

Evaluation of Fatty Acid Enrichment of Live Food for Yellowtail Snapper *Ocyurus chrysurus* Larvae

CYNTHIA K. FAULK AND G. JOAN HOLT

University of Texas Marine Science Institute, Fisheries and Mariculture Laboratory, 750 Channel View Drive,
Port Aransas, Texas 78373 USA

D. ALLEN DAVIS

Auburn University, Department of Fisheries and Allied Aquacultures, 204 Swingle Hall,
Auburn, Alabama 36830 USA

Abstract

The purpose of this study was to examine the effect of varying dietary levels of highly unsaturated fatty acids (HUFA) in live prey on the standard length, specific growth rate, survival, and fatty acid composition of yellowtail snapper *Ocyurus chrysurus* larvae. Two experiments were conducted utilizing rotifers and *Artemia* enriched with live algae (*Isochrysis galbana* or *Nannochloris oculata*) or commercial preparations (Aquagrow Advantage, Aquagrow Advantage plus Aquagrow arachidonic acid, and Algamac 2000). Larval growth and fatty acid composition were evaluated during the rotifer, *Brachionus plicatilis*, and *Artemia* feeding periods and survival rates were calculated at the termination of each trial (18 or 20 d after hatching). In general, prey enriched with the commercial products contained higher levels of docosahexaenoic acid, eicosapentaenoic acid, n-3 HUFA, and Σ HUFA than those enriched with live algae. The addition of arachidonic acid to the Aquagrow Advantage enrichment medium significantly increased the amount of this fatty acid in rotifers but not in *Artemia*. At the end of the growth trials, larval standard length was highest when larvae were fed prey enriched with *I. galbana* (6.4 mm) or commercial preparations (6.7–7.1 mm) versus *N. oculata* (5.2 mm). Furthermore, larvae fed prey enriched with commercial preparations had significantly ($P < 0.05$) higher survival rates (2.2–5.9%) than those fed prey enriched with live algae (1.1–1.4%). These results suggest that yellowtail snapper larvae require dietary levels of HUFA beyond those achieved by enriching prey with live *N. oculata* or *I. galbana*.

The snapper family is comprised of numerous species found in tropical and subtropical areas worldwide and represents important recreational and commercial fisheries in these areas (Anderson 1987). The National Marine Fisheries Service currently lists snapper stocks inhabiting United States coastal waters as overutilized indicating that they are being harvested at or beyond their maximum sustainable yield (NMFS 1999). One method of alleviating fishing pressures on snapper species is to provide alternate sources of production via mariculture. Although there is considerable interest in developing culture techniques for snapper, recent attempts have met with limited success (Davis et al. 2000). Additional information on broodstock nutrition, spawning, larval rearing, and juvenile grow-out is needed before commercial production of snapper is a viable alternative to wild caught fish.

Yellowtail snapper *Ocyurus chrysurus* range from North Carolina to southeastern Brazil in the western Atlantic Ocean and are also found in the Gulf of Mexico and the Caribbean (Hoese and Moore 1998). Captive broodstock of yellowtail snapper have been successfully spawned year-round through temperature and photoperiod manipulations providing a continual supply of yolk sac larvae for culture (Turano et al. 2000). However, high mortality during the early larval stage remains a substantial bottleneck for the production of seed stock and hence commercial culture. Previous yellowtail snapper rearing trials resulted in poor survival near day 16 post-hatch due to an apparent stress response which was especially severe during periods of handling (Turano et al. 2000). Similarly, Blaylock et al. (2000) reported high mortality rates for red snapper *Lutjanus campechanus* larvae around day 19 post-hatch.

The authors of these studies suggested that inadequate larval nutrition may contribute to the high mortality rates observed prior to the juvenile stage.

Highly unsaturated fatty acids (HUFA) such as arachidonic acid (ARA, 20:4 n-6), eicosapentaenoic acid (EPA, 20:5 n-3), and docosahexaenoic acid (DHA, 22:6 n-3) play an important role in stress tolerance, membrane fluidity, and proper development and functioning of neural and visual systems (Kanazawa 1997; Rainuzzo et al. 1997; Sargent et al. 1997). Marine fishes typically have a limited ability to convert shorter chain fatty acids to longer chain HUFA due to low activity of the necessary enzymes and must obtain these fatty acids through their diet (Ghioni et al. 1999). Rotifers and *Artemia* nauplii are commonly used as food items for first feeding marine fish larvae. However, they are naturally deficient in the HUFA needed for optimal larval growth and survival, making it necessary to enrich these feeds with essential fatty acids prior to offering them to the larvae (Sargent et al. 1997). To test the hypothesis that high mortality rates prior to the juvenile stage are due to a deficiency of HUFA, we examined the effect of enriching live feeds with different live algae and commercial preparations containing varying levels of HUFA on the growth and survival of yellowtail snapper larvae.

Materials and Methods

Yellowtail snapper eggs were obtained from F1 laboratory reared broodstock spawned in a recirculating system via manipulations of temperature and photoperiod (Turano et al. 2000) at the Fisheries and Mariculture Laboratory of the University of Texas Marine Science Institute in Port Aransas, Texas. The broodstock diet consisted of shrimp (80%), squid (10%), and fish from shrimp by-catch (10%). A lipid and vitamin supplement was added to the diet twice weekly at a rate of 2-mL supplement/100-g feed. The supplement was injected into the tails of the shrimp and consisted of 50-g AIN vitamin mixture 76 (ICN Biomedicals Inc., Irvine, California, USA) dissolved in 50 mL of menhaden oil (Omega Protein Inc., Houston,

Texas, USA). Salinity and temperature at the time of spawning averaged 34.8 ± 0.2 ppt and 25.0 ± 1.0 C, respectively.

Larval Rearing Trials

Fertilized eggs were collected from the broodstock tank and set in a solution of 100-ppm formalin for 30 min (Douillet and Holt 1994) and subsequently transferred to the culture tanks (3000/tank). Egg diameters were measured to the nearest 0.1 mm for at least 20 eggs per spawn ($N = 2$) using a Wild Heerbrugg stereomicroscope, Summa Sketch III digitizing tablet (GTCO CalComp, Inc., Columbia, Maryland, USA), and Sigma Scan software (Jandel Corporation, San Rafael, California, USA). Larvae hatched within 24 h post-fertilization, and yolk sac larvae averaged 2.3 ± 0.1 mm standard length (see Riley et al. 1995 for description of larval development). The pre-feeding stage lasted approximately 48 h, and larvae were fed enriched rotifers on days 3–15 after hatching. Rotifers were added to the rearing tanks twice daily to maintain a concentration of 3 rotifers/mL. Newly hatched *Artemia* nauplii were added once daily on days 12–14 at a concentration of 2/L. On days 14–20, enriched *Artemia* were initially added to the tanks in the morning at concentrations of approximately 3/L. During the day, the majority of enriched *Artemia* were removed from the tanks every 3 h due to consumption by the larvae or filtration through the biofilter. Therefore, similar amounts of *Artemia* were collected from the enrichment containers and added to the rearing tanks at 3-h intervals until the lights switched off in the evening. The prymnesiophyte alga *Isochrysis galbana* was added daily to all rearing tanks to maintain a concentration of approximately 30,000 cells/mL during the period of rotifer feeding.

In the first feeding study, larvae were reared in 150-L conical tanks equipped with internal biological filters as described by Craig et al. (1990). Tank salinities averaged 34.0 ± 0.5 ppt, temperature was maintained at 27.0 ± 1.0 C, and the photoperiod was 14-h light:10-h dark. Live prey were enriched as described below with a commercial enrichment Algamac 2000

(A2000; Aquafauna Bio-marine, Hawthorne, California, USA), live *I. galbana*, or live *Nannochloris oculata* with three replicate tanks per treatment.

The second feeding study was conducted in a recirculating system consisting of a series of 12 300-L circular tanks connected to a 1,000-L biological filter box. Water was pumped from the filter box to each rearing tank at a rate of approximately 2 L/min. Water flowed from each tank through an internal PVC pipe covered with nylon screen (48 or 250 μm) and back to the filter box, where it was filtered down to 10 μm with the use of a nylon filter bag. Tank salinities averaged 31.5 ± 0.5 ppt and temperature was maintained at 27.0 ± 1.0 C with the use of a Titan heat pump (Aqualogic, Inc., San Diego, California, USA). Photoperiod was 14-h light: 10-h dark. Live prey enrichments utilized during this trial included the following three commercial products: Aquagrow Advantage (Aq; ABN, Advanced Bionutrition Corporation, Columbia, Maryland, USA), 90% Aq and 10% Aquagrow ARA (Aq10; ABN), and A2000. Three replicate tanks were used for each treatment.

Live Prey Enrichments

Rotifers *Brachionus plicatilis* used in this study were obtained from a continuous culture raised on *N. oculata* and Baker's yeast. For each treatment, the required number of rotifers were harvested the day before feeding, placed in a 10-L plastic container (300 rotifers/mL), and enriched with either live algae or commercial products. *Artemia* cysts were incubated for 20 h at which time nauplii were separated from empty cysts and placed in a 10-L plastic container (50–100 nauplii/mL). Rotifers and *Artemia* were maintained at 30.0 ppt and 26.0 C during the enrichment period. Prey were enriched with the commercial products twice over a 16-h period (0 and 8 h) at doses of 0.3 g per 1×10^6 rotifers and 0.2 g per 100,000 *Artemia* nauplii. Prior to the second enrichment at 8 h, rotifers and *Artemia* were collected on a 55- or 150- μm nitex sieve, respectively, rinsed with clean seawater, and returned to the 10-L enrichment containers. This step was included

in the enrichment process in order to remove any unused enrichment material remaining in the containers from the first 8-h period. For the live algae enrichments, prey were provided with concentrations of approximately 3.5×10^6 cells/mL for *I. galbana* and 5×10^6 cells/mL for *N. oculata*.

Sample Collection and Analysis

Larvae ($N = 20$) were removed from each tank for measurements of standard length and subsequent calculations of specific growth rate (SGR) on days 11 and 18 after hatching for trial 1 and days 12 and 20 for trial 2. Larvae were collected in the morning prior to feeding. Following collection, larvae were anesthetized with 0.1% tricaine methanesulfonate (MS-222) and length measurements were made to the nearest 0.1 mm as described previously for egg diameter. SGR was calculated using the following equation:

$$\text{SGR } (\%/d) = 100(\ln L_F - \ln L_I)/T$$

where, L_F and L_I are the final and initial standard lengths and T is the number of days between measurements. SGR was calculated independently for the rotifer and *Artemia* feeding periods. During the rotifer feeding period, L_F was the mean standard length of 11- and 12-d old larvae for trials 1 and 2, respectively, and L_I was the mean standard length of day 1 larvae. During the *Artemia* feeding period, L_F was the mean standard length of 18- and 20-day old larvae and L_I was the mean standard length of 11- and 12-d-old larvae for trials 1 and 2, respectively.

The percent survival was calculated by dividing the initial number of day 1 larvae by the number of larvae remaining in each tank at the end of the study $\times 100$. Survival values were calculated on day 18 for trial 1 and day 20 for trial 2. An estimation of the number of day 1 larvae present in each tank was based on the number of eggs placed in the tank and the percent hatch. Hatch rates were determined by placing 20 eggs in each of three replicate 250-mL beakers and recording the number of larvae hatched in each beaker the following morning.

Enriched rotifers and *Artemia* were sampled twice during each trial for lipid analysis. En-

riched prey items were collected following the 16-h enrichment period on days 6 and 11 for rotifers and days 14 and 18 for *Artemia*. Larval samples ($N = 20$) were collected in the morning prior to feeding on days 11 and 18 after hatching for trial 1 and days 12 and 20 for trial 2. After collection, samples of prey and/or larvae were placed in a 50-mL cylinder (PVC) with a 48- μm nitex mesh bottom, rinsed three times with 20 mL of distilled water, and frozen at -80 C. Samples were subsequently lyophilized and stored at -80 C until lipid extraction could be performed.

Total lipids were cold extracted from lyophilized samples as described by Folch et al. (1957) using chloroform:methanol (2:1, v: v) containing 0.01% (w: v) butylated hydroxytoluene as an antioxidant. Total lipids were saponified in 0.5-M KOH, and fatty acid methyl esters (FAMES) were prepared by transesterification with 14% boron trifluoride in methanol following the procedure of Morrison and Smith (1964).

FAMES were analyzed on a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector and a Supelcowax 10 fused silica capillary column (30-m long, 0.53-mm internal diameter, 1.0- μm thickness; Supelco, Inc., Bellefonte, Pennsylvania, USA). Helium was used as a carrier gas at the rate of 4 mL/min and a split ratio of 25:1. Injector and detector temperatures were 250 and 260 C respectively. Oven temperature was held at 160 C for 5 min and rose to 220 C at a rate of 3 C/min where it was held for 30 min. FAME peaks were recorded with the use of a Tigre II analog/digital interface and Chrom Perfect Spirit software (Justice Laboratory Software, Palo Alto, California, USA). Individual peaks were identified by comparison to chromatograms of known standards (Supelco, Inc., Bellefonte, Pennsylvania, USA).

Statistical Analysis

A one-way ANOVA followed by a Tukey test for multiple comparisons of means was used to test for significant differences ($P < 0.05$) in standard length, SGR, survival, and fatty acid content for each rearing trial. Values used in the analyses were transformed as necessary to

meet the normality assumption of ANOVA. Tank means were entered into the analyses. Statistical analyses were performed using SYSTAT 10.0 (SPSS Inc., 2000, Chicago, Illinois, USA).

Results

The fatty acid composition of enriched rotifers and *Artemia* for trial 1 are given in Table 1. The level of DHA was substantially higher when both prey items were enriched with A2000 compared to either live *I. galbana* or *N. oculata*. As a result, rotifers and *Artemia* enriched with A2000 contained significantly higher amounts of Σ HUFA and n-3 HUFA and the ratio of DHA/EPA was also higher. No significant differences were found among enriched rotifers for EPA, ARA, n-6 HUFA, or the ratio of n-3/n-6 HUFA. Variations in the amount of EPA and ARA measured in enriched rotifers resulted in a significantly higher ratio of EPA/ARA in the *I. galbana* and *N. oculata* treatments compared to A2000. *Artemia* enriched with A2000 contained significantly more EPA and ARA than other treatments while no differences were found for the ratio of EPA/ARA. Total n-6 HUFA and the ratio of n-3/n-6 HUFA were highest in *Artemia* enriched with A2000.

In trial 2, the level of DHA in the rotifers was significantly higher when Aq was included in the enrichment process (Table 2). No significant differences in the amount of EPA or the DHA/EPA ratio were found among treatments. The addition of ARA to the Aq enrichment medium resulted in a significantly higher level of this fatty acid in the Aq10 enriched rotifers while the amount of ARA in rotifers enriched with A2000 was intermediate in value. As a result, the ratio of EPA/ARA for rotifers was lower in the Aq10 enrichment than that of Aq and A2000 rotifers. In general, the amount of n-3 and n-6 HUFA followed the pattern of DHA and ARA levels in enriched rotifers. Rotifers enriched with Aq had the highest level of both Σ HUFA and n-3 HUFA whereas those enriched with Aq10 contained the highest level of n-6 HUFA. In general, the fatty acid composition of enriched *Artemia* was similar among treatments with the exception of DHA.

TABLE 1. Selected fatty acid composition (% total lipid) of enriched rotifers and *Artemia* for trial 1. Within rows, values (mean \pm SEM; N = 2) in each prey type sharing the same superscript are not significantly different (P > 0.05).

Fatty acid	Rotifers			<i>Artemia</i>		
	A2000 ¹	<i>I. galbana</i>	<i>N. oculata</i>	A2000 ¹	<i>I. galbana</i>	<i>N. oculata</i>
18:2n-6	4.0 (0.1) ^a	14.3 (0.1) ^b	10.8 (1.4) ^a	4.4 (<0.1) ^a	6.1 (<0.1) ^b	5.8 (<0.1) ^c
18:3n-3	3.4 (<0.1)	8.9 (<0.1)	8.9 (0.1)	20.5 (0.1) ^a	23.3 (0.1) ^b	24.5 (0.2) ^c
20:4n-6	2.4 (<0.1)	1.9 (<0.1)	0.8 (0.8)	1.8 (<0.1) ^a	1.4 (0.3) ^b	0.9 (<0.1) ^b
20:5n-3	4.1 (0.1)	4.8 (0.1)	2.2 (1.9)	4.4 (<0.1) ^a	2.9 (<0.1) ^b	2.4 (<0.1) ^c
22:5n-3	2.1 (0.1)	2.5 (0.1)	0.9 (0.8)	0.1 (<0.1)	0.1 (<0.1)	0.1 (<0.1)
22:6n-3	24.9 (0.9) ^a	3.0 (0.1) ^b	nd ²	6.3 (<0.1) ^a	1.2 (0.1) ^b	0.1 (<0.1) ^c
Σ HUFA ³	36.2 (1.1) ^a	16.7 (0.1) ^b	7.0 (5.1) ^b	13.9 (0.1) ^a	7.6 (0.2) ^b	5.2 (0.2) ^c
n-3 HUFA ³	33.3 (1.2) ^a	14.3 (0.3) ^b	5.9 (4.1) ^b	12.1 (0.1) ^a	5.9 (0.1) ^b	4.2 (0.1) ^c
n-6 HUFA ³	2.9 (<0.1)	2.4 (0.4)	1.1 (1.0)	1.9 (0.1) ^a	1.6 (0.1) ^a	1.1 (<0.1) ^b
n-3/n-6	11.7 (0.5)	6.0 (1.0)	10.9 (6.4)	6.5 (0.2) ^a	3.7 (0.2) ^b	3.9 (0.2) ^b
DHA/EPA	6.1 (0.1) ^a	0.6 (<0.1) ^b	--	1.4 (<0.1) ^a	0.4 (<0.1) ^b	0.1 (<0.1) ^c
EPA/ARA	1.7 (0.1) ^a	2.5 (0.1) ^{ab}	2.8 (0.2) ^b	2.4 (<0.1)	2.1 (0.4)	2.6 (<0.1)

¹A2000 = *Algamac 2000*

²nd = not detected

³HUFA = highly unsaturated fatty acids defined as fatty acids with 20 or more carbon atoms and two or more double bonds.

TABLE 2. Selected fatty acid composition (% total lipid) of enriched rotifers and *Artemia* for trial 2. Within rows, values (mean \pm SEM; N = 2) in each prey type sharing the same superscript are not significantly different (P > 0.05).

Fatty acid	Rotifers			<i>Artemia</i>		
	A2000 ¹	Aq ²	Aq10 ³	A2000 ¹	Aq ²	Aq10 ³
18:2n-6	2.1 (<0.1) ^a	3.6 (0.1) ^b	3.6 (0.1) ^b	4.3 (<0.1)	4.2 (0.1)	4.3 (0.1)
18:3n-3	1.5 (<0.1) ^a	2.6 (0.1) ^b	2.0 (<0.1) ^c	20.6 (0.1)	20.2 (0.8)	21.0 (0.7)
20:4n-6	2.9 (<0.1) ^a	1.5 (<0.1) ^b	5.4 (0.3) ^c	1.5 (<0.1)	1.5 (0.3)	1.4 (<0.1)
20:5n-3	4.3 (0.1)	5.8 (0.1)	4.4 (0.5)	4.4 (<0.1)	4.1 (0.2)	4.1 (0.1)
22:5n-3	1.7 (0.1) ^a	2.1 (<0.1) ^b	1.7 (0.1) ^a	nd ⁴	nd ⁴	nd ⁴
22:6n-3	21.6 (0.1) ^a	29.1 (0.8) ^b	26.9 (0.1) ^b	5.3 (<0.1) ^a	5.9 (0.2) ^b	5.2 (<0.1) ^a
Σ HUFA ⁵	32.4 (0.3) ^a	39.9 (0.9) ^b	41.1 (1.3) ^b	12.1 (<0.1)	12.3 (0.1)	11.4 (0.1)
n-3 HUFA ⁵	29.3 (0.1) ^a	38.2 (1.0) ^b	34.7 (0.9) ^b	10.4 (<0.1) ^a	10.6 (0.4) ^a	9.9 (0.1) ^b
n-6 HUFA ⁵	3.1 (0.2) ^a	1.8 (0.1) ^b	6.4 (0.4) ^c	1.7 (<0.1)	1.6 (0.3)	1.5 (<0.1)
n-3/n-6	9.4 (0.4) ^a	22.0 (2.3) ^b	5.4 (0.2) ^c	6.1 (0.1)	6.6 (0.9)	6.6 (<0.1)
DHA/EPA	5.0 (0.1)	5.0 (0.1)	6.1 (0.7)	1.2 (<0.1) ^a	1.4 (<0.1) ^b	1.3 (<0.1) ^a
EPA/ARA	1.5 (0.1) ^a	4.0 (<0.1) ^b	0.8 (0.1) ^c	2.9 (0.1)	2.9 (0.7)	3.0 (0.1)

¹A2000 = *Algamac 2000*.

²Aq = *Aquagrow Advantage*.

³Aq10 = 90% *Aquagrow Advantage* + 10% *Aquagrow ARA*.

⁴nd = not detected.

⁵HUFA = highly unsaturated fatty acids defined as fatty acids with 20 or more carbon atoms and two or more double bonds.

TABLE 3. Selected fatty acid composition (% total lipid) of yellowtail snapper larvae reared during trial 1 at 11 and 18 days after hatching. Within rows, values (mean \pm SEM; N = 3) in each age group sharing the same superscript are not significantly different (P > 0.05).

Fatty acid	Day 11 larvae			Day 18 larvae		
	A2000 ¹	<i>I. galbana</i>	<i>N. oculata</i>	A2000 ¹	<i>I. galbana</i>	<i>N. oculata</i>
18:2n-6	3.3 (0.1) ^a	9.7 (0.5) ^b	8.9 (0.3) ^b	4.2 (0.1) ^a	7.6 (0.1) ^b	9.0 (<0.1) ^c
18:3n-3	1.3 (0.1) ^a	3.0 (0.1) ^b	4.1 (0.2) ^c	8.7 (0.5)	9.5 (0.6)	9.4 (0.1)
20:4n-6	3.9 (0.2)	3.4 (0.2)	3.8 (0.3)	3.6 (0.4)	3.5 (0.8)	3.2 (0.3)
20:5n-3	3.4 (0.4) ^a	4.6 (0.3) ^{ab}	5.2 (0.5) ^b	4.1 (0.6)	4.4 (0.2)	4.9 (0.1)
22:5n-3	3.3 (0.3) ^a	5.2 (0.3) ^b	5.7 (0.8) ^b	1.7 (0.1) ^a	2.9 (0.1) ^b	4.1 (0.1) ^c
22:6n-3	22.1 (1.3) ^a	8.8 (0.6) ^b	6.3 (0.3) ^c	11.5 (0.9) ^a	5.9 (<0.1) ^b	3.0 (0.2) ^c
Σ HUFA ²	34.0 (2.0) ^a	25.3 (1.7) ^b	24.1 (2.2) ^b	23.7 (1.2) ^a	20.9 (0.6) ^{ab}	18.4 (0.9) ^b
n-3 HUFA ²	30.0 (1.9) ^a	21.6 (1.4) ^b	19.7 (1.8) ^b	19.9 (0.9) ^a	16.2 (0.3) ^b	14.8 (0.5) ^b
n-6 HUFA ²	4.0 (0.2)	3.7 (0.3)	4.3 (0.4)	3.8 (0.3)	4.6 (0.8)	3.6 (0.4)
n-3/n-6	7.5 (0.3) ^a	5.9 (0.1) ^b	4.6 (0.1) ^c	5.2 (0.4)	3.8 (0.8)	4.1 (0.3)
DHA/EPA	6.6 (0.5) ^a	1.9 (<0.1) ^b	1.2 (0.1) ^c	2.9 (0.5) ^a	1.4 (0.1) ^b	0.6 (<0.1) ^c
EPA/ARA	0.9 (0.1) ^a	1.3 (<0.1) ^b	1.4 (0.1) ^b	1.2 (0.2)	1.4 (0.3)	1.5 (0.1)

¹A2000 = Algamac 2000.

²HUFA = highly unsaturated fatty acids defined as fatty acids with 20 or more carbon atoms and two or more double bonds.

TABLE 4. Selected fatty acid composition (% total lipid) of yellowtail snapper larvae reared during trial 2 at 12 and 20 d after hatching. Values are mean \pm SEM (N = 3). No significant differences were found among treatments (P > 0.05).

Fatty acid	Day 12 larvae			Day 20 larvae		
	A2000 ¹	Aq ²	Aq10 ³	A2000 ¹	Aq ²	Aq10 ³
18:2n-6	2.0 (<0.1)	3.0 (0.2)	3.0 (0.5)	3.6 (0.3)	3.7 (0.3)	4.0 (0.1)
18:3n-3	0.6 (<0.1)	0.7 (<0.1)	0.6 (0.1)	7.1 (0.7)	8.5 (0.8)	8.4 (0.6)
20:4n-6	3.3 (0.3)	3.4 (1.4)	4.9 (1.4)	4.4 (0.4)	3.5 (0.2)	3.6 (0.4)
20:5n-3	1.7 (0.3)	2.5 (0.2)	2.7 (0.3)	4.6 (0.4)	5.4 (0.6)	5.4 (0.3)
22:5n-3	1.1 (0.2)	1.5 (0.1)	1.5 (0.1)	0.7 (<0.1)	0.8 (0.1)	0.8 (<0.1)
22:6n-3	15.3 (2.2)	17.2 (1.0)	17.5 (0.5)	7.2 (1.0)	7.8 (0.9)	6.4 (0.5)
Σ HUFA ⁴	23.1 (3.3)	26.0 (0.9)	28.4 (0.8)	20.4 (1.1)	19.0 (0.2)	18.3 (0.1)
n-3 HUFA ⁴	19.3 (3.0)	22.0 (0.6)	22.8 (1.3)	15.5 (1.3)	15.0 (0.2)	14.0 (0.4)
n-6 HUFA ⁴	3.8 (0.3)	4.0 (1.5)	5.5 (1.4)	4.9 (0.4)	4.0 (0.2)	4.3 (0.4)
n-3/n-6	5.0 (0.4)	6.9 (2.0)	5.1 (2.0)	3.3 (0.5)	3.8 (0.2)	3.3 (0.4)
DHA/EPA	8.9 (0.6)	7.1 (0.5)	6.6 (0.4)	1.6 (0.3)	1.5 (0.4)	1.2 (0.1)
EPA/ARA	0.5 (<0.1)	1.0 (0.3)	0.8 (0.4)	1.0 (0.1)	1.6 (0.3)	1.5 (0.2)

¹A2000 = Algamac 2000.

²Aq = Aquagrow Advantage.

³Aq10 = 90% Aquagrow Advantage + 10% Aquagrow ARA.

⁴HUFA = highly unsaturated fatty acids defined as fatty acids with 20 or more carbon atoms and two or more double bonds.

Artemia enriched with Aq had significantly higher values for DHA and the DHA/EPA ratio compared to the Aq10 and A2000 enrichments. In addition, the amount of n-3 HUFA was significantly lower when *Artemia* were enriched with Aq10 but there were no significant differences in the amounts of ARA or n-6 HUFA among treatments.

The fatty acid composition of yellowtail snapper larvae reared during trials 1 and 2 are presented in Tables 3 and 4, respectively. In trial 1, the amount of individual fatty acids, n-3 HUFA, n-6 HUFA, and total HUFA generally reflected the patterns observed in the live prey with one major exception; the rotifers enriched with *N. oculata* had no detectable level of DHA but the larvae fed these rotifers had DHA as 6.3% of their total lipid. Larvae fed prey enriched with A2000 contained higher levels of DHA, Σ HUFA, and n-3 HUFA in their tissues than those fed on prey enriched with live algae. Although the addition of ARA to the Aq enrichment resulted in significantly higher levels of this fatty acid in rotifers, no significant increase in ARA or decrease in the ratio of EPA/ARA was detected in the tissues of 12-d-old larvae in trial 2.

Figure 1 shows the ratios between the DHA, EPA, and ARA levels in larval tissues and those in enriched prey items. The ratio of larval DHA content to prey DHA content for rotifers and *Artemia* enriched with *N. oculata* could not be calculated because only trace amounts of this fatty acid were detected in the prey items. Larvae fed rotifers enriched with high levels of HUFA (A2000, Aq, Aq10) contained relatively lower levels of these fatty acids in their tissues compared to dietary input. ARA ratios were an exception to this trend and were lower only in the tissues of larvae in the Aq10 treatment. The amount of HUFA in the tissues of larvae fed the *I. galbana* and *N. oculata* treatments was greater than the amount measured in the rotifers suggesting preferential retention of these fatty acids when dietary levels were low. Similarly, larvae fed exclusively on *Artemia* for several days had higher HUFA content in their tissues than in

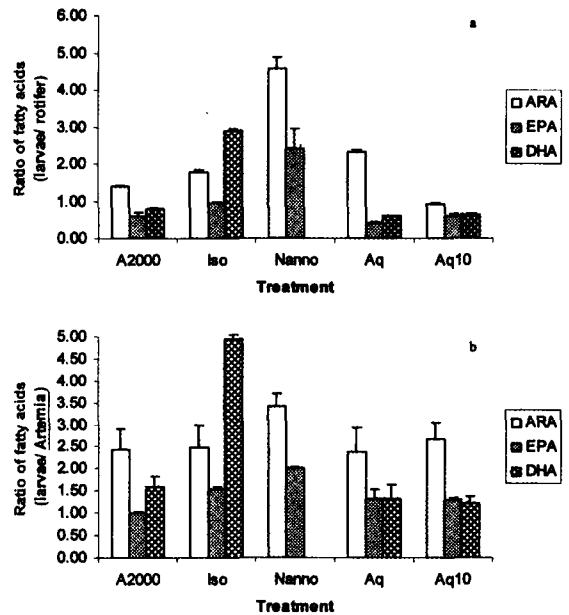


FIGURE 1. Relative proportion of DHA, EPA, and ARA in whole body tissue of yellowtail snapper larvae compared to dietary levels in enriched (a) rotifers and (b) *Artemia*. Ratios for DHA in the *N. oculata* treatment could not be calculated due to the low DHA content in the prey. Data are mean + SEM (N = 3).

the prey, especially in the *I. galbana* and *N. oculata* treatments.

In trial 1, the standard lengths of 11- and 18-d-old yellowtail snapper larvae fed prey enriched with *N. oculata* were significantly lower than those fed prey enriched with *I. galbana* or A2000 ($P < 0.05$; Table 5). SGR of larvae fed *N. oculata* enriched prey was lowest on day 11 after hatching, but on day 18 all treatments exhibited similar SGR ($P > 0.05$). Survival on day 18 after hatching was significantly lower for larvae fed prey enriched with *N. oculata* or *I. galbana* ($P < 0.05$). In trial 2, no significant differences in growth, SGR, or survival were found among larvae fed A2000, Aq, or Aq10 enriched prey items ($P > 0.05$; Table 6).

Discussion

The utilization of various enrichment products significantly affected the fatty acid composition of rotifers and to a lesser extent, *Artemia*. Overall, the commercial products produced

TABLE 5. Standard length (mm), SGR (%/d), and survival (%) of yellowtail snapper larvae fed rotifers and *Artemia* enriched with live algae or a commercial product during trial 1. Values (mean \pm SEM; N = 3) followed by the same superscript within each column are not significantly different ($P > 0.05$).

Treatment	Standard length		SGR		Survival	
	Age	11	18	11	18	
A2000 ¹		4.8 (0.1) ^a	6.7 (0.2) ^a	7.2 (0.2) ^a	4.8 (0.3)	5.9 (1.2) ^a
<i>I. galbana</i>		4.7 (0.1) ^a	6.4 (0.1) ^a	7.2 (0.1) ^a	4.2 (0.2)	1.4 (0.7) ^b
<i>N. oculata</i>		4.0 (0.1) ^b	5.2 (0.1) ^b	5.4 (0.3) ^b	4.0 (0.4)	1.1 (0.5) ^b

¹A2000 = Algamac 2000

TABLE 6. Standard length (mm), SGR (%/d), and survival (%) of yellowtail snapper larvae fed rotifers and *Artemia* enriched with commercial products during trial 2. Values are mean \pm SEM (N = 3). No significant differences were found among treatments ($P > 0.05$).

Treatment	Standard length		SGR		Survival	
	Age	12	20	12	20	
A2000 ¹		5.1 (0.1)	7.1 (0.3)	7.2 (0.2)	4.2 (0.8)	2.2 (0.3)
Aq ²		4.7 (0.1)	6.8 (0.2)	6.5 (0.3)	4.7 (0.1)	3.6 (1.1)
Aq10 ³		5.0 (0.1)	6.8 (0.4)	7.0 (0.1)	3.9 (0.8)	3.7 (0.5)

¹A2000 = Algamac 2000.

higher HUFA levels in prey than did either of the live algae enrichments (*N. oculata* or *I. galbana*). However, in all treatments the amount of DHA in enriched *Artemia* was considerably lower than that measured in the rotifers. Previous studies have shown that *Artemia* nauplii exhibit a lower incorporation efficiency of DHA than rotifers (Harel et al. 2002) and readily retroconvert DHA to shorter chain fatty acids including EPA (Navarro et al. 1999). The different patterns of fatty acid incorporation among rotifers and *Artemia* were also reflected in the larval tissues. After yellowtail snapper larvae had been feeding solely on *Artemia* for several days, the amount of DHA in larval tissue was reduced by approximately half that present during the rotifer feeding period.

The fatty acid composition of yellowtail snapper larvae generally reflected the patterns observed in the live prey. As the level of dietary DHA and total HUFA increased, a concurrent increase in these fatty acids was observed in the larval tissue 11 and 18 d after hatching (trial 1). However, when larvae were fed prey containing low HUFA levels (e.g., live algae enrichments) the amount of HUFA measured in larval tis-

ues was greater than the levels in the prey. These findings, consistent with other studies on marine fish larvae, result from preferential retention of DHA in larval tissues and/or chain elongation/desaturation of shorter chain fatty acids (Castell et al. 1994; Copeman et al. 2002). Copeman et al. (2002) evaluated the effects of live food enrichments on growth, survival, and fatty acid composition of larval yellowtail flounder *Limanda ferruginea*. The authors suggested that if the ratio of HUFA in the fish tissue to HUFA in the diet was greater than 1 this indicated preferential retention of HUFA, whereas a ratio less than 1 might indicate that the requirement for a particular fatty acid was being met by dietary levels in the prey. In this study, the tissue to diet HUFA ratios of yellowtail snapper larvae fed live algae (*N. oculata* or *I. galbana*) enriched rotifers were generally greater than 1, as were the *Artemia* fed larvae. If the ratio is a good indicator of prey quality, these data suggest that live algae enrichments did not meet minimum requirements of yellowtail snapper for essential fatty acids nor did any of the *Artemia* enrichments. However, rotifers enriched with Aq10 may be close to meeting

the HUFA requirements for yellowtail snapper larvae.

The enrichment of live prey with HUFA including ARA and DHA has been shown to enhance the growth and survival of several species of marine fishes (Rainuzzo et al. 1997; Bessonart et al. 1999). In this study, the SGR and standard length of 11-d-old yellowtail snapper larvae were improved when DHA increased from undetectable levels in rotifers enriched with *N. oculata* to 3% of the total lipid in rotifers enriched with *I. galbana*. A further increase in the amount of DHA in rotifers to 25% (A2000 enrichment) did not further increase growth but significantly improved overall survival. However, the SGR of larvae fed *Artemia* with low DHA enrichment (*N. oculata*; DHA = 0.1%) was not reduced at day 18 (compared to treatments with higher levels of DHA) suggesting that the effect of low levels of DHA in the diet on growth is most critical during the rotifer feeding period.

Koven et al. (2003) reported that the mortality of gilthead seabream larvae decreased in response to acute stressors with increasing levels of dietary ARA. We were unable to improve growth or survival by increasing the amount of ARA in rotifers (trial 2). However, any beneficial effects of increased ARA levels in the rotifers on survival may have been masked by the lack of significant differences in the amount of ARA in the enriched *Artemia*. Subsequent work is needed to increase the ARA content in *Artemia* to test this hypothesis.

The relative concentrations of HUFA such as DHA, EPA, and ARA also affect the growth and survival of marine fish larvae. DHA and EPA utilize the same enzymes to esterify fatty acids into phospholipid structures (Sargent et al. 1999), and an excess of EPA in cell membranes may result in decreased larval vitality (Watanabe 1993). Maximal growth and survival in this study was achieved when the ratio of DHA/EPA was approximately 5:1 in rotifers and 1.4:1 in *Artemia*. In rotifers and *Artemia* enriched with live algae or commercial preparations, high ratios of DHA/EPA were a direct reflection of higher levels of DHA rather than varying amounts of both fatty acids making

it difficult to separate the importance of high DHA levels vs. appropriate DHA/EPA ratios.

One goal of this study was to increase larval survival by decreasing the frequency and/or severity of mortality commonly observed in snapper larvae during the *Artemia* feeding period by providing increased amounts of HUFA via the diet. Daily observations throughout the experimental period indicated that larvae in all treatments exhibited signs of stress after they had been feeding exclusively on *Artemia*. Commencing on day 15 and 16 post-hatch, large numbers of larvae in all treatments were observed floating on the surface of the water and closer examination revealed severely over-inflated air bladders. These larvae never appeared to recover and were typically found dead on the bottom of the tank in the afternoon. We were not able to carry out stress tests that are typically used to investigate the impact of HUFA on stress resistance because of low overall survival at the end of the experiments. Thus, this study cannot directly address questions regarding the effect of varying HUFA levels on the vitality and resistance to stress in yellowtail snapper larvae. However, an apparent correlation between low dietary HUFA levels and survival is worth mentioning and will hopefully instigate further and more detailed research in this area. In trial 1, survival at day 18 was significantly lower in the *I. galbana* and *N. oculata* treatments compared to the A2000 treatment in which DHA and total HUFA levels were higher. The mortality event was more severe in larvae fed prey enriched with live algae, perhaps due to lower HUFA levels, and resulted in significantly reduced survival rates in these treatments.

The correlation between increasing HUFA levels in rotifers and *Artemia* and increasing growth and survival support our findings that the fatty acid profiles of enriched prey affected the outcome of this study. However, it is possible that the results of this study were also influenced by differences in the protein content or amino acid profiles of live prey fed the various enrichments. Although the survival rates of yellowtail snapper larvae remain low, they were significantly improved when commercial

products with high levels of n-3 HUFA and DHA/EPA were used to enrich their prey. Larval growth, measured as standard length, was also enhanced in larvae offered higher levels of dietary HUFA. *Artemia* enrichments never reached the HUFA levels produced in rotifers or those measured in dry feeds commonly used to raise marine fish larvae in our laboratory (Brinkmeyer and Holt 1998) and may simply not be an adequate food for yellowtail snapper larvae. Further research is needed to increase HUFA levels in enriched *Artemia* and to establish an early weaning protocol for this species to transition larvae from live prey to a formulated diet prior to the age at which larval mortality is highest.

Acknowledgments

This work was supported by contributions from the Sid W. Richardson Foundation and the Saltonstall – Kennedy Program (Grant NA07FD0173 from the National Oceanographic and Atmospheric Administration, U.S. Department of Commerce).

Literature Cited

- Anderson, W. D. Jr. 1987. Systematics of the fishes of the family Lutjanidae (Perciformes: Percoidae), the snappers. Pages 1-32 in J. J. Polovina and S. Ralston, editors. Tropical snappers and groupers: biology and fisheries management. West View Press, Boulder, Colorado, USA.
- Bessonart, M., M. S. Izquierdo, M. Salhi, C. M. Hernández-Cruz, M. M. González, and H. Fernández-Palacios. 1999. Effect of dietary arachidonic acid levels on growth and survival of gilthead sea bream (*Sparus aurata* L.) larvae. *Aquaculture* 179:265–275.
- Blaylock, R. B., K. M. Leber, J. M. Lotz, K. C. Stuck, and D. A. Ziemann. 2000. The US Gulf of Mexico Marine Stock Enhancement Program (USGMSEP): the use of aquaculture technology in “responsible” stock enhancement. *Bulletin of the Aquaculture Association of Canada* 100(3):16–22.
- Brinkmeyer, R. L. and G. J. Holt. 1998. Highly unsaturated fatty acids in diets for red drum (*Sciaenops ocellatus*) larvae. *Aquaculture* 161:253–268.
- Castell, J. D., J. G. Bell, D. R. Tocher, and J. R. Sargent. 1994. Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (*Scophthalmus maximus*). *Aquaculture* 128: 315–333.
- Copeman, L. A., C. C. Parrish, J. A. Brown, and M. Harel. 2002. Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. *Aquaculture* 210:285–304.
- Craig, S. R., S. J. Hatch, and G. J. Holt. 1990. Biological filter for conical tanks. *Progressive Fish Culturist* 52:61–62.
- Davis, D. A., K. L. Bootes, and C. R. Arnold. 2000. Snapper (Family Lutjanidae) culture. Pages 884-889 in R.R. Stickney, editor. *Encyclopedia of aquaculture*. John Wiley & Sons, Inc., New York, New York, USA.
- Douillet, P. A. and G. J. Holt. 1994. Surface disinfection of red drum (*Sciaenops ocellatus* Linnaeus) eggs leading to bacteria-free larvae. *Journal of Experimental Marine Biology and Ecology* 179:253–266.
- Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226:497–507.
- Ghioni, C., D. R. Tocher, M. V. Bell, J. R. Dick, and J. R. Sargent. 1999. Low C₁₈ to C₂₀ fatty acid elongase activity and limited conversion of stearidonic acid, 18:4(n–3), to eicosapentaenoic acid, 20:5(n–3), in a cell line from the turbot, *Scophthalmus maximus*. *Biochimica et Biophysica Acta* 1437:170–181.
- Harel, M., W. Koven, I. Lein, Y. Bar, P. Behrens, J. Stubblefield, Y. Zohar, and A. R. Place. 2002. Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. *Aquaculture* 213:347–362.
- Hoese, H. D. and R. H. Moore. 1998. *Fishes of the Gulf of Mexico, Texas, Louisiana, and adjacent waters*. Texas A&M University Press, College Station, Texas, USA.
- Kanazawa, A. 1997. Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. *Aquaculture* 155:129–134.
- Koven, W., R. van Anholt, S. Lutzky, I. B. Atia, O. Nixon, B. Ron, and A. Tandler. 2003. The effect of dietary arachidonic acid on growth, survival, and cortisol levels in different-age gilthead seabream larvae (*Sparus auratus*) exposed to handling or daily salinity change. *Aquaculture* 228:307–320.

- Morrison, W. R. and L. M. Smith.** 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research* 5:600–608.
- NMFS (National Marine Fisheries Service).** 1999. Our living oceans. Report on the status of U.S. living marine resources. Technical memorandum NMFS-F/SPO-41. U.S. Department of Commerce, Washington, D.C., USA.
- Navarro, J. C., R. J. Henderson, L. J. McEvoy, M. V. Bell, and F. Amat.** 1999. Lipid conversion during enrichment of *Artemia*. *Aquaculture* 174:155–166.
- Rainuzzo, J. R., K. I. Reitan, and Y. Olsen.** 1997. The significance of lipids at early stages of marine fish: a review. *Aquaculture* 155:103–115.
- Riley, C. M., G. J. Holt, and C. R. Arnold.** 1995. Growth and morphology of larval and juvenile captive bred yellowtail snapper *Ocyurus chrysurus*. *Fishery Bulletin* 93:179–185.
- Sargent, J., L. McEvoy, A. Estevez, G. Bell, M. Bell, J. Henderson, and D. Tocher.** 1999. Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture* 179:217–229.
- Sargent, J. R., L. A. McEvoy, and J. G. Bell.** 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture* 155:117–127.
- Turano, M. J., D. A. Davis, and C. R. Arnold.** 2000. Observations and techniques for maturation, spawning, and larval rearing of the yellowtail snapper *Ocyurus chrysurus*. *Journal of the World Aquaculture Society* 31:59–68.
- Watanabe, T.** 1993. Importance of docosahexaenoic acid in marine larval fish. *Journal of the World Aquaculture Society* 24:152–161.