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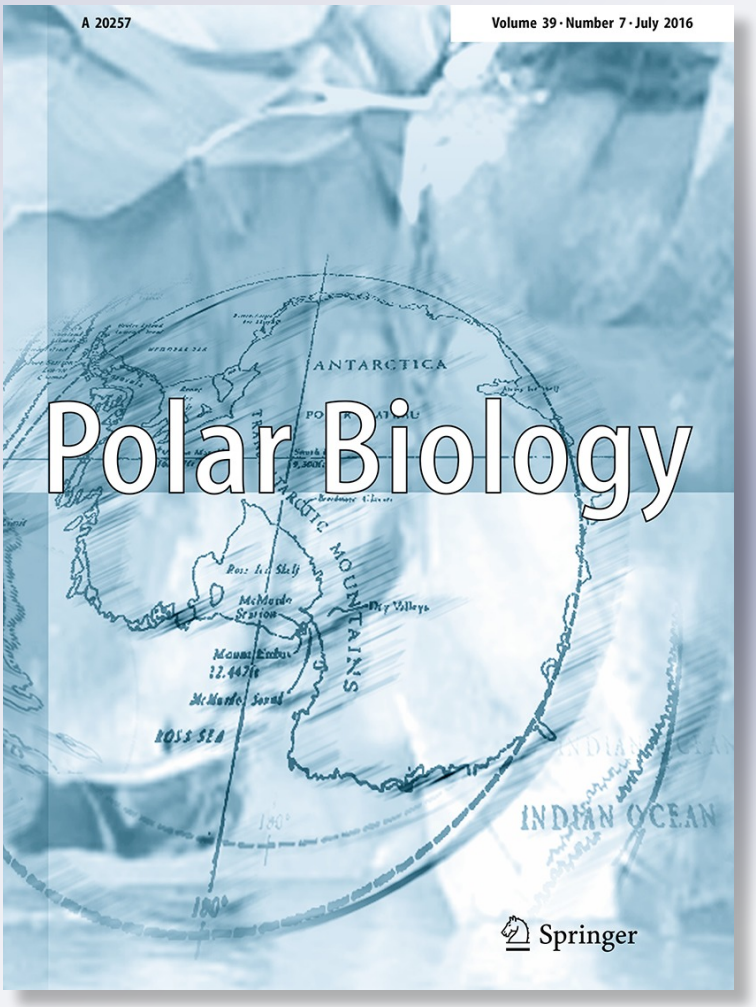

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ORIGINAL PAPER

Metabolic responses to salinity changes in the subantarctic notothenioid teleost *Eleginops maclovinus*

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Abstract *Eleginops maclovinus* is an endemic, subantarctic Notothenioidei species. This study examined the influence of different environmental salinities (5, 15, and 45 psu; and 32 psu as a control) on energy metabolism in *E. maclovinus* over a period of 14 days. Metabolite contents and enzymatic activities related to carbohydrate, amino acid, and lipid metabolisms were evaluated in metabolic (liver) and osmoregulatory (gill and kidney) tissues. At extreme salinities (5 and 45 psu), the liver showed a high consumption of energy reserves, mainly as amino acids and carbohydrates. Carbohydrate metabolism in the gills did not change under different salinities, but increased lactate levels were found, suggesting that this tissue may use lactate as an energy substrate. Amino acid metabolism in the gills decreased at 5 psu but increased at 45 psu, and lipid metabolism increased at 5 and 15 psu during the first days of the trial, indicating a possible use of lipids as energy. Kidney carbohydrate catabolism and amino acid metabolism increased after 14 days at 45 psu, while lipid metabolism did not vary in relation to salinity changes. Together, these results suggest that the liver is most affected by salinity changes, probably due to its role as a supplier of energetic substrates. The gills and kidney, osmoregulatory tissues, maintained their energy metabolism levels with minor modifications. In conclusion, *E. maclovinus* exhibits metabolic adjustments to adapt to different salinities, showing the best responses in isosmotic environmental salinities.

Keywords Carbohydrates · *Eleginops maclovinus* · Gills · Kidney · Lipids · Liver · Metabolism

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Introduction

Euryhaline teleosts can acclimate to a variety of salinity conditions. This adaptive capacity depends on several factors, including energy supply and demand in relation to osmoregulatory processes (Soengas et al. 2007). Importantly, successful acclimation to changes in salinity may require metabolic reorganization to meet increased energetic demands (Boeuf and Payan 2001; Soengas et al. 2007; Vargas-Chacoff et al. 2009a).

To adapt to different salinities, teleosts present osmoregulatory and metabolic adjustments in a number of tissues, such as the gills, kidney, and liver. For example, sodium and chloride transport across the gill epithelium adjusts to environmental salinity, exercising ion uptake in hypoosmotic water and ion excretion in hyperosmotic water (McCormick 2001). In turn, the kidney plays an active role in the extrusion of divalent ions and the elimination of excess water in hyperosmotic and hypoosmotic environments, respectively (Bijvelds et al. 1998; Kelly and Woo 1999; Kelly et al. 1999; Beyenbach 2000). Adaptive modifications to salinity require energy (i.e. ATP) and may alter overall energy requirements, and the kidney has an

additional function in changing intermediary metabolism (Soengas et al. 1994, 2007; Kelly and Woo 1999; Arjona et al. 2009). Finally, the liver is the main site of glycogen/glucose turnover, ammoniagenesis, fatty acid synthesis, and gluconeogenesis in teleosts (Peragón et al. 1998). Liver metabolism may become enhanced during osmotic adaptation to provide fuel for metabolic and osmoregulatory processes, especially in osmoregulatory tissues such as the gills and kidney (Vargas-Chacoff et al. 2009a, b; Costas et al. 2011).

Eleginops maclovinus (Valenciennes 1830) is a subantarctic Notothenioidei endemic to the Patagonian region of South America. Due to its high degree of euryhalinity, this species can be found in environments with different salinities, including the open sea, coastal waters, estuaries, and freshwater rivers (Pavés et al. 2005; Pequeño et al. 2010). Moreover, Reyes and Hune (2013) found *E. maclovinus* inhabiting intertidal pools isolated from the tidal flux for extended periods. In these pools, evaporation can increase the salinity up to 3–4 times higher than seawater (Nordlie 1985; Panfili et al. 2004, 2006; Tine et al. 2007). Another report in the osmoregulatory system of *E. maclovinus* studied the influence of acclimation to four different environmental salinities (5, 15, 32, and 45 psu) over 14 days (Vargas-Chacoff et al. 2014a), finding that specimens used allostatic changes to accommodate their osmotic system (McEwen and Wingfield 2003).

However, modifications in the intermediary metabolism related to osmoregulation have been poorly studied in subantarctic teleosts (Magnoni et al. 2013) and, to the best of our knowledge, these regulatory mechanisms have never been analysed in *E. maclovinus*. Therefore, the objective of the present study was to examine the influence of four different environmental salinities (5, 15, and 45 psu, plus a control group at 32 psu) on the energy metabolism of different tissues (liver, gills, and kidney) in juvenile *E. maclovinus* using a 14-day acclimation time-course experiment.

Materials and methods

Fish and experimental design

The same specimen and experimental procedures used in Vargas-Chacoff et al. (2014a) were applied in the present study. Briefly, juvenile specimens of *E. maclovinus* ($n = 104$) that were acclimated to seawater (SW; 32 psu) were randomly divided into four different groups (24 fish per group) and maintained in eight 500-l tanks (two tanks per group, 12 fish per tank) at different salinities.

Fish were kept at a density of 3.6 kg m^{-3}, in a recirculating tank system, and under natural photoperiod and temperature (12.0 ± 0.5 °C) conditions. Specimens of *E. maclovinus* (150 ± 5 g) were fed daily at 1 % of their body weight with commercial dry pellets (Skretting Nutrece Defense 100) containing 48 % protein, 22 % fat, 13 % carbohydrates, 8 % moisture, and 8.5 % ash.

Before subjecting fish to salinity challenges, eight fish were sampled as pre-transfer controls. Then, the SW-acclimated fish were divided as previously mentioned into four groups and subjected to one of the following salinities: low salinity water (LSW; 5 psu); medium salinity water (MSW; 15 psu); SW (32 psu, control group); or high salinity water (HSW; 45 psu). Samples were taken 1, 3, 7, and 14 days post-transfer (dpt).

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

Tissue sampling

Fish were netted and subjected to lethal doses of 2-phenoxyethanol (1 ml l^{-}) and cervical dislocation before tissues were sampled. The fish were weighed, and then a gill arch (dried with absorbent paper), a portion of the kidney, and the complete liver were extracted from each fish, frozen in liquid nitrogen, and stored at −80 °C.

Tissue metabolites and enzymatic activities

Frozen liver, kidney, and gill samples were finely minced in an ice-cooled Petri dish and divided into two aliquots to assess metabolite levels and enzymatic activities. To assess metabolite levels, the frozen tissues 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* (Eppendorf 5415R). The resulting supernatant was used to determine tissue metabolite levels. Tissue lactate and triglyceride levels were spectrophotometrically measured using commercial kits (Spinreact, Lactate Ref. 1001330 and Triglycerides Ref. 1001311). Tissue glycogen concentrations were assessed using the Keppler and Decker (1974) method. Glucose levels were obtained after glycogen breakdown (i.e. after subtracting free glucose levels) using a commercial kit (Spinreact, Glucose-HK Ref. 1001200). Total α-amino acid levels were assessed using the ninhydrin colorimetric method described by Moore (1968) and adapted to a microplate assay.

To assess enzymatic activities, 0.5 mM *p*-methylsulphonylfluoride (Sigma Chemical Co.) was added to the aliquots as dry crystals, and the mix was 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, and 250 mM sucrose. The homogenate was centrifuged for 30 min at 13,000×*g*, and the resulting supernatant was used in enzyme assays for fructose 1,6-bisphosphatase (FBP, EC 3.1.3.11), glycerol-3-phosphate dehydrogenase (G3PDH, EC 1.1.1.8), glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), glutamate dehydrogenase (GDH, EC 1.4.1.2), hexokinase (HK, EC 2.7.1.1), lactate dehydrogenase-oxidase (LDH-O, EC 1.1.1.27), alanine aminotransferase (ALA-AT, EC 2.6.1.2), and aspartate aminotransferase (ASP-AT, EC 2.6.1.1). Enzymatic activities were determined using a Microplate Reader (Multiscango, Thermo Scientific) and the Scan 3.2 software (Multiscango).

Enzyme reaction rates were determined by changes in the absorbance of NAD(P)H at 340 nm. The reactions were started by adding homogenates (15 µl) to a pre-established protein concentration, omitting the substrate in the control wells (final volume 275–295 µl), and allowing the reactions to proceed at 37 °C for 5–15 min. Protein levels were assayed in triplicate using the Pierce BCA Protein Assay Kit (#23225). Enzyme assays were carried out at initial velocity conditions. Previously described conditions were used for enzyme assays (Sangiao-Alvarellos et al. 2005a, b; Vargas-Chacoff et al. 2009a, b, 2014b, c). These conditions are as follows:

Hepatic, branchial, and renal FBP was assessed in 85 mM imidazole–HCl (pH 7.7), 0.5 mM NADP, and 5 mM $MgCl_2$ 6 H_2O; excess phosphoglucose isomerase and G6PDH (Sigma Chemical Co.); 0.1 mM fructose 1,6-bisphosphate was used as the substrate.

Hepatic, branchial, and renal G3PDH was assessed in 50 mM imidazole–HCl (pH 7.8) and 0.15 mM NADH; 0.2 mM DHAP was used as the substrate.

Hepatic, branchial, and renal G6PDH was assessed in 78 mM imidazole–HCl (pH 7.7), 5 mM $MgCl_2$, and 0.5 mM NADP; 1 mM glucose 6-phosphate was used as the substrate.

Hepatic, branchial, and renal GDH was assessed in 50 mM imidazole–HCl (pH 7.8), 250 mM ammonium acetate, 0.1 mM NADH, and 1 mM ADP; 80 mM α-ketoglutaric acid was used as the substrate.

Hepatic, branchial, and renal HK was assessed in 50 mM imidazole–HCl (pH 8), 5 mM $MgCl_2$, 0.15 mM NADP, and 1 mM ATP; excess G6PDH (Sigma Chemical Co.) and 135 mM glucose were used as the substrate.

Branchial and renal LDH-O was assessed in 50 mM imidazole–HCl (pH 8.5) and 2.5 mM NADP; 6.25 mM lactic acid was used as the substrate. Hepatic ALA-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; excess LDH (Sigma Chemical Co.) and 2.5 mM L-alanine as substrate.

Hepatic ALA-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; excess LDH (Sigma Chemical Co.) and 2.5 mM L-alanine as substrate.

Hepatic ASP-AT was assessed in 50 mM imidazole–HCl (pH 7.8), 10 mM α-cetoglutarato, and 0.32 mM NADH, 10.5 mM L-aspartate as substrate and enzymatic solution of MDH.

Statistical analyses

A Student's *t* test was used to evaluate the differences between tank replicates. Since there were no significant differences in data from replicate tanks, these data were pooled. The data were checked for normality, independence, and homoscedasticity before a two-way analysis of variance (ANOVA) was conducted. Data from the time-course trial were analysed by two-way ANOVA with salinity and time as the factors of variance. Two-way ANOVA was followed by the Tukey test to identify different groups. Logarithmic transformation of the data was performed when necessary to fulfil conditions for the parametric analysis of variance, but data are shown as decimal values for clarity. The groups were considered to be significantly different at a level of $p < 0.05$.

Results

No mortalities were observed in any group over the course of the experiment. The *p* values resulting from the two-way ANOVA of all assessed parameters are shown in Table 1. All significant differences are presented as compared to the SW control group.

Liver

Significant time effects were observed for all assessed metabolites and enzymatic activities ($p < 0.05$) (Table 1). Metabolic changes in the liver of fish acclimated to different environmental salinities are shown in Table 2 (metabolite concentrations) and Fig. 1 (enzymatic activities). Significant salinity effects were observed for glucose, glycogen, and total α-amino acid levels ($p < 0.05$) (Table 2), as well as for some enzymatic activities (G6PDH, G3PDH, GDH, and ALA-AT) ($p < 0.05$) (Fig. 1B, D, E, G).

The HSW group had increased glucose values 3 dpt ($p < 0.05$) (Table 2). Glycogen levels decreased in the LSW group at all sampled times and at 14 dpt in the HSW group (Table 2). Total α-amino acid levels decreased 7 dpt in the HSW group and 14 dpt in the LSW group (Table 2). Triglyceride values did not show differences between salinities (Table 2).

Table 1 *p* values from two-way ANOVA of parameters measured in liver, gill, and kidney

Tissue	Parameter	Salinity	Time	Salinity × time
Liver	Glucose levels	0.045	<0.001	NS
	Glycogen levels	NS	<0.001	<0.001
	Triglyceride levels	NS	NS	0.004
	Total α-amino acid levels	0.049	NS	0.032
	FBP activity	<0.001	<0.001	<0.001
	HK activity	<0.001	<0.001	<0.001
	G6PDH activity	NS	<0.001	0.002
	G3PDH activity	NS	<0.001	<0.001
	GDH activity	NS	<0.001	0.013
	ASP-AT activity	<0.001	NS	0.018
	ALA-AT activity	<0.001	NS	0.001
Gill	Glucose levels	<0.001	<0.001	NS
	Glycogen levels	<0.001	0.005	0.018
	Triglyceride levels	0.001	<0.001	<0.001
	Lactate levels	0.023	<0.001	<0.001
	Total α-amino acid levels	<0.001	<0.001	0.038
	FBPase activity	NS	NS	NS
	HK activity	NS	NS	NS
	G6PDH activity	<0.001	0.030	NS
	G3PDH activity	<0.001	NS	0.001
	GDH activity	0.004	<0.001	<0.001
	LDH-O activity	NS	NS	NS
Kidney	Glucose levels	<0.001	<0.001	NS
	Glycogen levels	<0.001	<0.001	0.018
	Triglyceride levels	NS	NS	NS
	Lactate levels	NS	NS	NS
	Total α-amino acid levels	<0.001	<0.001	<0.001
	FBPase activity	NS	NS	NS
	HK activity	NS	NS	0.042
	G6PDH activity	0.028	<0.001	<0.001
	G3PDH activity	0.006	NS	NS
	GDH activity	NS	NS	NS
	LDH-O activity	0.033	0.024	0.022

NS not significant

Enzyme activities related to carbohydrate metabolism presented different patterns of change. FBP activity gradually increased over time in the LSW group (Fig. 1A), but no significant differences were recorded. G6PDH activity decreased in the HSW group at 14 dpt (Fig. 1B). HK activity only increased in the LSW and MSW groups at 7 dpt ($p < 0.05$) (Fig. 1C). The activity of G3PDH, the major linking enzyme between carbohydrate and lipid metabolism, decreased in the MSW group from 7 dpt until the end of the trial ($p < 0.05$) (Fig. 1D). Amino acid metabolism-related enzymes (GDH, ASP-AT, and ALA-AT) showed different responses to salinity transfer (Fig. 1E–G). At 14 dpt, GDH activity increased in the HSW group but decreased in the LSW group. ASP-AT and ALA-AT activities increased at 3 dpt in the LSW group before returning to basal values for the remainder of the experiment.

Gills

Significant effects for salinity, time, and the interaction between these variables were observed in almost all assessed parameters (Table 1). Metabolic changes in the gills of fish acclimated to different environmental salinities are shown in Table 3 (metabolite concentrations) and Fig. 2 (enzymatic activities).

Glucose presented similar levels in all experimental conditions except in the HSW group, in which values

Table 2 Time course of changes in liver metabolites of *E. maclovinus* juveniles after transfer from sea water (32 psu) to low salinity water (5 psu), medium salinity water (15 psu), sea water (control group, 32 psu), and high salinity water (45 psu)

Time	Treatment (salinity psu)	Glucose (μmol g wet weight^{-1})	Glycogen (μmol glycosyl units g wet weight^{-1})	Triglyceride (μmol g wet weight^{-1})	Total α-amino acids (μmol g wet weight^{-1})
Day 0	Untreated	1.57 ± 0.20	4.26 ± 0.49	2.44 ± 0.34	125.04 ± 3.46
Day 1	5	2.19 ± 0.20	2.13 ± 0.53$^{\#}$	1.85 ± 0.50	142.22 ± 14.91^{a}
	15	2.39 ± 0.09	2.33 ± 0.26$^{+,\#}$	1.17 ± 0.08	131.01 ± 3.22
	32	1.70 ± 0.19	4.43 ± 0.72$^{+,\#}$	2.01 ± 0.32	128.97 ± 9.67
	45	1.88 ± 0.15	5.99 ± 0.80$^{a,+}$	2.57 ± 0.39^{a}	123.90 ± 10.51^{a,b}
Day 3	5	2.66 ± 0.53$^{+,\#}$	1.23 ± 0.23$^{\#}$	1.56 ± 0.42	136.57 ± 15.56^{a,b}
	15	2.56 ± 0.28$^{+,\#}$	3.46 ± 0.41$^{+,\#}$	2.17 ± 0.27	135.41 ± 9.97
	32	1.49 ± 0.14$^{\#}$	4.21 ± 0.55^{+}	2.09 ± 0.39	129.02 ± 5.49
	45	2.69 ± 0.19^{+}	5.30 ± 0.40$^{a,+}$	2.37 ± 0.24^{a}	133.20 ± 4.41^{b}
Day 7	5	2.82 ± 0.34$^{\#}$	2.32 ± 0.34$^{+,\#}$	1.72 ± 0.35	125.37 ± 9.64$^{a,b,+,\#}$
	15	2.57 ± 0.16$^{+,\#}$	4.70 ± 0.84$^{+,\#}$	1.60 ± 0.32	128.42 ± 7.01$^{+,\#}$
	32	1.65 ± 0.14^{+}	5.25 ± 0.29^{+}	2.24 ± 0.26	133.07 ± 1.24$^{\#}$
	45	2.69 ± 0.19$^{+,\#}$	4.40 ± 0.40$^{a,+,\#}$	2.33 ± 0.36^{a}	97.82 ± 6.13$^{a,+}$
Day 14	5	2.34 ± 0.43	1.47 ± 0.18$^{\#}$	2.08 ± 0.44	97.51 ± 7.63$^{b,\#}$
	15	2.13 ± 0.14	4.98 ± 1.11^{+}	2.42 ± 0.26	116.66 ± 10.15$^{+,\#}$
	32	1.68 ± 0.15	4.79 ± 0.45^{+}	1.97 ± 0.19	132.38 ± 6.97^{+}
	45	2.24 ± 0.07	0.58 ± 0.17$^{b,\#}$	0.80 ± 0.07^{b}	129.16 ± 4.05$^{a,b,+}$

Each point represents mean ± SEM of $n = 6$ fish per group in each sampling time

Different letters indicate significant differences ($p < 0.05$, two-way ANOVA test) between groups within the same salinity. Different symbols represent significant differences within the same time point

increased at 14 dpt (Table 3). Glycogen values in the LSW, MSW, and HSW groups decreased at 14 dpt (Table 3); however, the LSW group also presented differences compared to the control at 3 dpt. Lactate values were increased in all experimental groups at all time points, with the exception of 7 dpt (Table 3). Triglyceride levels decreased in the LSW and MSW groups at 1 and 7 dpt (Table 3), coinciding with increased G3PDH activity (Fig. 2D). Amino acid levels were increased in the HSW group over the entire trial, but only reached statistically significant differences at 14 dpt. In contrast, amino acid levels in the LSW and MSW groups were lower than the control over the entire experiment (Table 3).

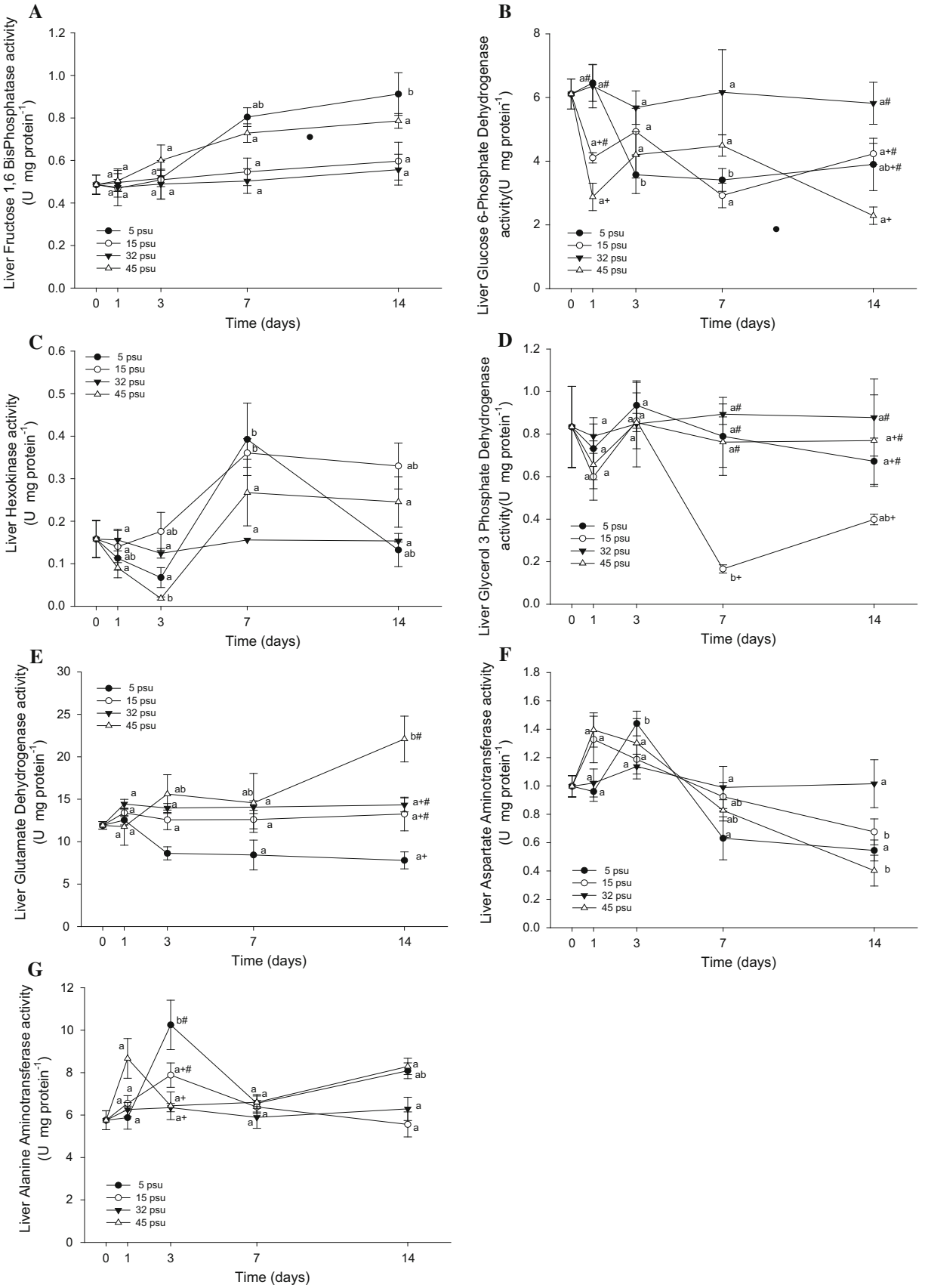
Enzyme activities related to carbohydrate metabolism presented different patterns of change. FBP and G6PDH activities were decreased in the MSW and HSW groups, respectively, but no significant differences were found (Fig. 2A, B, respectively). HK activity was unchanged by salinity (Fig. 2C). Branchial lipid mobilization was observed early in the trial, with increased G3PDH activity observed at 1 and 3 dpt in the LSW, MSW, and HSW groups (Fig. 2D). Regarding amino acid metabolism, GDH activity was decreased in iso- or hypoosmotic conditions over the course of the experiment (Fig. 2E). For lactate metabolism enzymes, LDH-O activity was not affected by any experimental salinities (Fig. 2F).

Kidney

A significant interaction between salinity and time was observed for the metabolite levels of glucose and total α-amino acids, as well as for the enzymatic activities of G6PDH, HK, and LDH-O ($p < 0.05$) (Table 1). Metabolic changes in the kidney of fish acclimated to different environmental salinities are shown in Table 4 (metabolite concentrations) and Fig. 3 (enzymatic activities).

Triglyceride and lactate levels were unaffected by environmental salinity changes (data not shown). Glucose values were lower in the LSW and MSW groups ($p < 0.05$) (Table 4). Total α-amino acid levels presented a clear pattern of variation due to experimental salinity and time, with higher levels observed in the LSW, MSW, and HSW groups. Increased α-amino acid levels were especially notable in the HSW group at 14 dpt ($p < 0.05$) (Table 4).

Regarding carbohydrate metabolism, G6PDH (Fig. 3B) and HK (Fig. 3C) activities increased in the HSW group at 14 dpt ($p < 0.05$) while decreasing on the same day in the LSW group. In contrast, FBP (Fig. 3A), G3PDH (Fig. 3D), and GDH (Fig. 3E) activities were unaffected by the salinity challenge. Activity of the lactate metabolism enzyme LDH-O decreased in the LSW group at 14 dpt ($p < 0.05$) (Fig. 3F).



◀**Fig. 1** Time course of changes in liver enzymatic activity of *E. maclovinus* juveniles after transfer from sea water (32 psu) to low salinity water (5 psu), medium salinity water (15 psu), sea water (control group, 32 psu), and high salinity water (45 psu). **A** FBP, **B** G6PDH, **C** HK, **D** G3PDH, **E** GDH, **F** ASP-AT, **G** ALA-AT. Each point represents mean ± SEM of $n = 6$ fish per group in each sampling time. *Different letters* indicate significant differences ($p < 0.05$, two-way ANOVA test) between groups with the same salinity. *Different symbols* represent significant differences within the same time point

Discussion

The subantarctic euryhaline teleost *E. maclovinus* can adapt its osmoregulatory system to a wide range of environmental salinities (Vargas-Chacoff et al. 2014a). Otolith and dietary shift data demonstrate the ecological flexibility of *E. maclovinus*. Indeed, this species exhibits complex growth ring depositions that are probably associated with large fluctuations in environmental temperature and salinity (Licandeo et al. 2006). Furthering knowledge on the effects of salinity in this species, the present study observed modifications in the main pathways related to energy metabolism in selected tissues of *E. maclovinus* under various salinity conditions.

Analysis of the results revealed two phases related to changes in salinity, adjustment, and long-term response. In the adjustment period (up to 3 dpt), fish underwent metabolic reorganization to cope with the osmotic demand imposed by a new environmental salinity. In the long-term response period (from 3 to 14 dpt), fish achieved a new allostatic status in regard to energy metabolism. Both of these phases were reported in the osmoregulatory response of this species after a similar salinity challenge (Vargas-Chacoff et al. 2014a). These similarities suggest a coordinated osmoregulatory and metabolic response in *E. maclovinus* under conditions of modified salinity, as has been described in other teleost fish species (Soengas et al. 2007).

Liver

The liver is a key metabolic organ in vertebrates, including teleosts (Mommsen et al. 1999), and its role is particularly important when considering that different metabolic routes are affected by environmental salinity. In *E. maclovinus* specimens, carbohydrate metabolism (FBP, G6PDH, and HK; Fig. 1A–C) underwent adjustment and long-term response periods following a change in salinity. The increased HK activity in the LSW and MSW groups at 7 dpt suggests the activation of glycolysis (see Vargas-Chacoff et al. 2014a). Moreover, the decreased activity of G6PDH, the pentose phosphate shunt, suggests an importance of glucose as a fuel source while also indicating lower glucose phosphorylation capacity, especially in the

Table 3 Time course of changes in gill metabolites of *E. maclovinus* juveniles after transfer from sea water (32 psu) to low salinity water (5 psu), medium salinity water (15 psu), sea water (control group, 32 psu), and high salinity water (45 psu)

Time	Treatment (salinity psu)	Glucose (μmol g wet weight^{-1})	Glycogen (μmol glycosyl units g wet weight^{-1})	Lactate (μmol g wet weight^{-1})	Triglyceride (μmol g wet weight^{-1})	Total α-amino acids (μmol g wet weight^{-1})
Day 0	Untreated	0.85 ± 0.09	0.75 ± 0.07	0.070 ± 0.01	1.24 ± 0.10	39.56 ± 1.39
Day 1	5	0.53 ± 0.03	0.83 ± 0.13a	0.074 ± 0.01$^{a,+,\#}$	0.53 ± 0.07$^{\#}$	30.98 ± 1.91$^{\#}$
	15	0.50 ± 0.06	0.83 ± 0.13^{a}	0.103 ± 0.01$^{a,b,\#}$	0.67 ± 0.08$^{a,\#}$	34.37 ± 2.75$^{a,b,\#}$
	32	0.65 ± 0.05	0.73 ± 0.06	0.059 ± 0.01^{+}	1.18 ± 0.17^{+}	37.64 ± 1.45$^{+,\#}$
	45	0.86 ± 0.09^{a,b}	0.71 ± 0.04^{a,b}	0.089 ± 0.02$^{a,b,+,\#}$	1.44 ± 0.11$^{a,+}$	47.31 ± 3.23$^{a,b,+}$
Day 3	5	0.66 ± 0.10	0.48 ± 0.03$^{b,\#}$	0.096 ± 0.01$^{a,b,+,\#}$	0.88 ± 0.05	31.32 ± 2.89$^{*,\#}$
	15	0.58 ± 0.09	0.55 ± 0.06$^{a,b,+,\#}$	0.143 ± 0.01$^{a,\#}$	0.94 ± 0.09^{a,b}	25.95 ± 0.57^{a,*}
	32	0.58 ± 0.02	0.79 ± 0.05$^{+,\#}$	0.056 ± 0.01^{+}	1.25 ± 0.09	36.91 ± 3.15$^{+,\#}$
	45	0.73 ± 0.05^{a}	0.87 ± 0.10$^{a,+}$	0.140 ± 0.01$^{a,\#}$	0.97 ± 0.06^{a}	47.21 ± 1.87$^{a,b,+}$
Day 7	5	0.48 ± 0.04	0.49 ± 0.06^{b}	0.106 ± 0.02^{a,b}	0.60 ± 0.07$^{\#}$	37.37 ± 2.30
	15	0.59 ± 0.04	0.47 ± 0.02^{b}	0.068 ± 0.01^{b}	0.80 ± 0.09$^{a,\#}$	42.34 ± 2.45^{b}
	32	0.58 ± 0.03	0.60 ± 0.07	0.060 ± 0.01	1.34 ± 0.06+	39.05 ± 3.20
	45	0.79 ± 0.04^{a,b}	0.51 ± 0.02^{b,c}	0.094 ± 0.02^{a,b}	1.33 ± 0.11$^{a,+}$	45.78 ± 1.13^{a}
Day 14	5	0.74 ± 0.06$^{\#}$	0.40 ± 0.04^{b}	0.141 ± 0.01$^{b,\#}$	0.99 ± 0.18	37.92 ± 0.62$^{\#}$
	15	0.66 ± 0.07$^{\#}$	0.45 ± 0.03^{b}	0.081 ± 0.02$^{a,b,+,\#}$	1.30 ± 0.13^{b}	39.50 ± 4.34$^{b,\#}$
	32	0.70 ± 0.07$^{\#}$	0.58 ± 0.04	0.058 ± 0.01^{+}	1.39 ± 0.12	41.01 ± 0.86$^{\#}$
	45	1.03 ± 0.06$^{b,+}$	0.47 ± 0.02^{c}	0.083 ± 0.02$^{b,+,\#}$	1.24 ± 0.04^{a}	58.67 ± 3.67$^{b,+}$

Further details as in footer of Table 2

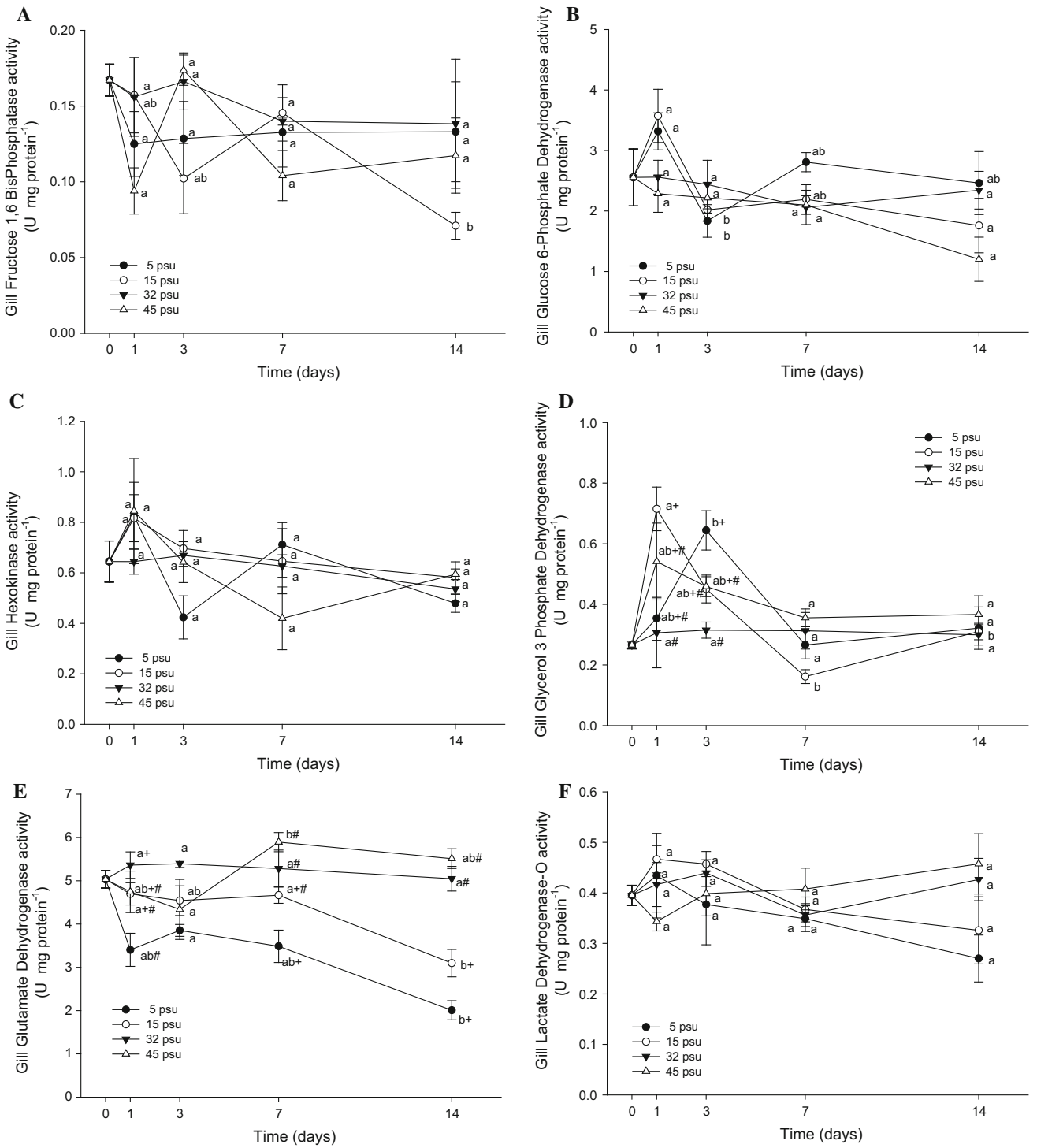


Fig. 2 Time course of changes in gill enzymatic activities of *E. maclovinus* juveniles after transfer from sea water (32 psu) to low salinity water (5 psu), medium salinity water (15 psu), sea water (control group, 32 psu), and high salinity water (45 psu). **A** FBP, **B** G6PDH, **C** HK, **D** G3PDH, **E** GDH, **F** LDH-O. Further details as in legend of Fig. 1

HSW group at 14 dpt (Laiz-Carrión et al. 2005a, b; Sangiao-Alvarellos et al. 2005b; Vargas-Chacoff et al. 2009a). The role of glucose as a metabolic fuel has great importance because members of the Notothenioidei suborder generally have limited access to dietary carbohydrates (Magnoni et al. 2013).

The enhanced gluconeogenic activity at 3 dpt in the LSW and HSW groups for ASP-AT and ALA-AT

Table 4 Time course of changes in kidney metabolites of *E. maclovinus* juveniles after transfer from sea water (32 psu) to low salinity water (5 psu), medium salinity water (15 psu), sea water (control group, 32 psu), and high salinity water (45 psu)

Time	Treatment (salinity psu)	Glucose (μmol g wet weight^{-1})	Glycogen (μmol glycosyl units g wet weight^{-1})	Total α-amino acids (μmol g wet weight^{-1})
Day 0	Untreated	0.71 ± 0.07	0.79 ± 0.07	30.70 ± 2.24
Day 1	5	0.56 ± 0.04$^{\#}$	0.83 ± 0.07^{a}	26.64 ± 4.05^{a}
	15	0.50 ± 0.05$^{\#}$	0.83 ± 0.17^{a}	31.83 ± 2.57^{a}
	32	0.65 ± 0.05$^{+,\#}$	0.73 ± 0.06	30.91 ± 2.19
	45	0.86 ± 0.09$^{a,b,+}$	0.71 ± 0.04^{a}	43.61 ± 3.95^{a}
Day 3	5	0.66 ± 0.10	0.48 ± 0.03^{b}	29.04 ± 4.26a$^{\#}$
	15	0.72 ± 0.06	0.57 ± 0.06^{a,b}	54.87 ± 3.30$^{b,+}$
	32	0.60 ± 0.02	0.79 ± 0.04	29.98 ± 2.73$^{\#}$
	45	0.73 ± 0.05^{a}	0.87 ± 0.10^{a}	62.46 ± 3.27$^{b,+}$
Day 7	5	0.48 ± 0.04$^{\#}$	0.50 ± 0.06^{b}	47.59 ± 3.56$^{b,+,\#}$
	15	0.59 ± 0.04$^{+,\#}$	0.47 ± 0.02^{b}	55.04 ± 3.17$^{b,\#}$
	32	0.61 ± 0.03$^{+,\#}$	0.67 ± 0.07	31.78 ± 1.86^{+}
	45	0.79 ± 0.04$^{a,b,+}$	0.51 ± 0.02^{b}	53.29 ± 3.18$^{a,b,\#}$
Day 14	5	0.74 ± 0.06$^{+,\#}$	0.40 ± 0.04^{b}	48.09 ± 5.73$^{b,\#}$
	15	0.66 ± 0.07$^{\#}$	0.45 ± 0.03^{b}	53.78 ± 5.57$^{b,\#}$
	32	0.75 ± 0.05$^{+,\#}$	0.63 ± 0.03	33.47 ± 1.02^{+}
	45	1.03 ± 0.06$^{b,+}$	0.41 ± 0.02^{b}	94.25 ± 11.54c*

Further details as in footer of Table 2

(Fig. 1F–G), together with the gradual increase in FBP activity (Fig. 1A) in these same groups over the entire period, is concordant with the depleted glycogen levels found at 14 dpt (Table 2) and the increased glycolytic potential of HK found at 7 dpt (Fig. 1C). GDH activity was notably increased 14 dpt in the HSW group (Fig. 1E), when glycogen reserves were depleted, suggesting that amino acids are mobilized during the long-term response period (Table 2). These data also indicate that the carnivorous *E. maclovinus* metabolizes amino acids derived from proteolysis or uses exogenous amino acids as a fuel source in conditions of extreme salinity. In a previous study, *E. maclovinus* acclimated to high salinity levels had increased plasma cortisol levels (Vargas-Chacoff et al. 2014a). Due to the proteolytic actions of this hormone (Wendelaar-Bonga 1997; Mommsen et al. 1999), protein catabolism could be inferred.

Lipid metabolism, assessed by G3PDH activity (Fig. 1D) and triglycerides levels (Table 2), did not significantly vary between salinity conditions. These results suggest that the high hepatic energy consumption observed in the LSW group during acclimation would be mainly fuelled by carbohydrates, whereas the HSW group demonstrated a clear role of amino acids in energy metabolism. Therefore, lipid metabolism appears to be less relevant during the first weeks of acclimatization to different environmental salinities in *E. maclovinus*. These results are in agreement with the effects described in *Sparus aurata*, another teleost fish (Sangiao-Alvarellos et al. 2003, 2005b; Laiz-Carrión et al. 2005b; Vargas-Chacoff et al. 2009a).

Gills

In the gills, carbohydrate metabolism (FBP, G6PDH, and HK) activity did not change as a result of modified environmental salinity (Fig. 2A–C), suggesting that glucose is mainly imported from the liver (Vargas-Chacoff et al. 2009a, 2014c). Regarding lactate metabolism, increased branchial lactate levels were observed, but LDH-O activity (Fig. 2F) did not increase. This indicates an enhanced pyruvate reduction rate derived from the aerobic metabolism of pyruvate. Pyruvate could be obtained from amino acids in the LSW group or transported from the bloodstream, supporting previous postulations that the gills may use lactate as energy in ionic pumps, including Na^{+}, K^{+}-ATPase (Polakof et al. 2006; Vargas-Chacoff et al. 2009a, b, 2014a). Soengas et al. (2007) indicated that achieving ionic and osmotic balance together with the excretion of nitrogenous products requires high metabolic activity in the gills.

Amino acid metabolism, evaluated through GDH activity and total amino acid levels (Fig. 2E, Table 3), presented differentiated responses depending on environmental salinity. The drop in GDH activity and amino acid levels in the LSW and MSW groups could mean diminished glutamate deamination due to protein catabolism. In

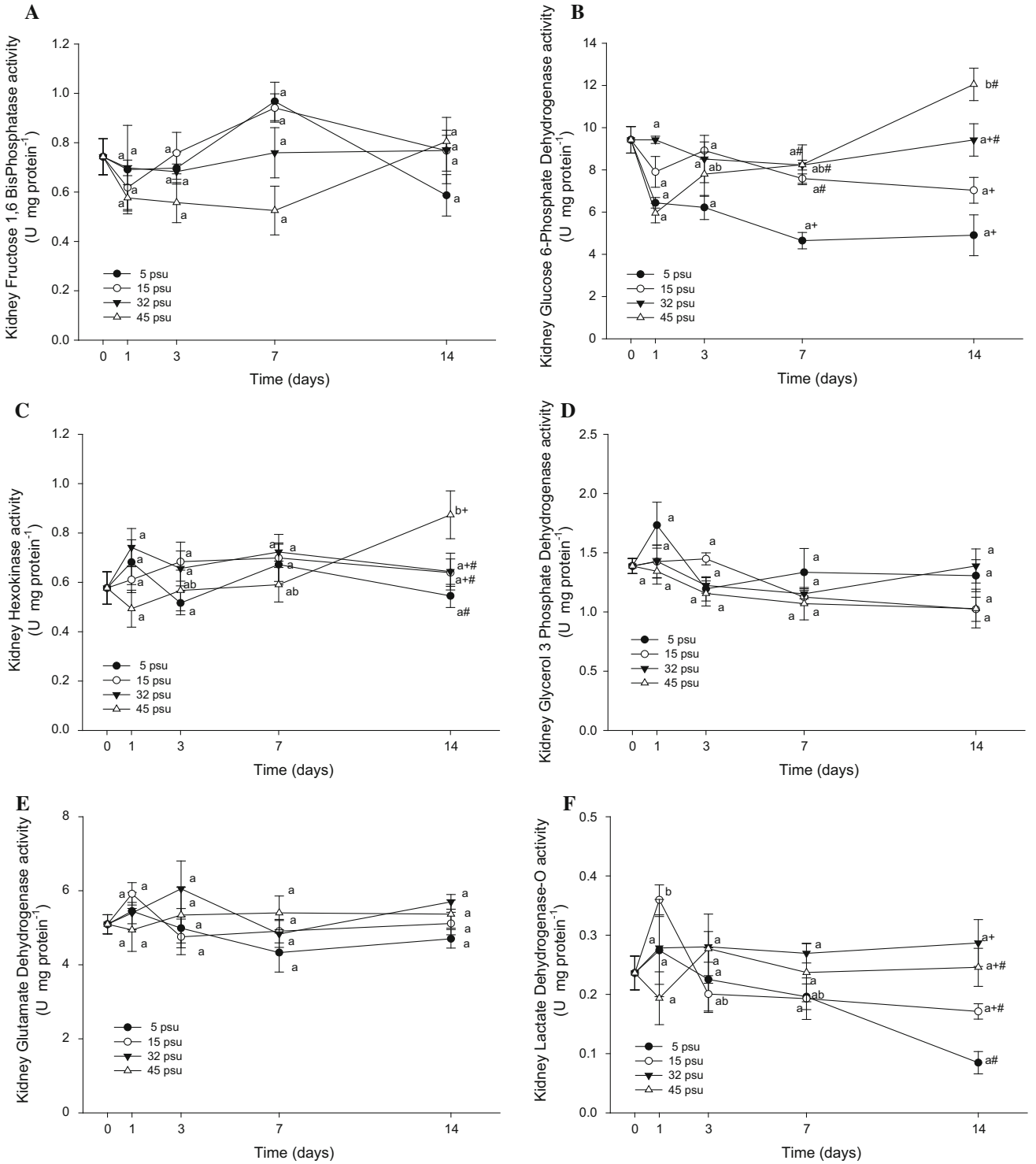


Fig. 3 Time course of changes in kidney enzymatic activities of *E. maclovinus* juveniles after transfer from sea water (32 psu) to low salinity water (5 psu), medium salinity water (15 psu), sea water (control group, 32 psu), and high salinity water (45 psu). **A** FBP, **B** G6PDH, **C** HK, **D** G3PDH, **E** GDH, **F** LDH-O. Further details as in legend of Fig. 1

contrast, increased GDH activity and amino acid levels in the HSW group may be linked their use as a source of energy. These data support the idea that under extreme environmental salinity conditions, the gills of *E. maclovinus* metabolize amino acids from other sources (e.g. liver or muscle).

Lipid metabolism, reflected by changes in G3PDH activity and triglycerides (Fig. 2D, Table 3), presented adjustment and long-term response periods in the LSW and MSW groups. Specifically in these groups, G3PDH activity increased, while the triglyceride concentrations decreased, indicating a possible use of lipid derivatives as an energy source during the first 3 dpt. These results are in contrast to prior evidence in *S. aurata*, where no role of lipids as an energy source in the gills was observed in specimens submitted to a salinity challenge (Sangiao-Alvarellos et al. 2003, 2005b).

Kidney

Numerous changes were found in several metabolic pathways in the kidney. Carbohydrate catabolism, based on changes in HK activity and glucose levels (Fig. 3C, Table 4), increased at 14 dpt in the HSW group. This increased HK activity indicates a greater capacity to phosphorylate exogenous glucose, which is in line with similar observations in *S. aurata* treated with the growth hormone (Sangiao-Alvarellos et al. 2005b). Parameters related to lactate metabolism, such as LDH-O activity (Fig. 3F), decreased in the LSW group at 14 dpt, but lactate levels showed no variations over the trial. Therefore, lactate might not be used by the kidney as energy. Similar results were presented for *S. aurata* subjected to different salinities, where no changes were found for either renal LDH-O activity or lactate levels (Sangiao-Alvarellos et al. 2005b).

Amino acid metabolism, assessed by total amino acid levels, showed varied free amino acid levels in the kidney due to changes in environmental salinity (Table 4). The absence of changes in the patterns of GDH and FBP (i.e. gluconeogenesis) activities suggests that amino acid levels may be related to other enzymes or routes (Fig. 3A, E, respectively). This, in turn, would produce increased glutamate deamination due to protein catabolism or, in the case of the HSW group, to the transport of amino acids from the bloodstream (Polakof et al. 2006). Moreover, the unchanged GDH and FBP activities could be related to greater synthesis of fatty acids in the kidney, as suggested by increased activity of the enzyme G6PDH. This enzyme activity could be fuelled by total α-amino acids from other tissues (Vargas-Chacoff et al. 2009a, b, 2014c).

Lipid metabolism, assessed by G3PDH activity and triglyceride levels (Fig. 3D, Table 4), did not show variations related to salinity changes, suggesting that lipids are not a relevant energy source for the kidney in these conditions.

In conclusion, extreme salinities (5 and 45 psu) elicited dramatic metabolic responses to fuel osmoregulatory tissues in *E. maclovinus*. Specifically, fish acclimated to 5 psu showed significantly decreased branchial GDH activity, indicating amino acid mobilization, while free amino acids increased in the kidney of fish exposed to 45 psu on day 14 post-transfer, thus supporting the idea that proteolysis fuels osmoregulatory capacities at extreme salinities. In the liver, a key metabolic tissue in teleosts, osmoregulatory imbalances (Vargas-Chacoff et al. 2014a) were counteracted in extreme salinities as early as day 3 post-transfer, as evidenced by increased ALA-AT and ASP-AT activities. Likewise, glycogen consumption was also evident in the liver at the end of the experimental trial. The decreased G3PDH activity observed in the liver at 15 psu suggested lipid accumulation in this experimental group, which is indicative of metabolite storage that would be related to the previously described best growth rates of this species (Vargas-Chacoff et al. 2014a). In summary, *E. maclovinus* is able to counteract steep salinity variations within a few days and is able to cope with osmoregulatory imbalances (Vargas-Chacoff et al. 2014a) through metabolic readjustments after 14 days, showing the best responses in isosmotic environmental salinities.

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Compliance with ethical standards

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

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

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