JOURNAL OF EXPERIMENTAL ZOOLOGY 307A:7–17 (2007)

The Metabolic Organization of a Primitive Air-breathing Fish, the Florida Gar (*Lepisosteus platyrhincus*)

NATASHA THERESE FRICK, JASON SCOTT BYSTRIANSKY, AND JAMES STUART BALLANTYNE*
Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

ABSTRACT The metabolic organization of the air-breathing Florida gar, *Lepisosteus platyrhincus*, was assessed by measuring the maximal activities of key enzymes in several metabolic pathways in selected tissues, concentrations of plasma metabolites including nonesterified fatty acids (NEFA), free amino acids (FAA) and glucose as well as tissue FAA levels. In general, *L. platyrhincus* has an enhanced capacity for carbohydrate metabolism as indicated by elevated plasma glucose levels and high activities of gluconeogenic and glycolytic enzymes. Based upon these properties, glucose appears to function as the major fuel source in the Florida gar. The capacity for lipid metabolism in *L. platyrhincus* appears limited as plasma NEFA levels and the activities of enzymes involved in lipid oxidation are low relative to many other fish species. *L. platyrhincus* is capable of oxidizing both D- and L-β-hydroxybutyrate, with tissue-specific preferences for each stereoisomer, yet the capacity for ketone body metabolism is low compared with other primitive fishes. Based on enzyme activities, the metabolism of the air-breathing organ more closely resembles that of the mammalian lung than a fish swim bladder. The Florida gar sits phylogenetically and metabolically in an intermediate position between the ''primitive'' elasmobranchs and the ''advanced'' teleosts. The apparently unique metabolic organization of the gar may have evolved in the context of a bimodal air-breathing environmental adaptation. *J. Exp. Zool. 307A:7–17, 2007.* © 2006 Wiley-Liss, Inc.

How to cite this article: Frick NT, Bystriansky JS, Ballantyne JS. 2007. The metabolic organization of a primitive air-breathing fish, the florida gar (*lepisosteus platyrhincus*). J. Exp. Zool. 307A:7–17.

The metabolic organization of the phylogenetically ''ancient'' elasmobranchs has been shown to be quite different compared with the more ''advanced'' teleost fishes. Elasmobranchs have a low capacity for extrahepatic lipid oxidation and appear to rely more heavily on ketone body metabolism than do teleost fishes (Zammit and Newsholme, '79; Singer and Ballantyne, '89; Ballantyne, '97). Studies on the metabolic organization of fish species that sit in an intermediate evolutionary position between the elasmobranchs and teleosts have revealed some interesting trends in the evolution of fish metabolism. Lake sturgeon (*Acipenser fulvescens*) are primitive chondrosteans and have a metabolic organization resembling both elasmobranchs and teleosts (Singer et al., '90). Sturgeon are comparable to teleosts with respect to fatty acid oxidation but their ketone body metabolism more closely resembles that seen in elasmobranchs (Singer et al., '90). The metabolic organization of another primitive fish, the bowfin (*Amia calva*), is similar to that of sturgeons with respect to the utilization of ketone bodies, suggesting this is a primitive metabolic feature of vertebrates (Singer and Ballantyne, '91). Teleosts have low levels of plasma ketone bodies in comparison to more primitive fish (Moon and Mommsen, '87; Singer et al., '90; Singer and Ballantyne, '91; LeBlanc et al., '95; Ballantyne, '97) and correspondingly, levels of β-hydroxybutyrate dehydrogenase (β-HBDH) in teleost tissues

Grant sponsor: N.S.E.R.C. Discovery grant.
*Correspondence to: Dr. J.S. Ballantyne, Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. E-mail: jballant@uoguelph.ca
Received 21 April 2005; Accepted 10 July 2006
Published online 8 November 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.a.338.

© 2006 WILEY-LISS, INC.
WILEY InterScience® DISCOVER SOMETHING GREAT

are low or nondetectable (LeBlanc and Ballantyne, '93; LeBlanc, '96), supporting the suggestion that ketone body metabolism is of greater importance in primitive fishes.

The reduced extrahepatic lipid oxidation found in elasmobranchs (Moon and Mommsen, '87; Singer and Ballantyne, '89; Ballantyne, '97), the intermediate capacity seen in the sturgeon (Singer et al., '90) and the bowfin (Singer and Ballantyne, '91) and the high levels seen in most teleosts (Newsholme and Crabtree, '86), suggests that more advanced fishes have an increased ability to utilize fatty acids as an energy source. However, Power et al. ('93) and LeBlanc et al. ('95) found that the primitive agnathans, *Geotria australis* and *Petromyzon marinus*, respectively, rely heavily on lipids as substrates for oxidation. This finding does not concur with previous suggestions that the ability to utilize fatty acids to a substantial degree is a characteristic of more advanced fishes. Power et al. ('93) concluded that the different degrees of lipid utilization seen in fish did not evolve in a particular phylogenetic sequence.

The gars (family Lepisosteidae) are a group of primitive air-breathing fish that share a common ancestry with *Amia*, as both belong to the infraclass Neopterygii (Graham, '97). The metabolic organization of species within the family Lepisosteidae remains virtually unknown, aside from a few endocrinology studies on the alligator gar (*Lepisosteus spatula*) (Pollock et al., '87, '88; Sherwood et al., '91). As facultative air breathers, gar depend mostly on their gills for oxygen uptake at low water temperatures; however, when water temperatures rise to 20–25°C, the increased demand for oxygen causes long-nose gar (*Lepisosteus osseus*) to utilize their modified swim bladder as an air-breathing organ (ABO) for 70–80% of the total oxygen requirement (Rahn et al., '71). Understanding the metabolic organization of the Florida gar (*Lepisosteus platyrhincus*) will thus contribute to our knowledge of the evolution of fish metabolism and the metabolic role of the lung in the transition to the terrestrial environment. To determine the relative importance of various metabolic substrates as energy sources in *L. platyrhincus*, we examined (1) the maximal activities of key enzymes from the major metabolic pathways in liver, red and white muscle, gill, heart and ABO, (2) the levels of circulating plasma metabolites including nonesterified fatty acids (NEFAs), free amino acids (FAAs) and glucose and (3) the levels of tissue (red and white muscle, heart and ABO) FAAs. We hypothesized *L. platyrhincus* would have a heightened capacity for ketone body metabolism and a reduced ability to metabolize lipids, compared to the more advanced teleost fishes.

MATERIALS AND METHODS

Animals

Gar (*L. platyrhincus*) (33.5–37.5 cm) were purchased from a local fish supplier and kept in freshwater at 25°C under a 12:12 L:D artificial photoperiod for ~2 weeks before being sacrificed. During this period, gar were fed goldfish ad libitum and all gar were observed to feed. Under these conditions, gar 'gulped' air for aerial respiration approximately once every 5 min. Food was withheld 2 days prior to sampling.

Tissue and plasma collection

Blood samples were taken from nonanesthetized animals by caudal puncture using heparinized syringes (500 U sodium heparin ml^{-1}) with an 18-gauge needle. Blood samples were centrifuged at 5,000*g*, at 4 °C for 5 min, and the plasma was quickly frozen and stored at −80°C. Fish were killed by a blow to the head and tissues (liver, red and white muscle, gill, heart and ABO) were rapidly excised (<3 min) and immediately frozen in liquid nitrogen and stored at −80°C for future analysis. This research was approved by the animal care committee at the University of Guelph and followed the Canadian council on animal care guidelines.

Enzyme analysis

Tissue preparation

Tissues (liver, red and white muscle, gill, heart and ABO) were thawed and homogenized in 50 $mmol\,l^{-1}$ imidazole buffer (pH 7.4 at 20°C), using a PT10 unit Polytron homogenizer (Kinematica Gmbh., Luzurn, Switzerland) with three 10 sec bursts separated by 30 sec on ice. All preparative procedures were carried out at 4°C. Homogenates were then centrifuged (16,200*g*, 4°C) for 10 min and the resulting supernatant was used directly in the enzyme assays. For the enzyme assays of citrate synthase (CS), glutamate dehydrogenase (GDH) and β-HBDH, 0.2% Triton X-100 (v:v) was added to the homogenate prior to centrifugation.

Enzyme assays

Maximal enzyme activities were determined using a Hewlett-Packard 8452A diode array spectrophotometer (Hewlett-Packard, Mississauga, Ontario) equipped with a temperature-controlled cell changer maintained at $25 \pm 0.1°C$ with a Haake G circulating water bath (Haake Buchler Instruments Inc., Saddlebrook, NJ). Reaction rates of most enzymes were determined by the change in absorbance of NADH or NADPH at 340 nm (millimolar extinction coefficient $\varepsilon_{340} = 6.22$). CS and carnitine palmitoyl transferase (CPT) were monitored at 412 nm using 5,5′ dithio-bis (2-nitrobenzoic acid) (millimolar extinction coefficient $\varepsilon_{412} = 13.6$). Cytochrome *c* oxidase (CCO) was monitored at 550 nm (millimolar extinction coefficient $\varepsilon_{550} = 28$) while succinyl coenzyme-A ketotransferase (SKT) was monitored at 310 nm (millimolar extinction coefficient $\varepsilon_{310} = 11.9$).

Conditions of the enzyme assays for 3-hydroxyacyl CoA dehydrogenase (HOAD) (E.C. 1.1.1.35), total CPT (I and II) (E.C. 2.3.1.21), malic enzyme (ME) (E.C. 1.1.1.40), CS (E.C. 4.1.3.7), hexokinase (HK) (E.C. 2.7.1.1), lactate dehydrogenase (LDH) (E.C. 1.1.1.27), fructose 1,6-bisphosphatase (FBPase) (E.C. 3.1.3.11), aspartate aminotransferase (Asp-AT) (E.C. 2.6.1.1), alanine aminotransferase (Ala-AT) (E.C. 2.6.1.2) and GDH (E.C. 1.4.1.3) are described in Singer et al. ('90). SKT (E.C. 2.8.3.2) followed the method described in Singer et al. ('90) except that the concentration of succinate was increased to $2\,mmol\,l^{-1}$. CCO (E.C.1.9.3.1) and D- and L-β-HBDH (E.C. 1.1.1.30) were measured using the methods of Blier and Guderley ('88) and LeBlanc and Ballantyne (2000), respectively. Pyruvate kinase (PK) (E.C. 2.7.1.40) was determined as described in Levesque et al. (2005), except that the concentration of KCl was increased to $50\,mmol\,l^{-1}$. Gutamine synthetase (GS) (E.C. 6.3.1.2) and phosphate-dependent glutaminase (PDG) (E.C. 3.5.1.2) were assayed according to the method of Chamberlin et al. ('91), except that the acidified solution in the PDG assay was centrifuged for 15 min at 16,200*g* at 4°C. All enzyme assays were optimized regarding substrate concentration.

The freeze-thaw stabilities of HOAD, CPT, CS, LDH and PK were determined by Sidell et al. ('87), while β-HBDH, ME, SKT, CCO, Ala-AT, Asp-AT, GS, PDG, GDH and FBP were confirmed for gar tissue in the current study. These preliminary results indicated HK was not freeze-thaw stable; therefore, the results presented here may be an underestimate.

Determination of plasma NEFAs

Methylation and determination of plasma NEFAs were performed as previously described in Singer et al. ('90). A gas chromatograph (Hewlett-Packard, HP5890A) fitted with a flame ionization detector and a DB-225 megabore fused silica column (Chromatographic specialties Inc., Brockville, Ont.) under isothermal conditions (200°C) using helium as a carrier gas was used to analyze NEFA. Fatty acid methyl esters were identified by comparing their retention times with lipid standards (Nu-Check Prep, Elysian, MN) and absolute amounts of these fatty acids were determined using an internal standard, heptadecanoic acid (17:0), which was added to the plasma sample prior to methylation.

Determination of FAAs

Plasma sample preparation and determination of plasma FAAs were as outlined in Barton et al. ('95). A high-performance liquid chromatograph (HPLC) (Hewlett-Packard, HP 1090 series II/L liquid chromatograph) equipped with a UV–visible series II diode array detector, an automatic injector and a narrow-bore ($20\,cm \times 2.1\,mm$) reversed-phase column (AminoQuant 79916AA-572, Hewlett-Packard) was used in both plasma and tissue FAA determination. Tissue FAA levels were determined using the method described in Frick and Wright (2002). Concentrations of amino acids are expressed in $nmol\,g\,wet\,weight^{-1}$ for tissues and in $nmol\,ml^{-1}$ for plasma.

Determination of plasma glucose concentrations

Plasma glucose concentrations were determined using a Sigma diagnostics kit (510-A) (Sigma Diagnostics, St. Louis, MO), which used glucose oxidase for an enzymatic determination of glucose levels. Values are expressed as the mean values of duplicate measurements of each plasma sample ($mmol\,l^{-1}$).

Chemicals

All chemicals used were purchased from Sigma Chemical Co. (Sigma-Aldrich Canada Ltd., Oakville, Ont., Canada) with the exception of the HPLC-grade methanol and acetonitrile (purchased from Fisher Scientific Ltd., Whitby, Ont., Canada), and

fatty acid standards (purchased from Nu-Check Prep Inc.).

Statistical analysis

Single-factor analyses of variance with Tukey's test were used to determine differences in individual enzyme activities and amino acid levels between tissues. Assumptions for normality were verified by generating appropriate residual plots. Data transformations (logarithmic and square root) were used when appropriate to meet the above assumptions. D- and L-β-HBDH enzyme activities were compared using a two-tailed Student's *t*-test. *P*-values <0.05 were considered statistically significant.

RESULTS

Maximal activities of several key enzymes in liver, red muscle, white muscle, gill, heart and ABO provide a quantitative and qualitative view of the metabolic organization of *L. platyrhincus* and are presented in Table 1. CS activity was significantly higher in the heart of gar compared to the liver, red and white muscles, gill and ABO. CCO activity was similar between heart, liver and red muscle tissues and significantly lower in white muscle. In liver tissue, the activity of L-β-HBDH was significantly greater than D-β-HBDH, while in the gill, the opposite was found. Neither L nor D-β-HBDH activities were detected in white muscle or heart tissues (Table 1). FBPase activity was significantly higher in the liver, compared to all other tissues and was not detectable in white muscle (Table 1). CPT activity was only detected in the heart and liver tissues. PDG and GDH activities were significantly higher in the heart and liver, respectively, compared to all other tissues (Table 1).

The absolute concentrations (nmol ml^{-1}) and percentages (by mole) of individual NEFAs in the plasma of *L. platyrhincus* are presented in Table 2. The total plasma NEFA concentration (C:14 and longer) was 606.6 nmol ml^{-1}. The dominant fatty

TABLE 1. Maximal enzyme activities in selected tissues of the Florida gar, L. platyrhincus

Enzyme	Activity (μmol g^{-1} min^{-1})					
	Liver	Red muscle	White muscle	Heart	Gill	ABO
Lipid catabolism						
HOAD	0.44±0.15	0.95±0.27^{a}	0.25±0.05	0.38±0.03	0.20±0.06^{b}	0.30±0.13
CPT	0.13±0.02^{a}	BLD	BLD	0.57±0.02^{b}	BLD	BLD
Lipid synthesis						
ME	0.06±0.02^{a}	0.24±0.04^{b}	0.08±0.01^{a}	1.15±0.06^{c}	0.06±0.01^{a}	0.19±0.02^{abd}
Ketone body metabolism						
β−HBDH (D)	0.06±0.02^{b*}	0.02±0.01^{a}	BLD	BLD	0.013±0.002^{a*}	BLD
β−HBDH (L)	0.12±0.01^{b}	0.03±0.004^{a}	BLD	BLD	0.003±0.001^{a}	0.05±0.002^{c}
SKT	3.92±0.52^{a}	0.38±0.08^{bc}	0.02±0.005^{b}	2.71±0.57^{ad}	0.57±0.08^{be}	1.71±0.28^{cde}
Oxidative metabolism						
CS	0.90±0.02^{c}	10.99±2.10^{a}	1.05±0.11^{c}	24.11±2.74^{b}	3.84±0.17^{c}	2.97±0.23^{c}
CCO	0.96±0.19^{a}	0.75±0.13^{abc}	0.12±0.01^{d}	0.96±0.09^{a}	0.27±0.02^{bd}	0.23±0.03^{bd}
Glycolysis						
HK	0.43±0.08^{b}	0.22±0.03^{b}	0.07±0.02^{b}	3.56±0.37^{a}	0.49±0.05^{b}	0.61±0.08^{b}
LDH	33.71±3.05^{a}	308.84±46.73^{b}	227.30±4.31^{c}	254.49±20.30^{b}	34.27±4.60^{a}	70.40±5.95^{a}
PK	10.06±2.20^{bc}	128.36±25.82^{ad}	182.96±34.10^{a}	144.25±9.04^{a}	35.10±3.06^{b}	64.60±5.11^{cbd}
Gluconeogenesis						
FBP	4.40±0.55^{a}	0.53±0.05^{b}	BLD	0.06±0.01^{b}	0.05±0.01^{b}	0.12±0.02^{b}
Amino acid metabolism						
GDH	27.19±3.26^{a}	2.61±0.46^{bc}	0.27±0.04^{b}	6.77±0.33^{c}	5.72±0.21^{bc}	1.73±0.24^{bc}
Asp-AT	23.44±2.45^{b}	45.03±7.18^{a}	6.26±1.16^{b}	88.40±7.36^{c}	10.59±0.79^{b}	23.65±2.44^{b}
Ala-AT	14.17±0.84^{a}	2.26±0.24^{b}	1.93±0.43^{b}	2.31±0.62^{b}	2.15±0.18^{b}	0.38±0.04^{b}
GS	0.15±0.02^{b}	0.14±0.01^{b}	0.07±0.01^{a}	0.16±0.02^{bd}	0.22±0.02^{cd}	0.12±0.02^{ab}
PDG	0.18±0.01^{bd}	0.21±0.04^{d}	0.02±0.002^{c}	0.46±0.01^{a}	0.11±0.01^{bcd}	0.08±0.005^{bc}

Note: Activities are expressed as the mean ± SEM (in μmol g tissue^{-1} min^{-1}). For a given enzyme, tissues labeled with no letters in common are significantly different ($P<0.05$); a common letter or the absence of a letter indicates no significant difference.
*indicates a significant difference between L- and D-β-HBDH within a given tissue. BLD = below the level of detection. For all other abbreviations see text.

TABLE 2. *Absolute amounts and proportions of individual nonesterified fatty acids (NEFAs) in the plasma of Florida gar, L. platyrhincus*

Fatty acid	Concentration (nmol ml^{-1})	Percentage (% by mole)
14:0	28.0 ± 1.0	4.6 ± 0.1
14:1	4.2 ± 0.8	0.7 ± 0.1
16:0	197.0 ± 9.3	32.5 ± 0.5
16:1	69.3 ± 14.0	11.2 ± 1.8
18:0	49.2 ± 3.1	8.2 ± 0.7
18:1	81.0 ± 2.3	13.4 ± 0.5
18:2(n6)	32.7 ± 1.3	5.4 ± 0.1
18:3(n3)	3.9 ± 0.8	0.6 ± 0.1
18:4(n3)	BLD	BLD
20:0	2.2 ± 0.2	0.4 ± 0.1
20:1	2.30 ± 0.4	0.4 ± 0.1
20:2(n6)	2.6 ± 0.4	0.4 ± 0.1
20:3(n6)	2.2 ± 0.2	0.4 ± 0.0
20:4(n6)	35.6 ± 5.2	5.9 ± 1.0
20:3(n3)	4.0 ± 2.0	0.7 ± 0.3
20:4(n3)	BLD	BLD
20:5(n3)	7.6 ± 0.8	1.2 ± 0.1
22:0	2.5 ± 2.5	0.4 ± 0.4
22:1	BLD	BLD
23:0	BLD	BLD
22:2(n6)	BLD	BLD
22:4(n6)	2.6 ± 2.6	0.5 ± 0.5
22:5(n6)	5.5 ± 2.8	0.9 ± 0.5
22:5(n3)	4.7 ± 0.8	0.8 ± 0.1
22:6(n3)	55.5 ± 2.4	9.1 ± 0.0
24:0	13.9 ± 13.9	2.3 ± 2.3
24:1	BLD	BLD
Total	606.6 ± 27.7	100.0
Total saturates	292.9 ± 15.8	48.3 ± 1.5
Total monoenes	156.7 ± 15.0	25.7 ± 1.4
Total polyenoic	157.0 ± 11.7	26.0 ± 2.3
(n3) Polyenoic	75.7 ± 4.9	12.5 ± 0.4
(n6) Polyenoic	81.3 ± 10.4	13.5 ± 2.1
(n3)/(n6)	0.9 ± 0.1	
(Monoenes/polyenoic)	1.0 ± 0.1	
Unsaturation index[1]	137.6 ± 7.8	
Mean chain length	17.7 ± 0.1	

Note: Values are presented as the mean ± SEM ($n = 5$). BLD = below level of detection.
[1] $\Sigma m_i n_i$; where m_i is the mole percentage and n_i is the number of carbon–carbon double bonds in the fatty acid.

acid was palmitic acid (16:0), accounting for 32% of total plasma NEFAs and 67% of the total saturates. Saturates were the major fatty acid class detected, accounting for 48% of the total plasma NEFAs. Monounsaturates accounted for 25% of the total plasma NEFAs, with oleic acid (18:1) contributing ~52% of the total monounsaturates and 13% of the total NEFAs. Polyunsaturates accounted for 25% of the total NEFAs, with 22:6(n3) contributing ~35% to this NEFA group. The n3/n6 and monoene/polyene ratios were 0.9 and 1.0, respectively. The unsaturation index was 137.6 and the mean carbon chain length was 17.7.

The absolute amounts of FAAs in the plasma (nmol ml^{-1}) and tissues (red and white muscles, heart and ABO) (nmol g^{-1}) of *L. platyrhincus* are presented in Table 3. All 21 FAAs measured were detected in both the plasma and tissues. The mean total plasma FAA concentration was 4,111 nmol ml^{-1}, with essential FAA contributing ~37% of the total FAA. Hydroxyproline was the dominant FAA in the plasma (~12%), followed by glutamine (~9%). Total FAA levels were highest in the heart tissue (30,712 nmol g^{-1}) and very similar between the other three tissues (red and white muscles and ABO). In all four tissues nonessential FAAs accounted for over 80% of the total with taurine being the most abundant. The mean plasma glucose level was 8.95 ± 0.91 mmol l^{-1}.

DISCUSSION

In general, *L. platyrhincus* has an enhanced capacity for carbohydrate metabolism as indicated by elevated plasma glucose levels and high activities of gluconeogenic and glycolytic enzymes relative to other fish species. Based on maximal enzyme activities, their capacity for lipid and oxidative metabolism appears limited while amino acid metabolism is similar to that of other primitive fishes. Contrary to our hypothesis gar do not appear to have an enhanced capacity for ketone body metabolism, as has been found in other primitive fish (Moon and Mommsen, '87; Singer et al., '90; Singer and Ballantyne, '91; LeBlanc et al., '95; Ballantyne, '97). The results of this study are compared to similar investigations on species representing other fish groups. For direct comparisons with species examined at different temperatures we assumed a Q_{10} of two. Although Q_{10} values may vary from two, this provides a way of comparing maximal enzyme reaction rates between studies conducted at different temperatures.

Oxidative metabolism

CS and CCO are good indicators of the oxidative capacity of a tissue. CS activity in *L. platyrhincus* was in the range reported for other primitive fish species (Singer et al., '90; Singer and Ballantyne, '91; LeBlanc et al., '95). Similar data for CCO activity in other primitive fish species are not available. CCO and CS activities in all tissues

TABLE 3. Concentrations of free amino acids (FAAs) in the plasma and selected tissues of the Florida gar, L. platyrhincus

	Plasma ($n=6$)	Red muscle ($n=7$)	White muscle ($n=7$)	Heart ($n=3$)	ABO ($n=6$)
Essentials					
HIS	65 ± 13^{b}	194 ± 25^{c}	80 ± 10^{bc}	$1{,}270 \pm 259^{a}$	146 ± 33^{c}
THR	349 ± 28^{a}	318 ± 67^{a}	376 ± 44^{a}	994 ± 186^{b}	523 ± 82^{a}
VAL	213 ± 30^{bc}	120 ± 17^{b}	134 ± 6^{b}	985 ± 189^{a}	317 ± 47^{c}
MET	68 ± 7^{a}	318 ± 18^{b}	306 ± 37^{b}	613 ± 128^{c}	246 ± 35^{b}
TRP	28 ± 3^{a}	222 ± 20^{b}	244 ± 15^{b}	657 ± 92^{b}	202 ± 28^{b}
PHE	47 ± 7^{b}	30 ± 6^{b}	54 ± 5^{bc}	252 ± 32^{a}	81 ± 12^{c}
ILE	153 ± 21^{c}	63 ± 7^{b}	77 ± 7^{b}	369 ± 55^{a}	151 ± 20^{c}
LEU	234 ± 33^{c}	111 ± 12^{b}	118 ± 9^{b}	467 ± 96^{a}	199 ± 23^{bc}
LYS	368 ± 73	600 ± 145^{a}	701 ± 381^{ad}	248 ± 53^{cd}	192 ± 24^{bc}
ARG	192 ± 48^{a}	254 ± 75	295 ± 180	613 ± 187^{b}	146 ± 14^{a}
Total essentials	$1{,}717 \pm 186^{a}$	$2{,}230 \pm 245^{a}$	$2{,}385 \pm 378^{a}$	$6{,}468 \pm 955^{b}$	$2{,}203 \pm 209^{a}$
Non essentials					
ASP	166 ± 25^{b}	1577 ± 182^{d}	597 ± 85^{a}	171 ± 19^{b}	65 ± 7^{c}
GLU	54 ± 8^{b}	271 ± 59^{a}	126 ± 24^{a}	$2{,}704 \pm 457^{c}$	753 ± 41^{d}
ASN	34 ± 8^{a}	31 ± 4^{a}	25 ± 2^{a}	$1{,}113 \pm 545^{b}$	114 ± 37^{c}
SER	165 ± 33^{ac}	157 ± 25^{ac}	124 ± 19^{a}	856 ± 382^{b}	215 ± 27^{c}
GLN	385 ± 39^{a}	461 ± 68^{a}	211 ± 19^{a}	$2{,}116 \pm 390^{b}$	579 ± 61^{a}
GLY	19 ± 7^{a}	729 ± 125^{b}	$1{,}226 \pm 318^{c}$	212 ± 36^{b}	70 ± 11^{d}
ALA	355 ± 66^{a}	639 ± 50^{b}	417 ± 52^{a}	772 ± 182^{b}	393 ± 20^{a}
TAU	284 ± 34^{b}	9848 ± 601^{a}	$8{,}761 \pm 917^{a}$	$13{,}252 \pm 2{,}365^{c}$	$8{,}670 \pm 1{,}298^{a}$
TYR	101 ± 9^{a}	127 ± 37^{ab}	101 ± 19^{a}	$1{,}037 \pm 253^{c}$	292 ± 63^{b}
HPR	726 ± 141^{a}	123 ± 59^{b}	412 ± 104^{ad}	684 ± 42^{a}	348 ± 51^{cd}
PRO	106 ± 43^{a}	15 ± 3^{b}	142 ± 48^{a}	$1{,}326 \pm 289^{c}$	623 ± 145^{d}
Total non-essentials	$2{,}395 \pm 205^{a}$	$13{,}976 \pm 386^{b}$	$12{,}141 \pm 739^{c}$	$24{,}243 \pm 4{,}149^{d}$	$12{,}121 \pm 1{,}271^{bce}$
TOTAL	$4{,}111 \pm 358^{b}$	$16{,}207 \pm 412^{a}$	$14{,}526 \pm 1{,}058^{a}$	$30{,}712 \pm 5{,}062^{c}$	$14{,}324 \pm 1{,}281^{a}$

Note: Levels are presented as the mean ± SEM, in $nmol\,ml^{-1}$ (plasma) or $nmol\,g^{-1}$ (tissue). Individual amino acid levels between tissues and plasma labeled with no letters in common are significantly different ($P < 0.05$); a common letter or the absence of a letter indicates no significant difference.

examined are quite low compared to the levels found in teleost fishes (Pelletier et al., '94; Battersby and Moyes, '98; West et al., '99). In general, primitive fishes appear to have a lower aerobic capacity than the more advanced teleosts (Suarez et al., '86; Ewart and Driedzic, '87; Sidell et al., '87; Moyes et al., '89).

In addition to functioning as a hydrostatic organ, the swim bladder of gar serves as an air-breathing tissue. In teleosts, where the role of the swim bladder is mainly to control buoyancy, the aerobic capacity (based on CCO and CS activities) is very low (Gesser and Fange, '71; Bostrom et al., '72; Ewart and Driedzic, '90). Gesser and Fange ('71) showed CCO activity in the swim bladder of teleosts was significantly lower than in white muscle, a tissue with an already low aerobic capacity. In the Florida gar both CS and CCO activities are higher in the ABO compared to white muscle, and are similar to levels found in gill tissue suggesting the ABO of gar is an aerobic tissue with a high metabolic capacity. Surprisingly, CS activity in the mammalian lung is similar to levels found in the gar ABO; however, CCO levels are higher in the mammalian lung (Murphy et al., '80; Rao, '83).

Carbohydrate metabolism

In *L. platyrhincus*, plasma glucose levels ($8.95\,mmol\,l^{-1}$) are generally higher compared to values reported in other teleost fish species (see Chavin and Young ('70) for a summary), and primitive fishes (i.e. *Acipenser fulvescens* ($3.5\,mmol\,l^{-1}$) (Gillis and Ballantyne, '96); *A. brevirostrum* (4.8–$6.3\,mmol\,l^{-1}$) (Jarvis and Ballantyne, 2003); *Scyliorhinus canicula* ($2.0\,mmol\,l^{-1}$) (Conlon et al., '94)) suggesting glucose may be an important fuel source in gar. FBPase is a nonequilibrium enzyme in the gluconeogenesis pathway and may be rate-limiting (Suarez and Mommsen, '87). High FBPase activity in the liver of gar is consistent with previous studies where the liver was found to be the major gluconeogenic tissue in marine (Moon and Mommsen, '87) and freshwater elasmobranchs (Singer and Ballantyne, '89), sturgeon (Singer et al.,

'90), bowfin (Singer and Ballantyne, '91) and many teleosts (Suarez and Mommsen, '87). The level of FBPase in the liver of the gar was high compared to levels reported in the bowfin (Singer and Ballantyne, '91), sturgeon (Singer et al., '90) and several teleosts (Cowey et al., '77; Jorgensen and Mustafa, '80; Knox et al., '80; Mommsen et al., '85; Suarez and Mommsen, '87). In addition, FBPase activity was detectable in the heart, gill and ABO. Assuming FBPase is rate-limiting in gar, it appears gar may have an enhanced capacity for glucose synthesis compared to most other fish species.

This high gluconeogenic capacity is complimented by high rates of glycolytic enzymes in most tissues. HK and PK are both considered limiting enzymes in the glycolytic pathway, and LDH is an endpoint reaction for anaerobic metabolism. HK activities in the various tissues of gar were similar to those found in the bowfin (Singer and Ballantyne, '91) except in heart where levels were ~3.4-fold higher in the gar. Compared to sturgeon (Singer et al., '90), sea lamprey (LeBlanc et al., '95) and several teleosts (Knox et al., '80), HK levels are relatively high in gar tissues, particularly in the heart where levels were up to 9-fold greater. Despite the possible loss in HK activity due to its sensitivity to freezing, the levels reported here are comparably high. PK activities in gar were higher than levels found in lamprey (LeBlanc et al., '95) and several teleosts (Knox et al., '80; Pelletier et al., '94; West et al., '99). The activities of LDH in red muscle, white muscle and heart of gar were much higher than those found in other primitive fish (Moon and Mommsen, '87; Singer et al., '90; Singer and Ballantyne, '91; LeBlanc et al., '95) and some teleosts (Knox et al., '80; Gerrits, '94; LeBlanc, '96), but low compared to several Amazonian teleost species (Almeida-Val and Hochachka, '95). Gas gland cells of fish swim bladder produce lactic acid to reduce pH, which acts to release oxygen (Pelster, '95). Correspondingly, the LDH to CS ratio of fish swim bladder is very high (530–1,300) (Ewart and Driedzic, '90; Walsh and Milligan, '93). In the gar ABO where this function appears to be reduced, the LDH:CS is considerably lower (~24), more comparable to the mammalian lung (9–16) (Murphy et al., '80; Mirejovska et al., '81).

These results indicate gar have a high capacity to synthesize and oxidize glucose compared to many fish species. This characteristic may be a result of living in an environment with limited oxygen as high LDH activities are also seen in hypoxia-tolerant Amazonian teleosts (Almeida-Val and Hochachka, '95). Although gar are able to breathe air, remaining submerged for long periods may be advantageous during hunting or to avoid predation. Under hypoxic conditions, a greater anaerobic capacity for energy production would be selected, resulting in a greater utilization of glucose as a major fuel source. This is in contrast to many fish species, which rely more heavily on amino acids and lipids as metabolic fuels.

Lipid metabolism

In general, the capacity for lipid catabolism in gar is low. HOAD is the limiting enzyme in the beta-oxidation of fatty acids and in the gar tissues examined, activities were similar to those reported in bowfin (Singer and Ballantyne, '91) and sturgeon (Singer et al., '90), but lower than levels found in lamprey (LeBlanc et al., '95) and several teleosts (Knox et al., '80; Ewart and Driedzic, '87; Pelletier et al., '94). CPT I catalyzes the rate-limiting step in carnitine-dependent oxidation of long-chain fatty acids (McGarry and Foster, '80). The assay used in this study does not distinguish between CPT I and II and indicates total CPT activity. Total CPT activity in the heart of gar was high compared to sturgeon (Singer et al., '90) and bowfin (Singer and Ballantyne, '91), but low when compared to several teleost species (Ewart and Driedzic, '87; Sidell et al., '87). Total CPT activity in the liver of gar was similar to bowfin (Singer and Ballantyne, '91), but again, was much lower than what is reported for several teleosts (West et al., '99). In general, the low levels of lipid catabolizing enzymes suggest the overall capacity for fatty acid oxidation is low and extrahepatic oxidation of lipids is limited. Extrahepatic oxidation of lipids is also reduced in the bowfin (Singer and Ballantyne, '91) and in both marine (Moon and Mommsen, '87) and freshwater (Singer and Ballantyne, '89) elasmobranchs.

Liver ME activity is thought to be involved in the generation of NADPH required for fatty acid synthesis in the liver (Henderson and Tocher, '87). The significantly lower ME activity in the liver of *L. platyrhincus* compared to the red muscle and heart suggests lipogenesis is limited in liver. The levels of ME in bowfin (Singer and Ballantyne, '91), lamprey (LeBlanc et al., '95) and little skate (Moon and Mommsen, '87) were also found to be lower in the liver compared to muscle and heart. The inverse situation was observed by Singer et al. ('90) in lake sturgeon where ME activity was

highest in the liver, and by Henderson and Tocher ('87) who suggested the liver is the most lipogenic tissue in teleosts. As it is unlikely that red muscle is involved in the synthesis of fatty acids, the high activity of ME found in the red muscle of several fish species (Moon and Mommsen, '87; Singer and Ballantyne, '91; LeBlanc et al., '95) suggests ME has other functions within red muscle. Chamberlin et al. ('91) proposed that ME in red muscle might be used to generate citric acid cycle intermediates during amino acid oxidation allowing for the complete cycling and generation of ATP solely from amino acid fuels.

Plasma NEFAs are the most metabolically active form of lipid in the blood of vertebrates and their levels indicate the degree to which fatty acids are used as a fuel source (Henderson and Tocher, '87). *L. platyrhincus* plasma NEFA levels reported here are the lowest for any nonelasmobranch fish (Singer et al., '90; Ballantyne et al., '93; LeBlanc et al., '95), but most closely resemble levels found in the bowfin (Singer and Ballantyne, '91). Plasma NEFA concentrations in elasmobranchs (Ballantyne et al., '93) are even lower than those reported here. Saturated fatty acids make up the greatest proportion of gar plasma NEFAs. This is similar to what is seen in the bowfin (Singer and Ballantyne, '91) and lamprey (LeBlanc et al., '95). In contrast, sturgeon plasma contains a relatively similar proportion of saturated and monounsaturated NEFAs (Singer et al., '90; Jarvis and Ballantyne, 2003) while Arctic char plasma NEFAs are more than 50% polyunsaturated (Barton et al., '95). Palmitoleic acid (16:1) comprised 11.2% of the total plasma NEFAs in *L. platyrhincus*, similar to what is found in both the bowfin (Singer and Ballantyne, '91) and sturgeon (Singer et al., '90), but high compared to several teleosts and elasmobranchs (Ballantyne et al., '93; McKinley et al., '93; Ballantyne et al., '96) where 16:1 typically accounts for only 3–6% of the total. The levels of n3–n6 polyunsaturated NEFAs are approximately equal in gar plasma, with 22:6n3, 20:4n6 and 18:2n6 accounting for 9.1%, 5.9% and 5.4% of the total, respectively. Plasma of freshwater fishes have a much lower n3:n6 NEFA ratio compared to marine fishes yet this ratio is typically greater than 1, even in freshwater fish (approximately 1.8 in bowfin (Singer and Ballantyne, '91) and sturgeon (Singer et al., '90), 2.0 in carp and 1.7 in Atlantic salmon (Henderson and Tocher, '87)). The reduced capacity for fatty acid oxidation or synthesis in gar tissues and the extremely low circulating plasma NEFA levels strongly suggest the capacity for lipid metabolism is limited in Florida gar.

Amino acid metabolism

Total plasma amino acid levels were more than double those reported in lamprey (LeBlanc et al., '95), but were low compared to those of teleosts (Plakas et al., '80; Gutierrez et al., '87; Barton et al., '95). Asp-AT and Ala-AT activities were low in gar compared to lake sturgeon (Singer et al., '90), bowfin (Singer and Ballantyne, '91) and the little skate (Moon and Mommsen, '87), but similar to what is seen in lamprey (LeBlanc et al., '95). Gar tissues have the lowest reported PDG and GS activities to date when compared with the tissues of other fish such as the bowfin and lake charr (Chamberlin et al., '91), lamprey (LeBlanc et al., '95) and the little skate (Ballantyne, '97). As has been reported for other fish species (Chamberlin et al., '91; Chamberlin and Ballantyne, '92; LeBlanc et al., '95), gar displayed a higher PDG activity than GS in both liver and red muscle, indicating net glutamine catabolism in these tissues. There is a net synthesis of glutamine in gar white muscle tissue, with a PDG to GS ratio of 0.17, similar to what is seen in the bowfin (PDG:GS = 0.88) (Chamberlin et al., '91) and in mammals (PDG:GS = 0.25) (Vatts et al., '99). The low levels of PDG and GS suggest glutamine is not an important source of energy in gar. Plasma glutamine levels in the gar are relatively high compared to other primitive fishes (agnathans, elasmobranchs and chondrosteans) and are similar to levels found in teleosts (Ballantyne, 2001) and this is consistent with a general trend towards an inverse relationship between tissue PDG and plasma glutamine levels. GDH activity was much higher in the liver (~9-fold) and heart (~3–10-fold) tissues of gar compared to that of other primitive fish (Singer et al., '90; Ballantyne et al., '93; LeBlanc et al., '95). Further study into the patterns of nitrogen metabolism of gar is needed to explain these observations.

Ketone body metabolism

β-HBDH, the enzyme that oxidizes the ketone body β-hydroxybutyrate to acetoacetate, was found to utilize both the L- and D-stereoisomers of β-hydroxybutyrate in several tissues of the Florida gar. LeBlanc ('96) found the teleost *Carassius auratus* capable of utilizing both the L- and D-stereoisomers of β-hydroxybutyrate. The ability of a primitive fish such as the gar, to

distinguish between the two stereoisomers and oxidize them at different rates suggests this ability may be present in other species of fish, both primitive and advanced. In gar liver the L- stereoisomer was utilized to a greater degree, opposite to what was found in goldfish (LeBlanc and Ballantyne, 2000) and in the elasmobranch, *Squalus acanthias* (J. Treberg, personal com.). In the liver of goldfish both D- and L-β-HBDH are considered cytosolic enzymes; however, in kidney tissue, D-β-HBDH was mainly mitochondrial (LeBlanc and Ballantyne, 2000). The activities of β-HBDH and SKT were found to be highest in the liver of the gar. This differs from the situation in several other primitive fish in which both SKT and β-HBDH were found to be higher in red muscle (Singer et al., '90; LeBlanc et al., '95), suggesting *L. platyrhincus* does not oxidize ketone bodies to any substantial degree in red muscle. Singer and Ballantyne ('91) suggested the oxidation of β-hydroxybutyrate as an energy source ''is an ancient vertebrate characteristic''. In general, primitive fish such as elasmobranchs (Moon and Mommsen, '87), lake sturgeon (Singer et al., '90), bowfin (Singer and Ballantyne, '91) and sea lamprey (LeBlanc et al., '95), were found to rely on ketone bodies to a greater degree than many teleosts (LeBlanc and Ballantyne, '93; LeBlanc, '96); however, Florida gar do not appear to fit into this proposed phylogenetic trend.

Evolutionary considerations

With respect to lipid metabolism, the Florida gar sits in an intermediate position between the ''primitive'' elasmobranchs and the ''advanced'' teleosts. The apparent heightened capacity of the Florida gar to synthesize and oxidize glucose sets it apart from most other fishes. This capability may be a result of the evolutionary pressures of living in a low-oxygen environment. Gars are well adapted to this environmental constraint through the evolution of their modified swim bladder and their heightened capacity for anaerobic glycolysis. The low rate of oxidative enzymes found in gar tissues also corroborates the idea that gar are adapted to live in an environment with limited oxygen availability. The metabolic organization of the gar ABO resembles the mammalian lung more so than a fish swim bladder based on the activities of several enzymes including PK, LDH and CS.

Understanding the physiology of this primitive fish group gives us valuable insight into the evolution of fish metabolism. The gars have been exposed to unique environmental pressures, which have shaped their particular evolution. The distinct metabolic organization of the gar is a product of the complex relationship between the evolutionary phylogeny of primitive fishes and the environmental conditions under which it lives.

ACKNOWLEDGMENTS

This research was funded by a N.S.E.R.C. Discovery grant to J.S. Ballantyne. Special thanks to P.L. LeBlanc for his technical guidance and scientific input into this study.

LITERATURE CITED

Almeida-Val VMF, Hochachka PW. 1995. Air-breathing fishes: metabolic biochemistry of the first diving vertebrates. In: Hochachka PW, Mommsen TP, editors. Biochemistry and molecular biology of fishes, Vol. 5. Amsterdam: Elsevier Science. p 45–55.

Ballantyne JS. 1997. Jaws: the inside story. The metabolism of elasmobranch fishes. Comp Biochem Physiol B 118:703–742.

Ballantyne JS. 2001. Amino acid metabolism. In: Wright PA, Anderson PM, editors. Fish physiology, Vol. 20: Nitrogen Excretion. New York: Academic Press Inc. p 77–107.

Ballantyne JS, Glemet HC, Chamberlin ME, Singer TD. 1993. Plasma nonesterified fatty acids of marine teleost and elasmobranch fishes. Mar Biol 116:47–52.

Ballantyne JS, Mercure F, Gerrits MF, Van Der Kraak G, McKinley S, Martens DW, Hinch SG Diewert RE. 1996. Plasma nonesterified fatty acid profiles in male and female sockeye salmon, *Oncorhynchus nerka*, during the spawning migration. Can J Fish Aquat Sci 53:1418–1426.

Barton KN, Gerrits MF, Ballantyne JS. 1995. Effects of exercise on plasma nonesterified fatty acids and free amino acids in Arctic char (*Salvelinus alpinus*). J Exp Zool 271: 183–189.

Battersby BJ, Moyes CD. 1998. Influence of acclimation temperature on mitochondrial DNA, RNA, and enzymes in skeletal muscle. Am J Physiol 275:R905–R912.

Blier P, Guderley H. 1988. Metabolic responses to cold acclimation in the swimming musculature in the lake white fish, *Coregonus clupeaformis*. J Exp Zool 246:244–252.

Bostrom SL, Fange R, Johansson RG. 1972. Enzyme activity patterns in gas gland tissue of the swim bladder of the cod (*Gadus morhua*). Comp Biochem Physiol B 43:473–478.

Chamberlin ME, Ballantyne JS. 1992. Glutamine metabolism in elasmobranch and agnathan muscle. J Exp Zool 264: 267–272.

Chamberlin ME, Glemet HC, Ballantyne JS. 1991. Glutamine metabolism in a holostean (*Amia calva*) and a teleost fish (*Salvelinus namaycush*). Am J Physiol 260:R159–R166.

Chavin W, Young JE. 1970. Factors in the determination of normal serum glucose levels of goldfish, *Carassius auratus*. Comp Biochem Physiol 33:629–653.

Conlon JM, Agius L, George K, Alberti MM, Hazon N. 1994. Effects of dogfish urotensin II on lipid mobilization in the fasted dogfish, *Scyliorhinus canicula*. Gen Comp Endocrinol 93:77–180.

Cowey CB, De La Higuera M, Adron JW. 1977. The effect of dietary composition and of insulin on gluconeo-

genesis in rainbow trout (*Salmo gairdneri*). Br J Nutr 38: 385–395.

Ewart HS, Driedzic WR. 1987. Enzymes of energy metabolism in salmonid hearts: spongy versus cortical myocardia. Can J Zool 65:623–627.

Ewart HS, Driedzic WR. 1990. Enzyme activity levels underestimate lactate production rates in cod (*Gadus morhua*) gas gland. Can J Zool 68:193–197.

Frick NT, Wright PA. 2002. Nitrogen metabolism and excretion in the mangrove killifish *Rivulus marmoratus*. I. Influence of environmental salinity and external ammonia. J Exp Biol 205:79–89.

Gerrits MF. 1994. Aspects of the energy metabolism of red muscle in Arctic char (*Salvelinus alpinus*). MSc thesis, University of Guelph, Guelph: Ontario 149 pp.

Gesser H, Fange R. 1971. Lactate dehydrogenase and cytochrome oxidase in the swim bladder of fish. Int J Biochem 2:163–166.

Gillis TE, Ballantyne JS. 1996. The effects of starvation on plasma free amino acid and glucose concentrations in lake sturgeon. J Fish Biol 49:1306–1316.

Graham JB. 1997. Air-breathing fishes: evolution, diversity and adaptation. London, UK: Academic Press.

Gutierrez J, Fernandez J, Geese M, Blasco J. 1987. Amino acid levels in whole blood and plasma of *Scyliorhinus canicula*. Comp Biochem Physiol A 87:57–61.

Henderson RJ, Tocher DR. 1987. The lipid composition and biochemistry of freshwater fish. Prog Lipid Res 26:281–347.

Jarvis PL, Ballantyne JS. 2003. Metabolic responses to salinity acclimation in juvenile shortnose sturgeon *Acipenser brevirostrum*. Aquaculture 219:891–909.

Jorgensen JB, Mustafa T. 1980. The effect of hypoxia on carbohydrate metabolism in flounder (*Platichthys flesus* L.)—II. High energy compounds and the role of glycolytic and gluconeogenic enzymes. Comp Biochem Physiol B 67: 249–256.

Knox D, Walton MJ, Cowey CB. 1980. Distribution of enzymes of glycolysis and gluconeogenesis in fish tissues. Mar Biol 56: 7–10.

LeBlanc PJ. 1996. Aspects of ketone body metabolism in the goldfish (*Carassius auratus*). MSc thesis, University of Guelph: Guelph, Ontario 110 pp.

LeBlanc PJ, Ballantyne JS. 1993. β-hydroxybutyrate dehydrogenase in teleost fish. J Exp Zool 267:356–358.

LeBlanc PJ, Ballantyne JS. 2000. Novel aspects of the activities and subcellular distribution of enzymes of ketone body metabolism in the live and kidney of the goldfish, *Carassius auratus*. J Exp Zool 286:434–439.

LeBlanc PJ, Gillis TE, Gerrits MF, Ballantyne JS. 1995. Metabolic organization of liver and somatic muscle of landlocked sea lamprey, *Petromyzon marinus*, during the spawning migration. Can J Zool 73:916–923.

Levesque HM, Bondy J, Short C, Ballantyne JS, Driedzic WR, Moon TW. 2005. Effects of seasonal temperature and photoperiod on Atlantic cod (*Gadus morhua*). II. Enzymes of intermediary metabolism. Can J Fish Aquat Sci 62: 2864–2873.

McGarry JD, Foster DW. 1980. Regulation of hepatic fatty acid oxidation and ketone body production. Annu Rev Biochem 49:395–420.

McKinley RS, Singer TD, Ballantyne JS, Power G. 1993. Seasonal variation in plasma nonesterified fatty acids of Lake sturgeon (*Acipenser fulvescens*) in the vicinity of hydroelectric facilities. Can J Fish Aquat Sci 50:13–20.

Mirejovska E, Bass A, Hurych J, Teisinger J. 1981. Enzyme changes during experimental silicotic fibrosis. II. Intermediary metabolism enzymes of the lungs. Environ Res 25: 434–440.

Mommsen TP, Walsh PJ, Moon TW. 1985. Gluconeogenesis in hepatocytes and kidney of Atlantic salmon. Mol Physiol 8: 89–100.

Moon TW, Mommsen TP. 1987. Enzymes of intermediary metabolism in tissues of the little skate, *Raja erinacea*. J Exp Zool 244:9–15.

Moyes CD, Buck LT, Hochachka PW, Suarez RK. 1989. Oxidative properties of carp red and white muscle. J Exp Biol 143:321–332.

Murphy B, Zapol WM, Hochachka PW. 1980. Metabolic activities of heart, lung and brain during diving and recovery in the Weddell seal. J Appl Physiol 48:596–605.

Newsholme EA, Crabtree B. 1986. Maximum catalytic activity of some key enzymes in provision of physiologically useful information about metabolic fluxes. J Exp Zool 239: 159–167.

Pelletier D, Dutil JD, Blier P, Guderly H. 1994. Relation between growth rate and metabolic organization of white muscle, liver and digestive tract in cod, *Gadus morhua*. J Comp Physiol 164B:179–190.

Pelster B. 1995. Metabolism of the swimbladder tissue. In: Hochachka PW, Mommsen TP, editors. Biochemistry and molecular biology of fishes, Vol. 4. Amsterdam: Elsevier Science. p 101–118.

Plakas SM, Katayama T, Tanaka Y, Deshimaru O. 1980. Changes in the levels of circulating plasma free amino acids of carp (*Cyprinus carpio*) after feeding a protein and an amino acid diet of similar composition. Aquaculture 21: 307–322.

Pollock HG, Kimmel JR, Hamilton JW, Rouse JB, Rawitch AB, Ebner KE, Lance V. 1987. Isolation and structures of alligator gar (*Lepisosteus spatula*) insulin and pancreatic polypeptide. Gen Comp Endocrinol 67:375–382.

Pollock HG, Kimmel JR, Ebner KE, Hamilton JW, Rouse JB, Lance V, Rawitch AB. 1988. Isolation of alligator gar (*Lepisosteus spatula*) glucagon, oxyntomodulin, and glucagon-like peptide: amino acid sequences of oxyntomodulin and glucagon-like peptide. Gen Comp Endocrinol 69: 133–140.

Power GW, Cake MH, Potter IC. 1993. Carnitine palmitoyltransferase activity is present and high in the muscle and liver of lampreys (Agnatha). J Exp Zool 266:157–162.

Rahn H, Rahn KB, Howell BJ, Gans C, Tenney SM. 1971. Air breathing of the garfish (*Lepisosteus osseus*). Respir Physiol 11:285–307.

Rao PVVP. 1983. Effects of intraperitoneal cadmium administration on mitochondrial enzymes in rat tissues. Toxicology 27:81–87.

Sherwood NM, Doroshov S, Lance V. 1991. Gonadotropin-releasing hormone (GnRH) in bony fish that are phylogentically ancient: reedfish (*Calamoichthys calabaricus*), sturgeon (*Acipenser transmontanus*), and alligator gar (*Lepisosteus spatula*). Gen Comp Endocrinol 84:44–57.

Sidell BD, Driedzic WR, Stowe DB, Johnston IA. 1987. Biochemical correlations of power development and metabolic fuel preference in fish hearts. Physiol Zool 60:221–232.

Singer TD, Ballantyne JS. 1989. Absence of extrahepatic lipid oxidation in a freshwater elasmobranch, the dwarf stingray *Potamotrygon magdalenae*: evidence from enzyme activities. J Exp Zool 251:355–360.

Singer TD, Ballantyne JS. 1991. Metabolic organization of a primitive fish, the bowfin (*Amia calva*). Can J Fish Aquat Sci 48:611–618.

Singer TD, Mahadevappa VG, Ballantyne JS. 1990. Aspects of the energy metabolism of Lake Sturgeon, *Acipenser fulvescens*, with special emphasis on lipid and ketone body metabolism. Can J Fish Aquat Sci 47:873–881.

Suarez RK, Mommsen TP. 1987. Gluconeogenesis in teleost fish. Can J Zool 65:1869–1882.

Suarez RK, Mallet MD, Daxboeck C, Hochachka PW. 1986. Enzymes of energy metabolism and gluconeogenesis in the Pacific blue marlin, *Makaira nigricans*. Can J Zool 64: 694–697.

Vatts P, Mukherjee AK, Kumria MML, Singh SN, Patil SKB, Rangnathan S, Sridharan K. 1999. Changes in the activity levels of glutamine synthetase, glutaminase and glycogen synthetase in rats subjected to hypoxic stress. Int J Biometerorol 42:205–209.

Walsh PJ, Milligan CL. 1993. Roles of buffering capacity and pentose phosphate pathway activity in the gas gland of the gulf toadfish *Opsanus beta*. J Exp Biol 176:311–316.

West JL, Bailey VMF, Almeida-Val AL, Sidell BD, Driedzic WR. 1999. Activity levels of enzymes of energy metabolism in heart and red muscle are higher in north-temperate-zone than in Amazonian teleosts. Can J Zool 77: 690–696.

Zammit VA, Newsholme EA. 1979. Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobranch fish. Biochem J 184:313–322.