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Effects of Parental Diet on Nutritional Composition of Yolk and Metabolic Programming in Southern Flounder

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Abstract

Fatty acids play a critical role in cellular functions and are vital to the growth and development of fish during early stages of life. The nutrients available to fish embryos and early larvae are dependent on recent maternal diet in certain fish species, including Southern Flounder (*Paralichthys lethostigma*). Variations in maternal diet can result in subsequent changes to metabolic functioning of offspring, such as capacity for nutrient absorption, which is indicative of metabolic programming. The aim of this study was to (1) investigate the effects of the maternal diet on the fatty acid profiles of eggs, and (2) determine whether Southern Flounder exhibit metabolic programming in the form of measured differences in larval fatty acid composition between spawns from two maternal diet treatment groups (shrimp or sardine). Results demonstrated direct diet-egg relationships for 11 fatty acids, with the majority of these fatty acids being higher in the shrimp diet and corresponding eggs. Analyses of larval fatty acid composition, however, did not reveal significant differences in any of the 27 fatty acids measured for any of the three larval stages sampled. Therefore, there was no evidence of metabolic programming in Southern Flounder based on comparisons of larval fatty acid

composition among parental diet treatments. This contrasts with prior studies that provided evidence for metabolic programming in marine teleosts.

Introduction

Lipids are a vital component of all living cells, playing an important role in membrane composition, metabolism, hormone production, cell signaling, and a source of energy (German, 2011; Calder, 2015). Fatty acids are the building blocks of lipids, and essential fatty acids (EFA) are those that cannot be synthesized by the body and must be obtained through the diet (Taşbozan & Gökçe, 2017). EFAs include omega-3 and omega-6 long-chain polyunsaturated fatty acids (PUFA), such as DHA (docosahexaenoic acid, 22:6n3). In fishes, EFAs are especially important during embryonic and larval development since the lipid-rich yolk in eggs is the sole source of nutrition for the newly hatched larvae, providing energy as well as materials for building new tissues during early growth and development (Sargent et al., 1995).

The nature of reproductive energy and nutrient allocation in different fish species affects egg composition and falls within a continuum of breeding strategies from income breeding to capital breeding, where fishes may utilize lipids and fatty acids that are stored in their tissues (capital) or from their recent diet (income) for vitellogenesis (Burns & Fuiman, 2020). Thus, maternal diet and nutrient allocation strategy are important factors that can affect the fatty acid composition of egg yolk (Taşbozan & Gökçe, 2017).

Southern Flounder, *Paralichthys lethostigma*, is an economically important species that is currently facing population declines throughout much of the U.S. Southeast Atlantic and Gulf of Mexico (Erickson et al., 2021; Smith et al., 2022). Southern flounder are classified as income breeders in nutritionally favorable conditions, meaning they can rapidly transfer recently

ingested nutrients into yolk (Burns & Fuiman, 2020). Therefore, lipids ingested by the broodstock (adult fish) directly affect egg quality (Rainuzzo et al., 1997).

Existing studies have further explored the mechanisms and extent of nutritional transfer from diet to eggs and offspring, focusing especially on essential fatty acids. The results of several studies on Southern flounder widely support the link between parental diet and offspring fatty acid composition, in that concentrations of certain fatty acids in the broodstock diet correlate with concentrations of certain fatty acids in eggs and larval bodies (Oberg & Fuiman, 2015; Burns & Fuiman, 2019; Burns & Fuiman, 2020; Bailey et al., 2023).

The concept of metabolic programming (or nutritional programming) describes how early nutrition can produce long-term metabolic effects on offspring, including their ability to absorb and retain nutrients in their own diet (Hou & Fuiman, 2020). This phenomenon has primarily been studied in mammals and is beginning to be studied in fishes, especially programming effects on growth, survival, and nutrient metabolism (Hou & Fuiman, 2020). Alteration of these metabolic pathways occurs during a critical window of high plasticity in development (Hou & Fuiman, 2020). Since yolk provides all early nutrition and its composition is affected by maternal diet, metabolic programming in offspring can be linked to parental diet.

A variety of methods have been employed to induce metabolic programming in fish experimentally, as well as how the resulting effects are measured. A study on Nile tilapia (*Oreochromis niloticus*) manipulated fry diet with high or low carbohydrate levels and detected permanent metabolic effects through analysis of mRNA and metabolites (Kumkhong et al., 2020). Evidence of metabolic programming mediated by parental diet has also been reported for Red Drum. It was shown that differing DHA content in the adult diet produced pronounced

antipredator behavioral differences in offspring and variations in fatty acid composition in eggs and larvae (Fuiman and Perez, 2015).

A diet-shift (shrimp vs. fish) study on red drum uncovered evidence of metabolic programming through corresponding egg and larval fatty acid composition differences, as well as alterations of metabolic pathways in larvae (Hou et al., 2022). Hou et al. (2022) found 15 out of 27 measured fatty acids in red drum eggs to be significantly correlated with fatty acid levels in the recent maternal diet. The considerable extent to which fatty acid contents of the maternal diet are reflected in eggs and offspring supports the use of fatty acid quantities in fish eggs and larval tissue as biomarkers to track lipid transfer and evaluate the functional consequences of variations in the maternal diet (Hou et al., 2020).

This study investigates how changes in parental diet affect fatty acid composition of eggs and whether those variations in egg composition affect the fatty composition of larvae as an indicator of metabolic programming in Southern Flounder. We expected that variations in fatty acid intake by adult southern flounder would produce corresponding variations in egg composition and hypothesized that variations in egg composition would produce larvae with different fatty acid compositions, indicating that their lipid metabolism is programmed by maternal diet. Our hypothesis aligns with findings of several prior studies that corroborate the existence of metabolic programming in some marine fishes mediated by maternally-derived fatty acids. The conclusions of this study can inform future aquaculture practices to address rising demands for food supply (Naylor et al., 2021) and conservation management strategies for restocking wild populations.

Methods

Southern flounder broodstock were maintained by staff at the Fisheries and Mariculture Laboratory (FAML) of the University of Texas Marine Science Institute in Port Aransas, TX within a recirculating aquaculture system. Temperature, salinity, and photoperiod were held constant (18℃, 32 ppt, 10 h:14 h light:dark). The experiment was carried out using two treatment groups of adult flounder in separate raceways. Since it is known that different fishery products like fish and shrimps have high variation in fatty acid patterns and contents (Mesa et al., 2021), we fed one raceway exclusively brown shrimp (*Farfantepenaeus aztecus*) and the other exclusively Spanish sardine (*Sardinella aurita*) to produce eggs with different fatty acid compositions. Strip spawning was conducted approximately every week, facilitated by Ovaprim synthetic hormone injections to stimulate ovulation.

Larvae were reared under water conditions of 30-34 ppt and 18-19 °C. All larvae were fed the same diet of live prey twice a day. From 5 to 30 days post-hatch (dph), they were fed enriched rotifers. Additionally, they were fed newly hatched *Artemia* from 23 to 25 dph and enriched *Artemia* from 26 to 55 dph in increasing concentrations as the larvae grew. The commercial enrichment product used for rotifers and *Artemia* was Algamac 3050 DHA 10 (Table 1).

Samples were taken of broodstock diets (sardine and shrimp), larval diets (rotifers and *Artemia*), flounder eggs, and flounder larvae at different life stages for fatty acid analysis. Larvae samples for each spawn were pooled—twenty individual larvae were sampled from each spawn at 27 dph, and 10 individuals were sampled at 41 and 55 dph. All egg and larva samples were photographed under a microscope, and standard length was measured using the ImageJ software (Version 1.54g, Schneider et al. 2012). All samples were rinsed with reverse osmosis (RO) water and stored in a -80℃ freezer for subsequent analysis.

To prepare for the biochemical assay, samples were lyophilized and homogenized. For diet samples, each homogenized sardine sample included 3 individuals, and shrimp samples consisted of 1 individual. Lipids were cold extracted using a solution of 2:1 chloroform:methanol (v/v). A known amount of an internal standard, tricosanoic acid (23:0), was incorporated to determine the amount of each fatty acid in the samples. The process of fatty acid methylation, consisting of saponification and transesterification, was facilitated by potassium hydroxide and 14% boron trifluoride, respectively. Fatty acid methyl esters were then dissolved in hexane to be run through a gas chromatograph (Shimadzu) using helium gas as a carrier. Resulting chromatograms allowed for the identification of 27 fatty acids in each sample by comparing peaks with commercial standards. Each fatty acid was quantified as mg g^{-1} dry weight of the sample, as well as the percentage of total fatty acids.

Quality checking of data and exploration of initial relationships was executed with principal component analyses (PCA). Statistical differences for diet, egg, and larva samples based on diet group were tested with Student's t-tests, and p-values were adjusted for multiple comparisons using the Benjamini-Hochberg method ($\alpha = 0.05$). All statistical analyses were conducted in R Studio (Version 2023.09.1+494) with the ggplot2 and tidyverse packages.

Results

Diet Fatty Acid Composition

Eleven samples of the sardine diet and 8 samples of the shrimp diet were analyzed to identify significant differences in fatty acid composition between the two treatments. Several differences in fatty acid composition were observed between the two broodstock diets (shrimp, sardine; Table 2). Student's t-tests showed that 22 out of 27 fatty acids analyzed were significantly different between shrimp and sardine. Nine of the 22 significantly different fatty

acids were at higher proportions in sardine than shrimp (14:0, 16:0, 16:1n7, 16:2n4, 18:3n3, 18:3n6, 18:4n3, 22:1n11, and 22:6n3), while 13 fatty acids were significantly higher in shrimp than sardine (15:0, 16:3n4, 17:0, 18:0, 18:1n7, 18:1n9, 18:3n4, 20:2n6, 20:3n3, 20:4n6, 20:5n3, 22:4n6, and 22:5n3; Table 2).

Egg Fatty Acid Composition

Thirty-nine females in the broodstock were strip-spawned to obtain 48 egg samples, which consisted of 20 samples from the shrimp treatment and 28 from the sardine treatment. Eighteen of the 27 fatty acids measured were significantly different between eggs produced by adult females exclusively fed either shrimp or sardine (Table 3). Three of the 18 significant fatty acids were higher in eggs from the sardine diet treatment eggs than the shrimp diet treatment (16:2n4, 22:5n6, and 22:6n3), while eggs from the shrimp diet treatment had a significantly higher amount of 15 fatty acids (14:0, 16:1n7, 16:3n4, 17:0, 18:1n9, 18:2n6, 18:3n3, 18:3n6, 20:2n6, 20:3n3, 20:4n3, 20:4n6, 20:5n3, 22:4n6, and 22:5n3).

Diet-Egg Relationships

When comparing fatty acid profiles of the broodstock diet and eggs, only direct (positive) relationships were considered, meaning fatty acids that were significantly different by diet treatment in both the diet and egg samples and had differences in the same direction. Eleven of 27 measured fatty acids that were significantly higher in the broodstock diet were also higher in the eggs produced from the same diet treatment and are thus directly related (Table 4).

Larval Fatty Acid Composition

Larvae at 27 dph were sampled from 10 spawns (shrimp: n = 6, sardine: n = 4, 20 individuals per sample) and showed relatively consistent fatty acid profiles between both diet treatment groups (Table 5). Student's t-tests revealed no significant differences by broodstock diet treatment in any of the 27 fatty acids measured for 27-dph larval body tissue. Larvae at 41 dph were sampled from 9 spawns (shrimp: n = 5, sardine: n = 4, 10 individuals per sample), and there were no significant differences in any fatty acids between the two broodstock diet groups (Table 6). Eleven spawns were sampled for 55-dph larvae (shrimp: n = 4, sardine: n = 7, 10 individuals per sample), and no significant differences were found between broodstock diet treatments (Table 7).

Larval Fatty Acid Ratios

Because a previous study on Southern flounder larvae identified a meaningful correlation between larval antipredator performance and larval ratios of DHA:ARA, as well as DHA:EPA (Burns & Fuiman, 2019), these two ratios were examined for differences based on diet treatment. In our study, no significant differences in DHA:EPA nor DHA:ARA by diet treatment group ($P > 0.5$) were found for 27-, 41-, and 55-dph larvae.

Discussion

Differences in the fatty acid levels of the flounder eggs directly corresponded to differences in the parental diet for 11 fatty acids, 6 of which are essential omega-6 or omega-3 PUFA (20:2n6, 20:4n6, 20:5n3, 22:4n6, 22:5n3, 22:6n3). Bailey et al. (2023) used the same diet treatments (shrimp and sardine) and found 8 direct diet-egg relationships (18:1n7, 16: 2n4, 16:3n4, 22:5n3, 22:6n3, 20:4n6, 22:5n6), 5 of which were PUFA (22:5n3, 22:6n3, 20:4n6,

22:5n6). Similar conclusions have also been found for another batch-spawning teleost, red drum, where it has been proven that fatty acid contents of eggs can differ significantly based on variations in the maternal diet (Fuiman and Perez, 2015; Hou et al., 2020). The considerable number of direct diet-egg relationships observed in our study affirms our expectations and corroborates the findings of previous studies of southern flounder as well as other species, thereby cementing the role of maternal diet on egg fatty acid composition (Oberg & Fuiman, 2015; Burns & Fuiman, 2019; Burns & Fuiman, 2020; Bailey et al., 2023).

Although the parental diet treatments generated the expected differences in egg fatty acid composition, signs of metabolic programming (measured as differences in fatty acid composition of the larval body tissue) were not present in larvae at any of the three larval stages observed. Exogenous nutrition (larval diet) was the same for all spawns, which ensured that the spawns differed only in maternally-derived endogenous nutrition for the larvae. We had hypothesized that the Southern flounder larvae would show different fatty acid profiles depending on the maternal diet, based on several previous studies that established ties between larval fatty acid composition and maternal diet (Fuiman & Ojanguren, 2011; Fuiman & Perez, 2015; Burns & Fuiman, 2019). However, there were no differences in larval fatty acid composition by broodstock diet, which contradicts our hypothesis that the fatty acid composition of the maternal diet affects the ability of offspring to accumulate certain fatty acids.

We also tested whether DHA:ARA and DHA:EPA ratios of larvae differed based on diet treatment, building upon the findings of Burns and Fuiman (2019) that these ratios were correlated with egg fatty acids instead. However, there was no significant difference between diet treatment for either ratio. Our results suggest that this type of metabolic programming may

not be present in Southern flounder or at least may not be detectable in fatty acids of larvae up to 55 dph.

The number of possible methods for evaluating the presence of metabolic programming in fish is extensive, ranging from genetic analyses to behavioral performance. Previous studies of metabolic programming in southern flounder and red drum included behavioral tests which demonstrated effects of programming on antipredator performance of larvae (Fuiman and Ojanguren, 2011; Burns and Fuiman, 2019). Such tests were not performed in the current study, but it is possible that metabolic programming effects may be more detectable in performance-based behavioral evaluations rather than biochemical assays. Therefore, future research on nutritional programming in southern flounder may benefit from evaluating several measures of larval quality, such as specific biomarkers (e.g. DHA), ratios of PUFA (e.g., DHA:ARA), or behavioral assessments of offspring fitness.

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Table 1. Fatty acid composition (% total fatty acids) of live prey items for larval feeding. Pre- and post- refer to samples taken before and after prey items received enrichment. Values are mean +/- 1 SD.

	Rotifers		*Artemia*	
Fatty Acid	Pre	Post	Pre	Post
14:0	4.90 ± 0.49	4.77 ± 0.48	0.88 ± 0.50	2.50 ± 0.92
15:0	0.38 ± 0.03	0.33 ± 0.03	0.16 ± 0.04	0.23 ± 0.04
16:0	20.22 ± 0.93	16.68 ± 1.43	10.87 ± 2.60	14.32 ± 1.70
16:1n7	10.05 ± 1.21	9.37 ± 1.74	2.44 ± 0.62	2.55 ± 0.13
16:2n4	0.49 ± 0.17	0.38 ± 0.11	0.18 ± 0.05	0.13 ± 0.02
17:0	0.45 ± 0.14	0.38 ± 0.09	0.56 ± 0.14	0.55 ± 0.03
16:3n4	0.35 ± 0.07	0.27 ± 0.06	0.70 ± 0.19	0.51 ± 0.07
18:0	2.13 ± 0.33	1.89 ± 0.36	4.01 ± 0.96	3.77 ± 0.47
18:1n9	3.98 ± 0.27	3.34 ± 0.47	17.36 ± 4.58	13.05 ± 1.66
18:1n7	2.41 ± 0.16	2.20 ± 0.31	6.16 ± 1.52	5.63 ± 0.57
18:2n6	4.66 ± 0.20	3.77 ± 0.28	5.67 ± 1.53	4.10 ± 0.54
18:3n6	0.44 ± 0.03	0.43 ± 0.04	0.30 ± 0.08	0.30 ± 0.01
18:3n4	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.02	0.05 ± 0.02
18:3n3	0.54 ± 0.18	0.46 ± 0.18	26.25 ± 7.21	18.26 ± 2.55
18:4n3	0.21 ± 0.02	0.28 ± 0.04	3.68 ± 1.10	2.31 ± 0.29
20:1n9	0.46 ± 0.07	0.47 ± 0.15	0.43 ± 0.11	0.36 ± 0.05
20:2n6	0.20 ± 0.04	0.19 ± 0.04	0.15 ± 0.04	0.14 ± 0.02
20:3n6	0.69 ± 0.19	0.63 ± 0.18	0.09 ± 0.04	0.19 ± 0.02
20:4n6	3.06 ± 0.19	3.32 ± 0.19	0.90 ± 0.51	2.05 ± 0.35
20:3n3	0.03 ± 0.01	0.05 ± 0.02	0.54 ± 0.13	0.46 ± 0.06
20:4n3	0.57 ± 0.05	0.70 ± 0.06	0.52 ± 0.14	0.62 ± 0.02
20:5n3	12.8 ± 2.76	12.11 ± 2.93	2.36 ± 1.01	4.41 ± 0.87
22:1n11	0.24 ± 0.07	0.20 ± 0.07	0.04 ± 0.01	0.05 ± 0.01
22:4n6	0.32 ± 0.04	0.30 ± 0.04	0.16 ± 0.04	0.23 ± 0.05
22:5n6	4.95 ± 0.82	7.05 ± 1.25	0.71 ± 1.36	4.65 ± 1.30
22:5n3	2.95 ± 0.42	2.65 ± 0.37	0.08 ± 0.09	0.30 ± 0.06
22:6n3	14.57 ± 2.40	21.67 ± 3.79	1.94 ± 3.67	12.58 ± 3.61

n	17	19	12	16

Table 1. Fatty acid composition (% total fatty acids) of Southern flounder broodstock diets. Values are mean +/- 1 SD. * denotes statistical significance.

Fatty Acid	Shrimp	Sardine
14:0 *	1.32 ± 0.62	7.36 ± 2.54
15:0 *	1.45 ± 0.25	0.77 ± 0.19
16:0 *	15.38 ± 1.01	22.88 ± 0.65
16:1n7 *	4.56 ± 0.49	7.29 ± 1.76
16:2n4 *	0.47 ± 0.13	1.17 ± 0.10
17:0 *	2.66 ± 0.17	1.02 ± 0.15
16:3n4 *	1.46 ± 0.17	0.76 ± 0.18
18:0 *	10.78 ± 0.98	5.55 ± 1.36
18:1n9 *	9.69 ± 1.19	6.30 ± 1.14
18:1n7 *	3.19 ± 0.21	2.6 ± 0.3
18:2n6	2.37 ± 0.95	2.05 ± 0.55
18:3n6 *	0.55 ± 0.09	0.76 ± 0.16
18:3n4 *	0.25 ± 0.04	0.06 ± 0.03
18:3n3 *	0.97 ± 0.53	2.11 ± 1.37
18:4n3 *	0.17 ± 0.05	1.11 ± 0.40
20:1n9	1.43 ± 0.71	0.81 ± 0.45
20:2n6 *	1.17 ± 0.18	0.16 ± 0.04
20:3n6	0.44 ± 0.15	0.35 ± 0.05
20:4n6 *	8.69 ± 1.38	1.75 ± 0.51
20:3n3 *	0.32 ± 0.20	0.06 ± 0.01
20:4n3	0.40 ± 0.10	0.38 ± 0.05
20:5n3 *	12.16 ± 0.99	8.59 ± 1.47
22:1n11 *	0.23 ± 0.06	0.89 ± 0.63
22:4n6 *	1.36 ± 0.34	0.25 ± 0.16
22:5n6	1.16 ± 0.08	0.86 ± 0.47
22:5n3 *	1.92 ± 0.43	1.42 ± 0.10
22:6n3 *	11.82 ± 0.99	16.18 ± 5.48
n	8	11

Table 3. Fatty acid composition (% total fatty acids) of Southern flounder eggs produced under two diet treatment groups. Values are mean +/- 1 SD. * denotes statistical significance.

Fatty Acid	Shrimp	Sardine
14:0 *	3.20 ± 0.40	2.72 ± 0.16
15:0	0.83 ± 0.07	0.82 ± 0.06
16:0	21.97 ± 1.62	22.65 ± 0.92
16:1n7 *	6.28 ± 0.89	5.29 ± 0.36
16:2n4 *	0.69 ± 0.14	1.07 ± 0.13
17:0 *	0.88 ± 0.14	0.79 ± 0.11
16:3n4 *	1.10 ± 0.27	0.61 ± 0.04
18:0	3.56 ± 0.36	3.75 ± 0.3
18:1n9 *	12.61 ± 2.00	10.65 ± 1.54
18:1n7	3.52 ± 1.01	2.97 ± 0.81
18:2n6 *	2.14 ± 0.39	1.18 ± 0.10
18:3n6 *	0.27 ± 0.04	0.21 ± 0.03
18:3n4	0.20 ± 0.08	0.17 ± 0.05
18:3n3 *	0.59 ± 0.10	0.34 ± 0.06
18:4n3	0.64 ± 0.37	0.64 ± 0.34
20:1n9	0.97 ± 0.43	0.74 ± 0.36
20:2n6 *	0.39 ± 0.10	0.19 ± 0.03
20:3n6	0.21 ± 0.03	0.18 ± 0.24
20:4n6 *	3.21 ± 0.55	1.89 ± 0.17
20:3n3 *	0.29 ± 0.08	0.14 ± 0.03
20:4n3 *	0.57 ± 0.13	0.44 ± 0.07
20:5n3 *	4.11 ± 0.49	3.34 ± 0.37
22:1n11	0.05 ± 0.06	0.03 ± 0.02
22:4n6 *	0.70 ± 0.23	0.44 ± 0.07
22:5n6 *	0.77 ± 0.09	1.17 ± 0.13
22:5n3 *	5.56 ± 0.61	4.02 ± 0.38
22:6n3 *	17.90 ± 3.79	28.08 ± 2.32
n	20	28

Table 4. T-test output of direct diet-egg relationships; p-value adjusted for using Benjamini-Hochberg correction method. Directly related fatty acids are indicated in bold and *.

	Diet			Eggs		
Fatty Acid	t	p-adjusted	Significance	t	p-adjusted	Significance
14:0	7.59	2.11E-05	*	5.03	8.72E-05	*
15:0	-6.49	5.11E-05	*	0.34	7.66E-01	ns
16:0	18.47	1.36E-08	*	-1.70	1.22E-01	ns
16:1n7	4.89	6.21E-04	*	4.69	1.75E-04	*
16:2n4*	**12.52**	**1.58E-07**	*****	**-9.40**	**1.59E-10**	*****
16:3n4*	**-8.48**	**1.35E-06**	*****	**8.02**	**3.56E-07**	*****
17:0*	**-21.53**	**7.07E-11**	*****	**2.46**	**2.87E-02**	*****
18:0	-9.72	1.59E-07	*	-1.97	7.61E-02	ns
18:1n7	-4.99	2.00E-04	*	2.01	7.40E-02	ns
18:1n9*	**-6.23**	**3.51E-05**	*****	**3.68**	**1.27E-03**	*****
18:2n6	-0.83	4.40E-01	ns	10.85	2.21E-09	*
18:3n3	2.51	3.14E-02	*	9.96	5.05E-10	*
18:3n4	-10.86	4.67E-07	*	1.61	1.37E-01	ns
18:3n6	3.70	3.03E-03	*	5.00	4.28E-05	*
18:4n3	7.60	3.20E-05	*	9.10	9.93E-01	ns
20:1n9	-2.16	6.29E-02	ns	1.86	9.19E-02	ns
20:2n6*	**-15.54**	**1.95E-06**	*****	**9.05**	**2.47E-08**	*****
20:3n3*	**-3.65**	**1.15E-02**	*****	**8.02**	**1.14E-07**	*****
20:3n6	-1.72	1.33E-01	ns	0.68	5.40E-01	ns
20:4n3	-0.52	6.13E-01	ns	3.89	1.04E-03	*
20:4n6*	**-13.52**	**1.95E-06**	*****	**10.35**	**3.12E-09**	*****
20:5n3*	**-6.32**	**2.11E-05**	*****	**5.92**	**2.65E-06**	*****
22:1n11	3.45	8.96E-03	*	1.35	2.14E-01	ns
22:4n6*	**-8.55**	**2.75E-05**	*****	**4.83**	**1.58E-04**	*****
22:5n3*	**-3.20**	**1.85E-02**	*****	**10.04**	**3.11E-10**	*****
22:5n6	-2.11	6.68E-02	ns	-12.74	2.94E-15	*
22:6n3*	**2.58**	**3.14E-02**	*****	**-10.66**	**1.59E-10**	*****

Table 2. Fatty acid composition (% total fatty acids) of 27-dph Southern flounder larvae produced under two diet treatment groups. Values are mean +/- 1 SD.

Fatty Acid	Shrimp	Sardine
14:0	1.90 ± 0.39	1.67 ± 0.4
15:0	0.33 ± 0.01	0.29 ± 0.05
16:0	17.13 ± 0.81	17.2 ± 1.93
16:1n7	4.82 ± 0.85	3.45 ± 0.64
16:2n4	0.61 ± 0.03	0.56 ± 0.30
17:0	0.53 ± 0.10	0.58 ± 0.05
16:3n4	0.82 ± 0.16	0.82 ± 0.23
18:0	7.38 ± 0.34	7.66 ± 2.51
18:1n9	5.17 ± 0.93	7.98 ± 3.97
18:1n7	3.75 ± 0.33	4.33 ± 1.14
18:2n6	4.80 ± 0.94	4.47 ± 1.49
18:3n6	0.37 ± 0.02	0.31 ± 0.03
18:3n4	0.09 ± 0.03	0.08 ± 0.03
18:3n3	1.69 ± 1.39	6.27 ± 8.80
18:4n3	0.29 ± 0.18	0.81 ± 1.03
20:1n9	0.63 ± 0.21	0.61 ± 0.17
20:2n6	0.83 ± 0.14	0.67 ± 0.40
20:3n6	0.63 ± 0.07	0.45 ± 0.17
20:4n6	4.22 ± 0.37	3.83 ± 1.22
20:3n3	0.32 ± 0.24	0.40 ± 0.16
20:4n3	0.54 ± 0.05	0.51 ± 0.11
20:5n3	7.27 ± 0.88	5.46 ± 0.82
22:1n11	0.10 ± 0.03	0.06 ± 0.03
22:4n6	0.51 ± 0.08	0.37 ± 0.12
22:5n6	5.24 ± 0.58	4.77 ± 0.79
22:5n3	4.34 ± 0.67	2.81 ± 1.71
22:6n3	19.63 ± 1.78	17.70 ± 5.48
n	6	4

Table 3. Fatty acid composition (% total fatty acids) of 41-dph Southern flounder larvae produced under two diet treatment groups. Values are mean +/- 1 SD.

Fatty Acid	Shrimp	Sardine
14:0	1.19 ± 0.08	1.20 ± 0.06
15:0	0.19 ± 0.02	0.18 ± 0.02
16:0	14.87 ± 1.76	15.02 ± 0.87
16:1n7	2.26 ± 0.08	2.26 ± 0.07
16:2n4	0.24 ± 0.06	0.25 ± 0.05
17:0	0.58 ± 0.05	0.57 ± 0.02
16:3n4	0.69 ± 0.11	0.66 ± 0.10
18:0	6.54 ± 0.85	6.70 ± 0.57
18:1n9	12.11 ± 1.17	12.14 ± 1.06
18:1n7	6.12 ± 0.35	6.00 ± 0.46
18:2n6	3.99 ± 0.24	3.87 ± 0.22
18:3n6	0.32 ± 0.02	0.31 ± 0.01
18:3n4	0.09 ± 0.01	0.08 ± 0.01
18:3n3	9.52 ± 2.87	9.95 ± 1.24
18:4n3	1.33 ± 0.39	1.41 ± 0.14
20:1n9	0.62 ± 0.10	0.57 ± 0.11
20:2n6	0.43 ± 0.08	0.40 ± 0.06
20:3n6	0.30 ± 0.04	0.27 ± 0.03
20:4n6	3.58 ± 0.63	3.44 ± 0.25
20:3n3	2.43 ± 0.48	2.40 ± 0.70
20:4n3	0.70 ± 0.08	0.70 ± 0.07
20:5n3	5.15 ± 0.34	4.82 ± 0.11
22:1n11	0.14 ± 0.03	0.12 ± 0.07
22:4n6	0.57 ± 0.12	0.48 ± 0.07
22:5n6	4.07 ± 0.70	4.21 ± 0.78
22:5n3	1.92 ± 0.54	1.75 ± 0.37
22:6n3	13.43 ± 2.45	13.71 ± 2.38
n	5	4

Table 4. Fatty acid composition (% total fatty acids) of 55-dph Southern flounder larvae produced under two diet treatment groups. Values are mean +/- 1 SD.

Fatty Acid	Shrimp	Sardine
14:0	1.07 ± 0.17	0.87 ± 0.20
15:0	0.18 ± 0.01	0.17 ± 0.01
16:0	13.83 ± 1.36	15.07 ± 1.71
16:1n7	1.96 ± 0.6	1.72 ± 0.41
16:2n4	0.17 ± 0.04	0.18 ± 0.06
17:0	0.59 ± 0.03	0.58 ± 0.03
16:3n4	0.61 ± 0.11	0.60 ± 0.18
18:0	6.03 ± 1.62	7.70 ± 1.90
18:1n9	12.99 ± 0.33	12.06 ± 1.14
18:1n7	5.92 ± 0.01	5.39 ± 0.79
18:2n6	3.98 ± 0.21	3.58 ± 0.65
18:3n6	0.30 ± 0.03	0.27 ± 0.04
18:3n4	0.08 ± 0.01	0.09 ± 0.01
18:3n3	12.28 ± 3.63	8.80 ± 4.49
18:4n3	1.51 ± 0.48	1.10 ± 0.62
20:1n9	0.42 ± 0.03	0.40 ± 0.06
20:2n6	0.24 ± 0.05	0.24 ± 0.03
20:3n6	0.24 ± 0.01	0.23 ± 0.01
20:4n6	3.69 ± 1.07	4.40 ± 1.02
20:3n3	1.56 ± 0.43	1.62 ± 0.35
20:4n3	0.63 ± 0.03	0.54 ± 0.11
20:5n3	4.28 ± 0.35	4.07 ± 0.25
22:1n11	0.08 ± 0.01	0.06 ± 0.03
22:4n6	0.57 ± 0.26	0.69 ± 0.29
22:5n6	4.74 ± 0.43	4.98 ± 0.56
22:5n3	1.48 ± 0.36	1.44 ± 0.13
22:6n3	14.25 ± 0.51	16.26 ± 3.00
n	4	7

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