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The metabolic response in fish to mildly elevated water temperature relates to species-dependent muscular concentrations of imidazole compounds and free amino acids

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ABSTRACT

Fish species show distinct differences in their muscular concentrations of imidazoles and free amino acids (FAA). This study was conducted to investigate whether metabolic response to mildly elevated water temperature (MEWT) relates to species-dependent muscular concentrations of imidazoles and FAA. Thirteen carp and 17 Nile tilapia, housed one per aquarium, were randomly assigned to either acclimation (25 °C) or MEWT (30 °C) for 14 days. Main muscular concentrations were histidine (HIS; $P < 0.001$) in carp versus *N*-α-acetylhistidine (NAH; $P < 0.001$) and taurine (TAU; $P=0.001$) in tilapia. Although the sum of imidazole (HIS +NAH) and TAU in muscle remained constant over species and temperatures ($P > 0.05$), (NAH+HIS)/TAU ratio was markedly higher in carp versus tilapia, and decreased with MEWT only in carp ($P < 0.05$). Many of the muscular FAA concentrations were higher in carp than in tilapia ($P < 0.05$). Plasma acylcarnitine profile suggested a higher use of AA and fatty acids in carp metabolism ($P < 0.05$). On the contrary, the concentration of 3-hydroxyisovalerylcarnitine, a sink of leucine catabolism, ($P=0.009$) pointed to avoidance of leucine use in tilapia metabolism. Despite a further increase of plasma longer-chain acylcarnitines in tilapia at MEWT ($P=0.009$), their corresponding beta-oxidation products (3-hydroxy-longer-chain acylcarnitines) remained constant. Together with higher plasma non-esterified fatty acids (NEFA) in carp ($P=0.001$), the latter shows that carp, being a fatter fish, more readily mobilises fat than tilapia at MEWT, which coincides with more intensive muscular mobilization of imidazoles. This study demonstrates that fish species differ in their metabolic response to MEWT, which is associated with species-dependent changes in muscle imidazole to taurine ratio.

1. Introduction

Exposure of fish to elevations in water temperature can occur due to global warming (Ficke et al., 2007) or because aquaculture is increasingly established in warm countries (FAO, 2014). Fish are poikilothermic ectotherms, whose metabolism and growth is influenced by the water temperature and also farming conditions such as food availability, uptake and utilization (Jobling, 1994). Elevations in water temperature increase basal metabolism that results in a higher energy demand for maintenance and less energy available for growth (Jobling, 1994). Previous studies have shown that elevated water temperature can lead to changes in nutrient metabolism; for example, it can result in an increased breakdown of amino acids (AA) in fish (Geda et al., 2012). However, there is uncertainty about whether these AA are derived from the diet or from free AA (FAA) concentrated in muscle.

Fish tissues, like other animals, contain FAA and non-protein nitrogenous compounds (imidazole compounds, taurine, trimethylamine oxide and other methylamine compounds) (Van Waarde, 1988). The imidazole compounds are the major non-protein nitrogenous constituents present in skeletal muscles of vertebrates (Crush, 1970; Abe, 1983a, 1983b). There are five imidazole compounds in skeletal muscle of fish: histidine (HIS), carnosine (β-alanyl-L-histidine), anser-

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ine (β-alanyl-L-methyl-L-histidine), ophidine (balenine; β-alanyl-3-methyl-L-histidine), and *N*-α-acetylhistidine (NAH) (Van Waarde, 1988; Yamada et al., 2009). The imidazole compounds have a number of biological roles such as H^+ buffer (Sewell et al., 1992; Abe, 2000), neurotransmitter (Petroff et al., 2001), non-enzymatic free-radical scavenger (Guiotto et al., 2005), antioxidant (Boldyrev et al., 2004) and blood glucose regulator (Sauerhofer et al., 2007).

The distribution and abundance of imidazole compounds vary among fish species. For instance, skeletal muscle of carp (*Cyprinus carpio*) contains higher levels of HIS (Van Waarde, 1988) whereas that of Nile tilapia (*Oreochromis niloticus*) has higher levels of NAH (Yamada et al., 1992). It is not fully elucidated why animals store high concentrations of the FAA and imidazole compounds in their muscles (Shiau et al., 1997). The reason for the difference in the distribution patterns of the imidazole compounds in the skeletal muscle among fish species is also not yet understood. To our knowledge, it has not been investigated whether the different muscular concentrations of FAA and imidazole compounds would determine the use of nutrients in metabolism of fish at elevated temperature.

When fish cannot store high amounts of body fat (e.g., carp store more fat than tilapia: Abdelghany and Ahmad, 2002), as in many terrestrial animals, readily available muscular concentrations of FAA and imidazole compounds might serve as a rapid source of energy. The species difference in muscular concentration of imidazole compounds and their metabolic response to elevated water temperature would relate to the difference in body fat concentration between species. Therefore, due to the aforementioned reasons, carp and Nile tilapia were used as model fish in the present study to investigate the metabolic response of fish to a mildly elevated water temperature (MEWT).

2. Materials and methods

2.1. Animals and experimental design

Thirteen carp *(Cyprinus carpio) and* 17 Nile tilapia *(Oreochromis niloticus)* were acclimated to laboratory conditions, in two batches (batch 1: 6 carp and 6 tilapia; batch 2: 7 carp and 11 tilapia) for two weeks. The fish were individually and randomly allocated in 63L-glass aquaria of 60×30×36 cm (JUWEL Aquarium, Rotenburg, Germany). The acclimated fish were fasted for 24 h, weighed (average initial body weight: 119 ± 4 g tilapia, 123 ± 9 g carp), distributed into the aquaria, and randomly assigned to two groups, a control ("T25") and treatment ("T30"). This experiment was set up using two species of fish under two levels of temperature, in a 2×2 factorial design. All experimental methods and procedures used in this study were approved by the ethics committee of animal experiments at Faculty of Veterinary Medicine, Ghent University. The work described has been carried out in accordance with EU Directive 2010/63/EU for animal experiments.

2.2. Feed, feeding protocol and elevated temperature

The fish were hand-fed (feed: Benelux NV, Wielsbeke - Ooigem, Belgium) (Table 1) for 14 days at a feeding rate of 1.5% of average wet body weight per fish per day twice at 10:00 and 15:00. The experimental diet was analyzed for proximate chemical analysis of dry matter (DM), moisture (M), crude protein (CP), crude fat (diethyl ether-extract, EE), crude fibre (CF) and ash (Table 1). The DM and M contents were determined by drying feed samples in freeze dryer for 24 h and heating in a forced air oven at 103 °C to a constant weight. The ash content was determined by combustion of the feed at 550 °C. The EE was analyzed with the Soxhlet method (ISO 1443, 1973). The CF was determined using the Association of Official Analytical methods (Method 962.09 and 985.29, 1995). The Kjeldahl method (ISO 5983–1, 2005) was used to determine CP (6.25× N). The percentage of nitrogen-free extract (NFE) was calculated as: NFE=100–(M+Ash+CP +EE+CF). The T25 group was managed at an acclimation temperature of 25 °C and the T30 group was managed at a constant MEWT of 30 °C, set-up at a rate of 5 °C per 60 h. All the aquaria were maintained at 12:12 h light-dark photoperiod using fluorescent lights controlled by timers.

Table 1
Chemical composition of the experimental diet[a] (on as fed basis).

Proximate composition (g/kg)	
Dry matter	922.00
Moisture	78.00
Crude protein	326.20
Crude ash	54.40
Crude fat	55.80
Crude fibre	31.30
NFE[b]	454.30
Amino acid composition (g/kg)	
Alanine	19.98
Arginine	19.90
Aspartic acid+Asparagine	31.62
Cysteine	5.46
Glutamic acid+Glutamine	67.55
Glycine	13.98
Histidine	8.11
Isoleucine	15.44
Leucine	34.21
Lysine	16.85
Methionine	6.28
Phenylalanine	18.46
Proline	21.18
Serine	16.51
Threonine	13.23
Tryptophan	3.56
Tyrosine	12.52
Valine	17.06

Vitamin and mineral premix added per kg feed (as given in the commercial feed technical sheet): retinol, 3 mg; vitamin C, 100 mg; cholecalciferol, 0.025 mg; vitamin E, 33 mg; calcium, 6 g; phosphorus, 6 g; sodium, 0.8 g; iron, 100 mg; copper, 2.5 mg; manganese, 15 mg; zinc, 50 mg; selenium, 0.25 mg.
List of ingredients reported in Aqua-KI in decreasing order of inclusion: Fish products, products and by-products of oil seeds, vegetal products and by-products, algae, AA, vitamins, minerals, calcium propionate, antioxidants, oils and fats.
[a] Aqua-KI feed, manufactured by Benelux NV, Wielsbeke – Ooigem, Belgium.
[b] NFE =100–(M+Ash+CP+EE+CF).

Daily feed intake was determined by removing any uneaten feed after 60 min from each aquarium, drying and weighing as previously described (Geda et al., 2015). All fish were fasted for 24 h during measurements of their initial and final body weights. Average daily measured water quality parameters were pH (8.2) (Merck KGaA, Darmstadt, Germany), dissolved oxygen (5.7 mg/L for T25, 5.3 mg/L for T30) (Hanna Instruments Srl, Nufalau, Romania), ammonium (< 0.10 mg/L) and nitrite (0.13 mg/L) (JBL GmbH and Co KG, Neuhofen/Pfalz, Germany).

2.3. Blood sampling, plasma collection and analysis

All fish were fasted for 24 h and were euthanized using an overdose of a benzocaine (ethyl 4-aminobenzoate) solution (1g/10 mL acetone) and subjected to blood sampling. Blood samples were collected from the heart (cardiac puncture) using a 1 mL syringe (Becton Dickinson S.A., Madrid, Spain) and a 26G needle (Becton Dickinson, Drogheda, Ireland) rinsed with heparin (LEO Pharma, Ballerup, Denmark). The blood in the heparinized plasma tubes was centrifuged at 1200×*g* and 5 °C for 10 min. The plasma samples were collected and stored at –20 °C until analyzed. Acylcarnitine profile of the plasma samples was determined using quantitative electrospray tandem mass spectrometry (Zytkovicz et al., 2001). For determination of non-esterified fatty acids (NEFA), the plasma samples were analyzed spectrophotometrically (EZ Read 400 Microplate Reader, Biochrom Ltd., Cambridge, United

Table 2
Selected skeletal muscle FAA in carp (n=13) and tilapia (n=17) at MEWT (mean, pooled SEM).

Amino acids (μmol/g)	T25		T30		Pooled	*P*		
	Carp	Tilapia	Carp	Tilapia	SEM	T	S	T×S
Alanine	2.615	2.277	2.147	2.461	0.121	0.573	0.963	0.203
Aspartate	0.209	0.968	0.157	0.704	0.083	0.178	< 0.001	0.362
Asparagine	0.305	2.905	0.165	1.851	0.275	0.119	< 0.001	0.228
Arginine	0.852	0.052	0.503	0.044	0.091	0.217	< 0.001	0.238
Ornithine	0.091	0.062	0.078	0.054	0.009	0.555	0.139	0.902
Leucine	0.165	0.087	0.161	0.118	0.008	0.180	< 0.001	0.097
Isoleucine	0.075	0.046	0.079	0.064	0.004	0.131	0.003	0.315
Valine	0.104	0.063	0.099	0.091	0.005	0.209	0.010	0.079
Glutamate (Glu)	0.784	0.607	0.785	0.623	0.038	0.918	0.032	0.919
Glutamine (Gln)	1.076	1.176	0.772	1.161	0.066	0.224	0.067	0.272
Glu:Gln	0.787	0.538	1.012	0.573	0.055	0.168	0.001	0.311
Glycine	4.399	8.328	2.816	7.876	0.480	0.047	< 0.001	0.257
Serine	0.687	0.177	0.469	0.159	0.051	0.086	< 0.001	0.143
Histidine (HIS)	9.148	1.147	7.424	0.779	0.733	0.065	< 0.001	0.223
NAH	0.006	1.260	0.056	1.319	0.148	0.784	< 0.001	0.982
Lysine	1.694	0.102	1.345	0.135	0.148	0.283	< 0.001	0.196
Phenylalanine	0.066	0.025	0.068	0.033	0.005	0.508	< 0.001	0.694
Tyrosine	0.068	0.030	0.062	0.038	0.005	0.865	0.001	0.418
Proline	1.837	1.816	0.640	0.642	0.166	< 0.001	0.972	0.966
Hydroxyproline	0.177	0.745	0.070	0.488	0.062	0.032	0.000	0.360
Taurine (TAU)	8.962	16.537	11.907	16.763	0.972	0.337	0.001	0.409
Threonine	0.931	0.668	0.552	0.580	0.040	0.001	0.074	0.029
Tryptophan	0.021	0.009	0.013	0.011	0.002	0.275	0.007	0.077
Ammonium	1.243	1.385	1.270	1.398	0.070	0.892	0.366	0.963
Urea	9.340	3.482	7.400	2.402	0.814	0.268	< 0.001	0.749
TFAA	44.884	43.977	39.077	39.816	0.831	0.002	0.955	0.583
NAH+HIS:TFAA	0.205	0.054	0.191	0.052	0.015	0.618	< 0.001	0.678
TAU:TFAA	0.201	0.375	0.306	0.422	0.023	0.021	< 0.001	0.327

MEWT, mildly elevated water temperature; T, temperature; S, species; T×S, T and S interaction; NAH, *N*-α-acetylhistidine; TFAA, total FAA.

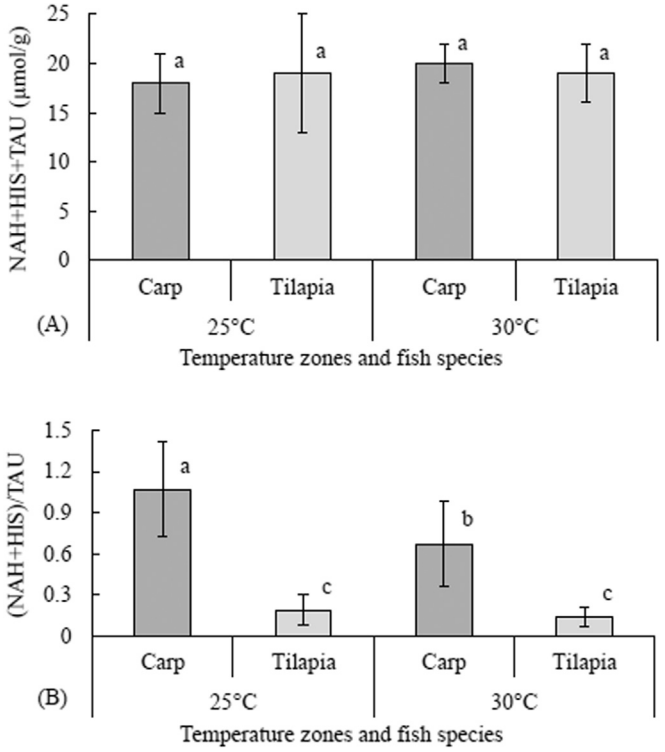

Fig. 1. Mean skeletal muscle imidazole (NAH and HIS) and TAU in carp (n=13) and tilapia (n=17) at acclimation (25 °C) and MEWT (30 °C): (A) sum of imidazole and taurine: temperature, species, and interaction effects, $P > 0.05$; (B) imidazole to taurine ratio: temperature effect, $P=0.012$; species effect, $P < 0.001$; interaction effect, $P=0.049$. [a,b,c]Mean values with different letters were significantly different ($P < 0.05$).

Kingdom) using a commercial analysis kit (Randox FA115) according to manufacturer's guidelines.

2.4. Skeletal muscle sampling and analysis

After the blood sampling, a sample of the dorsal muscle of about 4.5 cm^2 was taken on the left side of the body, 1 cm ventral to the base of the dorsal fin along the lateral line (Geda et al., 2015). The muscle samples were stored at −80 °C until analyzed. Determination of muscle tissue NAH and HIS concentrations was based on the reversed-phase HPLC (Waters Corporation) method (O'Dowd et al., 1990) with a slight modification (Breck et al., 2005). Muscle tissues were homogenized in 80% (v/v) ethanol and centrifuged at 2000*g* for 20 min. The supernatants were dried in a Termaks incubator (40 °C, normal atmosphere) (Termaks, Bergen, Norway), dissolved in phosphate buffer (pH 2.0) and filtered through a membrane filter (0.45 μm). An isocratic reverse phase HPLC was performed, using a 4.6 mm ID ×250 mm column with a silica-based packing (ZORBAX SB-C18, Agilent Technologies AS, Kolboth, Norway) and a Waters 600 E pump (Waters Corporation, Milford, MA, USA). A 0.1 M phosphate buffer (pH 2.0) was used as eluting solvent, with a flow rate of 0.6 mL/min. The concentrations of NAH and His were detected by UV absorbance (Waters 486–Tuneable Absorbance Detector, Waters Corporation) at 210 nm, using external standards.

For determination of free basic AA and total FAA (TFAA) concentrations in the muscle, tissue samples were prepared as described above. After complete drying, samples were dissolved in running buffer (Lithium Citrate Loading Buffer, 80-2038-10, Biochrom Ltd, Cambridge, UK) and AA content was determined by ninhydrin detection with Biochrom 20 Plus Amino Acid Analyser (Biochrom Ltd., Cambridge, United Kingdom) based on low pressure ion-exchange chromatography. Different gradient elution systems were used for identification of either TFAA profiles or, in a shortened version, the profile of free basic AA only. After post-column ninhydrin derivatiza-

Table 3
Selected plasma acylcarnitines in carp (n=13) and tilapia (n=17) at MEWT (mean, pooled SEM).

Carnitine ester (μmol/L)	T25		T30		Pooled	*P*-value		
	Carp	Tilapia	Carp	Tilapia	SEM	T	S	T×S
Free	6.686	4.766	6.912	4.880	0.388	0.818	0.012	0.940
Acetyl	3.617	1.312	3.575	1.173	0.282	0.816	< 0.001	0.901
Propionyl	0.256	0.041	0.263	0.063	0.245	0.660	< 0.001	0.834
Butyryl	0.207	0.126	0.173	0.090	0.016	0.242	0.008	0.976
3OH-butyryl	0.047	0.016	0.047	0.029	0.004	0.368	0.001	0.334
Hexanoyl	0.037	0.031	0.037	0.024	0.003	0.510	0.118	0.562
Octanoyl	0.457	0.033	0.032	0.024	0.003	0.039	0.073	0.685
Decanoyl	0.026	0.046	0.022	0.035	0.003	0.222	0.009	0.581
Dodecanoyl	0.037	0.034	0.035	0.040	0.003	0.817	0.876	0.602
3OH-dodecanoyl	0.013	0.009	0.010	0.006	0.001	0.323	0.169	0.968
Tetradecanoyl	0.026	0.036	0.018	0.048	0.003	0.654	0.001	0.066
3OH-tetradecanoyl	0.006	0.008	0.008	0.006	0.001	0.813	0.997	0.373
Tetradecenoyl	0.037	0.033	0.022	0.039	0.003	0.418	0.287	0.099
3OH-tetradecenoyl	0.011	0.010	0.010	0.008	0.001	0.205	0.205	0.726
Hexadecanoyl	0.054	0.066	0.040	0.095	0.006	0.404	0.001	0.021
3OH-hexadecanoyl	0.010	0.008	0.007	0.009	0.001	0.606	0.976	0.350
Hexadecenoyl	0.037	0.032	0.023	0.036	0.003	0.493	0.574	0.216
3OH-hexadecenoyl	0.011	0.007	0.007	0.008	0.001	0.337	0.337	0.175
Octadecanoyl	0.019	0.033	0.013	0.049	0.003	0.271	< 0.001	0.031
3OH-octadecanoyl	0.004	0.004	0.005	0.003	0.001	0.750	0.545	0.492
Octadecenoyl	0.111	0.083	0.060	0.130	0.010	0.895	0.253	0.011
3OH-octadecenoyl	0.006	0.006	0.005	0.006	0.001	0.996	0.783	0.722

MEWT, mildly elevated water temperature; T, temperature; S, species; T×S, T and S interaction.

tion, colorimetric detection was made at 570 and 440 nm (Waters 486, Waters Corporation).

2.5. Statistical analysis

Statistical analysis was conducted using SPSS version 20 (IBM Corporation, Armonk, NY, USA). All data were evaluated for normality using boxplot graphs, analyzed with Linear Mixed Model (LMM), and are expressed as means and standard error of the means (SEM). Because of inflated Type I error rate due to multiple comparisons of the main effects, a Bonferroni correction was applied as confidence interval adjustment. Statistical significance was accepted at $P < 0.05$. The effects of temperature, species and their interactions were managed as fixed factors; random effects of batch and aquarium nested within the batch were analyzed in the LMM:

$$Y_{ijklm} = \mu + T_i + S_j + (TS)_{ij} + B_k + (AB)_{l(k)} + \varepsilon_{ijklm}$$

where μ is the overall mean; T_i is the ith effect of temperature; S_j is the jth effect of species; $(TS)_{ij}$ is the ijth interaction effect between temperature and species; B_k is the kth effect of batch; $(AB)_{l(k)}$ is the klth interaction effect between batch and aquarium in which the effect of the aquarium is nested within that of the batch; and ε_{ijklm} is the random error.

3. Results

The results indicated that the main muscular FAA concentrations were HIS ($P < 0.001$) in carp; NAH ($P < 0.001$) and taurine (TAU) (P=0.001) in Nile tilapia, but temperature had no effect ($P > 0.05$) (Table 2; Fig. 1). The sum of imidazole (HIS+NAH) and TAU in the muscle remained constant over species and temperature zones ($P > 0.05$), whereas the (NAH+HIS)/TAU ratio was affected by the temperature×species interaction effects ($P < 0.05$) (Fig. 1). The concentrations of muscle FAA (Table 2) and plasma acylcarnitines (Table 3) were different for species with a few temperature×species interaction effects ($P < 0.05$). The level of muscular TFAA was significantly reduced (P=0.002) at MEWT. The concentration of 3-hydroxyisovalerylcarnitine was significantly affected by temperature×species interaction effects (P=0.009) (Fig. 2). The sum of the concentrations of 3-hydroxy-longer-chain acylcarnitines was constant while that of longer-chain acylcarnitines was affected by both species (P=0.009) and temperature×species interaction effects (P=0.009) (Fig. 3). The plasma NEFA concentration was higher in carp than in Nile tilapia (P=0.009) (Fig. 4).

4. Discussion

In the present study, acclimation temperature does not refer to the optimum temperature, which is different for carp and Nile tilapia. The present results confirm earlier findings that mild elevation of water temperature affects nutrient metabolism in fish (Geda et al., 2012, 2015). The study reported how the different muscular concentrations of FAA and imidazole compounds determine the use of nutrients in metabolism of fish at MEWT, a situation where changes in AA catabolism were triggered.

In Atlantic salmon exposed to normal (13 °C) or elevated (19 °C) temperature, both muscle and plasma TFAA concentrations were lower in the elevated temperature groups, measured at 4–24 h postprandially (Vikeså et al., 2015). This agrees with the MEWT-induced reduction of muscular TFAA in our study. Yet, the concentrations of muscle FAA profile were quite different between both species, with some increased or decreased concentrations both in tilapia and carp, of course, with some temperature-species interactions.

The most abundant amino acid in both carp and tilapia muscle was taurine with significant difference between the species, the higher concentration being in the tilapia. Taurine was also reported as the most abundant AA among the FAA profiles in marine animals (Park et al., 2002); its abundance in fishmeal was also documented (El-Sayed, 2013). Taurine can be synthesized from methionine and cysteine in fish (Goto et al., 2003). Taurine plays important roles in numerous biological processes such as antioxidation (free radical scavenge to reduce intracellular oxidation), osmoregulation (reduction of membrane permeability) and lipid metabolism (reduction of lipid peroxidation), thereby protecting tissue from oxidative injury (Lin and Xiao, 2006; Kim et al., 2008; Cheng et al., 2011; Zhu et al., 2011).

This study demonstrated that temperature-induced mobilization of muscular HIS and NAH pools seems to be compensated by TAU, possibly to safeguard the osmolytic capacity of these three compounds.

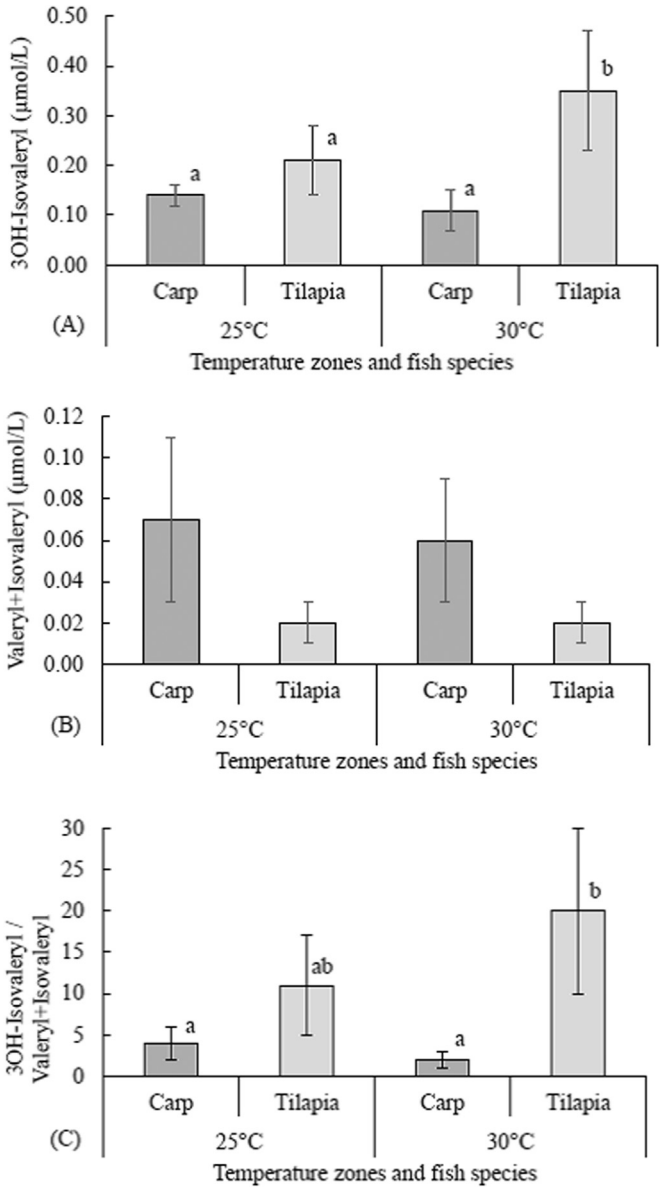

Fig. 2. Mean plasma 3-hydroxyisovalerylcarnitine, valerylcarnitine and their ratios in carp (n=13) and tilapia (n=17) at acclimation (25 °C) and MEWT (30 °C): (A) 3-hydroxyvalerylcarnitine: temperature effect, $P=0.070$; species effect, $P<0.001$; interaction effect, $P=0.009$; (B) valerylcarnitine: temperature effect, $P=0.717$; species effect, $P=0.007$; interaction effect, $P=0.724$; (C) ratio of 3-hydroxyvalerylcarnitine to valerylcarnitine: temperature effect, $P=0.209$; species effect, $P<0.001$; interaction effect, $P=0.090$. [a,b]Mean values with different letters were significantly different ($P<0.05$).

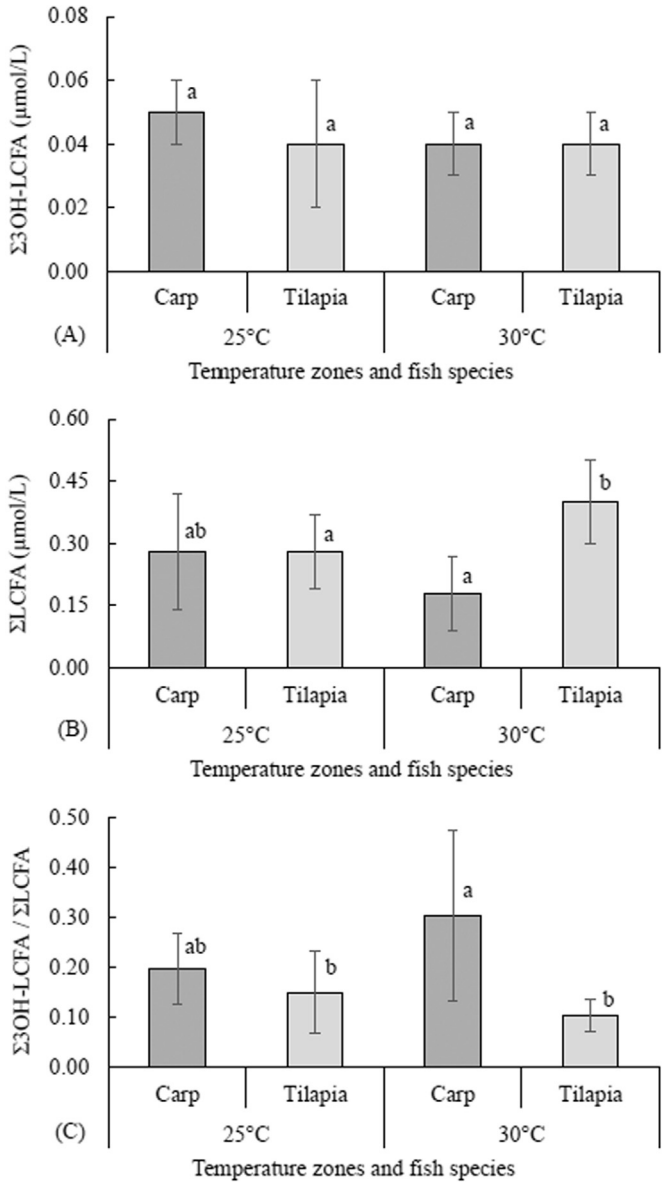

Fig. 3. Mean plasma 3-hydroxy-long-chain acylcarnitine and long-chain acylcarnitine and their ratios in carp (n=13) and tilapia (n=17) at acclimation (25 °C) and MEWT (30 °C): (A) sum of 3-hydroxy-long-chain acylcarnitines (3OH-tetradecanoyl, 3OH-tetradecenoyl, 3OH-hexadecanoyl, 3OH-hexadecenoyl, 3OH-octadecanoyl, 3OH-octadecenoyl): temperature, species, and interaction effects, $P>0.05$); (B) sum of long-chain acylcarnitines (tetradecanoyl, tetradecenoyl, hexadecanoyl, hexadecenoyl, octadecanoyl, octadecenoyl): temperature effect, $P=0.946$; species effect, $P=0.009$; interaction effect, $P=0.009$; (C) ratio of (A) to (B): temperature effect, $P=0.407$; species effect, $P=0.001$; interaction effect, $P=0.036$. [a,b]Mean values with different letters were significantly different ($P<0.05$).

The concentration of muscle free HIS was more than 8 fold higher in carp than tilapia, while muscle NAH concentration was higher in tilapia compared to carp. The NAH concentration in Nile tilapia muscle was lower than previously reported (Yamada et al., 1992, 1994, 2009), and only low concentrations were found in Cyprinidae (carps) as previously reported (Yamada et al., 2009). However, water temperature did not affect the concentration of NAH in the muscle, whereas the concentration of HIS tended to decrease in both species at the MEWT.

Histidine is an important buffer component in muscle (Abe and Okuma, 1991; Abe, 2000). It has been demonstrated that histidine and other imidazole compounds are released from muscle tissue during starvation (Shiau et al., 2001), yet the effect size is considerably different between the two species in the present study, with carp showing a greater extent of muscular free imidazole decrease than tilapia. The present data also suggest that any change in muscular concentration of imidazole compounds is compensated by taurine. Such compensations can be explained by the necessity of the fish to maintain osmolytic capacity in its muscles. Taurine is indeed known as an osmolyte (Zhang et al., 2006). Imidazole compounds have also been proposed to play a role in for instance osmotic balance, but also as antioxidant, as antiglycation agent or as precursor of urocanic acid as natural sunscreen for the skin (Ezzat et al., 2015).

The changes in (NAH+HIS)/TAU ratio are thus not necessarily related to nutrient use, nevertheless the fatty acid metabolism – as measured via the plasma acylcarnitine profile – showed specific species-temperature interactions. In particular, the longer-chain acylcarnitines, representing the use of fatty acids increased more in tilapia than in carp. This seems paradoxal at first instance with the lower fat stores in tilapia (about 3%) versus carp (about 6%) (Abdelghany and

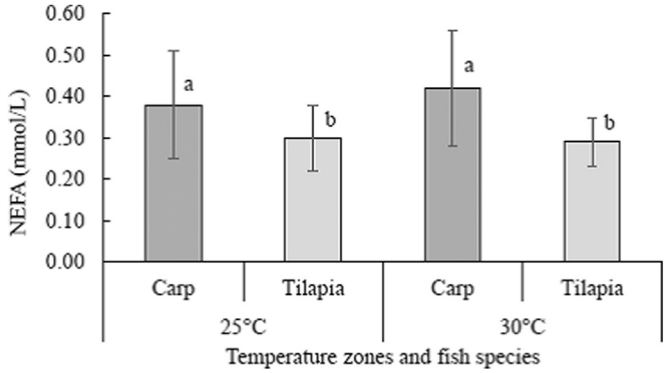

Fig. 4. Mean plasma non-esterified fatty acid (NEFA) in carp (n=13) and tilapia (n=17) at acclimation (25 °C) and MEWT (30 °C): temperature effect, P=0.765; species effect, P=0.009; interaction effect, P=0.536. [a,b]Mean values with different letters were significantly different ($P < 0.05$).

Ahmad, 2002), and the higher plasma NEFA concentration in carp compared to tilapia. However, the 3-hydroxy acylcarnitines of the corresponding fatty acids were unaltered, suggesting that fatty acids might accumulate as they are not pushed into fatty acid combustion to generate acetyl CoA. Therefore, this metabolic profile can be interpreted as a reduced use of fatty acids, especially given the distinctly higher concentrations of short-chain acylcarnitines in carp versus tilapia.

Tilapia seems to display a higher resistance to use AA in metabolism, with lower concentrations of the AA catabolites valeryl and isovaleryl carnitine in plasma, and a further increase of 3-hydroxyisovalerylcarnitine concentration in the plasma at MEWT. The corresponding 3-hydroxyisovaleryl CoA is a dead-end sink in the leucine catabolism, hence avoiding the use of leucine as substrate for acetyl CoA in the citric acid cycle. Although carp showed the highest levels of muscular free leucine, the MEWT tended to trigger relatively more leucine release in tilapia, yet without further promoting its use as an energy substrate.

The impaired metabolisation of both the longer-chain fatty acids and leucine would suggest that tilapia derives its acetyl CoA from another source. This study hypothesized that tilapia stores NAH because the hydrolyzed product would not only provide HIS, but also an acetyl moiety that could serve as source for acetyl CoA, but the lower concentrations of plasma acetylcarnitine do not support this.

In general, carp seems to have a more active nutrient metabolism than tilapia, seen in the higher concentrations of many acylcarnitines in the plasma. This can be explained by the higher thyroid metabolism in carp compared with tilapia (Geven et al., 2007). This might again relate to the higher HIS turnover in carp: HIS and in particular its metabolite histamine are known as stimulatory compounds (Abe, 1995; Li et al., 2009; Wu, 2009), hence might serve to increase metabolic rate.

The evolutionary reason for the observed differences between carp and tilapia was not covered in the present study, but the differences do not seem to be associated with for instance muscle fibre type. According to Turinsky and Long (1990) the typical difference found in FAA between fibre types in rats do not correspond with the difference found between the fish species in this study. This might be an interesting topic for further study. Muscle HIS concentration increases whereas muscle taurine decreases with growth in milkfish (Shiau et al., 1997) but the inverse occurs during starvation (Shiau et al., 2001), which supports the hypothesis that the higher imidazole to taurine ratio (Fig. 1) observed in carp compared with tilapia in this study might be explained by metabolic rate.

5. Conclusions

This first comparative study demonstrated the species-dependent metabolic response to a mild elevation of water temperature of the muscular concentrations of imidazoles and FAA in aquatic organisms. The study compared two fish species (carp versus tilapia) that clearly differed in muscular concentration profile and demonstrated that muscular concentrations of imidazoles and TAU in fish respond to water temperature in a species dependent manner. The present results point to a species-dependent relationship between nutrient use and muscular concentrations of imidazoles and FAA as a response to water temperature. The results are also expected to give rise to a range of follow-up studies by research groups active in the domains of imidazole-related compounds and global warming.

Conflict of interest

None of the authors has any conflicts of interest to declare.

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