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Estimating the quantitative essential amino acid requirements of striped bass *Morone saxatilis*, using fillet A/E ratios

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Abstract

In order to determine the essential amino acid requirements (EAA) of striped bass *Morone saxatilis*, fillets were analysed to ascertain the relative amino acid concentrations for determining A/E ratios ((EAA/total EAA) × 1000)). Analysis of the striped bass fillets yielded the following concentrations of essential amino acids (g kg^{-1}) and A/E ratios, respectively: arginine, 12.5, 115; histidine, 5.1, 47; isoleucine, 8.0, 74; leucine, 17.1, 157; lysine, 20.2, 186; methionine + cysteine, 9.2, 85; phenylalanine + tyrosine, 16.0, 147; threonine, 9.8, 90; tryptophan, 1.9, 18; and valine, 9.1, 84. In two experiments, diets with graded levels of EAA were fed to striped bass weighing 111 ± 3 g and 790 ± 122 g per fish, respectively. In both experiments, the dietary A/E ratios were maintained in the same relative concentrations as determined in the striped bass fillets. Statistical analysis of weight gains, feed conversions and nitrogen balance indicated significant differences ($P < 0.05$) between treatments. Non-linear regression analysis of the response criteria pooled from both experiments yielded the following estimates of dietary EAA requirements (g kg^{-1} dry diet) when digestible energy equalled 13.39 MJ kg^{-1} diet: arginine, 14; histidine, 6; isoleucine, 9; leucine, 19; lysine, 22; methionine + cysteine, 10; phenylalanine + tyrosine, 17; threonine, 11; tryptophan, 3; and valine, 10. The use of fillet A/E ratios allows for the rapid estimation of quantitative EAA requirements and the development of species specific diets for new aquaculture species. The data presented here are the first to simultaneously describe all the dietary EAA requirements for *M. saxatilis*.

KEY WORDS: A/E ratios, amino acid requirements, striped bass

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Introduction

Striped bass *Morone saxatilis* are important both as a game fish and a food fish. Generally, in the aquaculture industry, striped bass are crossed with the white bass *Morone chrysops* in order to produce a hybrid striped bass, which is the industry standard. Therefore, the striped bass female or male, depending on the hybrid produced (traditional vs. reciprocal cross), is an integral part of hybrid striped bass aquaculture. At present, only a few of the nutritional requirements for the pure striped bass have been established.

Since protein is the most costly portion of feeds, defining the quantitative essential amino acid (EAA) requirements of the aquacultural striped bass is of major economic importance. The complete quantitative EAA requirements have been established for only a limited number of cultured fish species, including rainbow trout *Oncorhynchus mykiss* (Ogino 1980), Nile tilapia *Oreochromis niloticus* (Santiago & Lovell 1988), catla *Catla catla* (Ravi & Devaraj 1991),common carp *Cyprinus carpio* (Nose 1979), Japanese eel, *Anguilla japonica*, (NRC 1993), channel catfish *Ictalurus punctatus* (NRC 1993), Chinook salmon *Oncorhynchus tshawytscha* (NRC 1993), coho salmon *Oncorhynchus kisutch* (Arai & Ogata 1993), chum salmon *Oncorhynchus keta* (Akiyama & Arai 1993), milkfish *Chanos chanos* (Borlongan & Coloso 1993) and white sturgeon *Acipenser transmontanus* (Ng & Hung 1995).

To date, most published amino acid requirements have been determined by individual dose–response feeding trials. This type of methodology requires a series of separate experiments to determine each of the EAA requirements. This is both time-consuming and very costly. As an alternative approach, a species tissue A/E ratio, (EAA/total EAA) × 1000), might be utilized to estimate amino acid requirements in an attempt to determine all 10 EAA requirements simultaneously.

Various investigators have used tissue A/E ratios to formulate early test diets for new species, providing the first estimates of a species EAA requirements. The concept of A/E ratios was introduced by Arai (1981) when formulating test diets for coho salmon. Fish fed diets supplemented with amino acids to simulate the A/E ratios of whole-body tissue demonstrated improved growth and feed efficiency. Ogata *et al.* (1983) based the dietary amino acid composition on the A/E ratios of whole cherry salmon fry, eyed cherry salmon eggs and white fish meal when formulating test diets for cherry salmon *Oncorhynchus masou* and amago salmon *Oncorhynchus rhodurus*. Diets supplemented with amino acids to simulate whole-body tissue resulted in higher growth rates for both species.

Cowey & Tacon (1983) showed a high correlation between the pattern of dietary amino acids required by carp, as determined by Nose (1979), and the pattern of amino acids in carp whole body tissue. Significant correlations were also found when the EAA requirements of channel catfish were regressed against the EAA composition of whole-body tissue (Wilson & Poe 1985). Brown (1995), assuming that a high correlation also existed with hybrid striped bass, used whole-body A/E ratios to establish a series of studies to quantify the complete EAA requirements for the hybrid, *Morone saxatilis* × *M. chrysops*.

Growth can be characterized by a net accretion of amino acids, with the pattern of amino acids required in the diet of growing animals bearing a close similarity to the pattern in their lean tissue (Fuller *et al.* 1989). This reflects the concept that the amino acid requirements of a growing animal are determined by the amino acid composition of the tissue proteins formed during growth and, furthermore, that an animal's amino acid requirements might first be deduced from the amino acid composition of its tissues (Mitchell 1950). Cowey & Luquet (1983) suggested that the EAA requirements of fish are related to the amino acid pattern in muscle protein, since muscle is the major product in immature growing fish. This suggestion is in line with observations in other animals. In the swine industry, the pattern of amino acids in skeletal muscle has been established as the model for an ideal protein and used to deduce the EAA requirements of pigs (Cole 1978 and Fuller *et al.* 1979).

The purpose of these studies was to provide an estimate of the minimum dietary requirements of the striped bass for the 10 essential amino acids, using the amino acid pattern (A/E ratios) of striped bass fillets as a model.

Materials and methods

Juvenile striped bass *M. saxatilis* were obtained from L. Curry Woods of the Department of Animal and Avian Sciences hatchery at the Crane Aquaculture Facility, Baltimore, MD, USA as fingerlings, and reared in circular fibreglass tanks to the sizes indicated for both experiments. All fish were injected with passive integrated transponders (PIT) for individual identification. In experiment 1, striped bass with an average weight (± SE) of 111 ± 3 g per fish were stocked in 1000 L tanks at 20 fish per tank. In experiment 2, striped bass weighing 790 ± 122 g per fish were stocked in 2200 L tanks at 26 fish per tank. For 1 month prior to starting both experiments all fish were fed a common commercial diet.

Fish were reared in tanks supplied with dechlorinated water in a flow-through system at a rate of two complete turnovers per day for both experiments. A calcium chloride and sodium chloride (9:1) solution was injected directly into the incoming water line at a continuous rate in order to achieve a minimum concentration of 36 mg L^{-1} calcium in the rearing tanks. A photoperiod of 12 h light and 12 h dark was maintained throughout both experiments. Water temperature was maintained at ≈ 23°C and recorded four times daily via a central computerized monitoring system (REES Scientific, Trenton, NJ, USA). All other parameters were monitored weekly throughout the experiments to ensure safe levels of pH, dissolved oxygen, ammonia, chlorine and Ca/hardness. Dissolved oxygen and temperature were measured with a YSI model 58 oxygen meter (Corning Incorporated, Corning, NY, USA). pH was monitored with a Corning model 250 Ion analyser. Chlorine, both free and total, and ammonia were measured using a Hach DR/700 colorimeter (Hach Company, Loveland, Co, USA). Calcium concentrations were determined with a Hach model HA-4P hardness test kit and by atomic absorption spectrophotometry using a Perkin-Elmer 5100 PC atomic absorption spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT, USA). In both experiments, the environmental conditions were maintained at safe levels, as outlined by Nicholson *et al.* (1990).

In experiment one, five semi-purified diets were fed in duplicate to 10 tanks of striped bass for a period of 12 weeks. Diets were formulated to provide a pattern of highly bioavailable amino acids with dietary A/E ratios identical to the A/E ratios of striped bass fillets. Diets were determined to be isonitrogenous by micro-Kjeldahl analysis and calculated as isoenergetic using published digestible energy (DE) values (NRC 1993). Striped bass fillets provided the primary source of intact protein in the five diets (Table 1).

Table 1 Composition of diets for determining the amino acid requirements of juvenile striped bass using fillet A/E ratios (experiment 1)[1]

	Diet				
Ingredients	1	2	3	4	5
Amino acid premix[2]	38	51	62	74	88
Calcium phosphate	25	25	25	25	25
Cellulose	0	7	13	20	29
Dextrin	290	290	290	290	290
Glutamic acid	274	254	237	218	195
Lignin sulphonate	10	10	10	10	10
Menhaden fish oil[3]	60	60	60	60	60
Menhaden fish solubles[3,4]	20	20	20	20	20
Mineral premix[5]	12	12	12	12	12
Sodium bicarbonate	110	110	110	110	110
Stay C[6]	1	1	1	1	1
Striped bass fillet	150	150	150	150	150
Vitamin premix[7]	10	10	10	10	10

[1]Ingredient concentrations expressed as g kg^{-1} fed.
[2]Contains (as g kg^{-1} premix): arginine, 115; histidine, 47; isoleucine, 74; leucine, 157; lysine, 185; methionine, 84; phenylalanine, 146; threonine, 90; tryptophan, 18; valine, 84.
[3]Zapata Corp., Reedville, VA, USA.
[4]50% Moisture.
[5]Contains (as mg kg^{-1} diet): KCl, 0.008; NaCl, 7.76; $MgSO_4$, 3.15; Fe citrate, 0.24; $MnSO_4$, 0.08; $ZnCO_3$, 0.12; $CuSO_4$, 0.004; KI, 0.0008; Na_2SeO_3, 0.00024.
[6]Calcium ascorbate 2 monophosphate: Hoffman–La Roche, Inc., Nutley, NJ, USA
[7]Contains (as mg kg^{-1} diet unless stated otherwise noted): choline chloride, 3465; inositol, 396; niacin, 153; α-tocopherol acetate, 45; calcium pantothenate, 50.4; riboflavin, 20.7; menadione sodium bisulphate, 9.9; thiamin, 12.6; pyridoxine-HCl, 12.6; cyanocobalamin (3000 mg g^{-1}), 5.8; folic acid, 5.4; retinyl acetate, 3.9; biotin, 4.5; cholecalciferol, 5 mg kg^{-1}; Ethoxyguin (antioxidant, Monsanto Corp., St. Louis, MO, USA), 125.

Approximately 3 kg of striped bass muscle was prepared as a dietary ingredient by filleting 2-year-old bass. Fillets were ground through a commercial meat grinder and homogenized. The homogenized fillets were then dried in a forced-air oven at 60°C overnight. Proximate analyses were performed on striped bass fillets prior to drying and diet formulation (Table 2). An incremental increase in dietary EAA was accomplished through the addition of a crystalline amino acid premix. Glutamic acid and cellulose were added to the diet to keep the diets isonitrogenous and isoenergetic, respectively. Diets were extruded through a commercial meat grinder (Hobart Corp., Troy, OH, USA), dried in an oven at 40°C overnight and stored in air-tight containers at 4°C.

In experiment 2, four diets were formulated with practical feed ingredients and fed in triplicate to 12 tanks of striped bass for a period of 12 weeks. As in experiment 1, the dietary pattern of amino acids were modelled on striped bass fillets (Table 3).

Table 2 Mean nutrient concentrations of striped bass *Morone saxatilis* fillets analysed[1]

Nutrients	g kg^{-1}	A/E ratio
Proximate analysis		
Ash	10.4	
Fat	37.3	
Moisture	745.9	
Protein	208.0	
Essential amino acids		
Arginine	12.5	115
Cysteine	2.7	25
Histidine	5.1	47
Isoleucine	8.0	74
Leucine	17.1	157
Lysine	20.2	186
Methionine	6.5	60
Phenylalanine	8.8	81
Threonine	9.8	90
Tryptophan[2]	1.9	18
Tyrosine	7.2	66
Valine	9.1	84

[1]$n = 2$.
[2]USDA (1987).

Owing to the practical nature of the ingredients used, diets could not be formulated as isonitrogenous nor could the A/E ratios be maintained exactly. Lysine was calculated to be the first limiting component in these diets. Thus, as the concen-

Table 3 Composition of practical diets for determining the amino acid requirements of striped bass (experiment two)[1]

	Diet			
Ingredient	1	2	3	4
Blood meal	20	20	35	50
Feather meal	0	45	70	100
Lysine HCl	0	10	10	12
Menhaden fish meal	165	220	230	220
Menhaden fish oil	80	80	75	70
Menhaden fish solubles	50	50	20	20
Mineral premix no. 3[2]	1	1	1	1
Stay C[3]	1	1	1	1
Vitamin premix no. 2[4]	15	15	15	15
Wheat bran	78	69	63	41
Wheat flour	190	180	180	180
Wheat middlings	400	315	300	290

[1]Ingredient concentrations expressed as g kg^{-1} fed.
[2]Contains (as mg kg^{-1} diet): $ZnSO_4$, 74.8; $MnSO_4$, 20.02; $FeSO_4.7H_2O$, 6.6; $CuSO_4$, 1.54; $C_2H_8N_2$–2HI, 9.99.
[3]Calcium ascorbate 2 monophosphate: Hoffman–La Roche, Inc., Nutley, NJ, USA.
[4]Contains (as mg kg^{-1} diet unless otherwise noted): myo-inositol, 132; niacin, 220.1; D-biotin, 0.6; D-pantothenic acid, 105.6; riboflavin, 52.8; vitamin K, 9.2; thiamin, 42.9; vitamin B_{12}, 0.0594; folic acid, 16.5; vitamin B_6, 30.9, vitamin A, 6600 IU kg^{-1}; vitamin E, 502 IU kg^{-1}; vitamin D_3, 1320 IU kg^{-1}; ethoxyquin (antioxidant), 99.

tration of lysine was varied in the diet so were the other essential amino acids at, or slightly higher than, the ratio defined by the fillet A/E ratios. Diets were again calculated to be isoenergetic using published DE values (NRC 1993). The diets for experiment two were mixed and extruded by Perdue Specialty Feeds, Catawissa, PA, USA, then stored at 4°C.

In both experiments, fish were fed twice daily Monday to Friday and once daily on Saturday and Sunday. During each feeding, fish were fed to satiation by hand and the total feed intake was recorded.

At the start of experiment one, 20 fish were killed by overdose of tricaine methanesulphonate (MS-222) (Argent, Redman, WA, USA) and stored at – 20°C for subsequent analysis of the whole carcass. At the start of both experiments fish were anaesthetized with 0.2 g L^{-1} MS-222 and weighed. The weights were recorded for individual fish according to the PIT identification. In experiment 1, all fish were weighed every 4 weeks following a 1-day fast until two periods of statistically different weight gains had been observed. The experiment lasted a total of 12 weeks. At the last sampling all fish were again weighed. Four fish per treatment, two samples per replication (tank), were killed and stored at – 20°C for subsequent analysis.

Experiment 2 followed the same sampling procedures as experiment 1, except that no fish were sacrificed for analysis. Weighing was again performed every four weeks following a one day fast for a period of 12 weeks. At the first and last sample period, all fish were weighed and the weights recorded accordingly. At the second and third sampling periods, 10 fish were randomly selected from each tank and weighed.

For both experiments, samples for chemical analysis were weighed and dried to obtain total moisture and then finely ground. Analysis of amino acids were performed on fillets and finished diets. Samples were hydrolysed in duplicate in 6 M HCl at 110 °C for 24 h before chromatographic separation using a Dionex D-600 amino acid analyser (Dionex, Smyrna, GA, USA). For methionine and cystine, samples were first treated with performic acid to oxidize methionine and cystine to methionine sulphone and cysteic acid before the hydrolysis (Moore & Stein 1963; Schram *et al.* 1954). Tryptophan, however, was degraded by the acid hydrolysis and could not be recovered by this method. Instead, literature values were used to estimate tryptophan in fillets and diet ingredients. Proximate analysis for protein, moisture, ether extract, and fibre in finished diets and fish carcasses were determined by micro-Kjeldahl analysis and standard methods for proximate analysis (AOAC 1984).

Growth indices were calculated using the following equations:

$$\text{Feed conversion ratio, FCR} = C/(W_t - W_o) \quad (1)$$

$$\text{Protein efficiency ratio, PER} = (W_t - W_o)/PF \quad (2)$$

$$\text{Apparent nitrogen utilization, ANU} = (ND/NI) \times 100 \quad (3)$$

where C is total food intake over the experiment (g), W_o is the initial weight of fish (g), W_t is the final weight of fish (g), PF is crude protein fed (g), ND is nitrogen deposition (g) and NI is nitrogen intake (g).

Data were analysed by analysis of variance mixed model procedures (SAS 1992). Pair-wise contrasts were used to identify significant differences at the 5% level between the means of the dependent variable for different amino acid levels. Non-linear procedures were used to fit two regression lines to identify the dietary amino acid levels corresponding to the joint-point of the lines. The non-linear regression model was adapted from the model described in the SAS System for Regression, Non-linear Models, Fitting Splines with Unknown Knots (SAS 1991). The model used fitted the slope of the first line, the dependent variable value for a second line with zero slope, and the dietary amino acid level at the joint-point of the two lines (Dougall *et al.* 1996).

Results

Experiment 1

Analysis of the experimental diets indicated that the dietary A/E ratios were maintained in a pattern simulating fillet A/E

Table 4 Amino acid and protein composition of semi-purified diets used for determining the amino acid requirements of juvenile striped bass (experiment 1)[a]

	Diet				
Amino acid	1	2	3	4	5
Arginine	11.1	12.2	11.9	14.8	16.5
Cysteine	1.2	1.3	1.2	1.4	1.3
Histidine	4.5	5.1	4.3	6.1	6.6
Isoleucine	7.0	7.9	9.1	9.4	10.3
Leucine	15.0	17.0	17.2	20.2	22.1
Lysine	17.8	19.7	19.1	23.8	26.6
Methionine	6.6	7.6	7.9	9.3	10.4
Phenylalanine	10.2	11.7	12.0	14.8	16.6
Threonine	9.4	9.7	10.1	11.5	12.7
Tryptophan[2]	2.1	2.4	2.7	2.9	3.3
Tyrosine	3.9	4.0	3.9	4.0	3.8
Valine	8.0	9.1	10.4	10.7	12.0
Crude protein	360	360	360	360	360

[1]Concentrations expressed as g kg^{-1} dry diet.
[2]Tryptophan is a calculated value based on ingredient composition.

Table 5 Mean growth performance of striped bass after 12 weeks when fed diets containing graded levels of essential amino acids (experiment 1)

	Diet					
	1	2	3	4	5	Pooled SEM
Initial weight (g)	104 a	106 a	115 a	116 a	113 a	8
Final weight (g)	140 a	147 a	157 ab	174 ab	182 b	10
Weight gain (g)	36 a	41 a	42 a	58 b	69 b	4
Individual feed consumption (g day^{-1})	1.20 a	1.25 ab	1.35 ab	1.50 ab	1.55 b	0.10
FCR[1]	2.91 a	2.69 a	2.82 a	1.94 b	1.90 b	0.19
PER[2]	0.86 a	0.95 a	0.88 a	1.10 b	1.29 b	0.05
N intake (g)	6.9 a	7.2 ab	7.6 ab	8.4 ab	8.6 b	0.5
N retention (g)	1.2 a	1.0 a	1.2 a	2.2 b	2.5 b	0.2
ANU[3] (%)	17.1 a	14.3 a	14.9 a	25.9 b	29.0 b	1.5

Means (n = 2) in the same row not sharing the same letters are statistically different (P < 0.05).
[1]Feed conversion ratio.
[2]Protein efficiency ratio.
[3]Apparent nitrogen utilization.

ratios. However, there were virtually no differences between the EAA profiles of diets two and three (Table 4). This could only be explained as an error in diet preparation. Because diet three had a similar amino acid profile and similar growth performance, it was not entered into the subsequent regression analysis. Statistical analysis of mean growth performance indicated significant differences (P < 0.05) between treatments for the response criteria. The weight gains, FCR, PER, N-retention, and ANU of fish fed diets one, two and three were significantly lower than that of those fish fed diets four and five. Statistical separation of the means also occurred, but to a lesser extent, for final weights, individual feed consumption and nitrogen intake (Table 5).

Table 6 Amino acid and protein composition of practical diets used in experiment two for determining the amino acid requirements of juvenile striped bass[1]

	Diet			
Essential amino acids	1	2	3	4
Arginine	15.9	16.1	18.5	19.5
Cysteine	3.6	4.1	4.7	4.2
Histidine	7.1	7.5	7.7	8.3
Isoleucine	9.8	10.5	11.9	12.4
Leucine	17.9	18.7	22.1	24.2
Lysine	15.7	18.1	24.6	26.5
Methionine	5.6	5.7	6.7	7.9
Phenylalanine	12.0	11.9	13.3	17.5
Threonine	9.6	10.1	11.8	12.7
Tryptophan[2]	2.9	3.1	3.3	3.4
Tyrosine	6.4	6.8	8.0	9.7
Valine	13.5	14.3	17.4	19.2
Crude protein	310	330	380	420

[1]Concentrations expressed as g kg^{-1} dry diet.
[2]Tryptophan is a calculated value based on ingredient composition.

Table 7 Mean growth performance of striped bass after 12 weeks when fed practical diets containing graded levels of essential amino acids (experiment 2)

	Diet				
	1	2	3	4	Pooled SEM
Initial weight (g)	799 a	794 a	803 a	791 a	16
Final weight (g)	1019 a	1034 a	1152 b	1164 b	35
Weight gain (g)	220 a	240 a	349 b	373 b	26
Individual feed consumption (g day^{-1})	6.0 a	6.3 a	7.7 b	7.6 b	0.2
FCR[1]	2.32 a	2.25 a	1.87 ab	1.74 b	0.16

Means (n = 3) in the same row not sharing the same letters are statistically different (P < 0.05).
[1]Feed conversion ratio.

Experiment 2

Amino acid analysis of the diets fed in experiment 2 are given in Table 6. Significant differences (P < 0.05) between treatments did exist for final weights, weight gain, individual feed consumption and FCR. Growth performance of fish fed diets one and two was significantly lower than fish fed diets three and four, which demonstrated superior final weights, weight gains and individual feed consumption. Significant differen-

Table 8 Minimum available dietary essential amino acid requirements of juvenile striped bass (*Morone saxatilis*) as determined by fillet A/E ratios and non-linear regression analysis of response criteria

Amino acids	g kg^{-1} diet	mg kJ^{-1} DE
Arginine	14	1.046
Histidine	6	0.448
Isoleucine	9	0.672
Leucine	19	1.419
Lysine	22	1.643
Threonine	11	0.822
Tryptophan[1]	3	0.224
Valine	10	0.747
TAAA[2]	17	1.270
TSAA[3]	10	0.747

[1]Tryptophan was calculated based on ingredient composition.
[2]Total aromatic amino acids (phenylalanine + tyrosine).
[3]Total sulphur amino acids (methionine + cysteine).

ces were also determined for FCR, with differences occurring between diets two and four (Table 7).

Growth performance data for experiment 2 was regressed against the dietary lysine concentration, with all other essential amino acids being in the A/E ratio defined by the fillets. Published bioavailability data for the ingredients used in experiment 2 indicate an average amino acid bioavailability of 85% (NRC 1993). This bioavailability was factored into the EAA requirements estimated from experiment 2. Diets fed in experiment 1 were considered to be 100% available owing to purified nature of the diets. Non-linear regression analysis of the growth performance data from both experiments was pooled to yield our estimates of the minimum EAA requirements for the striped bass (Table 8). The quantitative EAA requirements are expressed as mg kJ^{-1} DE as well as g kg^{-1} diet because the quantity of dietary EAA required varies with the energy content of the diet.

Discussion

In experiment 1, fish with an average weight of 111 g were chosen to represent a late first-year, fast growing, production fish. After 8 weeks on experimental feed, significant differences were noted between treatments. At 12 weeks, it was apparent that amino acid limitation had occurred in fish fed diets one, two and three. Although diet five showed slightly better growth and protein utilization there were no statistical differences ($P < 0.05$) between diets four and five. This, together with the regression analysis, indicates that diet five is statistically excessive in amino acids and that these higher levels of amino acids would not be cost effective.

Experiment 2 was designed with the purpose of verifying the results of experiment 1 and also to test the determined EAA requirements on larger fish fed a more practical diet. Fish with an average weight of 790 g were chosen to represent the final grow-out stage in a normal production cycle. By verifying the validity of applying the EAA requirements previously determined, lower protein diets might be formulated to reduce the high dietary costs in the final grow-out period.

The results of studies using the amino acid patterns of fish eggs and body tissue proteins with aquacultured fishes have been encouraging. By formulating diets with amino acid patterns similar to body tissues, improved growth and feed efficiency have been demonstrated (Rumsey & Ketola 1975; Arai 1981; Ketola 1982; Ogata *et al.* 1983). Brown (1995) used whole-body A/E ratios in conjunction with published quantitative requirements to predict the amounts of other essential amino acids required by hybrid striped bass. The EAA pattern of whole-body tissue for the hybrid correlated well ($r = 0.86$) with established EAA requirements. A high correlation of EAA requirements for established aquaculture species to the whole-body tissue of that species has also been demonstrated with common carp (Cowey & Tacon 1983), channel catfish (Wilson & Poe 1985), milkfish (Borlongan & Coloso 1993) and white sturgeon (Ng & Hung 1995). When established EAA requirements for hybrid striped bass (Griffin *et al.* 1992, 1994; Keembiyehetty & Gatlin 1992, 1993), rainbow trout (NRC 1993) and channel catfish (NRC 1993) were regressed against the EAA composition of the respective fillets (USDA 1987), we found correlations for each species to be significant: $r = 0.90$, $r = 0.97$ and $r = 0.93$, respectively (B.C. Small & J.H. Soares unpublished data). This is not unexpected since muscle protein accretion may be the largest single component of the EAA requirements in mature fish. Houlihan *et al.* (1986) demonstrated that the great majority of synthesis in white muscle, which comprises 50–60% of the mass of the fish, results in growth. They determined that efficiency of protein growth (growth/synthesis) in muscle was ≈ 76%.

The experiments presented here suggest several similarities among the EAA requirements for the striped bass and its hybrids. The total sulphur amino acid requirement of 10 g kg^{-1} of diet (Keembiyehetty & Gatlin 1993), the arginine requirement of 15 g kg^{-1} of diet (Griffin *et al.* 1994) and threonine requirement of 9 g kg^{-1} of diet (Keembiyehetty & Gatlin 1997) for hybrid striped bass closely agree with the striped bass requirements presented here (10 g kg^{-1} TSAA, 14 g kg^{-1} arginine and 11 g kg^{-1} threonine). However, the difference in the estimated lysine requirement for pure striped bass and the published lysine requirement for the hybrids is considerable: 22 g kg^{-1} of dry diet for *M. saxatilis* and 14 g kg^{-1} for hybrids (Griffin *et al.* 1992; Keembiyehetty & Gatlin 1992). To date, differences in the nutritional requirements of the striped bass and its hybrids have proven to be minimal. Although there is a good correlation between the estimated amino acid requirements presented in this paper and the published amino acid requirements for striped bass hybrids, the high lysine requirement for the pure striped bass cannot yet be explained. Research is being carried out in our laboratory to better understand lysine metabolism in the striped bass using traditional dose–response methods, determining lysine requirements for a number of parameters and by studying amino acid turnover in various tissues.

A particular species' tissue protein A/E ratios do not yield the total amount of each amino acid required in the diet of the fish. However, a considerable amount of literature has demonstrated that tissue amino acid patterns can serve as a valuable index in formulating test diets and early species' specific diets for aquaculture. We used striped bass fillet amino acid patterns as a model to increase the levels of each dietary essential amino acid in proportion to fillet A/E ratios, essentially performing a dose–response feeding trial of all the essential amino acids in one experiment. The high degree of correlation of the quantitative requirements derived by comparing muscle or whole-body EAA with the EAA requirements of aquaculture species lends merit to the use of fillet or whole-body A/E ratios as a first step in determining a new species' EAA requirements. By using this method, we were able to provide the first estimate of all 10 essential amino acid requirements for the striped bass in semi-purified and practical diets. Use of a fish species' fillet amino acid pattern to estimate amino acid requirements allows for the rapid development of nutritionally adequate diets without underestimating EAA requirements or incorporating excessive dietary protein, thereby enabling the reduction of production costs early in the development of new species for aquaculture.

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