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ORIGINAL ARTICLE

Different environmental temperatures affect amino acid metabolism in the eurytherm teleost Senegalese sole (*Solea senegalensis* Kaup, 1858) as indicated by changes in plasma metabolites

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Abstract Senegalese sole (*Solea senegalensis*) is a eurytherm teleost that under natural conditions can be exposed to annual water temperature fluctuations between 12 and 26°C. This study assessed the effects of temperature on sole metabolic status, in particular in what concerns plasma free amino acid changes during thermal acclimation. Senegalese sole maintained at 18°C were acclimated to either cold (12°C) or warm (26°C) environmental temperatures for 21 days. Fish maintained at 18°C served as control. Plasma concentrations of cortisol, glucose, lactate, triglycerides, proteins, and free amino acids were assessed. Cold acclimation influenced interrenal responses of sole by increasing cortisol release. Moreover, plasma glucose and lactate concentrations increased linearly with temperature, presumably reflecting a higher metabolic activity of sole acclimated to 26°C. Acclimation temperature affected more drastically plasma concentrations of dispensable than that of indispensable amino acids, and different acclimation temperatures induced different responses. Asparagine, glutamine and ornithine seem to be of particular importance for ammonia detoxification mechanisms, synthesis of triglycerides that may be used during homeoviscous adaptation and, to a lesser extent, as energetic substrates in specimens acclimated to 12°C. When sole is acclimated to 26°C taurine, glutamate, GABA and glycine increased, which may suggest important roles as antioxidant defences, in osmoregulatory processes and/or for energetic purposes at this thermal regimen. In conclusion, acclimation to different environmental temperatures induces several metabolic changes in Senegalese sole, suggesting that amino acids may be important for thermal acclimation.

Keywords Acclimation · Dispensable amino acids · Homeoviscous adaptation · *Solea senegalensis* · Temperature

Introduction

Body temperature is perhaps the most important physiological variable affecting the performance of ectotherms. Among these animals, eurytherms can tolerate wide temperature fluctuations. In temperate to subpolar zones, eurytherms can dynamically change the range of thermal tolerance between summer and winter (Pörtner et al. 2005). Water temperature changes have immediate effects on eurythermal aquatic animals, due to the high rate of heat exchange between these animals and ambient water (Stevens and Sutterlin 1976). In fact, thermal acclimation involves expression of allozymes, modifications of cell membranes composition, and/or metabolic alterations in

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the intracellular medium that may be reversible or not (Angilletta et al. 2002). For instance, in response to decreasing temperature, one common strategy is to increase enzymatic tissue activity by inducing synthesis of more copies of the enzyme to compensate for the effects of low thermal energy on enzyme rates (Somero 2004). The influence of different temperature regimes on some enzyme activities related to energy metabolism have been recently investigated in eurytherm teleosts (Couto et al. 2008; Vargas-Chacoff et al. 2009a, b), including Senegalese sole (*Solea senegalensis*) (Arjona et al. 2010).

In fish, an optimal temperature range with optimal conditions for growth, reproduction and disease tolerance exist, and exposure to temperatures above or below this range induces a stress response that can compromise these processes (Cossins et al. 1995). Therefore, thermal stress occurs when the water temperature exceeds the optimal temperature range, thus initiating changes that disturb normal physiological functions resulting in increased demand for energy production, changes in metabolic rate and even a potential decrease in individual endurance (Wendelaar Bonga 1997; Portz et al. 2006). These altered physiological functions often translate in an allostatic state (McEwen and Wingfield 2003). The metabolic changes associated to acclimation to different environmental temperatures show different patterns which depend on the species studied (Vargas-Chacoff et al. 2009a, b; Arjona et al. 2010). Similarly, temperature also affects the metabolism of amino acids in fish. The two main transaminases (glutamate oxaloacetate and glutamate pyruvate transaminases) are responsive to temperature change in fish (Jürss 1979). Seasonal variations have been described for plasma amino acids in the winter flounder (*Pseudopleuronectes americanus*), decreasing total amino acid concentrations in winter while higher concentrations were observed during summer (Squires et al. 1979). Furthermore, Woo (1990) reported an increase in total plasma amino acid concentrations of warm acclimated red seabream (*Pagrus major*), while total amino acid concentrations in gills and kidney of gilthead seabream (*Sparus aurata*) were significantly higher during cold acclimation (Vargas-Chacoff et al. 2009b). Moreover, metabolic changes associated with low temperature (e.g. elevated lipogenesis) may also influence amino acid metabolism in fish (Ballantyne 2001).

Senegalese sole is a marine teleost that inhabits coastal and estuarine areas, which are subjected to wide changes in environmental salinity and temperature (Imsland et al. 2003a). In nature, Senegalese sole is relatively abundant in water temperatures between 13 and 28°C (Vinagre et al. 2006). In aquaculture, it is commonly raised in semi-extensive earthen ponds and land-based facilities along the southern coast of the Iberian Peninsula where they are exposed to annual water temperature fluctuations between 12 and 26°C (Dinis et al. 1999; Imsland et al. 2003a). In a previous study, the implication of Senegalese sole acclimation to different environmental temperatures on thyroidal status and osmoregulatory system has been determined (Arjona et al. 2010).

Regardless the vast literature concerning metabolic changes during acclimation and/or exposure to different temperatures, few studies have focused on the effects of environmental temperature on plasma amino acid concentrations in fish. Therefore, the purpose of the present study was to assess the effects of acclimation to different environmental temperatures in the eurytherm Senegalese sole. The study focused on changes in plasma cortisol, free amino acids and other metabolites as indicators of the eventual metabolic adjustments. The results will be discussed with special attention to amino acid metabolism after exposure to temperatures encountered by this species in common culture conditions.

Materials and methods

Experimental procedures

Senegalese sole juveniles were provided by Planta de Cultivos Marinos (C.A.S.E.M., Universidad de Cádiz, Puerto Real, Cádiz, Spain) and transferred to the wet laboratories at the Faculty of Marine and Environmental Sciences (Puerto Real, Cádiz). Fish were acclimated to the new facilities for 14 days in flat-bottom flow-through tanks (400 L; salinity: 38 ppt; temperature: 17–18°C; natural photoperiod: January, 2007). Thereafter, thirty six Senegalese sole juveniles (94.5 ± 40.4 g; mean ± standard deviation) were anaesthetized with 2-phenoxyethanol (0.5 mL L^{-1}; Sigma-Aldrich, Germany), weighed, measured and evenly distributed over six flat-bottom tanks filled with 250 L of recirculating seawater. Fish were maintained under the same conditions previously described, with the exception of water temperature. Water temperature was either maintained at 18°C (Control group) or slowly changed by 1°C/day until the final acclimation temperature (12 and 26°C) was reached. Each treatment was performed in duplicate. Following a 7-day acclimation period, fish were maintained at the final acclimation temperature for 21 days before sampling. Previous studies showed that a period of 2–3 weeks is adequate to reach complete acclimation and a new steady state in eurythermal teleosts (Campbell and Davies 1975; Woo 1990; Goldspink 1995). Water quality parameters (hardness and levels of O_2, CO_2, H_2S, NO_2^-, NO_3^-, NH_4^+, Ca^{2+}, Cl_2, suspended solids) were monitored continuously throughout the experiment and no major changes were observed. Fish were daily fed by hand with commercial dry pellets (Dibaq-Diproteg SA, Segovia, Spain; 48% crude protein, 6% carbohydrates, 25% crude fat and 11.5% ash; 20.2 MJ kg^{-1} of feed) to apparent satiety. This resulted in a daily ration of about 1% body weight at the beginning of the experiment. Fish were fasted for 24 h before sampling since plasma cortisol and glucose concentrations may be affected by feeding (Arends et al. 1999). All experimental procedures complied with the Guidelines of the European Union Council (86/609/EU) and of the University of Cádiz (Spain) for the use of laboratory animals.

At the end of the experimental period, fish from each tank were quickly netted at a time and anaesthetized with 2-phenoxyethanol (1 mL L^{-1}; Sigma-Aldrich). Blood samples were withdrawn from the caudal vein by puncturing with a 1 mL syringe rinsed with a solution containing 25,000 U ammonium heparin per 3 mL 0.9% NaCl. The blood collection lasted less than 3 min in order to avoid cortisol increase due to manipulation during sampling (Arends et al. 1999). After each tank sampling, stored blood was centrifuged at $10{,}000\times g$ during 3 min at 4°C. The collected plasma was frozen in liquid nitrogen and stored at –80°C until assayed. All fish from each tank were weighed and measured, and liver was dissected and weighed.

Analytical procedures

Plasma cortisol was determined by radioimmunoassay (RIA) as described by Rotllant et al. (2006). Briefly, 50 µL of plasma samples were diluted in 950 µL phosphate buffer containing 1 g L^{-1} gelatin, pH 7.6 and denatured at 80°C for 1 h. Duplicate aliquots (100 µL) of diluted denatured plasma were then used in the assay.

Glucose, lactate and triglycerides analyses were performed on plasma samples using commercially available Spinreact kits (Glucose HK Ref. 1001200; Lactate Ref. 1001330; Triglycerides Ref. 1001311), adapted to 96-well microplates. Plasma protein was determined in 1:50 (v/v) diluted plasma samples using the bicinchoninic acid (BCA) Protein Assay Kit (Pierce #23225, Rockford, USA). Bovine serum albumin served as a standard. These assays were run on a Bio Kinetics EL-340i Automated Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA) using DeltaSoft3 software for Macintosh (BioMetallics Inc., NJ, USA).

Plasma samples for free amino acid analysis were deproteinised by centrifugal ultrafiltration (10 kDa cut-off, $2{,}500\times g$, 20 min, 4 °C). After deproteinisation, samples were pre-column derivatized with phenylisothiocyanate (PITC; Pierce), using the PicoTag method (Waters, USA) described by Cohen et al. (1989). External standards were prepared along with the samples, using physiological amino acid standard solutions (acid/neutral and basics from Sigma) and a glutamine solution. Norleucine was used as an internal standard. Samples and standards were analysed by High Performance Liquid Chromatography (HPLC) in a Waters Reversed-Phase Amino Acid Analysis System equipped with a PicoTag column (3.9 × 300 mm), using the conditions described by Cohen et al. (1989). Resulting peaks were analysed with the Breeze software (Waters).

Data analysis

For each treatment, relative growth rate (RGR) and hepatosomatic index (HSI) were calculated as follows:

RGR $(\%\ \mathrm{day}^{-1}) = (e^{g} - 1) \times 100$, with: $g = [\ln(W_2) - \ln(W_1)] \times \mathrm{days}^{-1}$, where, W_1 and W_2 were the initial and final wet weights, respectively;

$$\mathrm{HSI}\ (\%) = (\text{liver weight}) \times (\text{final wet weight})^{-1} \times 100.$$

The results were expressed as mean ± standard deviation (SD). Data were analysed for normality (Kolomogorov–Smirnov test) and homogeneity of variance (Levene's test) and, when necessary, these requisites were achieved by log-transformation, but data are shown untransformed for clarity. Data between duplicate tanks were previously tested by *t* test. In the absence of significant difference between tanks, data from the same treatment was pooled together and analysed by one-way analysis of variance (ANOVA) using the computer package SPSS for Windows 15.0. Therefore, the experimental unit considered for each treatment was the fish. When significant differences were obtained from the ANOVA, multiple comparisons were carried out performing Tukey–HSD mean comparison test. The level of significance used was $P \leq 0.05$ for all statistical tests.

Results

Survival at the end of the experimental period was 100 % for fish maintained at 18 and 26°C and 92% for fish acclimated to 12°C. There were no significant differences between duplicate tanks within each treatment for all data analysed. Fish acclimated to 12°C presented negative RGR and weight gain after four weeks of experiment, which was significantly lower than that from fish maintained at 18 and 26°C. In addition, a noticeable decrease in feed intake at cold temperature was observed. In fact, specimens acclimated to 12°C did not react to the feed provided and subsequently displayed a low feed intake. Moreover, a significantly higher HSI was observed in fish maintained at 18°C when compared to fish acclimated to 12 and 26°C (Table 1).

Plasma cortisol concentrations showed a U-shaped relationship respect to environmental temperature, with

Table 1 Relative growth rate (RGR) and hepatosomatic index (HSI) in *S. senegalensis* acclimated to 12 and 26°C, or maintained at 18°C for 21 days

Temperature (°C)	RGR (% day^{-1})	HSI (%)
12	-0.04 ± 0.02^{a}	0.94 ± 0.28^{a}
18	0.76 ± 0.11^{b}	1.25 ± 0.25^{b}
26	0.81 ± 0.16^{b}	0.86 ± 0.10^{a}

Values are mean ± SD ($n = 11$–12). Different letters indicate significant differences among treatments for the same parameter

values significantly higher (9-fold) in specimens acclimated to 12°C than in fish maintained at 18 and 26°C, while fish acclimated to 26°C showed significantly higher concentrations (2-fold) than fish at 18°C. Plasma glucose and lactate values presented a direct relationship respect to acclimation temperature with concentrations significantly higher in soles acclimated to 26°C than at 12°C. However, plasma triglycerides values showed an inverse relationship, being all the groups statistically different. Plasma total protein concentrations were not significantly different among treatments, while total plasma free amino acids concentrations were significantly higher in specimens acclimated to both 12 and 26°C than in specimens maintained at 18°C (Table 2).

A detailed picture on plasma levels of individual free amino acids showed that temperature affected more drastically plasma concentrations of dispensable than that of indispensable amino acids and that different exposure temperatures induced different responses (Fig. 1a, b). Therefore, asparagine, glutamine and ornithine presented significantly higher concentrations in fish acclimated to 12°C than at the other experimental temperatures, while glutamate, taurine, and alanine increased significantly in fish acclimated to 26°C. Glycine and aspartate were significantly higher in fish held at 26°C respect to that maintained at 18°C. Plasma concentrations of γ-amino-n-butyric acid (GABA) were significantly lower at 18°C than in fish acclimated to cold or warm temperatures, being the concentrations at 26°C higher than at 12°C. Plasma tyrosine values increased significantly in specimens maintained at 18°C than in fish acclimated to 12°C (Fig. 1a). Regarding indispensable amino acids, plasma concentrations of lysine were threefold higher in fish maintained at 12°C than in fish held at 18 and 26°C, while tryptophan had threefold lower concentrations in fish acclimated to 26°C than at the other temperatures tested. No significant differences were found for the remaining indispensable amino acids (Fig. 1b).

Discussion

Effects of acclimation to 12°C

In this study, loss of appetite and lower growth rates were observed in specimens exposed to cold temperature, as already reported in this species (Arjona et al. 2010) and other teleosts (Beckman et al. 2000; Larsen et al. 2001). However, the observed decrease in HSI from specimens acclimated to 12°C in the current study was surprising. Cold acclimation is normally characterized by hepatic lipid deposition as a result of high liver uptake of circulating lipids from peripheral fat (Ibarz et al. 2007), and the same pattern appears to happen in cold acclimated Senegalese sole (Arjona et al. 2010). In the current study, this lower HSI could be due to the fact that specimens acclimated to 12°C presented a low feed intake. Thus, the 12°C regimen may have a confounding effect of a situation close to starvation. In fact, several teleosts showed a significant decrease in HSI after feed deprivation (Soengas et al. 1996; Polakof et al. 2006), including Senegalese sole (Costas et al. 2011a).

Plasma cortisol concentrations from fish acclimated to 12°C were approximately ninefold higher than in fish maintained at 18°C, suggesting that cold acclimation under these experimental conditions induced the activation of the hypothalamic pituitary-interrenal axis (Wendelaar Bonga 1997). Similarly, Mozambique tilapia (*Oreochromis mossambicus*) acclimated to 20°C increased plasma cortisol concentrations (around threefold) respect to control fish maintained at 28°C (Fiess et al. 2007). Interestingly, when Senegalese sole is exposed at 12°C plasma cortisol concentrations remain higher than at 26°C. However, in a previous study Senegalese sole submitted to similar thermal regimens did not show any significant plasma cortisol variations (Arjona et al. 2010).

Table 2 Plasma cortisol, metabolites and total free amino acid (FAA) concentrations in *S. senegalensis* acclimated to 12 and 26°C, or maintained at 18°C for 21 days

Temperature (°C)	Cortisol (ng ml^{-1})	Glucose (mM)	Lactate (mM)	Triglycerides (mM)	Proteins (mg ml^{-1})	FAA (μM)
12	12.0 ± 5.6^{c}	2.1 ± 0.4^{a}	0.2 ± 0.1^{a}	10.0 ± 1.9^{c}	41.9 ± 2.3	$1{,}042.7 \pm 126.3^{b}$
18	1.3 ± 2.1^{a}	2.4 ± 0.5^{ab}	0.3 ± 0.1^{a}	5.3 ± 1.7^{b}	41.6 ± 3.0	765.8 ± 152.8^{a}
26	2.8 ± 1.9^{b}	3.1 ± 1.1^{b}	0.5 ± 0.2^{b}	2.9 ± 1.6^{a}	39.9 ± 4.2	$1{,}084.5 \pm 235.7^{b}$

Values are mean ± SD ($n = 11$–12). Different letters indicate significant differences among treatments for the same parameter

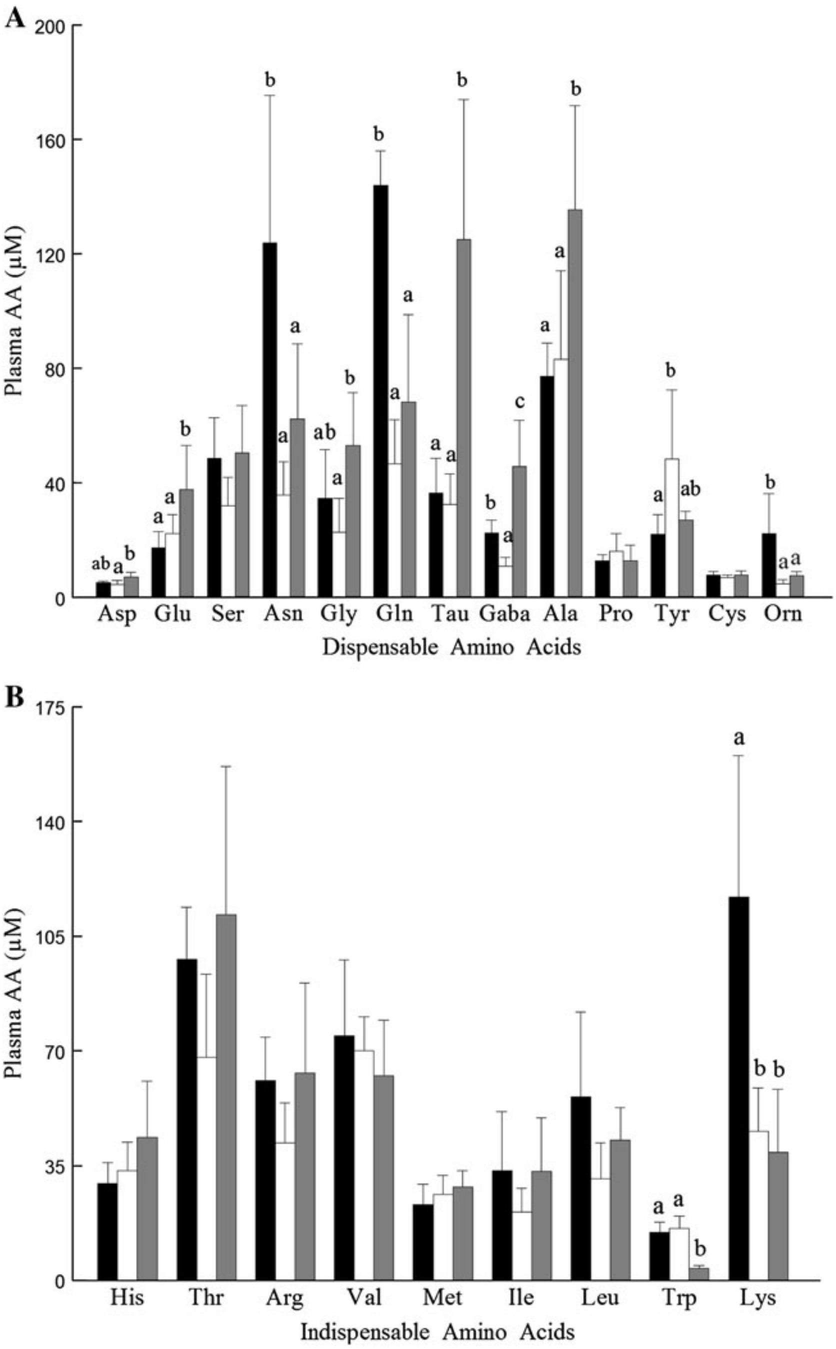

Fig. 1 Dispensable (**a**) and indispensable (**b**) plasma free amino acid concentrations in *S. senegalensis* acclimated to 12 (*filled square*) and 26°C (*open square*), or maintained at 18°C (*open square*) for 21 days. Values are mean ± SD ($n = 6$). *Different letters* indicate significant differences among treatments for the same amino acid ($P < 0.05$)

Therefore, in the current study environmental temperature could have modulated the rate at which plasma cortisol concentrations increased and/or the allostatic adaptation. Green sturgeon acclimated to 11°C or maintained at 19°C showed similar cortisol concentrations after being submitted to an acute stress, however a delayed synthesis of cortisol and its subsequent clearance was observed in specimens acclimated to 11°C (Lankford et al. 2003). In the present study, the increased cortisol concentrations observed in fish acclimated to 12°C could also be explained due to the possible near-starvation condition already mentioned. High cortisol concentrations were also observed in feed-deprived fish compared to fed groups (Polakof et al. 2006; Mancera et al. 2008), including Senegalese sole (Costas et al. 2011a). Cortisol presents a clear catabolic role in teleosts, playing a functional role in mobilising energy during starvation (Mommsen et al. 1999). However, plasma free amino acid patterns for both indispensable and dispensable amino acids observed in this study were very different to that reported by Costas et al. (2011a) in food-deprivated specimens. Therefore, we believe that changes in plasma metabolites and free amino acids are also temperature depending, even if the near-starvation condition may interfere to some extent.

Temperature may differentially affect the metabolism of specific amino acids as well as the overall importance of amino acids as energy sources (Ballantyne 2001). In the present study, increased plasma asparagine, glutamine and ornithine concentrations were observed in Senegalese sole acclimated to 12°C. These dispensable amino acids may have accumulated in plasma as a result of peripheral proteolysis due to a catabolic action of cortisol, and thus could have been funnelled for energy production in liver and other tissues. This hypothesis is supported by the fact that asparagine and glutamine are two of the most important amino acids for oxidation in fish liver (Jürss and Bastrop 1995). In addition, glutamine and ornithine are easily transaminated into glutamate since these dispensable amino acids are members of the "glutamate family" (Brosnan 2000), and glutamate transdeamination is the main pathway of amino acid oxidation in fish liver (Ballantyne 2001). An increase in total plasma dispensable amino acid concentrations was observed in gilthead seabream submitted to low temperature and presented as the "winter syndrome" (Gallardo et al. 2003). Both the cortisol-induced proteolysis and amino acid catabolism have been linked to ammonia production (Mommsen et al. 1999; Ip et al. 2001). Glutamine synthesis is an important pathway for ammonia detoxification in several fish species (Ip et al. 2001) and an increase in plasma glutamine concentrations in Senegalese sole juveniles either feed-deprived (Costas et al. 2011a) or chronically exposed to exogenous ammonia has been previously observed (Pinto et al. 2007). Although glutamine synthesis is energetically expensive (Randall and Tsui 2002), this strategy presents an advantage, being this dispensable amino acid utilized as an oxidative substrate. Therefore, this pathway appears to be of special importance in Senegalese sole acclimated to cold temperature, which could explain the threefold increase of plasma glutamine concentrations. The increased levels of plasma asparagine and ornithine (urea cycle metabolite) concentrations may also result from ammonia removal mechanisms and as a consequence of increased use of amino acids as energy substrates in cold acclimated Senegalese sole.

In the present study, plasma triglycerides values showed an inverse linear relationship respect to acclimation temperature, being approximately twofold higher in fish acclimated to 12°C than in fish maintained at 18°C, and in agreement with that reported previously for specimens of Senegalese sole under similar thermal regimens (Arjona et al. 2010). This agrees with the homeoviscous adaptation to cold where in order to maintain the appropriate fluidity of biological membranes, remodeling of membrane lipid composition occurs (Hazel 1997). Thus, the observed increase in plasma triglycerides in the current study is probably caused by an effect of cold temperature per se as it has been previously suggested for this species (Arjona et al. 2010). These increased plasma triglycerides concentrations are also congruent with the hypothesised increase in use of amino acids as energy substrates in Senegalese sole acclimated to 12°C. Asparagine and glutamine have been reported to be used as carbon source for triglycerides synthesis in liver (Nye et al. 2008). Therefore, the accumulating plasma concentrations of these two dispensable amino acids suggest that they may also be funnelled for hepatic triglycerides synthesis. Moreover, increased unsaturated fatty acid contents have been previously observed in several tissues in cold acclimated fish (Gallardo et al. 2003; Ibarz et al. 2005, 2007; Vargas-Chacoff et al. 2009b), including Senegalese sole (Arjona et al. 2010). The increase in plasma triglycerides in the present study is probably due, at least partially, to the augmented use of amino acids as energy substrates. In fact, even if sole as other fish (Somero 2004) show a decreased metabolic activity in response to cold, probably they use amino acids preferentially to triglycerides as energy substrates. However, a lipolytic action of cortisol that could also increase plasma triglycerides concentrations cannot be discarded.

The only indispensable amino acid showing changes in its plasma concentration due to thermal acclimation is lysine. The increased lysine concentrations observed in Senegalese sole acclimated to 12°C may result from its eventual poorer utilisation as energy substrate at this temperature. Lysine has acetoacetyl-CoA as its single entry point into energy metabolism, and this conversion may be inhibited due to accumulation of acetyl-CoA (allosteric end-product inhibition) as a result of high abundance of ketoacids due to increased amino acids deamination (Babu et al. 1989). Although not significant, Gallardo et al. (2003) also observed increased plasma lysine values in gilthead seabream submitted to cold environmental temperature. Interestingly, Lalouette et al. (2007) observed an increase of several indispensable amino acids, including lysine, resulting from proteolysis in cold exposed adult beetles (*Alphitobius diaperinus*).

Effects of acclimation to 26°C

Plasma cortisol concentrations from fish acclimated to 26°C were approximately twofold higher than in fish held at 18°C, in agreement with that previously reported in fish submitted to warm acclimation (Arends et al. 1998; Metz et al. 2003; Choi et al. 2007). However, this augmentation in plasma cortisol from the present study is low and most likely linked to a new allostatic equilibrium after warm acclimation (McEwen and Wingfield 2003). In fact, basal cortisol concentrations in plasma of unstressed Senegalese sole are reported to be around 1–6 ng/mL (Arjona et al. 2010; Costas et al. 2011a, b).

In this study, plasma glucose concentrations increased linearly with temperature. Since this parameter has been correlated positively with temperature in acclimated fish (Fiess et al. 2007; Vargas-Chacoff et al. 2009a, b), temperature-related differences observed in this study for this metabolite may reflect variations in the metabolic activity of Senegalese sole acclimated to different temperatures. In a previous study, Senegalese sole acclimated to 26°C also showed significantly higher plasma glucose concentrations when compared with specimens maintained at 19°C (Arjona et al. 2010). Warm acclimation temperature is known to increase basal metabolic rates in fish (Johnston and Dunn 1987). Results from the current study also showed plasma lactate concentrations around twofold higher in fish acclimated to 26°C than fish maintained at 18°C. This increased plasma lactate concentrations are most likely related to the higher metabolic activity at this thermal condition, as it has been shown by other species (Vargas-Chacoff et al. 2009a, b).

Similar to that observed in cold acclimated fish from the current study, total plasma free amino acid concentrations also increased in Senegalese sole acclimated to 26°C. However, these changes are only attributed to augmented concentrations of dispensable amino acids, which appear to present different roles at this thermal regimen. In fact, increased plasma alanine and glycine concentrations were observed in warm acclimated, specimens probably as result of their reduced utilization for energy purposes. These dispensable amino acids are preferential substrates for liver gluconeogenesis in fish (Ballantyne 2001). Therefore, gluconeogenesis is probably allosterically reduced due to increased plasma glucose concentrations in fish acclimated to 26°C. Moreover, warm acclimated Senegalese sole also showed an augmentation in plasma aspartate and glutamate concentrations. These two dispensable amino acids are important energy substrates for fish, being the pivotal molecules in transamination processes. In fact, glutamate transdeamination is the main pathway of amino acid oxidation in fish liver (Ballantyne 2001). Therefore, accumulation of these two dispensable amino acids most likely results from augmented transamination processes to allow for an increased utilization of amino acids as energetic substrates, due to the increased metabolic activity in specimens acclimated to 26°C.

Results from the present study also showed as fish acclimated to 26°C presented threefold lower plasma tryptophan concentrations. Tryptophan is the precursor of serotonin, which has been suggested to participate in the physiological and biochemical responses that occur during thermal acclimation in teleosts (Tsai and Wang 1997). In fact, warm water temperature induced an increase in serotonergic activity in fish (Sebert et al. 1985; De Boeck et al. 1996), suggesting an augmentation of tryptophan metabolism. Therefore, results from the present study make tempting to speculate that tryptophan uptake increased in brain of fish acclimated to 26°C due to an increased serotonin synthesis. However, further studies are necessary in order to probe this hypothesis.

Warm acclimated Senegalese sole presented significantly higher plasma GABA, glutamate, glycine and taurine concentrations. Intrahypothalamic microinjection with a high dose of GABA resulted in the selection of warmer water in tilapia, suggesting its function as modulator in temperature selection (Tsai et al. 2002). Glutamate and its decarboxylation product (GABA) are neurotransmitters present at high concentrations in fish brain (Li et al. 2009). Fiess et al. (2007) also observed significantly higher concentrations of taurine and glycine in brain of tilapia submitted to warmer temperature when compared to cold acclimated fish. In addition, glycine and taurine directly scavenge oxygen free radicals (Fang et al. 2002), which have been correlated positively with temperature (Parihar et al. 1997). In addition, changes in environmental temperature are also known to impair the osmoregulatory capacity in teleosts (Imsland et al. 2003b; Metz et al. 2003; Fiess et al. 2007), including Senegalese sole (Arjona et al. 2010). Since GABA, glycine and taurine are regarded as organic osmolytes for cell volume regulation (Yancey 2005), a possible role of these dispensable amino acids in osmoregulatory balance is also proposed for Senegalese sole specimens acclimated to 26°C. Therefore, taurine, glutamate, GABA and glycine may have important roles as antioxidants, neurotransmitters and/or organic osmolytes related to warm acclimation in Senegalese sole. Alternatively, taurine and GABA may just accumulate in plasma as they cannot serve as energy substrates as the other amino acids.

Conclusion

Results from the present study are consistent with an increasing reliance on carbohydrates for energy metabolism in sole acclimated to 26°C, presumably reflecting a higher metabolic activity of sole acclimated to this thermal condition, whereas lipid substrates could be utilized more at cold temperatures. Particularly, plasma triglycerides appear to have an important role in fish acclimated to 12°C during homeoviscous adaptation of cell membranes. Moreover, cold and warm acclimation may differentially affect metabolism of specific dispensable amino acids, as well as the general contribution of dispensable amino acids as energy sources. Asparagine, glutamine and ornithine seem to be of particular importance in specimens acclimated to 12°C since they are mainly used for ammonia detoxification mechanisms, triglycerides synthesis and, to a lesser extent, as energetic substrates. However, a different scenario occurs during warm acclimation in sole where taurine, glutamate, GABA and glycine present important roles as antioxidant defences, as well as for osmoregulatory processes and/or energetic purposes. The present study confirms that amino acids are more than building blocks for protein synthesis, showing different metabolic roles during thermal acclimation to cold or warm environments.

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