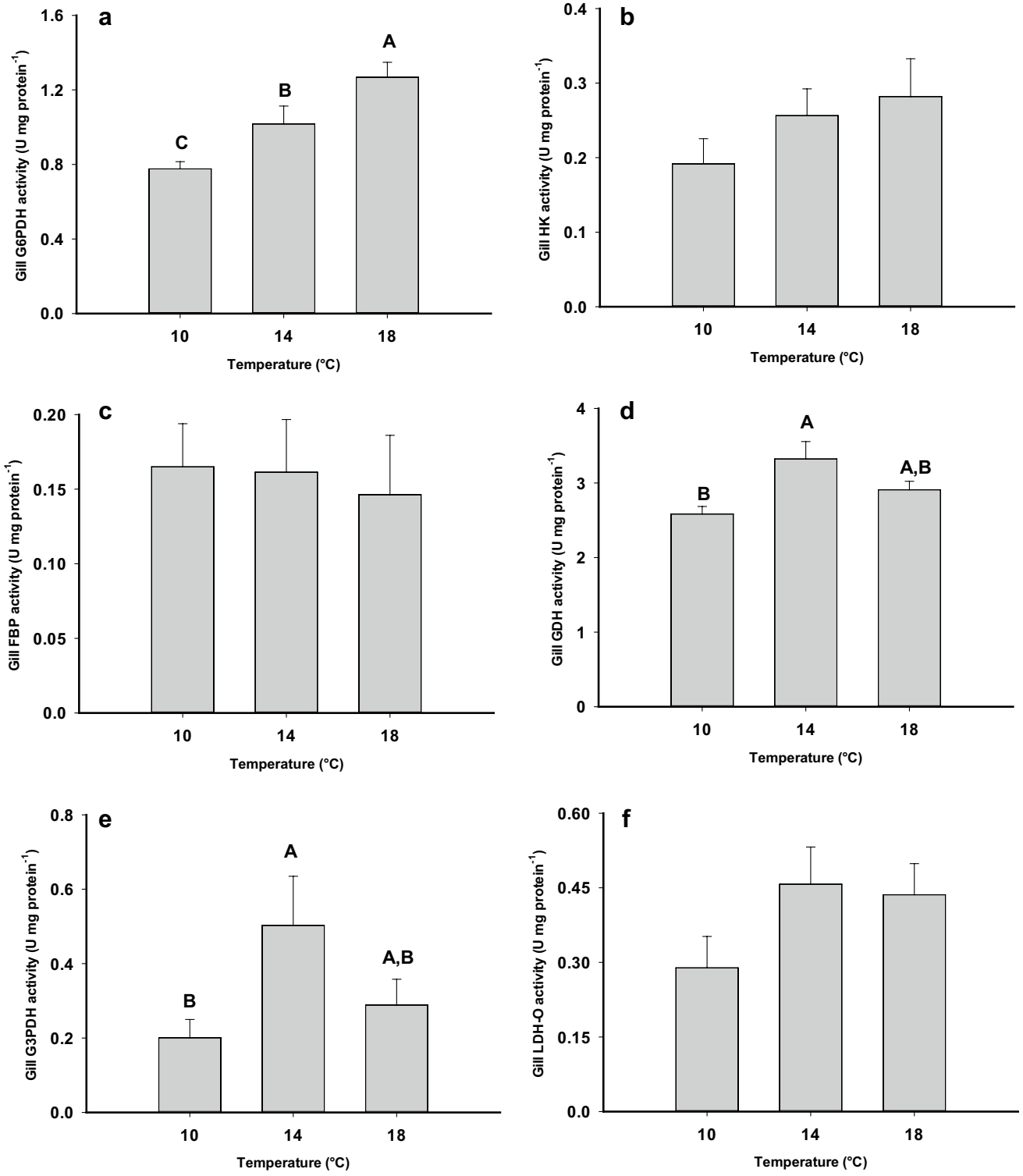

Fig. 3 Gill activities of the intermediate metabolism enzymes **a** G6PDH, **b** HK, **c** FBP, **d** GDH, **e** G3PDH, and **f** LDH-O in *Eleginops maclovinus* acclimated for 2 weeks to 10 °C (control), 14 and 18 °C. Data are shown as the mean ± SEM ($n = 14$ per group). Different letters indicate significant difference between the temperature groups ($P < 0.05$, one-way ANOVA followed by Tukey's test)

Table 5 Kidney metabolites in *Eleginops maclovinus* juveniles acclimated to 10 °C (control), 14, and 18 °C for 14 days

Kidney (μmoles g wet weight^{-1})	Temperature		
	10 °C	14 °C	18 °C
Glycogen	6.62 ± 0.99A	4.49 ± 0.71A,B	2.92 ± 0.36B
Glucose	5.35 ± 0.19	5.23 ± 0.31	5.96 ± 0.34
Proteins	87.34 ± 2.17	84.86 ± 1.61	84.75 ± 2.58
Total α-amino acids	108.15 ± 11.83A	65.31 ± 4.66B	68.63 ± 6.81B
Lactate	0.38 ± 0.08	0.43 ± 0.07	0.44 ± 0.07
Triglycerides	0.31 ± 0.14	0.31 ± 0.08	0.36 ± 0.06

Different letters indicate significant difference between groups ($P < 0.05$, one-way ANOVA followed by Tukey's test)

in organs with osmoregulatory functions. This increase is likely related to an incorporation of more reducing power (NADPH) and nucleotide precursors (ribose-5-phosphate), which are needed for biosynthetic processes and transcription, respectively. Glycolysis in the gills and kidney, as determined by lactate levels and the activities of HK and LDH, showed no significant differences. Similar results for LDH enzymatic activity levels in the gills have been reported for other Antarctic notothenioids during acclimation to higher temperatures (Enzor et al. 2017). Therefore, this metabolic pathway might not be affected by the assessed temperature changes, probably since oxygen concentration in the water did not significantly decrease, thereby preventing greater lactate levels and, eventually, an increase in the glycolytic pathway. Notably, the presently applied methodology did not detect hepatic lactate levels or enzymatic LDH activity, a result strongly suggesting the lack of a Cori cycle for *E. maclovinus*. These results align with those reported for gilthead seabream (*Sparus aurata*) (Vargas-Chacoff et al. 2009)

Gluconeogenic activity, as determined by enzymatic FBP activity, exhibited no statistically significant difference in any of the tested organs. However, a relative increase in FBP activity was found in both the liver and kidney at 14 and 18 °C, which may reflect the greater demand by some tissues for glucose as an oxidative substrate. Similar results were reported in *E. maclovinus* adults (Magnoni et al. 2013) and in male gilthead seabream (Gomez-Milan et al. 2007).

Lipid metabolism

Lipid metabolism, evaluated through enzymatic G3PDH activity and triglycerides levels, showed significant differences in the liver (only G3PDH activity) and gills (G3PDH activity and triglycerides levels). Hepatic enzyme activity of G3PDH showed significant difference between the control temperature (10 °C) and the two experimental temperatures (14 and 18 °C), which may be due to a differential, temperature-dependent use of glycerol-3-phosphate. In cold environmental temperatures, some teleosts accumulate lipids in the liver (Ibarz et al. 2007; Magnoni et al. 2013). Glycerol-3-phosphate is used as a precursor for the synthesis of triglycerides in the liver, which could explain the low levels of enzymatic activity of G3PDH recorded at higher temperatures. An interesting avenue for future studies would be to evaluate if this pattern is maintained in *E. maclovinus* at temperatures lower than 10 °C. Another possibility is that, at 10 °C or lower, *E. maclovinus* synthesizes glycerol-3-phosphate in the liver via the 3-carbon intermediate DHAP pathway, thereby generating large amounts of glycerol, as has been reported in other species (Driedzic and Ewart 2004; Lewis et al. 2004). The physicochemical characteristics of glycerol allow this compound to accumulate in large quantities without damaging cellular components, which would allow this metabolite to act as a cryoprotectant or chemical chaperone (Driedzic and Ewart 2004). Of note, triglycerides levels in the liver did not present significant differences between the presently evaluated temperatures. These results are similar to those reported for darkbarbel catfish (*Pelteobagrus vachellii*) (Qiang et al. 2017) and Antarctic fish (Enzor et al. 2017), suggesting that the liver uses other metabolites, such as amino acids or glycolytic substrates, as a source of energy when acclimating to higher temperatures. Gill triglyceride levels increased at 18 °C, while enzymatic G3PDH activity was highest at 14 °C. These results, along with the increased enzymatic activity of branchial G6PDH, reflect an increase in fatty acid biosynthesis. This increased biosynthesis likely increases triglycerides levels, which could modify the membrane environment by regulating the kinetic properties of certain enzymes in the gills, such as NKA, at higher environmental temperatures (Bystriansky and Ballantyne 2007).

Amino acid metabolism

The energy requirements of teleosts are largely maintained by dietary proteins/amino acids, and environmental temperature can differentially affect the metabolism of specific amino acids (Ballantyne 2001; Kaushik and Seiliez 2010). In the present study, the enzymes of amino acid metabolism showed significant differences in the three tested organs,

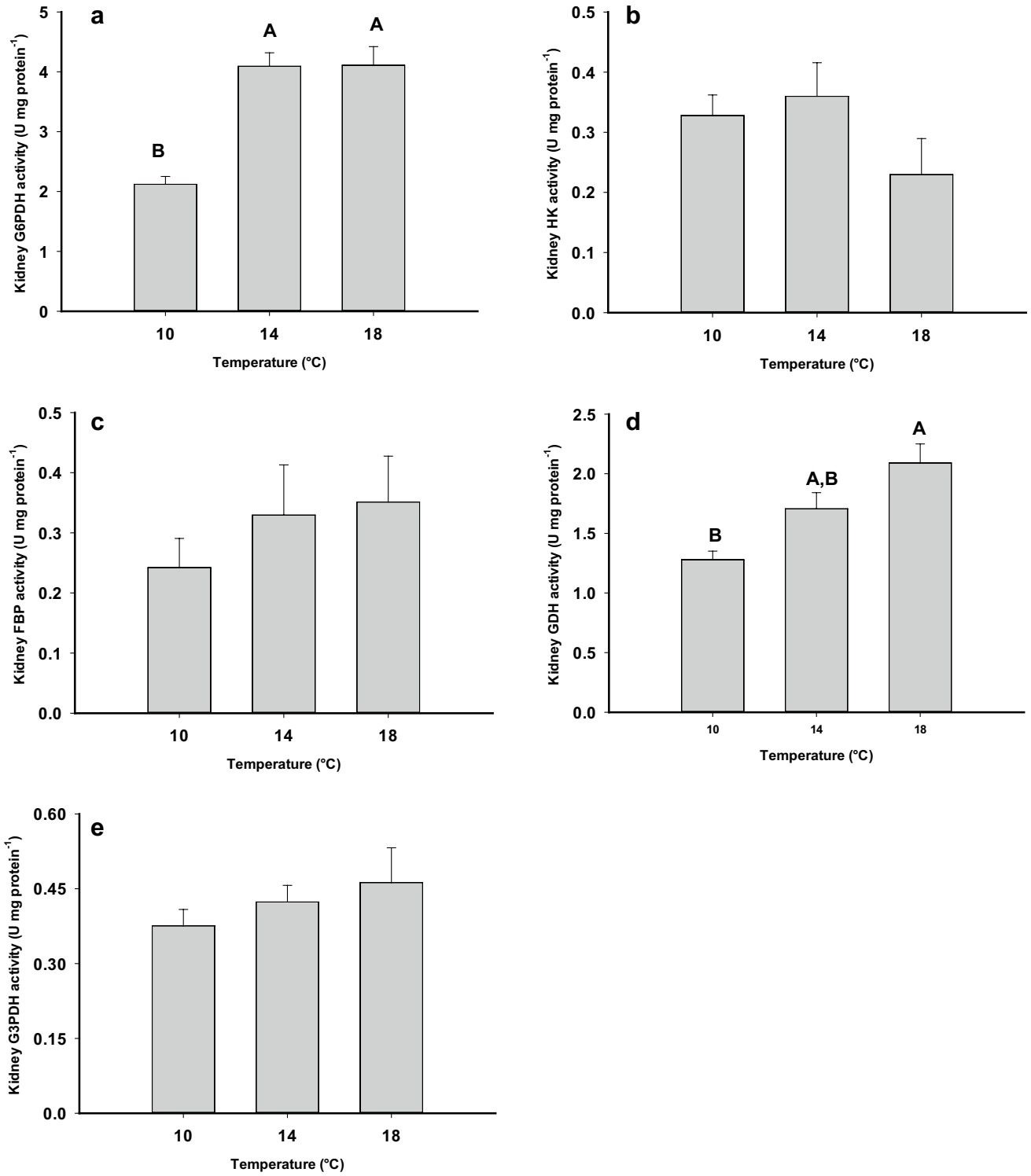

Fig. 4 Kidney activities of the intermediate metabolism enzymes **a** G6PDH, **b** HK, **c** FBP, **d** GDH, and **e** G3PDH in *Eleginops maclovinus* acclimated for 2 weeks to 10 °C (control), 14, and 18 °C. Data are shown as the mean ± SEM (n = 14 per group). Different letters indicate significant difference between the temperature groups ($P < 0.05$, one-way ANOVA followed by a Tukey's test)

indicating a strong modification of amino acid metabolism in *E. maclovinus* as a result of temperature. In *E. maclovinus*, the highest hepatic enzyme activity levels of GDH and Asp-AT were found at higher temperatures, thus reflecting a greater use of amino acids as an energy source to sustain the increased hepatic metabolism generated by environmental

temperatures of 14 and 18 °C. Additionally, increased amino acid metabolism leads to an increase in ammonium ions that are transformed by the liver through a two-step process of transdeamination by Asp-AT and GDH, which is reflected by the higher activity levels of these enzymes at high temperatures (Wilkie 1997). The enzymatic activity of Ala-AT, in contrast, evidenced lower enzyme activity at temperatures of 14 and 18 °C, which could be due to a decreased use of alanine in energy processes, with use of this amino acid in gluconeogenesis processes (Ballantyne 2001). Similar results have been reported in Senegalese sole (*Solea senegalensis*) (Costas et al. 2012) and gilthead seabream (Gomez-Milan et al. 2007). Interestingly, hepatic amino acid levels only decreased at 14 °C, suggesting higher amino acid oxidation at 14 °C and the possible use of other metabolites as an energy source at 18 °C

In the gills, GDH activity was highest at 14 °C. This was concomitant with a decrease in amino acid and protein levels. These results indicate an increased metabolization of amino acids derived from the proteolysis of this organ at 14 °C. In turn, amino acids in the gills were probably metabolized from sources other than plasma (e.g., liver or muscle) at 18 °C. In the kidney, enzymatic GDH activity linearly increased with temperature, while total amino acid levels in this organ were significantly lower at 14 and 18 °C. These results are consistent with an increased use of amino acid catabolism to obtain the energy required by the kidney under temperature-induced increases in metabolism.

Conclusion

The presented comparative data regarding the thermal sensitivities of key intermediate metabolism and osmoregulation enzymes support that the sub-Antarctic *E. maclovinus* is quite eurythermal within a range of 10–18 °C, evidencing a new allostatic state through a marked reorganization of intermediary metabolism components. This trait could be important for adapting to global warming (Bozinovic and Pörtner 2015). The obtained results indicate that *E. maclovinus* would suffer less direct impact from climate change. Indeed, the predicted average temperature increase in this century due to climate change is 1.8–4 °C (IPCC 2014), which should only cause some changes to the intermediate metabolism of *E. maclovinus*. In this hypothetical scenario, amino acid, carbohydrates, and lipid metabolisms will be affected, producing different tissue-dependent responses. In turn, the main effects to osmoregulation should be an increase in the gluconeogenic pathway and extrusion of nitrogenous waste, as suggested by the recorded increases in enzymatic GDH and NKA activities. Our results are the first to characterize the effects of temperature on the intermediary metabolism and osmoregulatory capacity of *E. maclovinus*. The short-term approach used in the present study serves as an indicator of the physiological performance of *E. maclovinus* in the face of a climate change scenario. However, the acclimation potential of this fish should be confirmed by using a long-term approach to assess the impact of higher environmental temperatures on fitness and physiological performance. Fully comprehending the response of animals to thermal changes requires not only an understanding of the acute responses, but also of the potentially compensatory acclimation responses (Pörtner et al. 2006). Knowing the variation of adaptive response in different fish species is important for evaluating the biochemical strategies of different organisms and for predicting the metabolic costs that will determine how climate change will affect organisms (Tomanek 2010).

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Compliance with ethical standards

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Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval All the applicable international, national, and/or institutional guidelines for the care and use of animals were followed as mentioned at the beginning of the Materials and Methods section.

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