

Changes in free amino acid profile of red snapper *Lutjanus campechanus*, eggs, and developing larvae

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Abstract The free amino acids (FAA) profile was determined for newly fertilized eggs and resultant larvae from wild-caught red snapper *Lutjanus campechanus* induced to spawn with hCG. Yolk sac and oil globule volumes of eggs and larvae were monitored over time from digital photographs. FAA profiles of the eggs and larvae were measured in picomoles (pmol) of FAA/mg of eggs by HPLC. Newly fertilized eggs had a mean total FAA content of 21.72 ± 3.55 nmoles/egg (92.81 ± 9.71 nmoles/mg eggs). Leucine, valine, lysine, and isoleucine were the most abundant essential FAA comprising 35.9% of the total FAA. Alanine, serine, asparagine, and glycine were the most abundant non-essential FAA comprising 34.2% of the total FAA. At 24 h post-hatch (hph) the mean total FAA had decreased by 81% since egg fertilization. The bulk of the FAA decrease was between the time of hatch and 12 hph. Only $8.5 \pm 1.5\%$ of the initial concentration in fertilized

eggs of isoleucine, $9.7 \pm 2.5\%$ of arginine, and $9.9 \pm 2.0\%$ of threonine remained at 12 hph. Among the non-essential FAA, alanine dropped the most by 12 hph with 4.6% of the concentration found in a recently fertilized egg remaining, while cysteine had increased $254.7 \pm 26.2\%$. The yolk sac volume decreased rapidly in the first 12 hph and was further reduced $77.0 \pm 2.5\%$ from 12 to 24 hph. The oil globule depletion rate was a more linear decline from fertilized egg to 36 hph.

Keywords Free amino acids · Red snapper, *Lutjanus campechanus* · Marine fish eggs · Marine fish larva · Aquaculture

Introduction

Red snapper *Lutjanus campechanus* is an important marine fish in the Gulf of Mexico utilized by the commercial fishing fleet as well as recreational fishermen. Because of its popularity, high market value, and the inability for domestic landings to meet consumer demand, there has been considerable interest in culturing the species for stock enhancement and food fish production (Laidley et al. 2003; Watanabe et al. 2005). However, poor viability of eggs and larvae is a major bottleneck in red snapper culture (Papanikos

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et al. 2003). Red snapper larvae develop rapidly, with yolk reserves gone and little of the oil globule remaining when the larvae are ready to begin exogenous feeding (Williams et al. 2004). Throughout yolk absorption, free amino acid pools are being depleted and are at very low levels by the time first feeding commences (Rønnestad et al. 1999). During conversion from endogenous reserves to exogenous feeding marine fish larvae, mass mortality often occurs (Fyhn 1989). Lack of appropriate food sources (Fyhn 1989) and late-developing digestive tracts (Rønnestad et al. 1999) at this critical developmental stage may be the cause of fatality for many marine fish larvae.

Free amino acids (FAA) are available to the developing fish embryo as building blocks of protein. Essential amino acids (EAA), those that the fish has to take in dietarily for survival, include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Barrows and Hardy 2001). Important non-essential amino acids (NEAA), those that the body can synthesize, include alanine, aspartic acid, asparagine, cysteine, glycine, glutamate, proline, serine, and tyrosine. Taurine, another nonessential amino acid is utilized as an important osmolyte but is not incorporated in proteins. The FAA pool in marine pelagic eggs and yolk-sac larvae provides an energy source for the developing embryo until it can begin feeding exogenously (Fyhn and Serigstad 1987; Fyhn 1989; Sivaloganathan et al. 1998; Rønnestad et al. 1998; Parra et al. 1999; Ohkubo and Matsubara 2002).

The limited amount of endogenous reserves remaining at the time of first feeding of red snapper larvae (Williams et al. 2004), make the quality of those reserves critical to survival. As specific free amino acids most critical for larval development are identified, it may be possible as part of brood stock management to increase their abundance in the egg and in turn provide more to the developing larvae. Food organisms high in a critical FAA can be given to developing larvae to improve growth and survival. The first step is to understand the nature of the free amino acid pool in red snapper eggs and how it changes as the larvae develop. The objectives of this study were to describe the free amino acid profile of ovulated red snapper eggs and larvae and to determine the change in abundance as red snapper larvae develop.

Materials and methods

Brood stock collection and spawning

Red snapper brood stock were collected from the Gulf of Mexico, near Gulf Shores, AL, and taken to the Claude Petet Mariculture Center in Gulf Shores, AL. Six females with an average weight of 2.28 ± 0.44 kg were injected intramuscularly with human chorionic gonadotropin (hCG) at a rate of 1,100 IU/kg body weight and males were given 550 IU/kg of body weight. Female red snapper were held at 26.5°C and 30.9 ppt salinity in a common recirculating system in 266-l aquaria with no more than three fish per tank. Ovulation and egg stripping took place approximately between 21 and 32 h after injection. Eggs of a given female were stripped directly in seawater, and simultaneously fertilized with sperm from one male. Three spawns were obtained and the resultant eggs and larvae from each spawn were monitored and then sampled and analyzed individually.

Egg and larval management

Fertilized eggs were held in a pan for ~5 min and then transferred to a 2-l graduated cylinder. The volume of floating fertilized eggs produced from each strip-spawned female was measured, triplicate samples taken and counted to determine the number of eggs/ml. About 30 ml of fertilized floating eggs were gently transferred to 60-l aquaria for incubation. The water exchange rate per aquarium was 100 ml/min from a recirculating system where water passed through a sand filter to remove suspended solids and a biofilter before returning to the incubators. Water temperature was maintained at 26.5°C. Salinity was 30.9 g/l and pH was 7.9 at the time eggs were transferred. Total ammonia nitrogen and nitrite nitrogen were measured and both remained below 0.1 mg/l during the incubation and hatching periods. The photoperiod during egg incubation and larval development was 24 h light:0 h dark. After hatching, dead eggs and egg debris on the bottom of the aquaria were carefully siphoned out of the water. Larvae were left in the incubators until approximately 36–48 h post-hatch (hph) when they were transferred to the larval rearing system in a greenhouse.

Egg weights

Immediately after egg fertilization, a sample of ~20 eggs from each spawn was collected, excess moisture removed and weighed to the nearest 0.1 ± 0.2 mg. The average egg weight was calculated by subtracting the tare weight from the total combined weight of the slide and eggs; then dividing the difference by the total number of eggs on the slide.

Egg morphometrics

After water-hardening and right before hatching, a sample of eggs from each spawned female was photographed utilizing a digital Olympus Camedia-3040 Zoom camera mounted on an Olympus SZ-PT stereo dissecting scope (Olympus America Inc., Melville, NY, USA). Egg and oil globule diameters were measured from the photographs using image-analysis software, Image Pro Plus (Media Cybernetics Inc., Silver Spring, MD, USA).

Larval morphometrics

Larvae were photographed laterally with the digital camera mounted on the stereo dissecting scope at 12, 24, and 36 hph. Larval body (total body length and post-anal body depth) and yolk-sac dimensions were measured utilizing the image-analysis software. Calculations as detailed by Williams et al. (2004) were utilized to determine yolk-sac volumes. Post-anal body depth was the linear measurement beginning from the ventral surface and ending at the dorsal surface at the position immediately posterior to the anus as viewed laterally.

Percent hatch and percent survival

After hatching, the percent hatch of eggs from each brood fish was based on volumetric counts. A total of ten counts, 10-ml each, were taken for each aquarium, the highest and lowest counts discarded and the average of the remaining eight counts was used to calculate the percent hatch. The percent hatch calculation was based on the total known number of larvae per aquarium divided by the total known number of fertilized eggs per aquarium multiplied by 100.

Larval survival was measured right before transfer based on volumetric counts made in the same manner used for determining percent hatch. The percent larval survival at transfer was calculated by dividing the total number of larvae per aquarium right before transfer by the total number of larvae per aquarium right after hatch multiplied by 100.

Eggs and larvae FAA analysis

Eggs and larvae were collected at various time intervals for FAA analysis. The eggs were placed on a piece of 125- μ m nylon mesh backed with absorbent paper toweling to wick away excess moisture from the eggs outer surfaces. Immediately afterward, the eggs were transferred to a pre-weighed cryovial. The cryovial was reweighed to determine the weight of the contents. The average weight of the eggs, calculated by the method previously mentioned, was used to calculate how many eggs were in the cryovial.

A sample of larvae, suspended in water, was gently collected into a Petri plate and carefully counted. A known number of larvae, ~200–240/sample, were collected on a clean piece of 125- μ m nylon mesh netting. The larvae were then gently washed off the mesh with a small amount of HPLC-grade distilled water into a clean watch glass then gently transferred by a micropipette to a cryovial. No larvae weights were recorded.

The cryovials and their contents were immediately frozen in liquid nitrogen. Within 2 months the samples were subsequently transported in a container with dry ice and transferred to an ultralow-temperature freezer maintained at -80°C . FAA profiles of the eggs and larvae were measured in picomoles (pmol) of FAA/mg of eggs by HPLC utilizing a modification of the Waters Pico-Tag method for solid tissues as suggested by the Waters, Millipore Corporation Manual No. 88140 (1986). A clean 1-ml graduated tissue grinder was pre-tared using an electronic Mettler analytical balance (Mettler-Toledo Inc., Columbus, OH, USA). Approximately 50 mg of tissue was placed in the tissue grinder and reweighed to record the exact amount to the nearest $0.1 \text{ mg} \pm 0.2 \text{ mg}$. Next, 300 μ l of 0.1 N HPLC-grade HCl containing 0.4-mM internal standard (methionine sulfone) was added to the tissue grinder contents and the total volume of the contents was noted. The tissue was immediately homogenized thoroughly.

The homogenate was transferred to a microcentrifuge tube and centrifuged for 15 min at 4°C, $1,500 \times g$. Afterwards, without disturbing the pellet, $\sim 300 \mu\text{l}$ of the supernatant was transferred to a Millipore 0.5-ml micro-centrifuge 10000 NMWL filter unit (Millipore, Billerica, MA, USA) and centrifuged at $4,500 \times g$ for 30 min (4°C) to deproteinize the sample. Next, $10 \mu\text{l}$ of the ultrafiltrate from each sample was transferred to sample-specific glass drying tubes. Additionally, $30 \mu\text{l}$ of standard solution (12.5% acid and neutral amino acids (A-6407, Sigma, St. Louis, MO), 12.5% basic amino acids (A-6282, Sigma, St. Louis, MO), 34% HPLC grade distilled water, 50% 0.4 mM methionine sulfone in 0.1 N HCl) was placed in a separate glass drying tube. The glass drying tubes were vacuum dried utilizing a Pico Tag Work Station (Waters Corporation, Milford, MA, USA). Afterwards, $10 \mu\text{l}$ of the redry solution (40% methanol, 40% 1.0 M sodium acetate, 20% triethylamine) was added to each glass drying tube and they were vacuum dried again. Next, $20 \mu\text{l}$ of derivatizing solution (70% methanol, 10% HPLC-grade water, 10% triethylamine, 10% phenylisothiocyanate) was added to each glass drying tube. The tubes were vortexed and allowed to stand at room temperature for 10 min and were subsequently vacuum dried for a third time. The glass drying tubes with contents were stored in an air-tight container and held at -50°C until ready to be analyzed by the high-performance liquid chromatography (HPLC) system. On the day of analysis, the glass drying tubes were each reconstituted with $100 \mu\text{l}$ of Pico-Tag diluent (WAT088119, Waters Corporation, Milford, MA, USA) and vortexed. Afterwards, $10 \mu\text{l}$ of the standard and $10 \mu\text{l}$ of each sample was injected into the HPLC system to measure the free amino acid profiles. Millenium³² (v. 3.05.01, Waters Corporation, Milford, MA, USA) was the software package utilized to analyze the chromatogram.

Statistical analysis

SPSS 11.5 for Windows, version 11.5 (SPSS, Inc., Chicago, IL, USA) was the statistical package used for data analysis. Levene's test for equality of variances was used to check the assumption that variances were equal. Differences in mean values of morphometrics or FAA concentrations between time intervals were analyzed by 1-way ANOVA if the data

were normally distributed; otherwise the non-parametric Kruskal–Wallis test was utilized. The Bonferroni method was used for post-hoc analysis. In the text, means are given as mean \pm standard deviation (SD).

Results

Reproduction

Fifty percent of the females injected ovulated, with ovulation occurring 21–32.5 h post-injection. Fertilization rates of floating eggs (Table 1) were typically high, ranging from 86.7 to 100%. The mean hatch rate of eggs was $32.5 \pm 13.3\%$ with a mean larval survival to 36–48 hph of $69.2 \pm 19.8\%$. The fecundity averaged $67,587 \pm 26,642$ eggs/kg.

FAA analysis

Ten essential and ten non-essential FAA were quantified in recently fertilized eggs (Table 2). The mean total FAA content was 21.72 ± 3.55 nmoles/egg (92.81 ± 9.71 nmoles/mg eggs). Leucine, valine, lysine, and isoleucine were the most abundant essential FAA, comprising 35.9% of the total. The most abundant (34.2% of total) non-essential FAA were alanine, serine, asparagine, and glycine. Alanine was the most abundant FAA overall (3.15 nmol/egg)

Table 1 Percent egg fertilization, percent hatch, and percent survival of larvae at transfer (36–48 hph) for each incubator tank

Brood number	Percent fertilization	Percent hatch	Percent survival
1 ^a	86.7	51.0	41.6
1	87.8	41.3	56.6
2	90.9	19.0	78.2
3	100	23.2	79.1
3	100	27.9	90.7
Mean	93.1 ± 6.5	32.5 ± 13.3	69.2 ± 19.8

Mean values by treatment are the mean percent \pm SD

^a Brood stock ID # values that are the same represent separate incubator aquaria for a specific female that produced enough eggs for more than one aquarium (in total, three females spawned). Percent fertilization, percent hatch, and percent survival at transfer were calculated based on each aquarium

Table 2 Mean concentration (nmol/individual) of essential and non-essential free amino acids (FAA) in recently ovulated eggs, in eggs just prior to hatch, and in larvae at 12 and 24 h post-hatch (hph) produced by three female red snapper

FAA concentration (nmol/individual)	Ovulated egg Mean \pm SD	Pre-hatch egg	12 hph	24 hph
Leucine	2.60 \pm 0.29	2.30 \pm 0.99	0.38 \pm 0.02	0.23 \pm 0.05
Valine	1.98 \pm 0.26	1.99 \pm 0.67	0.45 \pm 0.05	0.10 \pm 0.03
Lysine	1.66 \pm 0.30	1.48 \pm 0.77	0.26 \pm 0.05	0.12 \pm 0.02
Isoleucine	1.55 \pm 0.19	1.43 \pm 0.58	0.13 \pm 0.02	0.05 \pm 0.01
Threonine	1.11 \pm 0.19	0.70 \pm 0.34	0.11 \pm 0.02	0.10 \pm 0.01
Arginine	1.06 \pm 0.15	0.62 \pm 0.24	0.10 \pm 0.03	0.06 \pm 0.01
Histidine	0.60 \pm 0.10	0.51 \pm 0.21	0.15 \pm 0.04	0.15 \pm 0.03
Methionine	0.55 \pm 0.07	0.52 \pm 0.23	0.15 \pm 0.05	0.13 \pm 0.03
Phenylalanine	0.34 \pm 0.05	0.32 \pm 0.11	0.15 \pm 0.04	0.24 \pm 0.01
Tryptophan	0.14 \pm 0.02	0.14 \pm 0.06	0.08 \pm 0.02	0.10 \pm 0.01
Essential FAA	11.58 \pm 1.60	9.99 \pm 4.18	1.98 \pm 0.24	1.29 \pm 0.12
Alanine	3.15 \pm 0.69	2.11 \pm 1.09	0.14 \pm 0.02	0.16 \pm 0.03
Serine	1.74 \pm 0.38	1.29 \pm 0.66	0.17 \pm 0.04	0.16 \pm 0.04
Asparagine	1.64 \pm 0.31	1.21 \pm 0.61	0.17 \pm 0.03	0.10 \pm 0.03
Glycine	0.90 \pm 0.18	0.86 \pm 0.34	0.20 \pm 0.04	0.22 \pm 0.05
Proline	0.79 \pm 0.13	0.60 \pm 0.35	0.07 \pm 0.01	0.02 \pm 0.01
Taurine	0.69 \pm 0.11	1.00 \pm 0.21	1.04 \pm 0.22	1.08 \pm 0.16
Tyrosine	0.56 \pm 0.13	0.56 \pm 0.21	0.38 \pm 0.05	0.48 \pm 0.08
Glutamic acid	0.50 \pm 0.06	0.29 \pm 0.09	0.26 \pm 0.01	0.28 \pm 0.05
Aspartic acid	0.08 \pm 0.02	0.07 \pm 0.03	0.09 \pm 0.03	0.01 \pm 0.03
Cysteine	0.07 \pm 0.03	0.06 \pm 0.06	0.18 \pm 0.02	0.27 \pm 0.01
Non-essential FAA	10.13 \pm 1.95	8.06 \pm 3.62	2.69 \pm 0.30	2.85 \pm 0.44
Total FAA	21.72 \pm 3.55	18.05 \pm 7.78	4.68 \pm 0.53	4.14 \pm 0.56

contributing 14.5% of the FAA pool of recently fertilized eggs.

From the time of fertilization to 24 hph the mean total FAA concentration per individual egg or larva decreased (Fig. 1). The bulk of the FAA decrease was from just prior to hatch to 12 hph. By 24 hph, 19.1 \pm 3.1% of the FAA pool remained for the larvae (Table 3). All essential FAA concentrations in eggs and yolk-sac larvae declined over time with the exception of tryptophan and phenylalanine (Table 3). Relative to the quantity present in ovulated eggs, 11.1 \pm 1.8% of the essential FAA concentrations remained at 24 hph. The most dramatic reductions were of isoleucine, valine, and arginine, with 3.4 \pm 0.9, 5.0 \pm 1.7, and 6.1 \pm 0.6%, respectively, remaining at 24 hph.

Most non-essential FAA concentrations in eggs and yolk-sac larvae declined over time with the exceptions of cysteine, aspartic acid, taurine, and

tyrosine (Table 3). The greatest decline in non-essential FAA was by proline, alanine, and asparagine with 3.2 \pm 1.0, 5.0 \pm 1.0, and 6.4 \pm 1.7%, respectively, of the quantities found in ovulated eggs remained at 24 hph. Cysteine showed a 393.3 \pm 20.3% increase by 24 hph, while aspartic acid, taurine, and tyrosine appeared to fluctuate over time.

Morphometrics

Mean wet egg weight soon after fertilization was 0.26 \pm 0.03 mg with a mean egg diameter 800.8 \pm 17.92 μ m. The mean egg diameter just prior to hatch was 803.7 \pm 14.01 μ m. The average oil globule diameter from eggs with only one oil globule was 130.7 \pm 8.37 μ m. The oil globule was 16.3 \pm 1.0% of the egg diameter at that point and was 15.2 \pm 0.89% just prior to hatch ($P = 0.19$). Standard lengths of larvae at 12 hph ranged from

Fig. 1 Percent change in yolk-sac volume from recently hatched larvae to 36 h post-hatch, change in oil globule volume from recently ovulated egg to 36 h post-hatch, and change in essential free amino acids (FAA) and non-essential FAA concentrations from recently ovulated egg to 24 h post-hatch

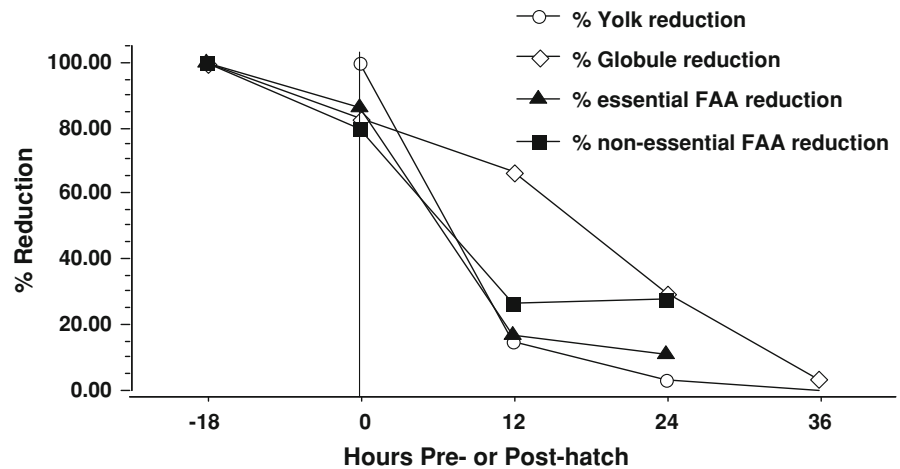


Table 3 Percentage (mean \pm SD) of essential (FEAA) and non-essential free amino acids (NEFAA) remaining just prior to hatch, and at 12 and 24 h post-hatch (hph) relative to FAA values of recently ovulated red snapper eggs (i.e., 100%) from three female red snapper

FAA	Pre-hatch (%)	12 hph (%)	24 hph (%)
Arginine	57.8 \pm 22.5	9.7 \pm 2.5	6.1 \pm 0.6
Histidine	84.6 \pm 35.4	25.7 \pm 7.1	24.4 \pm 4.3
Isoleucine	91.8 \pm 37.2	8.5 \pm 1.5	3.4 \pm 0.9
Leucine	88.5 \pm 38.2	14.5 \pm 0.9	9.0 \pm 1.9
Lysine	88.8 \pm 46.4	15.9 \pm 3.3	7.0 \pm 1.1
Methionine	94.9 \pm 42.0	27.7 \pm 9.1	24.1 \pm 4.8
Phenylalanine	95.6 \pm 32.7	46.0 \pm 12.0	71.6 \pm 3.3
Threonine	63.2 \pm 30.3	9.9 \pm 2.0	8.9 \pm 0.5
Tryptophan	103.5 \pm 45.4	59.0 \pm 16.9	74.5 \pm 10.8
Valine	100.4 \pm 33.7	22.8 \pm 2.5	5.0 \pm 1.7
Mean FEAA	86.3 \pm 36.2	17.1 \pm 3.1	11.1 \pm 1.8
Alanine	67.0 \pm 34.6	4.6 \pm 0.6	5.0 \pm 1.0
Asparagine	73.8 \pm 37.2	10.4 \pm 1.6	6.4 \pm 1.7
Aspartic acid	87.4 \pm 40.4	105.1 \pm 3.8	85.8 \pm 32.3
Cysteine	80.5 \pm 86.8	254.7 \pm 26.2	393.3 \pm 20.3
Glycine	95.6 \pm 37.6	22.1 \pm 4.0	24.3 \pm 5.3
Proline	75.5 \pm 43.9	8.3 \pm 1.2	3.2 \pm 1.0
Serine	75.5 \pm 43.9	8.3 \pm 1.2	3.2 \pm 1.0
Taurine	144.0 \pm 30.3	151.1 \pm 31.1	155.6 \pm 22.9
Tyrosine	100.9 \pm 37.7	68.0 \pm 8.5	86.8 \pm 13.6
Mean NEFAA	79.5 \pm 36.1	26.6 \pm 4.5	28.1 \pm 4.7
Mean T. FAA	83.1 \pm 36.1	21.5 \pm 3.7	19.1 \pm 3.1

2.46 mm to 2.87 mm. The mean standard length of larvae decreased over time from 12 to 24 hph ($P = 0.010$) and from 12 to 36 hph ($P < 0.001$),

but did not change between 24 and 36 hph ($P = 0.902$).

The yolk-sac decreased in volume between the three time points as measured by one-way ANOVA or the non-parametric Kruskal–Wallis test with $<1\%$ of the 12 hph volume remaining at 36 hph. The mean oil globule volume decreased from $1.20 \pm 0.285 \times 10^{-3} \text{ mm}^3$ for recently fertilized eggs to $0.04 \pm 0.025 \times 10^{-3} \text{ mm}^3$ for 36 hph larvae. At 36 hph, mouthparts were well developed but the mouth was not yet open.

Discussion

Newly fertilized red snapper eggs (0.80 mm egg diameter) with a mean total FAA content of 21.7 nmol/egg have less FAA than many other marine fish. Rønnestad and Fyhn (1993) summarized the total FAA content in the eggs of several marine species. They observed that the total FAA content/egg varied widely among species, ranging from 30 nmol/egg in Atlantic mackerel *Scomber scombrus* to 2,300 nmol/egg in Atlantic halibut *Hippoglossus hippoglossus*. These large differences were attributed to the differences in egg size, where Atlantic mackerel has a 1.2 mm egg diameter and Atlantic halibut has a 3.0 mm egg diameter. Sivaloganathan et al. (1998) measured the FAA profile of Asian sea bass *Lates calcarifer* with a similar size egg as red snapper, and found a total FAA content of 25.3 nmol/egg.

Alanine (3.2 nmol/egg), leucine (2.6 nmol/egg), valine (2.0 nmol/egg), serine (1.7 nmol/egg), lysine

(1.7 nmol/egg), asparagine (1.6 nmol/egg), and isoleucine (1.6 nmol/egg) were the most abundant FAA in ovulated eggs of red snapper. Similar results have been found in other marine finfish species that produce pelagic eggs such as European sea bass *Dicentrarchus labrax* (Rønnestad et al. 1998), Atlantic halibut (Finn et al. 2002), Senegal sole *Solea senegalensis* (Parra et al. 1999), Asian sea bass, (Sivaloganathan et al. 1998), and Barfin flounder *Verasper moseri*, (Matsubara and Koya 1997). In contrast, demersal ovulated eggs from Lump sucker *Cyclopterus lumpus*, Common mummichog *Fundulus heteroclitus*, and Ballan wrasse *Labrus bergylta* were dominated by taurine (Thorsen et al. 1993).

Amino acid profiles have been suggested as indices of amino acid requirements for fish (Wilson and Poe 1985; Brown 1995; Saavedra et al. 2006). Such profiles should not only be considered from a composition perspective but also in regard to utilization rates. FAA contributes to protein synthesis as well as providing a source of energy for developing embryos and larvae (Watanabe and Kiron 1994; Rønnestad et al. 1999). Based on abundance in ovulated eggs and the magnitude of decline by 24 hph, valine and isoleucine are very important in red snapper larval development (Table 3). Valine was the second most abundant essential FAA in the recently ovulated egg but by 24 hph its percentage decline was the second greatest (95%) of the essential FAA. Isoleucine was the fourth most abundant FAA initially and by 24 hph its abundance had declined to a greater percent (96.6%) than any essential FAA. In the larger slower-developing eggs of striped trumpeter *Lutris lineata*, Brown et al. (2005) found a total FAA concentration of 16.3 µg/egg (dry wet) and 3.4 µg/larvae at 1 day post-hatch (dph). Leucine, the most abundance essential FAA, had dropped to 14.6% by 1 dph of the original concentration (2,151 ng/egg) and to 1.63% by the time of first feeding at 5 dph. Dayal et al. (2003) found a total of 13.0 µg FAA/egg in recently fertilized Asian sea bass eggs and 9.36 µg in recently hatched larvae. Leucine, lysine, arginine, and valine were, in order, the most abundant essential FAA in the eggs. In recently hatched larvae, the concentration of valine had dropped the most (43.7%) and lysine the least (27.8%). At 2 dph, prior to first feeding, valine had dropped to 55.5% of the concentration in eggs, with lysine dropping the least (29.7%).

In red snapper eggs, alanine, serine, and asparagine were the most abundant non-essential FAA (Table 2), but only 4.6, 9.5, and 10.4% of the respective initial concentrations remained in recently hatched larvae. Brown et al. (2005) found that glutamine and alanine, the most abundant non-essential FAA in striped trumpeter eggs were reduced to 12.9 and 8.0% of the original quantities by 1 dph with 7.9% of each remaining at 5 dph. Dayal et al. (2003) found that alanine, glutamic acid, and serine were the most abundant non-essential FAA in Asian sea bass eggs, with 84.5, 77.9, and 75.3%, respectively, of the initial concentration remaining in recently hatched larvae.

Timing of FAA utilization varies by species. The red snapper appears to utilize FAA to a large extent during the egg and the earliest yolk-sac stages (Fig. 1). European sea bass (Rønnestad et al. 1998), turbot *Scophthalmus maximus*, plaice *Pleuronectes platessa*, and gilthead seabream *Sparus aurata* (Rønnestad and Fyhn 1993) utilize their FAA pools during the same development time frame as red snapper. Rønnestad and Fyhn (1993) found that lemon sole *Parophrys vetulus* and Atlantic cod experience a decrease in the FAA pool during both the egg and yolk-sac larval stages, while the Atlantic halibut larvae undergo a major decline in FAA reserves only during the yolk-sac stage.

In red snapper, there was no apparent preference in the utilization of NEFAA versus EFAA during the egg stage (Fig. 1). However, from just prior to hatch and thereafter there was a slight preference to utilize EFAA over NEFAA. Ohkubo and Matsubara (2002), working with barfin flounder eggs that take 9 days to hatch, found the greatest reduction in NEFAA occurred during the last few days prior to egg hatching. Reduction of EFAA began at a similar time prior to hatch but continued for several days post-hatch. Rønnestad et al. (1993) found that during the egg stage NEFAA were selectively used in Atlantic halibut. In contrast, Rønnestad and Fyhn (1993) found no selectivity for NEFAA over EFAA in Atlantic cod.

Generally, most individual FAA measured during this study decreased in the eggs and larvae over time, however the abundance of some remained stable or increased. Cysteine, a non-essential free amino acid increased over time (Table 3). Transsulfuration allows cysteine to be synthesized from serine and

the methionine sulfur atom, and cysteine can be metabolized into taurine (Stipanuk and Watford 2000). Taurine concentrations remained relatively unchanged during the egg and yolk-sac larvae stages of red snapper. Similar trends have been seen in many marine species that produce pelagic eggs such as cod (Fyhn and Serigstad 1987), Asian sea bass (Sivaloganathan et al. 1998), European sea bass (Rønnestad et al. 1998), and Senegal sole (Parra et al. 1999). Finn et al. (2002) noted that the amount of taurine per individual oocyte changed little in comparison to other FAA during oocyte hydration for Atlantic halibut and suggested that taurine may have some other role than for oocyte hydration. Takeuchi et al. (2002) suggested that taurine may be an essential nutrient for larval and juvenile fish. Several authors have shown that fish growth was improved when diets were supplemented with taurine (Martinez et al. 2004; Gibson-Gaylord et al. 2007; Kim et al. 2007; Lunger et al. 2007).

The overall rate of reduction in FAA more closely follows the rate of reduction of the yolk-sac than the oil globule (Fig. 1). There was little change in oil globule diameter in the egg from just after ovulation to just before hatch. From hatch to 36 hph, the globule was reduced at a relatively steady rate with $3.3 \pm 2.28\%$ of the pre-hatch volume remaining at 36 hph. The globule continues to be reduced past that point. Williams et al. (2004) reported that $<2\%$ of the globule remained just prior to functional mouthparts (46 hph) in red snapper. Yolk-sac utilization was more rapid. Williams et al. (2004) found that red snapper had no apparent yolk sac remaining by the time mouthparts were functional. Using the yolk sac volume at the time of hatch from Williams et al. (2004) and the volumes determined in this study at 12, 24, and 36 hph, the utilization rate of yolk sac and FAA were similar (Fig. 1). Fyhn and Govoni (1995) reported similar correlations in FAA decline and yolk-sac absorption for Atlantic menhaden *Brevoortia tyrannus* and spot *Leiostomus xanthurus*.

Conclusions

Red snapper eggs at time of fertilization are small (0.800 mm) with a total FAA pool of 21.7 nmole/egg. Alanine, leucine, valine, serine, lysine, asparagine, and isoleucine were the most abundant FAA at

that time. Red snapper appear to utilize FAA to a large extent during the egg and the earliest yolk-sac stages with no apparent preference in the utilization of NEFAA versus EFAA during the egg stage. The overall rate of reduction in FAA more closely follows the rate of reduction of the yolk sac than the oil globule. Based on abundance in ovulated eggs and the magnitude of decline by 24 hph, valine and isoleucine are very important in red snapper larval development.

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