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ABSTRACT

The jawless fish, the sea lamprey (*Petromyzon marinus*), spends part of its life as a burrow-dwelling, suspension-feeding larva (ammocoete) before undergoing a metamorphosis into a free swimming, parasitic juvenile that feeds on the blood of fishes. We predicted that animals in this juvenile, parasitic stage have a great capacity for catabolizing amino acids when large quantities of protein-rich blood are ingested. The sixfold to 20-fold greater ammonia excretion rates (J_{Amm}) in postmetamorphic (nonfeeding) and parasitic lampreys compared with ammocoetes suggested that basal rates of amino acid catabolism increased following metamorphosis. This was likely due to a greater basal amino acid catabolizing capacity in which there was a sixfold higher hepatic glutamate dehydrogenase (GDH) activity in parasitic lampreys compared with ammocoetes. Immunoblotting also revealed that GDH quantity was 10-fold and threefold greater in parasitic lampreys than in ammocoetes and upstream migrant lampreys, respectively. Higher hepatic alanine and aspartate aminotransferase activities in the parasitic lampreys also suggested an enhanced amino acid catabolizing capacity in this life stage. In contrast to parasitic lampreys, the twofold larger free amino acid pool in the muscle of upstream migrant lampreys confirmed that this period of natural starvation is accompanied by a prominent proteolysis. Carbamoyl phosphate synthetase III was detected at low levels in the liver of parasitic and upstream migrant lampreys, but there was no evidence of extrahepatic (muscle, intestine) urea production via the ornithine urea cycle. However, detection of arginase activity and high concentrations of arginine in the liver at all life stages examined infers that arginine hydrolysis is an important source of urea. We conclude that metamorphosis is accompanied by a metabolic reorganization that increases the capacity of parasitic sea lampreys to catabolize intermittently large amino acid loads arising from the ingestion of protein rich blood from their prey/hosts. The subsequent generation of energy-rich carbon skeletons can then be oxidized or retained for glycogen and fatty acid synthesis, which are essential fuels for the upstream migratory and spawning phases of the sea lamprey's life cycle.

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Introduction

The complex life cycle of the sea lamprey (*Petromyzon marinus*) is characterized by a prolonged burrow-dwelling phase lasting 3–7 yr, in which they subsist as suspension-feeding ammocoetes (larvae) before undergoing a complex metamorphosis into freely swimming, juvenile parasitic lampreys that feed on the blood of fishes (for review, see Youson 1980). Following the parasitic phase, sea lampreys cease feeding, migrate upstream (upstream migrants), spawn, and then die (Beamish and Potter 1975). Metamorphosis leads to profound changes in internal and external body structure, as characterized by the development of an oral disc and rasping tongue and eyes, along with a major reorganization of the liver, gastrointestinal tract, kidneys, and gills (Youson 1980; Rovainen 1996).

The objective of this investigation was to test the hypothesis that such life stage–dependent changes in body structure, activity level, mode of feeding, and dietary status alter patterns of amino acid catabolism and nitrogenous waste (N-waste)

production in sea lampreys. Indeed, postmetamorphic elevations of metabolic rate, along with a switch to a protein-rich diet of blood, may be reflected by greater rates of N-waste production and excretion during the parasitic stage of the sea lamprey's life cycle. Because the upstream migration is a nontrophic period, upstream migrant sea lampreys also have to endure several months without food (Beamish and Potter 1975; Larsen 1980; Emelyanova et al. 2004).

Although mechanisms of N-waste production were previously examined in migrating Pacific lamprey (*Lampetra tridentata*; Read 1968), sea lamprey ammocoetes (Wilkie et al. 1999), and feeding parasitic sea lampreys (Wilkie et al. 2004), it is not known how patterns of amino acid catabolism and N-waste production change at different stages of the lamprey life cycle. Thus, the first objective of this investigation was to compare N-waste excretion patterns during each of the sea lamprey's major life stages by measuring rates of ammonia (J_{Amm}) and urea excretion (J_{Urea}) in ammocoete, postmetamorphic (not yet feeding), parasitic, and upstream migrant sea lampreys, plus plasma ammonia and urea concentrations. The lamprey's capacity to produce N-wastes at different life-history stages was evaluated by comparing the activities of key enzymes of amino acid metabolism in the liver, muscle, and intestine. Key amino acids were also measured to determine how changes in the size of the amino acid pool or the quantity of specific amino acids influenced amino acid catabolism, J_{Amm} and J_{Urea} at different life stages.

Although ammocoetes and parasitic lampreys excrete approximately 10%–30% of their N-waste as urea (Wilkie et al. 1999, 2004), there is little to suggest that the ornithine urea cycle (OUC) is quantitatively important in lampreys. On the basis of enzymatic determinations of OUC activity, Read (1968) suggested that upstream migrant lampreys lacked the ability to produce urea de novo via the OUC, speculating that the majority was produced through the breakdown of arginine. Wilkie et al. (1999) demonstrated that urea is quantitatively important in ammocoetes, but the key OUC enzyme, carbamoyl phosphate synthetase III (CPS III), was not detected. More recently, low levels of CPS III activity were detected in the livers of parasitic sea lampreys but were insufficient to account for significant J_{Urea} (Wilkie et al. 2004). However, recent studies have shown that extrahepatic sites such as the muscle and/or intestine possess significant OUC enzyme activity (CPS III and ornithine carbamoyl transferase [OCT]) in many fishes, including the little skate (*Raja erinacea*; Steele et al. 2005), spiny dogfish (*Squalus acanthias*; Kajimura et al. 2005), ureogenic Lake Magadi tilapia (*Alcolapia grahami*; Lindley et al. 1999), gulf toadfish (*Opsanus beta*; Julsrud et al. 1998), larval trout (*Oncorhynchus mykiss*; Korte et al. 1997; Steele et al. 2001), and largemouth bass (*Micropterus dolomieu*; Kong et al. 1998). Thus, another objective was to test the hypothesis that extrahepatic sites potentially contributed to urea production in sea lampreys by comparing the intramuscular and intestinal activities of key OUC and other enzymes associated with urea production to measurements made in the liver of ammocoete, parasitic, and upstream migrant sea lampreys.

Material and Methods

Experimental Animals and Setup

Sea lamprey (*Petromyzon marinus*) ammocoetes were collected from Duffin's Creek, Ontario, using pulsed DC electrofishing (Smith Root model 12A) and transported to the University of Toronto at Scarborough in insulated containers containing aerated water. The animals were held in well-aerated 50-L aquaria, in which the bottom was lined with 10 cm of sand to serve as burrowing substrate, receiving dechlorinated tap water (T = 12°–15°C; hardness = 0.7 mmol L^{-1} as $CaCO_3$; pH = 7.9) at a rate of 300–500 mL min^{-1}. The ammocoetes were fed a weekly maintenance diet of baker's yeast (approximately 2 g per ammocoete; Holmes and Youson 1994).

A subset of ammocoetes entered metamorphosis and, following metamorphosis, were transferred to a 500-L Living Stream (Frigid Units). No attempts were made to feed lamprey undergoing metamorphosis because they do not feed at this time (Youson 2003). Following metamorphosis, however, the animals were allowed to feed on domestic rainbow trout (*Oncorhynchus mykiss*; mass = 100–200 g) purchased from Linwood Acres Trout Farm, Campbellcroft, Ontario. The health of the trout was frequently monitored, and when they lost equilibrium, they were quickly netted and euthanized with an overdose of buffered tricaine methanesulfonate (MS-222) anesthetic (Syndel Laboratories), as approved by the University of Toronto at Scarborough and Mount Allison University Animal Care Committees. Most of the parasitic lampreys continued feeding until experiments commenced 2 mo later.

Upstream migrant (prespawning) sea lampreys were captured in Duffin's Creek or the Humber River (near Toronto) and held in groups of 20–30 in 80-L aquaria receiving water at a rate of 1.0 L min^{-1} and a temperature of 12°–15°C. At the time of experimentation, care was taken to verify that all animals remained in prespawning condition by monitoring the development of secondary sex characters for each gender. It was not possible to feed the upstream migrant sea lampreys because they do not feed during this life stage (Larsen 1980).

Experimental Protocol

Nitrogenous waste excretion rates ($J_{N\text{-}waste}$) were determined in ammocoete (N = 24; mass = 3.53 ± 0.03 g), postmetamorphic (N = 5; 1.46 ± 0.25 g), parasitic (N = 8; 5.04 ± 0.74 g), and upstream migrant (N = 20; 248.6 ± 11.1 g) lampreys held individually in darkened flux chambers (T = 12°–14°C). Ammocoetes and parasitic lampreys were fasted 7–10 d before experiments to better compare basal levels of N-waste production and excretion. This approach was also better suited

for making similar comparisons to the naturally starving upstream migrant lampreys. The volume of each flux chamber reflected the size of each animal; smaller ammocoetes and parasitic lampreys were held in 200–500-mL flux chambers, and the larger upstream migrants were held in 3.0-L chambers. Each chamber was fitted with an externally mounted sampling port, which permitted the experimenter to collect water samples (10 mL at 0, 4, 8, and 12 h) with minimal disturbance to the animal inside. Excretion rates were determined over three consecutive 4-h flux determination periods to eliminate artifacts introduced by possible diurnal rhythms. Since excretion rates were stable over this period, only mean excretion rates are reported. Additional details are found in Wilkie et al. (1999, 2004).

Following flux determinations, the lampreys were anesthetized with 1.5 g L^{-1} MS222 buffered with 3.0 g L^{-1} $NaHCO_3$ for 3–5 min (Wilkie et al. 1999). Weights were then determined and blood collected from an incision through the heart using nonheparinized capillary tubes (Wilkie et al. 1999). Blood samples were centrifuged at 10,000 *g* for 5 min, and the plasma was collected and frozen at −80°C until analyzed for ammonia, urea, and amino acids. An incision was then made along the midventral line of the animal, exposing the liver and intestine, which were removed and snap frozen in liquid N_2 using precooled aluminum tongs. After removing the skin, muscle was excised from the anteriolateral trunk and preserved in an identical manner. All tissues were stored at −80°C until analyzed for amino acid levels or enzyme activities.

Analytical Techniques

Measurements of ammonia (J_{Amm}) and urea excretion (J_{Urea}) were based on the accumulation of each waste product in the water during each flux determination period, after correcting for the animal's weight and water volume. Total ammonia (the sum of NH_3 and NH_4^+) in the water was determined using the salicylate-hypochlorite assay (Verdouw et al. 1978), while plasma ammonia was determined enzymatically (glutamate dehydrogenase [GDH]) using a commercial kit (Sigma). Water and plasma urea concentrations were determined using the diacetyl monoxime reaction (Crocker 1967).

Amino acids were quantified in the plasma, liver, and muscle of ammocoete, parasitic, and upstream migrant lampreys by high-performance liquid chromatography using a Varian Star 9012 solvent delivery system and 9100 Autosampler, a Waters M420 fluorescence detector, and a reverse-phase C18 Whatman partisphere cartridge column. Plasma was deproteinized using an equal volume of 8% perchloric acid, neutralized with K_2CO_3, placed on ice for approximately 5 min, and centrifuged at 10,000 *g* at 4°C for 10 min. For amino acid quantification, 25 μL of supernatant was injected onto the column, following precolumn derivatization with an equal volume of o-ophthaldialdehyde (OPA). Pieces of liver (approximately 20 mg) and muscle (approximately 100 mg) were prepared in a similar manner. Individual amino acid identities and quantities were based on relative retention times and the fluorescence peak areas of the OPA–amino acid derivatives when compared with commercially prepared amino acid standards (Sigma; catalog AA-S-18). Sixteen amino acids were quantified, including the essential amino acids arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine and the nonessential amino acids alanine, aspartate, glutamate, glutamine, glycine, serine, and tyrosine.

Enzyme activities were determined on tissue homogenates prepared from frozen tissues (liver, muscle, intestine) using assay conditions and protocols that were optimized for lamprey tissues (Wilkie et al. 1999, 2004) using previously described methodology (Mommsen and Walsh 1989; Barber and Walsh 1993; Walsh 1996). Briefly, pieces of tissue (20–100 mg) were transferred to a chilled 1.5-mL centrifuge tube and ground for 30 s in 4 vols of homogenization buffer (pH 7.5; 20 mmol L^{-1} KH_2PO_4, 10 mmol L^{-1} HEPES, 0.5 mmol L^{-1} EDTA, 1 mmol L^{-1} dithiothreitol; 50% glycerol) using a PowerGen model 125 tissue homogenizer. Following homogenization, the samples were centrifuged 3 min at 10,000 *g* and 4°C, and activities were determined on the supernatant using a 96-well plate spectrophotometer at the appropriate wavelengths (Molecular Devices, Spectramax 340). Maximal enzyme activities were determined for GDH (in the amination direction), glutamine synthetase (GS), glutaminase (Glnase), alanine aminotransferase (AlaAt), aspartate aminotransferase (AspAt), and the OUC enzymes CPS III, OCT, and arginase (Arg). It should also be noted that uridine triphosphate was included in the reaction buffer for CPS III determinations to inhibit CPS II, which can lead to overestimates of CPS III activity (Anderson 1995). All enzyme activities are expressed as micromoles of product formed per minute per gram of wet tissue at 20°C (μmol min^{-1} g^{-1}).

Western blotting was used to test the hypothesis that hepatic GDH protein quantity increased following metamorphosis. Frozen livers (−80°C) from ammocoete, parasitic, and upstream migrant sea lampreys were homogenized 1 min on ice in 4 vols of homogenization buffer (composition: 50% glycerol; 10 mmol L^{-1} HEPES; 20 mmol L^{-1} K_2HPO_4; 0.5 mmol L^{-1} EDTA; 1 mmol L^{-1} dithiothreitol; pH 7.5) containing the protease inhibitor PMSF (2 mmol L^{-1}), which was then diluted fivefold to 10-fold in deionized water, boiled 5 min, cooled, and pulsed 30 s in a microcentrifuge. Total protein content of the supernatant was quantified using a commercial kit (Bicinchoninic Acid Protein Kit; Sigma Diagnostics BCA-1). Proteins were then separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE; 140 V for 1 h) using 4%–20% TrisHCl Ready Gels (BioRad) after equal amounts of protein (10 μg) were added to each respective well. Prestained broad-range molecular weight markers (Sigma; catalog C3437; 205,000–6,500 Da) were used, while pure bovine liver GDH (10 μg μL^{-1}; Boerhinger Mannheim, catalog 127 710) was used to confirm antibody detection and to provide a reference standard for subsequent

GDH quantitation. The gel was then placed in transfer buffer (TBS-T; composition: 49 mmol L^{-1} TRIS; 39 mmol L^{-1} glycine; 20% methanol; 0.4% SDS) for 30 min with agitation. Proteins were transferred onto a polyvinylidene difluoride membrane using semidry blotting at 13 V for 0.5 h, blocked 1 h at 4°C in TBS-T containing 5% nonfat skim milk, and incubated overnight with polyclonal antirat GDH (Rockland; catalog 200-4158) diluted 1 : 5,000 in 5% milk-TBS solution containing 0.05% TWEEN-20 (TBS-T). After washing the membrane in TBS-T and alkaline phosphatase buffer (composition: 0.1 mol L^{-1} TRIS HCl; 0.1 mol L^{-1} NaCl; 5 mmol L^{-1} $MgCl_2$; adjusted to pH 9.5), blots were incubated 1 h (with agitation) with antirabbit secondary antibody conjugated with alkaline phosphatase at 1 : 10,000 times in TBS-T containing 5% milk, rinsed 2 × 5 min in TBS-T, equilibrated with alkaline phosphatase buffer for 5 min, and finally incubated in developing solution (composition: 20 mL TBS66 μL nitroblue tetrazolium; 66 μL 5-bromo-4-chloro-3-indolyl phosphate) for 5 min at room temperature. Relative amounts of GDH were quantified by comparing the intensity of bands between ammocoete, parasitic, and upstream migrant lampreys using a Fluor-S Multi-Imager (Bio Rad) and Quantity One software (Bio Rad). Preliminary experiments using this protocol revealed that the antirat GDH antibody used detected shark (*Squalus acanthias*), eel (*Anguilla rostrata*), and lamprey GDH.

Calculations and Statistics

It is well known that metabolic processes show negative allometric scaling with body size (Hochachka and Somero 2002) and that these relationships extend to rates of nitrogen excretion in fishes (Brett and Groves 1979). Because the animals in this study ranged from 1.1 (postmetamorphic lamprey) to 363 g (upstream migrant lamprey), possible allometric relationships were identified by constructing log-log plots of whole body J_{Amm} or J_{Urea} versus body mass (M; in grams) for all lampreys studied and by performing linear regression analysis (least squares linear regression) to yield the following relationship:

$$\log \text{ whole body } J_{Amm} \text{ (or } J_{Urea}) = \log a + b \log M, \quad (1)$$

where whole body J_{Amm} or J_{Urea} are expressed in nmol N h^{-1}, a equals the y-intercept, and b is the mass exponent (or slope of the regression line) of the relationship. Size effects identified using this calculation were then removed by analysis of residuals using an approach similar to that described by Cutts et al. (2001), which enabled us to determine whether changes in whole body J_{Amm} and/or J_{Urea} were truly due to life stage. Such data were reported as the mean residual excretion rate (rJ_{Amm} or rJ_{Urea}) ±95% confidence interval (CI), where positive rJ_{Amm} or rJ_{Urea} indicated higher than expected whole body excretion rates and negative values revealed rates that were lower than expected (e.g., Cutts et al. 2001).

All other data presented are expressed as means ± 1 SEM and were analyzed using one-way ANOVA. When significant variation was observed, a Tukey-Kramer post hoc test was used to compare pairs of life-history stages. Where data were not normally distributed, the ANOVA was followed by a nonparametric Dunnet's test. The level of significance for all data was $P < 0.05$. Statistical analysis was performed using commercial software (SAS JMP ver. 4.0, SAS Institute, or InStat ver. 3.0, Graphpad).

Results

Ammonia and Urea Excretion

Ammonia was the major nitrogenous waste (N-waste) excreted at all life stages of the sea lamprey life cycle, accounting for approximately 80%–90% of total $J_{N\text{-waste}}$ (Fig. 1). However, $J_{N\text{-waste}}$ markedly varied at different stages of the sea lamprey life cycle. In ammocoetes, J_{Amm} and J_{Urea} were approximately 30 and 5 nmol N g^{-1} h^{-1}, respectively. However, after metamorphosis, J_{Amm} and J_{Urea} were 20-fold and 10-fold greater, respectively (Fig. 1). Approximately 2–3 mo after commencing feeding, J_{Amm} and J_{Urea} in parasitic lampreys (that had been starved 7–10 d) were approximately 50% lower compared with the postmetamorphic animals but still fivefold to 10-fold higher than rates measured in the ammocoetes (Fig. 1). In the upstream migrants that had entered the nontrophic, terminal prespawning phase of their life cycle, overall rates of $J_{N\text{-waste}}$ were 55% lower than rates measured in the much smaller parasitic lampreys because of comparable declines in both J_{Amm} and J_{Urea} (Fig. 1).

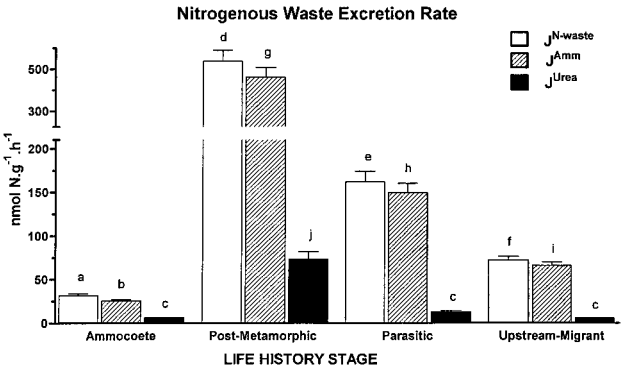

Figure 1. Differences in the basal (nonfeeding) rates of total nitrogenous waste excretion ($J_{N\text{-waste}}$; *open bars*), ammonia excretion (J_{Amm}; *hatched bars*), and urea excretion (J_{Urea}; *solid bars*) in ammocoete ($N = 24$), postmetamorphic ($N = 5$), parasitic ($N = 8$), and upstream migrant ($N = 20$) sea lampreys (*Petromyzon marinus*). Data presented as means + 1 SEM. Statistically significant differences in $J_{N\text{-waste}}$ at different life stages are denoted by a different letter, while nonsignificant values are denoted by a common letter. Life stage–dependent statistical significance was indicated in an identical manner for J_{Amm} and J_{Urea}. Significance was determined at $P < 0.05$.

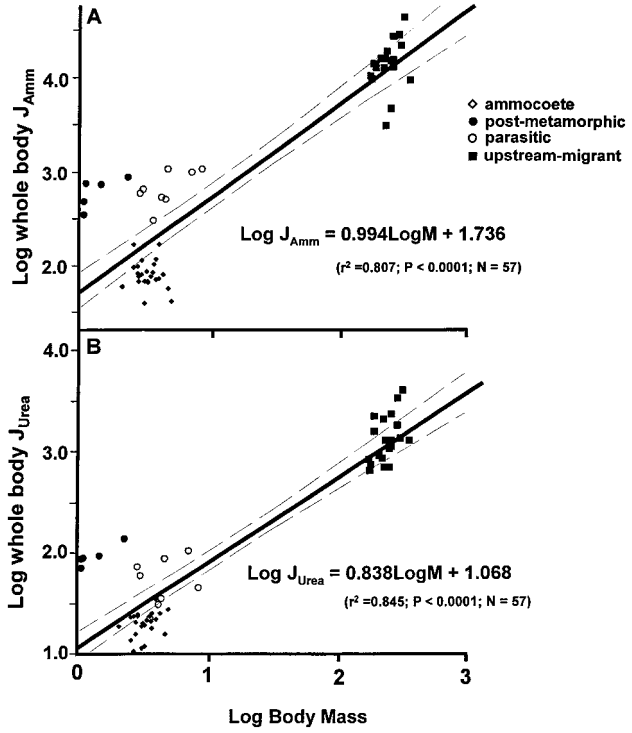

Figure 2. Log-log plot of whole body (*A*) J_{Amm} and (*B*) J_{Urea} of ammocoete (*diamonds*; $N = 24$), postmetamorphic (*solid circles*; $N = 5$), parasitic (*open circles*; $N = 8$), and upstream migrant (*solid squares*; $N = 20$) sea lampreys versus body weight. The line of best fit was determined using least squares linear regression, which was presented ±95% confidence interval (*dashed lines*). The mean residual excretion rates (rJ_{Amm} and rJ_{Urea}) were then determined for each life stage to remove the confounding effects that body size has on rates of excretion.

To assess the potential effects that life stage–dependent differences in body size had on J_{Amm} and J_{Urea}, log-log plots of J_{Amm} and J_{Urea} versus body mass were constructed (Fig. 2). This analysis revealed that there were significant linear relationships for log whole body J_{Amm} versus log body mass (Fig. 2*A*; $r^2 = 0.807$; $F = 229.7$; $P < 0.0001$) and for log whole body J_{Urea} versus log body mass (Fig. 2*B*; $r^2 = 0.845$; $F = 300.3$; $P < 0.0001$). The mass exponent describing J_{Amm} was 0.994 (95% CI = ±0.132; $J_{Amm} = 54.5M^{0.994}$; Fig. 2*A*), suggesting a proportional relationship between body size and J_{Amm}. The mass exponent describing J_{Urea} was 0.838 (95% CI = ±0.097; $J_{Urea} = 11.7M^{0.838}$; Fig. 2*B*), suggesting J_{Urea} scaled allometrically.

Analysis of residuals for J_{Amm} revealed significant differences between all pairs of life-history stages; postmetamorphic ($rJ_{Amm} = 0.915 \pm 0.183$; $N = 5$) and parasitic sea lampreys ($rJ_{Amm} = 0.420 \pm 0.145$; $N = 8$) had higher J_{Amm} than predicted by their body size, while ammocoetes had lower than expected J_{Amm} ($rJ_{Amm} = -0.369 \pm 0.084$; $N = 24$), and J_{Amm} in upstream migrants could be predicted based on body size alone ($rJ_{Amm} = 0.046 \pm 0.091$; $N = 20$; Fig. 2*A*). Similar analysis of J_{Urea} (Fig. 2*B*) revealed no significant differences in J_{Urea} between parasitic and migrant lampreys, but all other pairs of life stages were significantly different. Postmetamorphic lampreys had higher J_{Urea} than predicted by body size alone ($rJ_{Urea} = 0.791 \pm 0.156$; $N = 5$), while ammocoetes had J_{Urea} that was lower ($rJ_{Urea} = -0.228 \pm 0.071$; $N = 24$); J_{Urea} could be predicted for parasitic ($rJ_{Urea} = 0.078 \pm 0.123$; $N = 8$) and upstream migrant lampreys ($rJ_{Urea} = 0.044 \pm 0.123$; $N = 20$) using body size alone.

There were also life cycle–dependent changes in internal ammonia and urea stores. In ammocoetes, the plasma ammonia concentration was approximately 378 μmol N L^{-1}, but concentrations were 80% higher in parasitic lampreys and then subsequently declined by 57% in the upstream migrants (Fig. 3). Urea concentrations followed a similar trend in which the urea concentration averaged 280 μmol N L^{-1} in ammocoetes but was more than twofold greater in parasitic and upstream migrant lampreys (Fig. 3).

Free Amino Acid Pools in Plasma, Liver, and Muscle

Total free plasma amino acid (FAA) concentrations were not significantly different between ammocoete, parasitic, and upstream migrant lampreys, nor were there significant differences in the concentrations of essential amino acids (EAA; Table 1). However, individual amino acid concentrations, such as the nonessential amino acids aspartate, alanine, glutamate, and glycine, were two to three times greater in the plasma of parasitic lampreys compared with ammocoetes. Ammocoetes had 2.5-

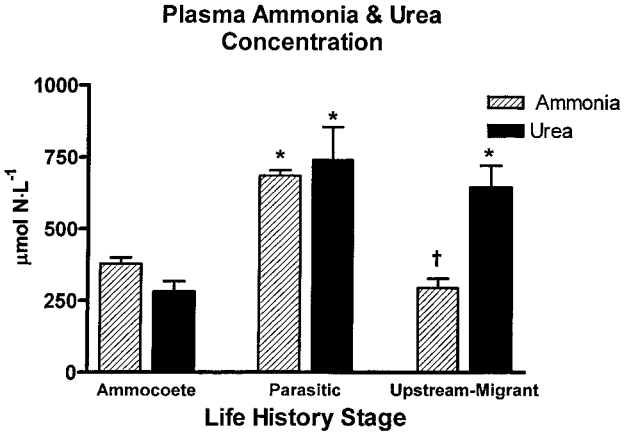

Figure 3. Differences in the plasma ammonia (*hatched bars*) and urea (*solid bars*) concentrations of ammocoete ($N = 13$), parasitic ($N = 15$), and upstream migrant ($N = 13$) sea lampreys. Data presented as means + 1 SEM. Asterisks denote statistically significant differences in ammonia and urea concentrations between ammocoetes and parasitic and upstream migrant lampreys, while daggers indicate significant differences between parasitic and upstream migrant lampreys at $P < 0.05$.

Table 1: Amino acid concentrations (μmol g^{-1} wet weight) in the plasma of ammocoete, parasitic, and upstream migrant sea lampreys

Amino Acid	Ammocoete ($N = 8$)	Parasitic ($N = 6$)	Upstream Migrant ($N = 6$)
Alanine	.079 ± .008	.228 ± .080*	.066 ± .012
Arginine	.072 ± .005	.054 ± .008	.052 ± .006
Aspartate	.007 ± .005	.016 ± .002*	.004 ± .002
Glutamine	.029 ± .007	.069 ± .020	.122 ± .020*
Glutamate	.007 ± .005	.029 ± .002*	.001 ± .000**
Glycine	.085 ± .016	.167 ± .027*	.093 ± .010
Histidine	.119 ± .013	.045 ± .003*	.016 ± .004*
Isoleucine	.050 ± .006	.078 ± .008*	.072 ± .007
Leucine	.087 ± .011	.180 ± .011*	.110 ± .013**
Lysine	.097 ± .008	.134 ± .015	.166 ± .023*
Methionine	.032 ± .005	.047 ± .002	.028 ± .005
Phenylalanine	.024 ± .003	.040 ± .002	.058 ± .007*
Serine	.057 ± .014	.068 ± .006	.059 ± .011
Threonine	.149 ± .123	.067 ± .007*	.071 ± .007*
Tyrosine	.034 ± .003	.029 ± .003	.037 ± .007
Valine	.099 ± .012	.180 ± .010	.095 ± .014
Total free amino acids	1.027 ± .105	1.355 ± .092	1.049 ± .056
Total essential amino acids	.729 ± .063	.751 ± .047	.667 ± .036

Note. Essential amino acids in italic.

* Statistically significant difference from ammocoetes ($P < 0.05$).

** Statistically significant difference from parasitic sea lampreys ($P < 0.05$).

fold greater concentrations of the EAAs histidine and threonine (Table 1). Although upstream migrant lampreys had not likely fed for at least 3–4 mo (see "Discussion"), plasma amino acids, including EAAs, were similar to concentrations measured in the parasitic lampreys. One exception was glutamate, which was approximately 30-fold greater in the plasma of the parasitic lamprey (Table 1).

The more or less constant plasma amino acid concentrations in the plasma were in marked contrast to the liver, where total FAAs in parasitic lampreys were more than threefold higher than those in ammocoetes (Table 2). However, the total FAAs were about 25% lower in the upstream migrant compared with the parasitic lampreys. The largest changes observed were the nonessential amino acids alanine, aspartate, and glutamate, which were fivefold to 10-fold greater in the livers of parasitic lampreys than in ammocoetes (Table 2). Some of these amino acids also differed significantly between the parasitic and upstream migrant lampreys, in which the respective concentrations of alanine and glutamate were fourfold and twofold greater in parasitic lampreys. However, aspartate was approximately twofold greater in the upstream migrant lampreys (Table 2). Glutamine concentrations were approximately 30% lower in the liver of upstream migrant lampreys than in parasitic lampreys (Table 2). In contrast to the total FAAs, total EAA concentrations were comparable in parasitic and upstream migrant lampreys but were approximately 30% lower in ammocoetes (Table 2). Notably, arginine was threefold greater in the liver of parasitic and upstream migrant sea lampreys than in ammocoetes (Table 2).

The total FAA pool was 1.5–2.0 times greater in the muscle of upstream migrant lampreys than in the muscle of ammocoetes and parasitic lampreys, respectively (Table 3). This result was mainly due to significant increases in the nonessential amino acids alanine, aspartate, glutamate, and glycine. Arginine was the only EAA to increase in upstream migrant lampreys, where it was approximately threefold greater than concentrations measured in ammocoetes and parasitic lampreys (Table 3). However, respective total free EAAs in the muscle were 70% lower in parasitic lampreys and 40% lower in upstream migrant lampreys than they were in ammocoetes. This result was mainly due to significantly lower concentrations of histidine, methionine, phenylalanine, threonine, and valine (Table 3).

Activities of Amino Acid Catabolizing Enzymes and Glutamate Dehydrogenase Quantification

The maximum activity of enzymes associated with amino acid deamination was influenced by life-history stage. The activity of GDH, which promotes the oxidative deamination of glutamate to ammonia and α-ketoglutarate, was sixfold greater in the liver of parasitic lampreys compared with ammocoetes (Fig. 4*A*). A similar, less pronounced pattern was observed in the

Table 2: Amino acid concentrations (μmol g^{-1} wet weight) in the liver of ammocoete, parasitic, and upstream migrant sea lampreys

Amino Acid	Ammocoete ($N = 9$)	Parasitic ($N = 6$)	Upstream Migrant ($N = 7$)
Alanine	.268 ± .037	2.622 ± .127*	.636 ± .054*,**
Arginine	.228 ± .053	.679 ± .049*	.623 ± .114*
Aspartate	.046 ± .029	.174 ± .052*	.420 ± .068*,**
Glutamine	.473 ± .074	.923 ± .260	.288 ± .052**
Glutamate	.321 ± .145	2.592 ± .395*	1.393 ± .238*,**
Glycine	.354 ± .052	1.924 ± .106*	1.832 ± .393*
Histidine	.072 ± .028	.045 ± .026	.037 ± .005
Isoleucine	.054 ± .006	.102 ± .019*	.090 ± .011
Leucine	.085 ± .008	.154 ± .025	.272 ± .068*
Lysine	.248 ± .036	.189 ± .026	.137 ± .0190
Methionine	.101 ± .024	.085 ± .022	.064 ± .013
Phenylalanine	.044 ± .004	.061 ± .006	.097 ± .014*
Serine	.696 ± .191	.641 ± .097	.595 ± .092
Threonine	…	…	…
Tyrosine	.198 ± .018	.084 ± .004*	.200 ± .048**
Valine	.129 ± .013	.135 ± .010	.168 ± .018
Total free amino acids	3.317 ± .529	10.409 ± .750*	6.847 ± .507*,**
Total essential amino acids	.961 ± .078	1.449 ± .054*	1.482 ± .183*

Note. Essential amino acids in italic.

* Statistically significant difference from ammocoetes ($P < 0.05$).

** Statistically significant difference from parasitic sea lampreys ($P < 0.05$).

intestine and muscle of parasitic lampreys, where GDH activities were two to four times greater than in ammocoetes. In upstream migrants, GDH activities in the liver and intestine were significantly reduced compared with the parasitic animals but were comparable to activities measured in the ammocoete liver (Fig. 4*A*). GDH activity in the muscle of the upstream migrants was comparable to activities measured in parasitic lampreys but fourfold greater than rates in ammocoete muscle (Fig. 4*A*).

Similar trends were also observed for ammocoete and parasitic lamprey hepatic AlaAT and AspAt, in which the activities of each enzyme were twofold to threefold greater in the parasitic lampreys (Fig. 4*B*, 4*C*). Differences in these two parameters were less obvious between parasitic and upstream migrants, where hepatic AlaAT and AspAt activity were similar between the two life stages (Fig. 4*B*, 4*C*). In the intestine, AlaAt activity was twofold higher in parasitic lampreys than in ammocoetes and upstream migrant lampreys (Fig. 4*B*). However, intestinal AspAt activity was 80% lower in parasitic animals than it was in ammocoetes and upstream migrants (Fig. 4*C*). AlaAt in muscle was slightly higher in upstream migrant compared with parasitic animals (Fig. 4*B*), while AspAt was about 50% higher in the parasitic phase compared with the ammocoetes and upstream migrant stages (Fig. 4*C*).

Because of the pivotal role of hepatic GDH in amino acid catabolism and ammonia generation in vertebrates, Western blots were performed to determine whether the observed increases in GDH activity in parasitic lampreys could be explained by greater quantities of hepatic GDH or, alternatively, by post-translational modifications (Fig. 5). Indeed, GDH protein quantity was greatest in the parasitic phase, where the relative amounts of GDH were 10-fold and threefold greater than in ammocoetes and upstream migrant lampreys, respectively (Fig. 5). It should also be noted that the polyclonal antibody used for this analysis, which was raised against rat GDH, produced two bands in liver homogenates taken from all major stages of the lamprey life cycle (Fig. 5*B*) but single bands in the eel, dogfish, and rat liver (data not shown).

GS activity was relatively low in the muscle of parasitic and upstream migrant lampreys (Fig. 6*A*). However, GS activity was 10-fold higher in the liver of parasitic lampreys than in the liver of upstream migrants (Fig. 6*A*). While Glnase activity was relatively low in the muscle, its activity was highest in the liver of upstream migrant lampreys, where Glnase activity was threefold greater than in the liver of parasitic lampreys (Fig. 6*B*).

Arg was also detected in the liver and muscle in ammocoetes (data not shown) and upstream migrants (Fig. 6*C*), but at relatively low levels. In contrast, Arg activities were approximately 20-fold greater in the liver of parasitic animals (Fig. 6*C*). Notably, Arg activity was below detection in the muscle of the parasitic lamprey (Fig. 6*C*).

Table 3: Amino acid concentrations (μmol g^{-1} wet weight) in the muscle of ammocoete, parasitic, and upstream migrant sea lampreys

Amino Acid	Ammocoete ($N = 8$)	Parasitic ($N = 8$)	Upstream Migrant ($N = 6$)
Alanine	.313 ± .038	1.397 ± .110*	2.008 ± .088*,**
Arginine	.392 ± .127	.471 ± .149	1.209 ± .161*,**
Aspartate	.095 ± .032	.036 ± .012	.389 ± .095 **
Glutamine	.315 ± .027	.320 ± .074	.602 ± .141
Glutamate	.309 ± .052	.641 ± .153	1.613 ± .312*
Glycine	.370 ± .045	.494 ± .085	2.062 ± .113*,**
Histidine	.228 ± .038	.032 ± .010*	.007 ± .006*
Isoleucine	.129 ± .017	.069 ± .011	.100 ± .023
Leucine	.194 ± .026	.115 ± .021	.162 ± .028
Lysine	.401 ± .101	.191 ± .034	.266 ± .120
Methionine	1.010 ± .108	.093 ± .029*	.092 ± .019*
Phenylalanine	.175 ± .027	.042 ± .009*	.108 ± .018*
Serine	.487 ± .028	.079 ± .025*	.255 ± .081
Threonine	.976 ± .193	.107 ± .025*	.275 ± .069*
Tyrosine	.197 ± .038	.043 ± .006*	.100 ± .012
Valine	.339 ± .048	.110 ± .019*	.144 ± .023*
Total free amino acids	5.930 ± .478	4.240 ± .467	9.390 ± .957*,**
Total essential amino acids	3.845 ± .334	1.230 ± .189*	2.362 ± .357*

Note. Essential amino acids in italic.

* Statistically significant difference from ammocoetes ($P < 0.05$).

** Statistically significant difference from parasitic sea lampreys ($P < 0.05$).

Hepatic versus Extrahepatic Urea Production

Although the key enzyme of the OUC, CPS III, was detected in the liver of upstream migrant lampreys (mean activity = 0.004 ± 0.001 μmol min^{-1} g^{-1}; $N = 8$), OCT was below detection (data not shown). CPS III and OCT were also below detection in the muscle and intestine of ammocoete, parasitic, and upstream migrant lampreys (data not shown).

Discussion

The greater mass-specific rates (expressed in nmol N g^{-1} h^{-1}) of J_{Amm} and J_{Urea} in postmetamorphic and parasitic sea lampreys compared with ammocoetes likely reflect a true elevation of N-waste production rates and amino acid catabolism capacity (Fig. 1). This premise is supported by two lines of evidence. First, whole body J_{Amm} and J_{Urea} (expressed as nmol N h^{-1}) increased with body mass when all life stages of the sea lamprey were considered (Fig. 2), as expected. However, when the effects of body mass were removed using residual analysis, whole body J_{Amm} and J_{Urea} were lower than expected in ammocoetes but greater than expected in postmetamorphic and parasitic lampreys (Fig. 2), suggesting the increases were truly life stage dependent. Second, unlike J_{Amm} and J_{Urea} in this study, most mass-specific rates (e.g., excretion rates, oxygen consumption) and enzyme activities in animals, with the exception of anaerobic glycolysis in fish locomotory muscle, decrease with body size (Brett and Groves 1979; Hochachka and Somero 2002). In other words, had excretion rates followed the allometric relationships of other animals, mass-specific rates would have decreased, not increased. Thus, the increases in mass-specific excretion rates, as well as tissue enzyme activities, observed following metamorphosis reflect real life stage differences.

The much lower $J_{N\text{-}waste}$ of burrow-dwelling ammocoetes compared with parasitic and upstream migrant lampreys is likely associated with their low metabolic rate (Potter and Rogers 1972; Holmes and Lin 1994; Wilkie et al. 2001), which is about 50% lower than in comparably sized lampreys immediately following metamorphosis (Lewis 1980). Because the ammocoete diet is mainly detritus of low nutritive value, digestion takes much longer in this life stage (Sutton and Bowen 1994). This factor, plus lower energy demands (Lewis 1980), is likely accompanied by lower basal rates of amino acid catabolism. The low activities of amino acid catabolizing enzymes such as GDH and AlaAt in liver, intestine, and muscle further support this hypothesis.

Following metamorphosis, amino acid catabolizing capacity is markedly elevated when sea lampreys ingest large amounts of protein-rich blood as they parasitize fishes. Indeed, parasitic sea lampreys in the size range examined (5–10 g) consume blood at rates ranging from 2.0% to 16.3% of their body weight per day on a dry weight basis while feeding on salmonids (Farmer et al. 1975). Thus, if the water content of trout whole

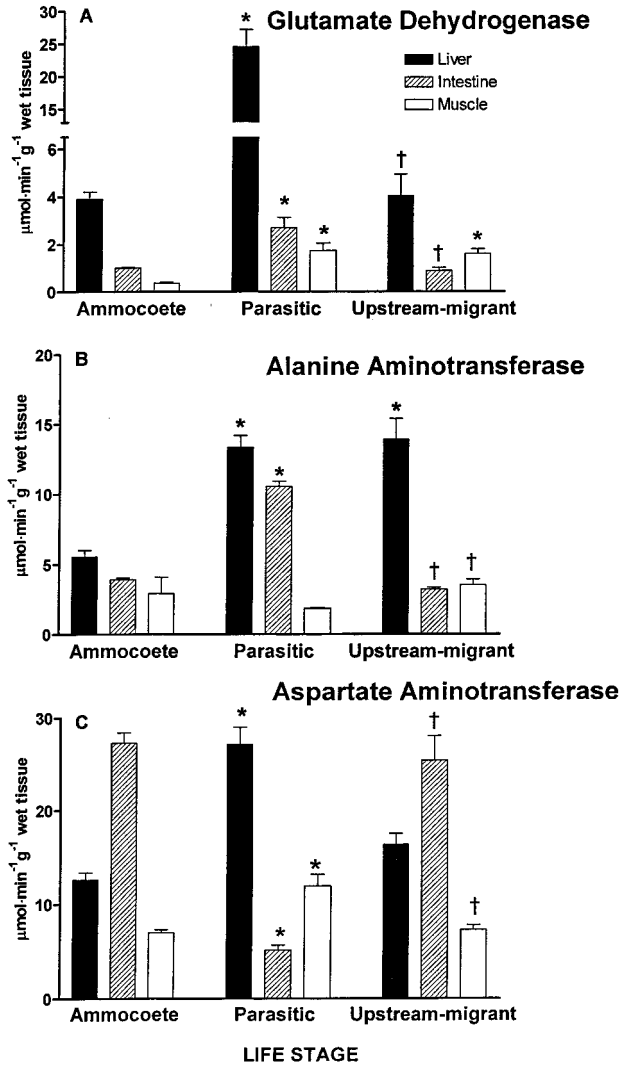

Figure 4. Maximum enzyme activities of (*A*) glutamate dehydrogenase, (*B*) alanine aminotransferase, and (*C*) aspartate aminotransferase in the liver (*solid bars*), intestine (*hatched bars*), and muscle (*open bars*) of ammocoete ($N = 7$), parasitic ($N = 7$–9), and upstream migrant ($N = 7$–9) sea lampreys. For each respective tissue, asterisks denote statistically significant differences in enzyme activities between ammocoetes and parasitic and upstream migrant lampreys, while daggers indicate significant differences between parasitic and upstream migrant lampreys at $P < 0.05$.

blood was 85% (Farmer 1980) and the protein content 82 g L^{-1} (D. Carapic and M. P. Wilkie, unpublished data), the protein content of a blood meal would be 55% on a dry weight basis. Assuming each gram of protein contains 0.16 g N (Wood 2001), the total N ingested would be 1.75–14.25 mg N g^{-1} d^{-1}, the equivalent of 125,000–1,000,000 nmol N g^{-1} d^{-1}. Thus, a feeding lamprey may ingest five times the protein or N load as an intensively feeding salmon (2.56 mg g^{-1} protein day^{-1} or 183,000 nmol N g^{-1} d^{-1}; Wood 2001).

A high capacity to deaminate surplus amino acids would permit parasitic lampreys to liberate and retain carbon skeletons that could be oxidized or used for gluconeogenesis or lipogenesis. Several observations support this "elevated amino acid catabolism hypothesis." First, respective basal rates of $J_{N\text{-waste}}$ were sixfold to 20-fold greater in postmetamorphic and parasitic lampreys compared with ammocoetes. Greater total metabolic demands alone cannot explain this increase, because mass-specific oxygen consumption is no more than twofold greater in parasitic animals than in ammocoetes (Beamish 1973; Lewis 1980). Second, postprandial J_{Amm} by parasitic lampreys increases 10-fold to 25-fold after ingestion of blood from domestic trout or basking sharks (*Cetorhinus maximus*) before returning to basal (nonfeeding) rates after 3–7 d (Wilkie et al. 2004). In contrast, postfeeding $J_{N\text{-waste}}$ increases only twofold to sixfold in intensively feeding hatchery-reared sockeye salmon (*Oncorhynchus nerka*; Brett and Zala 1975) and trout (Alsop and Wood 1997; Martin et al. 2003). Third, the activity and quantity of hepatic GDH in parasitic lampreys is several-fold greater compared with ammocoetes and upstream migrants (Figs. 4*A*, 5). Because the parasitic lampreys were not fed for 7–10 d, this greater GDH activity is likely life-history stage dependent and not related to actual feeding per se. Greater

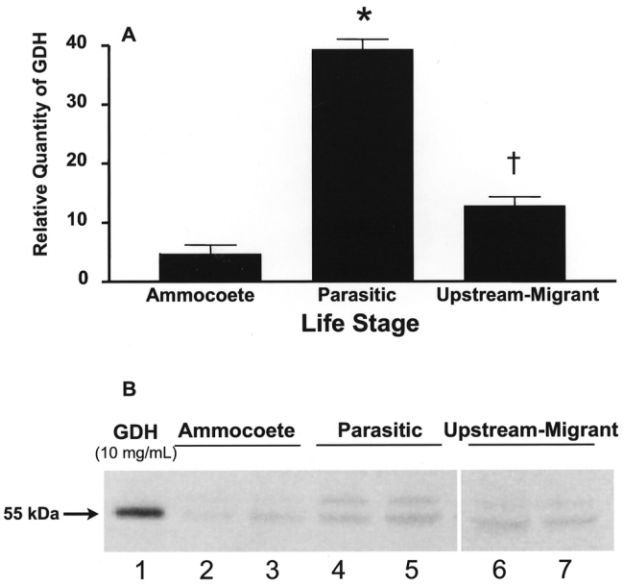

Figure 5. *A*, Relative quantity of glutamate dehydrogenase (GDH) in the liver of ammocoete ($N = 3$), parasitic ($N = 4$), and upstream migrant ($N = 4$) sea lampreys evaluated using densitometry. Asterisks denote statistically significant differences in values between ammocoetes and parasitic and upstream migrant lampreys, while daggers indicate significant differences between parasitic and upstream migrant lampreys at $P < 0.05$. *B*, Representative immunoblots depicting GDH expression in ammocoete (lanes 2, 3), parasitic (lanes 4, 5), and upstream migrant (lanes 6, 7) lampreys using antirat GDH. Lane 1 is loaded with 10 μg μL^{-1} of pure mammalian (bovine) GDH.

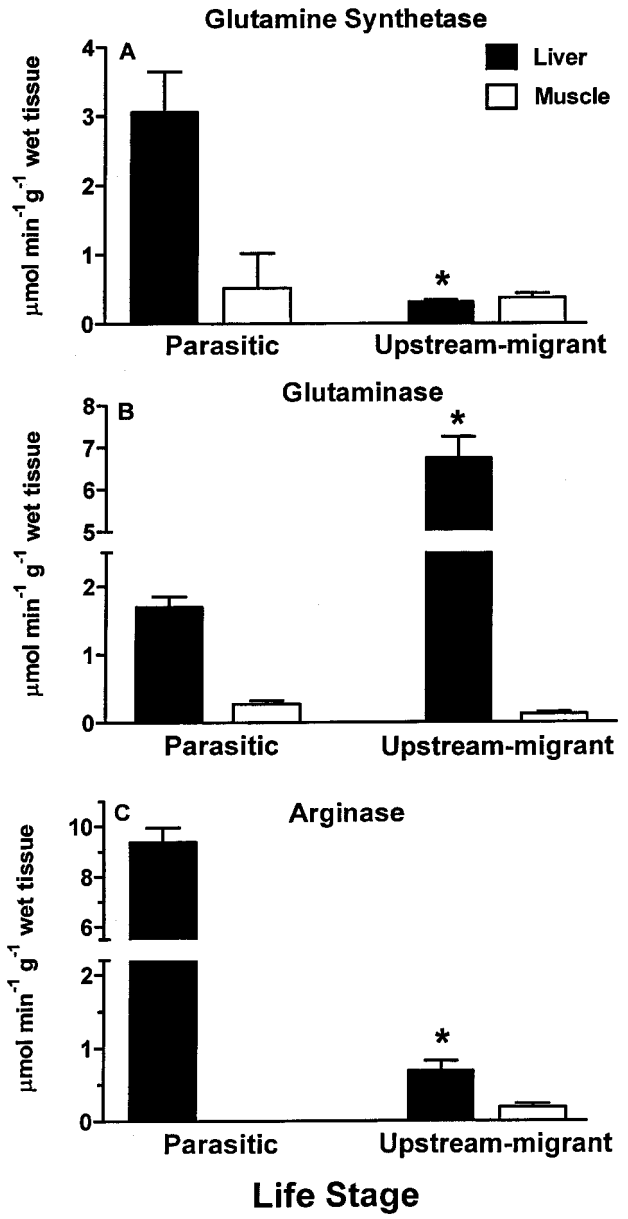

Figure 6. Maximum enzyme activities of (*A*) glutamine synthetase, (*B*) glutaminase, and (*C*) arginase in the liver (*solid bars*) and muscle (*open bars*) of parasitic ($N = 7–9$) and upstream migrant ($N = 7–9$) sea lampreys. For each respective tissue, asterisks denote statistically significant differences in enzyme activities between parasitic and upstream migrant lampreys at $P < 0.05$. Note that in *C*, arginase was below the level of detection in the muscle of parasitic lampreys.

overall GDH activity in this life stage probably ensures that there is sufficient capacity to catabolize intermittently large postprandial amino acid loads when opportunistic parasitic lampreys locate their prey/hosts.

The increased activities of hepatic AlaAt and AspAt, which supply glutamate for the GDH complex (Walton and Cowey 1982; Mommsen and Walsh 1992), are also consistent with a greater amino acid catabolizing capacity. The greater free glutamate and alanine concentrations in the liver (Table 2) relative to the ammocoete and upstream migrant stages also suggest a greater glutamate generation capacity in the parasitic lampreys. It should be emphasized that because the parasitic lampreys were starved 7–10 d before experiments, these differences in enzyme activities and amino acid concentrations would likely have been greater if sampling took place immediately after feeding. Evidence supporting this prediction includes the 10-fold to 25-fold greater J_{Amm} observed in parasitic lampreys after feeding (Wilkie et al. 2004) and observations that aminotransferase activities are positively correlated with protein loading in some fishes (Gallagher et al. 2001; Martin et al. 2003). Further investigation is therefore required to determine whether transaminase and GDH activities, as well as amino acid concentrations, in parasitic lampreys are even greater immediately after feeding.

Despite the early divergence of agnathan and gnathostome fishes (Forey and Janvier 1993), upstream migrant lampreys and migrating salmonids use very similar strategies of energy production and storage during this period of natural starvation. These include reliance on accumulated lipid (Bentley and Follet 1965; Beamish et al. 1979; Jonsson et al. 1997; Youson 2003; Kiessling et al. 2004), the sparing of muscle glycogen (Bentley and Follett 1965; Beamish et al. 1979), and muscle proteolysis (Beamish et al. 1979; Mommsen et al. 1980; Emelyanova et al. 2004). This study also demonstrates that the handling of amino acids arising from proteolysis is similar to patterns seen in migrating salmonids (Mommsen et al. 1980; Kiessling et al. 2004). The twofold greater free amino acid concentration in the muscle of upstream migrant compared with parasitic lampreys (Table 3) was therefore likely indicative of significant proteolysis in the former. Although plasma amino acid pools gradually increase during migration in the sockeye salmon (Mommsen et al. 1980) and in overwintering, nonfeeding river lamprey (*Lampetra fluviatilis*; Emelyanova et al. 2004), plasma amino acid concentrations in upstream migrant lampreys were similar to concentrations in ammocoetes and parasitic lampreys. Because the upstream migrant sea lampreys were sampled only once, we do not know how plasma amino acid concentrations were temporally influenced during this life stage, but this should be investigated.

Lipid oxidation is vital to satisfy the naturally starving sea lamprey's energy requirements during upstream migration and spawning (Bentley and Follet 1965; Beamish et al. 1979). However, shifts in protein and amino acid catabolism patterns are also necessary to spare glycogen and satisfy routine energy demands during this period. The decrease in hepatic GDH activity (Fig. 4) and quantity (Fig. 5) in upstream migrant lampreys suggests the liver's amino acid catabolizing capacity was lower in relation to the parasitic phase. However, hepatic AlaAt and AspAt activities were comparable to those in the parasitic phase

(Fig. 4), suggesting that the liver's transamination capacity was unaltered. Because alanine is the major gluconeogenic substrate in fishes (Ballantyne 2001), maintaining AlaAt activity could be essential for maintaining hepatic gluconeogenic capacity (LeBlanc et al. 1995) during natural starvation in upstream migrants.

As first proposed in the migrating sockeye salmon (Mommsen et al. 1980; French et al. 1983; Mommsen 2003), alanine is likely the major carrier of amino acid carbon and nitrogen to gluconeogenic and ammoniogenic tissues such as the liver in the upstream migrant lamprey. In this model, the majority of free amino acids liberated by muscle proteolysis are transaminated to various citric acid cycle intermediates, converted to pyruvate, and then transformed to alanine via AlaAt. The alanine is then transported to the liver, where it is transaminated to glutamate, yielding pyruvate that is either oxidized or used for gluconeogenesis. Three observations in this study support this "alanine shuttle" model in upstream migrant sea lampreys. First, free alanine concentrations in the muscle of upstream migrant lampreys were greater than in the parasitic phase and comparable to increases measured in white and red muscle of migrating sockeye salmon (Mommsen et al. 1980). Second, the AlaAt activity in the muscle of upstream migrant lampreys was twofold greater than in parasites, lying between values reported for the red muscle and white muscle of the sockeye (Mommsen et al. 1980). Finally, glutamate concentrations were threefold greater in the muscle of upstream migrant than in parasitic lampreys. For the "alanine shuttle" model to be viable, such glutamate would be essential for driving alanine formation via AlaAt.

One possible source of glutamate is through the hydrolysis of glutamine via Glnase. LeBlanc et al. (1995) reported that Glnase activities were one to three orders of magnitude greater than GS in the muscle and liver of upstream migrant sea lamprey, suggesting that net glutamine catabolism in these tissues led to net glutamate formation. While the Glnase activities in the muscle and liver of upstream migrant sea lampreys in this study (Fig. 6) were comparable to those reported by LeBlanc et al. (1995), the tissue GS activities we report were fivefold to 20-fold greater. However, it should be noted that the GS assay used in this study measured glutamyl transferase activity, not synthetase activity, and was therefore an indirect index of actual GS activity (Shankar and Anderson 1985; Walsh 1996). Previous studies have demonstrated that the ratio of transferase : synthetase activity is about 15 : 1 (Shankar and Anderson 1985; Walsh 1996). When GS activities are corrected using this ratio, the GS activities of both liver and muscle are approximately 0.03 μmol min^{-1} g^{-1} in upstream migrant lampreys. As a result, the actual Glnase : GS ratio in both the liver (~320 : 1) and muscle (~5 : 1) of upstream migrants favors net glutamine catabolism, or glutamate production, in agreement with LeBlanc et al. (1995).

This study demonstrates that urea accounts for about 10%–20% of total N-waste excretion at all major stages of the sea lamprey's life cycle (Fig. 1). Read (1968) detected traces of urea in the urine of upstream migrant Pacific lampreys and attributed it to arginine hydrolysis. Indeed, the threefold greater free arginine concentrations in the muscle of upstream migrant sea lampreys compared with other life stages (Table 3) suggest significant arginine was generated from muscle proteolysis. Although detected at low levels, the Arg activity in the muscle of upstream migrants (Fig. 6) would likely be sufficient to generate small amounts of urea from this larger, excess arginine pool. In addition, relatively high hepatic arginine concentrations, plus significant Arg activity, likely contributed to urea formation in the liver of the upstream migrants (Table 2; Fig. 6). In contrast, Arg activity was below detection in the muscle of parasitic lampreys (Fig. 6*C*). However, the much higher Arg activities in the liver of parasitic lampreys (Fig. 6*C*) compared with other life stages, plus high concentrations of arginine, suggest there may be significant hepatic urea production via this route. Unlike the situation in upstream migrants, arginine is likely acquired from the diet as parasitic sea lampreys feed on the blood of fishes (Wilkie et al. 2004). Indeed, greater dietary arginine loads have been reported to increase plasma urea and J_{Urea} in rainbow trout and turbot (*Psetta maxima*; Fournier et al. 2003). An additional explanation is that J_{Urea} by parasitic lampreys arises from the coingestion of urea as they ingest blood from teleosts or perhaps elasmobranchs (Wilkie et al. 2004). For instance, plasma urea concentrations may approach 5 mmol N L^{-1} in trout (Wilkie and Wood 1991) and range from 600 to 700 mmol N L^{-1} in elasmobranchs (Schmidt-Nielsen et al. 1972; Wood et al. 1995).

Urea production in the sea lamprey does not appear to be due to the OUC. Key OUC enzymes such as CPS III and OCT were either not present or not detectable in the liver, as in ammocoetes (Wilkie et al. 1999), or, in the case of parasitic (Wilkie et al. 2004) and upstream migrant lampreys, active only at low levels. Nor does it appear that extrahepatic tissues contribute to OUC-derived urea production in the lamprey due to the absence of CPS III and OCT activity in muscle and intestine. Nevertheless, the possibility that OUC enzymes are activated during earlier stages of embryonic or larval development—or even during metamorphosis, when there is a major reorganization of the kidneys, liver, and gills (Youson 1980; Rovainen 1996)—remains to be elucidated. Indeed, CPS III and OCT activities are greatest in the tissues of larval embryonic cod and trout before declining in later life stages (Wright et al. 1995; Chadwick and Wright 1999).

The life history and body structure of the lamprey has been highly conserved for at least 350 million years (Forey and Janvier 1993). Part of this success is due to adaptations such as a highly specialized oral disc and rasping tongue and the secretion of the anticoagulant lamphredin (Farmer 1980), which allows parasitic lampreys to attach to and feed on the blood of fishes (Rovainen 1996). We demonstrate that the onset of the parasitic

phase is also accompanied by a metabolic reorganization that increases the sea lamprey's capacity to deaminate amino acids arising from such protein-rich blood meals. In particular, the greater activities of GDH, AlaAT, and AspAt in the liver ensure there is sufficient capacity to catabolize intermittently large postprandial amino acid loads when opportunistic parasitic lampreys locate their prey/hosts. This allows parasitic lampreys to liberate and retain carbon skeletons that can be oxidized or stored as glycogen and lipids, the essential fuel reserves needed for upstream migration and ultimately spawning.

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