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Effect of dietary fatty acids on the body tissues of larval and juvenile cobia and their prey

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Abstract

Polyunsaturated fatty acids (PUFAs) have been used as biomarkers in pelagic ecosystems although previous studies have failed to quantify the timing of conservation of dietary PUFAs in pelagic fishes and invertebrates. Here we investigated the influence of diet upon the timing of conservation of PUFAs throughout multiple trophic exchanges in larval and juvenile cobia (*Rachycentron canadum*) and their prey. Cobia, rotifers (*Brachionus plicatilis*), and *Artemia* (*A. franciscana*) were fed laboratory processed or natural diets resembling prey and dietary modification of fatty acid signatures was quantified using two-source mixing models. Specimens were collected throughout the experiment to track dietary influences over time. Cobia larvae underwent significant dietary modification of PUFAs after 24 h and conserved >90% of dietary PUFAs after an average of 6 days. Similar results were identified in juvenile cobia as significant dietary modification of PUFAs took place after 3 days and >90% were conserved after an average of 12 days. In addition, no significant ontogenetic changes in PUFA signatures were identified in juvenile cobia throughout the 30-day experiment. PUFA signatures in prey items (rotifers and *Artemia*) underwent significant dietary modification in 24 h, with over 90% incorporation after 5–7 days. Results from this study support the premise that fatty acids are promising dietary indicators and may be useful for future studies examining trophic relationships in marine ecosystems and habitat use of marine fishes.

Keywords: Cobia; Feeding; Polyunsaturated fatty acids; *Rachycentron canadum*; Turnover

1. Introduction

Understanding feeding history is essential for evaluating ecosystem performance since energetic relationships within marine ecosystems can have long-term implications for fisheries resource management (Iverson et al., 1997; McGrady-Steed et al., 1997; Brown et al., 1999; Hanson and Chouinard, 2002; Jennings et al., 2002). Although a number of

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techniques have been used to examine feeding habits (i.e., direct observation, scat analysis, and stomach contents analysis), these approaches have fundamental biases and inherent drawbacks when applied to aquatic environments (Brule and Rodriguez Canche, 1993; Bowen, 2000). Consequently, alternative approaches have been sought to indirectly quantify trophic interactions and overcome the deficiencies of past studies.

It has long been recognized that storage lipids, particularly fatty acids, are influenced by diet (e.g., Cowey et al., 1976; Kanazawa et al., 1979). Fatty acids are long-chained, typically unbranched carboxylic acids, and are frequently the largest component of lipids, a prevalent substance in most marine organisms (Gurr et al., 2002). Polyunsaturated fatty acids (PUFAs) are often used as dietary indicators since they cannot be created de novo, are seldom modified by marine organisms due to biochemical limitations, and are typically the most common fatty acids in marine ecosystems (Raclot et al., 1998; Hastings et al., 2001; Graeve et al., 2002; Gurr et al., 2002). Previous studies attempting to quantify PUFA transfer in individual species and among multiple trophic levels have been conducted and suggest that diet influences PUFA signatures in the tissues of consumers (Fraser et al., 1989; Kirsch et al., 1998). However, these studies fell short of quantifying the timing and extent of dietary PUFA incorporation into consumer tissues. Therefore, empirical data regarding the quantified exchange of dietary PUFAs in marine fish and invertebrates during early life, including the effects of ontogeny, are needed before PUFAs can be properly applied to retrospectively determine feeding patterns of aquatic consumers.

Cobia (*Rachycentron canadum*) is a large, pelagic fish that is primarily distributed throughout warm temperate, subtropical and tropical waters worldwide except for the eastern Pacific, with substantial concentrations located in the Gulf of Mexico (Shaffer and Nakamura, 1989). Adult cobia are absent along the continental shelf between March and October, and are believed to move into tropical waters to over winter (Ditty and Shaw, 1992). Spawning in the Gulf of Mexico typically occurs offshore on or just beyond the edge of the continental shelf between April and October, though eggs and larvae have been collected from estuarine environments in Florida (Dawson, 1971; Ditty and Shaw, 1992). Although natural history and migration patterns of adult cobia have been investigated, little is known about their ecology during the early life (Meyer and Franks, 1996). Larvae and juveniles are found in pelagic waters (65–134 m isobath) and have been collected within the upper 1 m of the water column; however, due to lack of information on the early life history of cobia, recruitment is considered to be low in the GOM (Ditty and Shaw, 1992). Additionally, habitat use in larvae and juveniles including during recruitment is currently unknown, although cobia are thought to associate with drifting *Sargassum* throughout several life history stages especially during early life (Ditty and Shaw, 1992).

The purpose of the present study was to quantify the timing and extent of dietary PUFA transfer in marine organisms across several trophic levels. Specific objectives were to determine the dietary influence on PUFA composition of consumers (larval and juvenile cobia) and their prey [rotifer (*Brachionus plicatilis*), *Artemia* (*A. franciscana*)]. The effect of diet on the tissue composition of consumers and their prey was investigated to determine whether differences occur in the incorporation of dietary fatty acids by micro-invertebrates and marine fishes.

2. Materials and methods

2.1. Larval cobia experiment

Cobia eggs were obtained from broodstock spawned in a recirculating system at the Fisheries and Mariculture Laboratory of the University of Texas Marine Science Institute (UTMSI) in Port Aransas, Texas. Eggs (1600/tank) were subsequently placed in 150-L conical rearing tanks ($n=3$) equipped with mechanical filters (air-driven water through filter floss) to maintain water quality. Experimental tank conditions matched spawning conditions (temperature 26.1 ± 0.3 °C, salinity 31.0 ± 0.5‰, dissolved oxygen 6.6 ± 0.3 mg/L, photoperiod 12:12-h light/dark). After 24 h, egg chorion and undeveloped eggs were removed from the bottom of the conical tanks using siphons. Larvae were initially sampled ($n=100$ per tank) to obtain baseline values at day 3 post-hatch, when yolk sacs were determined to be almost

completely absorbed, marking the onset of exogenous feeding; day 4 post-hatch was the first day (day 1) of the feeding experiment. Cobia were fed enriched rotifers (*B. plicatilis*) during days 1–4 of the experiment at an average density of 3–5 rotifers/ml following Faulk and Holt (2003). Larvae were then fed enriched *Artemia* during days 5–21 of the experiment at an average density of 1–2 nauplii/ml. Rotifers and newly hatched *Artemia* for use in the larval cobia study were enriched with AlgaMac-2000 (Aquafuana Bio-Marine Inc.) twice (0800, 1600) over a 16-h period at 0.1 g/500,000 rotifers and 0.3 g/200,000 nauplii following Faulk and Holt (2003). Samples of rotifers ($n=3$ samples of 150,000 per tank) and *Artemia* ($n=3$ samples of 5000 per tank) were collected prior to feeding cobia larvae on each day of the experiment to assess daily variation in fatty acid signatures of diet. Larvae were collected prior to feeding from each tank on days 6 ($n=50$ per tank), 9 ($n=25$ per tank, 12 ($n=15$ per tank), 15 ($n=15$ per tank), 18 ($n=10$ per tank), 21 ($n=10$ per tank), and 24 ($n=10$ per tank). Collected samples of rotifers, *Artemia*, and cobia larvae were rinsed with distilled water in a 50-ml cylinder (PVC) with a 48-μm nitrex bottom, transferred to pre-weighed glass vials and frozen at −80 °C.

2.2. Juvenile cobia experiment

Cobia larvae were reared following the previously described protocol for 24 days (3 days endogenous feeding, 21 days fed rotifer/*Artemia*). Larvae were then transferred into a large 1600-L tank with flow-through, aerated, filtered seawater, and reared on a combination of live *Artemia* and Rangen microparticulate feed (salmon starter crumbles #2, 1.0–1.4 mm) for a period of 10 days. Juvenile cobia (≥34 days) were then fed a control diet of Rangen feed over a period of 15 days for standardization purposes. Juveniles were sampled ($n=5$/day) at days 34, 37, 40, 43, 46, and 49 (post-hatch), measured (mm total length), blotted dry, weighed (±0.0001 g), and placed in high-density polyethylene bags, and frozen at −80 °C to obtain baseline PUFA signatures. Individuals ($n=360$) were then randomly caught by dip-net and stocked (30 fish/tank) into 12 square, 120-L tanks, with flow-through, aerated, filtered seawater. Juvenile cobia were then fed either a Rangen microparticulate diet (control) or one of the three treatment diets (fish-based [*Brevoortia patronus*], shrimp-based [*Farfantapenaeus aztecus*], or squid-based [*Lolliguncula brevis*]). All diet species were collected from Galveston Bay, Texas during the same 7-day period. Diets were ground frozen with hand-held Braun mixers, freeze-dried for 24 h, and then ground a second time into fine particulate form.

Proximate composition analysis (lipid, protein, ash) was conducted on the three natural diets (fish, shrimp, squid). Samples were freeze-dried in a Labconoco lyophilizer for 24 h to determine water content. Total lipid was then quantified through petroleum ether extraction in a Soxhlet extractor for 24 h (Dobush et al., 1985). Samples of lean dried material were then incinerated in a muffle furnace for 12 h at 550 °C to determine ash and ash-free dry lean mass (AFDLM). AFDLM is composed of 94% protein and is typically a good estimate of protein composition (Montevecchi et al., 1984). Carbohydrate content was assumed to be negligible in these diets and not analyzed in the present study. Proximate composition values for Rangen microparticulate feed were provided by Rangen Inc., Buhl, ID.

Juveniles were fed treatment diets at 5–7% of their body weight per day for an additional 15 days. Replicate tanks ($n=3$) were used for each diet, and all tanks were maintained at 27 °C for the duration of the experiment. Three to five cobia were randomly sampled from each tank at 52, 55, 58, 61, and 64 days (post-hatch) and daily feeding rations were modified according to weight gain. Individuals were measured (mm total length), blotted dry, weighed (±0.0001 g), placed in high-density polyethylene bags, and frozen at −80 °C. A 12:12-h light/dark photoperiod was utilized throughout the study. Temperature, salinity, and dissolved oxygen were measured daily. Salinity ranged between 31‰ and 33‰ (mean = 31.72 ± 0.62); temperature ranged between 26 °C and 28 °C (mean = 27.55 ± 0.23); dissolved oxygen ranged between 6.0 and 7.1 mg/L (mean = 6.4 ± 0.6). ANOVA identified no tank effects for salinity ($p=0.168$), temperature ($p=0.868$), or dissolved oxygen ($p=0.211$).

2.3. Rotifer/Artemia experiment

Rotifers were obtained from a continuous culture raised on *Nannochloris occulata* and yeast. *Artemia*

cysts were incubated at 26.0 °C and 28.0‰ for 20 h, and then separated from empty cysts. Both rotifers and *Artemia* were stocked into 19-L clear plastic tanks ($n=3$ per species) at a density of 300/ml and 100/ml, respectively. Tank conditions were manipulated to match the water quality of subsequent cobia feeding experiments. Initial samples of untreated rotifers and *Artemia* ($n=3\times150$ ml per tank) were collected to determine baseline levels of PUFAs. Consumers were enriched with AlgaMac-2000 (Aquafuana Bio-Marine Inc.) twice (0800, 1600) daily at 0.1 g/500,000 rotifers and 0.3 g/200,000 *Artemia*. Water was aerated and exchanged (1/3 volume) daily to maintain proper tank conditions. Replicate samples ($n=3\times150$ ml per tank) of rotifers and *Artemia* were collected each morning prior to enrichment for 5 and 7 days, respectively. Collected samples were rinsed with distilled water in a 50-ml cylinder (PVC) with a 48-μm nitrex bottom, transferred to pre-weighed glass vials and frozen at −80 °C. ANOVA identified no tank effects for salinity ($p=0.075$, 0.355), temperature ($p=0.396$, 0.494), or dissolved oxygen ($p=0.068$, 0.392) in either rotifer or *Artemia* experiments, respectively.

2.4. Fatty acid analysis

Lipid from whole larval cobia, juvenile muscle tissue, and homogenized prey was extracted in duplicate aliquots of chloroform/methanol (2:1; v:v) after Folch et al. (1957) as modified by Iverson et al. (2001). Fatty acid methyl esters were prepared directly by transesterification from ≤100 mg of pure extracted lipid (filtered and dried over anhydrous sulfate), with 0.5 N sulfuric acid in methanol plus dichloromethane following the Hilditch procedure (Iverson et al., 1992). Analysis of methyl esters was run in duplicate using temperature-programmed gas chromatography according to Iverson (1993) on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30 m × 0.25 mm internal diameter column coated with 50% cyanopropyl polysilohexane (0.25 μm film thickness; J&W DB-23; Folsom, CA, USA) and linked to a computerized integration system (Turbochrome 4 software, PE Nelson) (Iverson et al., 1997). Identification of fatty acids and isomers was determined by calibrating gas chromatography data with known standards (Nu Check Prep., Elysian, MN, USA). Individual fatty acids were converted to mass percent of total fatty acids using conversion factors from Ackman (1972, 1991) after accounting for the contribution of butylated hydroxytoluene (BHT).

2.5. Data analysis

Dietary contribution of individual fatty acids were expressed as a function of relative biomass increase to assess the effects of changes in whole body tissue turnover on fatty acid composition, W_R:

$$W_R = \frac{W_t}{W_i} \tag{1}$$

where W_t is the weight of subjects at time t and W_i is the weight of subjects at day 0.

Two-source mixing models are commonly utilized to determine contribution from two potential prey sources (prey a and prey b) to a predator (Vander Zanden and Rasmussen, 2001). In the present study mixing models were used to determine fatty acid (FA) contribution from two dietary sources (e.g., diet A versus diet B) to a subject (cobia, rotifer, *Artemia*):

$$\%\ \text{FA contribution}_{\text{diet a}} = \left(\%\ \text{FA}_{\text{subject}} - \%\ \text{FA}_{\text{diet b}}\right) / \left(\%\ \text{FA}_{\text{diet a}} - \%\ \text{FA}_{\text{diet b}}\right) \times 100 \tag{2}$$

and

$$\%\ \text{FA contribution}_{\text{diet b}} = 100 - \%\ \text{FA contribution}_{\text{diet a}} \tag{3}$$

where (diet a) represents the initial FA values in (day 0), (diet b) represents the FA values for each experimental diet, and (subject) represents the FA values in cobia, rotifer, and *Artemia* at each stage of the experiment.

2.6. Statistical analysis

Analysis of variance (ANOVA) was used to determine tank effects for temperature, salinity, and dissolved oxygen. Multivariate analysis of variance (MANOVA), ANOVA, and Tukey's honestly significant difference (HSD $\alpha=0.05$) tests were used to investigate differences in PUFA composition of the 4

experimental diets, and the PUFA composition of individuals from replicate tanks. Fatty acid data were arcsine-transformed before analyses of variance to correct for their binomial distribution (percentages) (Zar, 1998). MANOVA, ANOVA, and Tukey's post hoc tests were used to test for significant differences among collected samples based upon individual PUFAs and PUFA signatures for prey (rotifers and *Artemia*), cobia larvae, and cobia juveniles. Additionally, MANOVA, ANOVA, and Tukey's (HSD) tests were used to determine whether significant differences in PUFA signatures were caused by ontogenetic factors during early life by comparing individuals fed the control diet throughout the course of the experiment. Statistical tests were preformed with statistical packages SPSS 8.0 (SPSS Inc. Chicago, Illinois).

3. Results

Of the 67 individual fatty acids identified during analysis, 7 PUFAs (18:2$n-6$ [linoleic], 18:3$n-3$ [α-linolenic], 20:4$n-6$ [arachidonic, AA], 20:5$n-3$ [eicosapentaenoic, EPA], 22:5$n-6$ [docosapentaenoic, DPA], 22:5$n-3$ [docosapentaenoic, DPA], and 22:6$n-3$ [docosahexaenoic acid, DHA]) were used in most analyses for prey (rotifer and *Artemia*), and larval cobia feeding experiments to reduce the number of variables because they were the most abundant and found to be indicators of diet in a previous study (Turner and Rooker, in press). 18:3$n-3$ was not used as a variable in the juvenile cobia experiment due to minimal levels found in each of the diets. Further reference to 'PUFA signatures' is based upon these 7 fatty acids; 6 for the juvenile cobia experiment.

3.1. Larval cobia experiment

No significant differences in PUFA signatures were detected among larval cobia from replicate tanks (MANOVA, $F=0.94$, $p=0.528$); therefore, replicate samples were combined for statistical testing. Significant differences were identified among PUFA signatures of newly hatched cobia larvae, enriched rotifers, and enriched *Artemia* at day 0 (MANOVA, $F=56.31$, $p<0.001$). Additionally, PUFA signatures of newly hatched cobia larvae were significantly different than 24-day-old larvae (MANOVA, $F=22.0$, $p<0.001$). Univariate (ANOVA) tests indicated that levels of 18:2$n-6$ and 18:3$n-3$ increased significantly after cobia larvae began feeding on rotifers while 22:5$n-6$, 22:5$n-3$, and 22:6$n-3$ decreased significantly ($p<0.001$). Levels of 20:4$n-6$ and 20:5$n-3$ were higher while on the rotifer diet than after the change from rotifers to *Artemia* on day 8 ($p<0.001$). However, all 7 PUFAs exhibited changes that were consistent with levels present in each diet. PUFA signatures in cobia larvae resembled dietary levels (>95% dietary contribution) in an average of 6 days being fed rotifers/*Artemia* (Fig. 1). Significant differences between PUFA

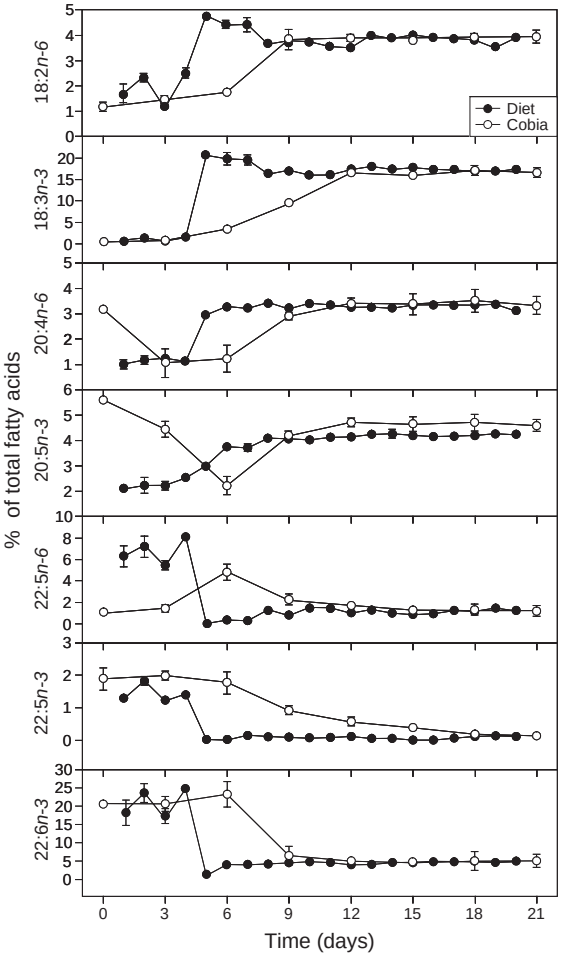

Fig. 1. Percent composition of individual polyunsaturated fatty acids 18:2$n-6$, 18:3$n-3$, 20:4$n-6$, 20:5:$n-3$, 22:$n-5$, 22:5$n-3$, and 22:6$n-3$ in cobia (*Rachycentron canadum*) (○) and diet (●) throughout the 21-day experiment.

composition of enriched rotifers and *Artemia* were primarily caused by differential dietary contribution rates after 1 day of enrichment. Biomass increase (W_R) was related to tissue turnover (Fig. 2). Four days after the change from rotifers to *Artemia* (day 9), cobia larvae exhibited a biomass increase of 17.54, a tissue turnover of over 4-fold since first feeding. During the same period, dietary contribution of 18:2*n*−6 in larvae was over 95% and levels of contribution in both 20:4*n*−6, and 22:6*n*−3 were greater than 85%. Subsequently, percent dietary contribution of 20:5*n*−3 and 22:5*n*−3 was greater than 75% during the same period, while 18:3*n*−3 exhibited less than 60% turnover. Dietary contribution of 22:5*n*−6 was low (0.9%) by day 9, although the large difference in levels of 22:5*n*−6 between rotifers to *Artemia* may have affected these calculations. Dietary contribution of 18:2*n*−6, 18:3*n*−3, 20:4*n*−6, 20:5*n*−3, and 22:6*n*−3 were over 95% by day 12, at which point larvae showed a W_R of 25.6. By day 21, dietary contribution of all 7 individual PUFAs were greater than 95%, at which point larvae showed a W_R of 111.7, over a 6-fold increase in tissue turnover since first feeding.

3.2. Juvenile cobia experiment

Lipid, protein (AFDLM), and ash differed among the four diets (Table 1). Lipid composition was

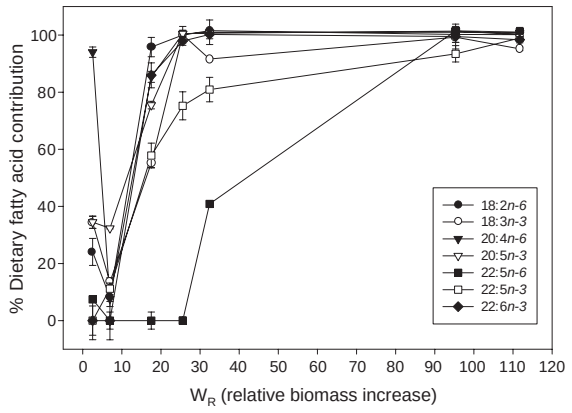

Fig. 2. Percent dietary contribution of individual fatty acids in larval cobia (*Rachycentron canadum*) as a function of relative biomass increase (W_R). Mean values are reported for (●) 18:2*n*−6, (○) 18:3*n*−3, (▼) 20:4*n*−6, (▽) 20:5*n*−3, (■) 22:5*n*−6, (□) 22:5*n*−3, and (◆) 22:6*n*−3.

significantly higher in the fish-based diet than either of the other diets. Protein was lower in the shrimp-based diet than the fish-based diet or squid-based diet, while levels of ash were lower in the squid-based diet than either of the other diets ($p<0.001$). Levels of lipid, protein, and ash were all lower in the control diet.

Juvenile (34-day-old) cobia ranged between 53.5 and 64.0 mm TL (mean = 57.7 ± 5.6 mm) and between 1.3 and 2.5 g in weight (mean = 1.8 ± 0.7 g). Growth varied significantly by date of collection and diet (ANOVA, $p<0.001$). Weight specific growth rate averaged 8.4% per day during the 15 days previous to the change in diet, while after the next 15 days, significant differences were identified in growth rates among individuals fed the control, fish-based, shrimp-based, and squid-based diets (ANOVA, $p<0.001$), which averaged a relative increase of 10.9%, 6.6%, 8.6%, and 10.5% per day, respectively. Juvenile cobia fed the control and squid-based diets were significantly larger and heavier than individuals fed the other two diets throughout the remainder of the study (ANOVA, $p<0.001$). Average relative biomass (W_R) per day was significantly greater in juveniles fed the control and squid-based diets (both 1.12) than individuals fed fish-based (1.07) or shrimp-based (1.09) ($p<0.001$); no significant differences were detected between juveniles fed control and squid-based diets.

No significant differences in individual PUFAs were detected among juvenile cobia from replicate tanks (MANOVA) for each of the four dietary treatments: control ($F=1.17$, $p=0.303$), fish-based diet ($F=0.52$, $p=0.888$), shrimp-based diet ($F=0.80$, $p=0.627$), squid-based diet ($F=1.91$, $p=0.074$). Juvenile cobia initially fed a control diet for 15 days were characterized by low levels of 20:4*n*−6, 22:5*n*−3, and 22:5*n*−6 (1.9%, 0.1%, and 0.6%, respectively) and high levels of 18:2*n*−6, 20:5*n*−3, and 22:6*n*−3 (8.6%, 8.3%, and 10.6%, respectively).

Juveniles fed different diets (Table 1) acquired significantly different PUFA signatures at day 49 to day 61 (MANOVA, $F=27.62$, $p<0.001$). Univariate tests (ANOVA) indicated that after the dietary change, significant differences were identified in all individual PUFAs among diets ($p<0.001$). Subsequently, juveniles fed the fish-based diet contained significantly higher levels of 20:5*n*−3, 22:5*n*−3, and 22:6*n*−3,

Table 1
Mean (±S.D.) dry mass[a] proximate composition and individual polyunsaturated fatty acids in experimental diets utilized during juvenile cobia feeding trials

Diet	Control	Fish-based	Shrimp-based	Squid-based
% Lipid	16.28 (±1.42)	31.02 (±3.43)	10.38 (±1.73)	10.98 (±3.93)
% Ash	8.30 (±0.56)	10.70 (±1.58)	16.09 (±1.32)	5.32 (±2.64)
% AFDLM	47.09 (±1.71)	58.28 (±2.71)	73.52 (±1.24)	83.70 (±3.55)
18:2*n*−6	8.55 (±0.40)	1.01 (±0.27)	0.42 (±0.15)	2.54 (±0.01)
20:4*n*−6	1.90 (±0.30)	1.03 (±0.14)	5.69 (±0.37)	4.61 (±0.31)
20:5*n*−3	5.49 (±0.52)	12.76 (±0.88)	12.11 (±0.77)	15.76 (±0.55)
22:5*n*−3	0.58 (±0.05)	0.32 (±0.20)	1.17 (±0.19)	0.99 (±0.28)
22:5*n*−6	1.53 (±0.09)	3.02 (±0.44)	3.53 (±0.51)	3.45 (±0.22)
22:6*n*−3	10.78 (±0.70)	9.15 (±1.01)	3.45 (±0.22)	32.99 (±1.29)

[a] Dry mass (wet values = dry value × [1 − proportion of water]).

individuals fed the shrimp-based diet contained significantly higher levels of 20:4*n*−6, 20:5*n*−3, 22:5*n*−6, and 22:5*n*−3, while juveniles fed the squid-based diet contained significantly higher levels of all individual PUFAs included in the study.

Mixing models were used to assess the timing and extent of dietary contribution of PUFA signatures in juvenile cobia, and significant changes in dietary PUFA contribution occurred in as few as 3 days (MANOVA, $F=7.38$, $p<0.001$). Univariate tests (ANOVA) indicated that all individual PUFAs exhibited different contribution rates ($p<0.001$) at day 49. Individuals fed the three diets had significantly different contribution rates at days 52 and 58, although rates were not significantly different between individuals fed the shrimp-based and squid-based diets on days 55 and 61. Juveniles fed the squid-based diet exhibited the largest increase in average dietary fatty acid contribution by day 49 (37.7%), compared with 36.2% (shrimp-based) and 32.1% (fish-based). This trend continued at each stage of the experiment, as at days 52, 55, 58, and 61 juvenile cobia fed the squid-based diet contained an average of 67.9%, 91.6%, 100%, and 100% of dietary PUFAs, respectively. During the same period, juveniles fed the shrimp-based diet averaged very similar levels of dietary PUFA contribution (67.4%, 87.5%, 98.4%, and 100%), while individuals fed the fish-based diet averaged much lower contribution levels (58.1%, 82.3%, 93.4%, and 96.4%) of dietary PUFAs, respectively.

Dietary contributions of individual fatty acids were plotted in relation to relative biomass increase (Fig. 3A–F). In all PUFAs analyzed, juvenile cobia fed the shrimp-based diet exhibited a larger increase in dietary contribution as biomass doubled ($W_R=2$) than individuals fed the other diets. Average contribution rates of individual PUFAs in juvenile cobia fed different diets throughout the study were significantly different (MANOVA, $F=3.60$, $p=0.013$). Univariate tests (ANOVA) indicated that all individual PUFAs exhibited different average contribution rates among diets (all $p<0.01$). Percent contribution of 18:2*n*−6 for juvenile cobia fed the shrimp-based diet was approximately 38% at a W_R of 2, while individuals fed fish-based and squid-based diets had significantly lower levels of approximately 21% and 25%, respectively (Fig. 3A). Similarly, dietary contribution of 20:4*n*−6, 20:5*n*−3, 22:5*n*−6 and 22:5*n*−3 in juvenile cobia were significantly higher in individuals fed the shrimp-based diet (percent contribution levels 57–74%), than in juveniles fed fish-based and squid-based diets (approximately 38–50% and 33–49%, respectively) (Fig. 3B–E). Dietary contribution of 22:6*n*−3 in juvenile cobia fed the shrimp-based diet was very high at a W_R of 2 (94%), although still significantly higher than levels in individuals fed the fish-based and squid-based diets (53% and 54%, respectively) (Fig. 3F).

3.3. Rotifer/Artemia experiment

Rotifers and *Artemia* initially contained high levels of 18:2*n*−6 and 18:3*n*−3 and lower levels of 22:5*n*−3 and 22:6*n*−3. No significant differences in individual PUFAs were detected among rotifer or *Artemia* samples from replicate tanks (MANOVA, $F=0.55$, $p=0.859$, $F=1.02$, $p=0.468$, respectively);

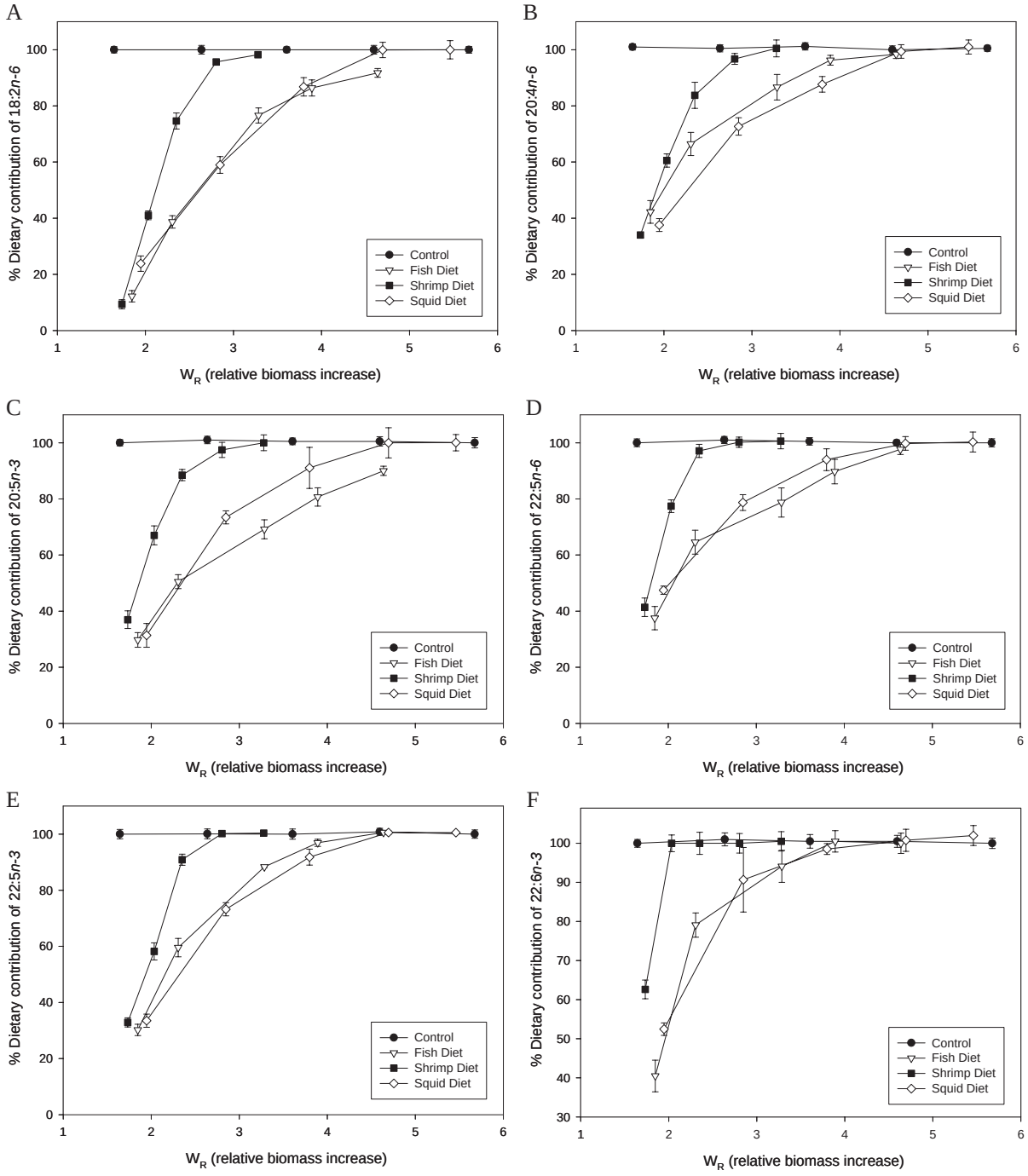

Fig. 3. Percent dietary contribution of (A) 18:2*n*−6, (B) 20:4*n*−6, (C) 20:5*n*−3, (D) 22:5*n*−6, (E) 22:5*n*−3, (F) 22:6*n*−3 in juvenile cobia (*Rachycentron canadum*) fed control (●), fish-based (▽), shrimp-based (■), and squid-based (◇) diets throughout the 15 days after dietary switch.

therefore, samples were pooled for analyses throughout each experiment. Significant differences were identified in rotifer samples among days 0 to 5 (MANOVA, $F=22.17$, $p<0.001$) and in *Artemia* samples among days 0–7 (MANOVA, $F=70.42$, $p<0.001$). Univariate (ANOVA) tests indicated that levels of 20:4$n-6$, 20:5$n-3$, 22:5$n-6$, 22:5$n-3$, and 22:6$n-3$ significantly increased after rotifers were enriched with AlgaMac-2000, while levels of 18:2$n-6$ and 18:3$n-3$ significantly decreased ($p<0.001$). Similarly, levels of 20:4$n-6$, 22:5$n-6$, 22:5$n-3$, and 22:6$n-3$ increased significantly in *Artemia* after enrichment while levels of 18:2$n-6$ and 18:3$n-3$ decreased significantly ($p<0.001$). Percent dietary contribution of 18:2$n-6$, 18:3$n-3$, 20:5$n-3$, 22:5$n-6$, 22:5$n-3$, and 22:6$n-3$ increased significantly by day 1 in both rotifers (24.1–85.0%) and *Artemia* (5.3–58.1%) and continued to increase steadily throughout each experiment. In contrast, enriched individuals did not show significant dietary contribution of 20:4$n-6$ in either experiment. Levels of 6 PUFAs in rotifers were comparable with levels in AlgaMac-2000 (>84% dietary contribution) by day 5 (Fig. 4A). Similarly, levels of 4 PUFAs in *Artemia* (18:2$n-6$, 18:3$n-3$, 20:5$n-3$, and 22:5$n-3$) were comparable with levels in AlgaMac-2000 (>88% dietary contribution) by day 7 of enrichment, while individuals exhibited lower dietary contribution of 22:5$n-6$ and 22:6$n-3$ (63% and 68%, respectively) (Fig. 4B).

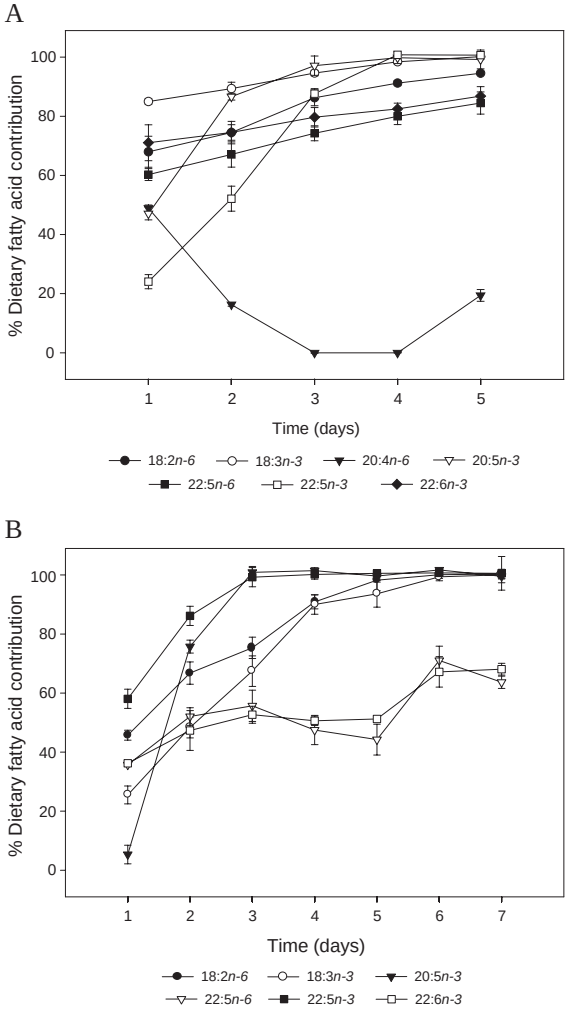

Fig. 4. Dietary contribution of PUFA signatures within rotifers (A) throughout the 5-day study and *Artemia* (B) throughout the 7-day study. A and B show percent dietary contribution of individual fatty acids in rotifers and *Artemia* for 5 and 7 days after diet switch, respectively. Symbols represent individual fatty acids on each day after diet switch: (●) 18:2$n-6$, (○) 18:3$n-3$, (▼) 20:4$n-6$, (▽) 20:5$n-3$, (■) 22:5$n-6$, (□) 22:5$n-3$, and (◆) 22:6$n-3$.

4. Discussion

Results from the present study demonstrate that dietary PUFA contribution to the lipid stores in cobia (larvae and juveniles) was significant after as few as 24–72 h and increased with additional feeding events. Larval and juvenile cobia in the present study contained large amounts of dietary PUFAs, demonstrating that PUFA composition of cobia was the result of dietary history. Percentages of individual PUFAs in both larval and juvenile cobia changed significantly after a change in diet, and this finding is consistent with the concept that lipid composition of marine fishes reflects dietary sources of PUFAs (Castell et al., 1994; Ibeas et al., 1996; Tucker et al., 1997; Kirsch et al., 1998). Still, past studies have failed to quantify the extent of such dietary exchanges, since many investigated the alteration of PUFA composition in tissues of cultured species to enhance growth, survival, and marketability (Watanabe, 1993; Ibeas et al., 1996). For example, Faulk and Holt (2003) found that larval cobia require high levels of dietary PUFAs for proper growth and survival; however, they did not examine the effect of dietary PUFA transfer upon

composition of PUFA signatures. In the present study, the extent of dietary contribution in all individual PUFAs sampled (18:2$n-6$, 20:4$n-6$, 20:5$n-3$, 22:5$n-6$, 22:5$n-3$, and 22:6$n-3$) in cobia larvae and juveniles was extensive, averaging of 25–35% after 3 days and >98% after 15–21 days, indicating that dietary PUFAs were very reflective of recent dietary changes. Since PUFAs of the $n-3$ and $n-6$ series (e.g., 20:4$n-6$, 20:5$n-3$, and 22:6$n-3$) cannot be synthesized de novo and are necessary for proper development, it appears that they are acquired directly from diet by cobia, and this finding is consistent with previous research on marine fishes (Watanabe, 1993; Evjemo et al., 1997; Copeman et al., 2002).

Experimental data from the present study suggests that dietary PUFAs are transferred rapidly and efficiently in micro-invertebrates and cobia larvae and juveniles. Previous studies investigating the timing of fatty acid transfer in marine organisms indicate that significant alterations of fatty acid signatures occur in consumer tissues over the course of several weeks. For example, Kirsch et al. (1998) determined throughout two feeding studies of adult Atlantic cod (*Gadus morhua*) that significant changes in fatty acid signatures occurred within 3 weeks after a change in diet. However, data from the present study was limited to larval and juvenile fishes and did not include adults, and thus relationships between rate of dietary PUFA incorporation and growth rate must be ascertained before such studies can be compared. Growth rate of juvenile cobia in the present study averaged 1.23 mm^{-1}/day for days 49 through 64 (post-hatch), which is comparable to the growth rate of several species of fast-growing pelagic fishes during the first year of life including *Seriola dummerili*, *Coryphaena hippurus*, and *Thunnus albacares* (Stequert et al., 1996; Massuti et al., 1999; Thompson et al., 1999; Rivera and Appeldoorn, 2000; Wells and Rooker, 2004). Therefore, the rate of PUFA transfer and incorporation in juvenile pelagic fishes would be comparable to levels identified in juvenile cobia in the present study. Although adult fish were not included in the present study, a comparison of the growth rates of juveniles and adults should facilitate an approximate characterization of dietary PUFA transfer in adults. Growth rates in adult cobia (2–5 years) tend to be considerably slower (0.35–0.50 mm^{-1}/day), possibly affecting the metabolic rate, the rate of biomass increase (W_R), and presumably the rate of dietary PUFA transfer and incorporation as well (Franks et al., 1999; Franks and Brown-Peterson, 2002). Since growth rate in adult cobia may be as much as 3–4 times slower than in larvae and juveniles, it stands to reason that the transfer and incorporation of dietary PUFAs in adult cobia may take as much as 3–4 times longer than in cobia during early life, as a significant change in PUFA signatures after a diet change in adult cobia may take 3–4 times the duration found in juvenile fish. Therefore it appears that dietary PUFAs are transferred rapidly in larval and juvenile cobia (at a correspondingly reduced rate in adult fishes) indicating that PUFAs could be used to represent very recent dietary histories of cobia among several different age classes.

PUFA signature data from juvenile cobia appears to demonstrate a connection between growth, relative increase in biomass, and dietary PUFA composition. Juvenile cobia fed the squid-based diet were the largest, exhibited the greatest increase in relative biomass, and obtained the greatest amount of dietary PUFAs. Nevertheless, percent contribution of dietary fatty acids in relation to biomass increase was greater in individuals fed the shrimp-based diet, which was similar to results from a previously conducted feeding study of juvenile red drum (Turner and Rooker, in press). It appears that contribution of dietary fatty acids occurred at a relatively greater rate in individuals fed the shrimp-based diet since they were accumulating biomass more slowly, and thus ingested a larger amount of dietary fatty acids per degree of biomass change. For instance, juvenile cobia fed the shrimp-based diet had 38% of dietary contributed 18:2$n-6$ at a W_R of 2, while individuals fed the fish-based and squid-based diets exhibited levels of 21% and 25%, respectively, at the same W_R. However, juvenile cobia fed the shrimp-based diet doubled biomass ($W_R=2$) after three feeding events (day 52 of the experiment), while those fed the fish-based diet doubled biomass after two feeding events (day 49), and individuals fed the squid-based diet doubled biomass after one feeding event (less than three days). Since squid-based diet contained significantly more protein than either of the other diets, and protein growth is directly related to protein consumed (McCarthy et al., 1994; Mommsen, 1998), it is not surprising that cobia fed the squid-based diet

exhibited a proportionally higher growth rate. Dietary PUFAs have been linked to somatic growth in marine fishes and invertebrates, especially 20:5$n-3$ and 22:6$n-3$ (Copeman et al., 2002; Koueta et al., 2002; Navarro and Villanueva, 2003; Woods, 2003), albeit it appears that the amount of total ingested PUFA influences dietary contribution rates of individual fatty acids and that dietary levels of protein primarily affect biomass increase.

Although factors controlling development during early life in marine fishes and invertebrates could influence the transfer of dietary PUFAs, no ontogenetic effect upon PUFA signatures was detected in juvenile cobia. Levels of individual PUFAs in cobia fed the control diet did not significantly change throughout the 30-day experiment, indicating that preferential deposition of particular fatty acids may be outweighed by the quantity and multiplicity of PUFAs available for utilization from diet. Information relating to the effects of ontogeny upon dietary PUFA transfer and deposition in marine fishes is lacking; however, it appears that the short-term ontogenetic results identified in the present study are similar to those identified in similar studies of marine invertebrates. For example, Navarro and Villanueva (2003) found that PUFA signatures in *Octopus vulgaris* did not change when fed a constant diet for 30 days, indicating that factors controlling ontogeny had no effect upon transfer of dietary PUFAs. Due to the limited scope of the present study, additional experiments are needed to determine the effects of ontogenetic changes upon dietary PUFA transfer and deposition in marine organisms.

In the present study, change in PUFA signature after dietary change was much faster in micro-invertebrates but were not as efficiently transferred as in marine fish. For example, dietary PUFA contribution in rotifers and *Artemia* was significant after as few as 24 h and increased with further enrichment. However, all PUFAs did not track enrichment as well as observed in trials on cobia. Specifically, levels of 20:4$n-6$ was not very reflective of diet in either rotifers or *Artemia*, with dietary contribution levels reaching 0% in many situations. The remaining 6 PUFAs were found to be excellent dietary tracers and incorporated large amounts of dietary PUFAs quickly, indicating that PUFAs could be transferred among several trophic levels rapidly and efficiently, similar to the results from past studies. Fraser et al. (1989) investigated the transfer of fatty acids in a marine food chain containing phytoplankton, zooplankton, and a larval herring (*Clupea harengus* L.) and found that peaks in levels of individual fatty acids during a phytoplankton bloom could be identified weeks later in zooplankton and fish larvae. In the present study, diet-related changes in PUFA signatures of consumers were more rapid and PUFA signatures in tissues of micro-invertebrates and larval and juvenile cobia were reflective of diet within 1–3 days of dietary change, respectively. These results demonstrate that fatty acid signatures can be transferred across three trophic levels (producers, primary, and secondary consumers) within as few as 5 days, indicating that shifts within PUFA signatures at lower levels of marine ecosystems can quickly affect PUFA signatures of higher-order consumers.

Since results from the present study suggest that PUFA signatures represent natural indicators of diet, they could therefore provide significant information relating to energy flow, habitat use, and population differentiation in individual species and marine ecosystems. Fatty acids have been used to track dietary shifts in natural populations, which could ultimately be used to identify changes in energy flow in marine consumers and entire ecosystems. For example, previous studies have identified significant changes in PUFA signatures caused by temporal shifts in diet among key organisms and the transfer of organic matter between adjacent food webs, which could have confounding effects upon patterns of energy flow within entire ecosystems (Hargeby et al., 1994; Fileman et al., 1998; Fukuda and Naganuma, 2001; Link and Garrison, 2002). Habitat selection is a critical component of successful growth, survival, and recruitment in marine fishes and invertebrates (Behrents, 1987; Levin, 1998; Hall and Rudstam, 1999) and patterns of habitat use have been identified using fatty acids. Although studies have typically focused upon large-scale changes in fatty acid signatures occurring between freshwater and marine food webs (Steffens, 1997; De Silva et al., 1998; Kao et al., 2002), subtle differences among various microhabitats could be identified to track small-scale movement patterns of fauna, similar to studies using stable isotopes (Herzka and Holt, 2000). Lastly, fatty acids signatures could be used to

characterize population differences based upon recent dietary histories (Beddingfield and McClintock, 1998; Hoff, 2000; Logan et al., 2000; Reimchen and Nosil, 2001), similar to other environmental indicators used to discriminate subpopulations such as chemical signatures in the hard parts (otoliths) of fishes (Rooker et al., 2001, 2003). Therefore, it appears that fatty acids might enable a further understanding of the dynamics of dietary shifts in trophic food webs, which is essential for evaluating ecosystem performance, and can have long-term implications for fisheries resource management (Hanson and Chouinard, 2002; Jennings et al., 2002).

Finally, results from the present study indicate that significant turnover in diets of lab-reared marine fish was particularly rapid (1–3 days), especially during their early life history. The utilization of PUFAs as dietary tracers in laboratory studies have been likened to processes occurring in trophic food webs; however, rarely are empirical and field data used together in order to trace the extent of long-term feeding events in open-ocean systems. To test the application of these results in a marine ecosystem, samples of wild caught juvenile cobia ($n=5$) found associated with floating *Sargassum spp*., an endemic secondary consumer shrimp (*Leander tenuicornis*), and the dominant source of dietary organic matter in the *Sargassum* community (POM-phytoplankton) (Rooker et al., 2004; Turner, 2004) and were collected from the western Gulf of Mexico (Port Aransas, Texas) in July of 2001 and 2002. PUFA signatures of this primary producer, secondary consumer, and juvenile cobia and indicated that fatty acids were similar among individuals from each subsequent level of the food chain. Correspondingly, these trends were found to follow the same pattern as identified in lab reared specimens (enrichment, *Artemia*, cobia) indicating that the utilization of PUFA signatures as biomarkers has promise for retrospective determination of recent dietary interactions among consumers in marine ecosystems (Fig. 5A and B).

In summary, results from the present study indicate that PUFAs are valuable biomarkers and can be used to determine retrospective feeding histories in larval and juvenile cobia, and their prey (rotifers and *Artemia)*. It appears that PUFA signatures are transferred into micro-invertebrates and larval and juvenile cobia very efficiently with a

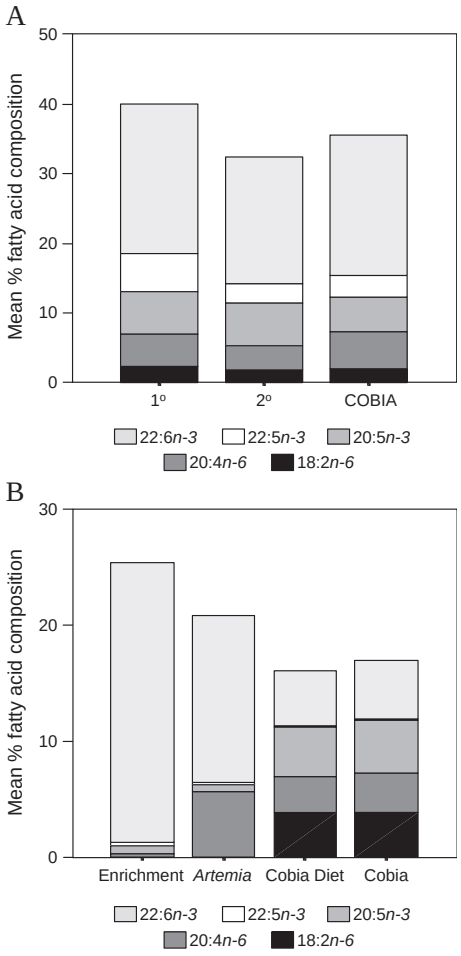

Fig. 5. Mean percent composition of fatty acids throughout several trophic levels in field (A) and lab (B) samples. In A, stacked bars represent PUFA signatures in POM/phytoplankton (1°), *Leander tenuicornis* (2°), and wild-caught juvenile cobia, all collected from Sargassum communities. In B, stacked bars represent PUFA signatures in enrichment (AlgaMac-2000), *Artemia* (enriched for 7 days), cobia diet (*Artemia* enriched for 1 day), and cobia (lab reared for 21 days).

dietary composition of >98% after several days, possibly attributable to the essential nature of PUFAs. In all species examined, significant dietary modification of PUFA signatures occurred in as few as 1–3 days, demonstrating the use of PUFAs for identifying very recent trophic relationships. Additionally, no ontogenetic changes were identified in PUFA signatures of juvenile cobia, indicating that diet is the primary source of PUFA composition these marine fish during early life.

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