Nutritional programming by maternal diet alters offspring lipid metabolism in a marine teleost

Zhenxin Hou · **Xiyuan Lu** · **Stefano Tiziani** ·
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Abstract Nutritional programming — the association between the early nutritional environment and long-term consequences for an animal — is an emerging area of research in fish biology. Previous studies reported correlations between maternal provisioning of essential fatty acids to eggs and the whole-body fatty acid composition of larvae reared under uniform conditions for red drum, *Sciaenops ocellatus*. This study aimed to further investigate the nutritional stimulus and the consequences of nutritional programming by feeding adult red drum several distinct diets and rearing larvae under uniform conditions until 21 days post-hatching when larval lipid and fatty acid compositions were assessed. Different maternal diets produced eggs with distinctive lipid and fatty acid compositions, and despite receiving the same larval diet for almost 3 weeks, larvae showed differences in total fatty acid accumulation and in retention of highly unsaturated fatty acids (HUFA). Specifically, larvae reared from a maternal diet of shrimp generally showed elevated levels of fatty acids in the initial steps of the n-3 and n-6 HUFA biosynthetic pathways and reduced levels of fatty acid products of the same pathways, especially in triglyceride. Furthermore, the variations in larval fatty acid accumulation induced by maternal diet varied among females. Lipid metabolism altered by parental diet may have consequences for larval physiological processes and behavioral performance, which may ultimately influence larval survival.

Keywords Nutritional programming · Lipid metabolism · Fatty acid · Maternal nutrition · Larval fish

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Introduction

Nutritional programming — the association between the early nutritional environment and long-term consequences for an animal — has been principally studied in mammalian models (Symonds et al. 2009; Ozanne 2015). In recent years, similar long-term

effects of early nutrition, from both endogenous (maternally derived) and exogenous (larval feeding) nutrients, have been reported in fishes (Panserat et al. 2019; Hou and Fuiman 2020). The documented consequences from nutritional programming include growth, survival, neural development, and nutrient metabolism (Vagner et al. 2007; Lund et al. 2012; Morais et al. 2014; Fuiman and Perez 2015; Izquierdo et al. 2015).

Structures and functions of many organ systems begin to develop during the endogenous feeding period (embryonic and early larval stages), when there is substantial developmental plasticity. This makes the maternally derived nutrients in the eggs a potential source of nutritional stimuli that could permanently program offspring metabolism. Contrary to the growing number of investigations of programming via nutritional intervention at first feeding (Vagner et al. 2007, 2009; Lund et al. 2012), only a few studies have examined programming via maternal nutrition. For example, differences in the levels of polyunsaturated fatty acids (PUFA) in the maternal diet of Senegalese sole *Solea senegalensis* resulted in differentially expressed genes related to biosynthesis of highly unsaturated fatty acids (HUFA) in larvae and altered growth rate in juveniles (Morais et al. 2014). Zebrafish *Danio rerio* that were fed diets deficient in one-carbon micronutrients (vitamin B and methionine) produced offspring that had elevated hepatic lipid content and altered expression of lipid metabolism genes as adults (Skjærven et al. 2018).

Fuiman and Perez (2015) were among the first to document a relationship between embryonic availability of docosahexaenoic acid (DHA; 22:6n-3) and accumulation of DHA and behavioral performance in later larvae. They showed that tissue DHA content was about two times greater in red drum *Sciaenops ocellatus* larvae that came from eggs with high DHA concentrations compared to larvae from eggs with lower levels of DHA, after larvae had fed on a high-DHA diet for several weeks (Fuiman and Perez 2015). In addition, the differential accumulation of DHA was associated with aspects of larval performance that are critical to survival, such as growth, routine swimming, and the escape response (Fuiman and Ojanguren 2011; Fuiman and Perez 2015; Perez and Fuiman 2015). However, while egg and larval DHA levels were implicated, respectively, as the stimulus and consequence of nutritional programming in red

drum, the previous study did not examine the coordinated variations in the lipid classes and associated fatty acids in eggs and larvae, which could provide more insight into the alterations in offspring lipid metabolism. The objective of this study was to further investigate the nutritional stimulus and the consequences of the putative nutritional programming in red drum by assessing the lipid and fatty acid composition of larvae that were derived from different maternal diets but raised under uniform conditions.

Methods and materials

To achieve the objective, we conducted two experiments. The goal of the first experiment was to reduce the number of candidates for nutritional programming stimuli by producing spawns from a variety of maternal diets. Programming of larval lipid metabolism was assessed by larval lipid composition at 21 days post-hatching (dph). After tentatively identifying the diet containing the stimulus (a maternal diet consisting of shrimp only), a second experiment was conducted to verify the programming effect of the shrimp diet on larval lipid metabolism while controlling for differences among females.

Broodstock care and diet treatments

Broodstock were maintained at the University of Texas Marine Science Institute, Fisheries and Mariculture Laboratory in Port Aransas, TX. Fish were induced to spawn naturally by photothermal regulation (Arnold et al. 1977) and were held in 12,000- or 16,000-L recirculating tanks at a controlled temperature (24–28 °C), salinity (30–38 ppt) and photoperiod (11:13 L:D during spawning). Fish were fed until satiation 3–5 times per week. Different egg lipid compositional profiles were obtained by manipulating broodstock diets, following previous feeding protocols (Fuiman and Perez 2015).

In Experiment 1, two broodstock tanks (MT1, MT7; names identify tanks and are not related to experimental treatments) were fed one of the five diets: (1) shrimp *Litopenaeus setiferus* or *Farfantepenaeus aztecus*, (2) Spanish sardine *Sardinella aurita*, (3) Atlantic mackerel *Scomber scombrus*, (4) squid *Loligo opalescens*, or (5) beef liver supplemented with squid (Table 1). A total of 28 spawns ($n=4$–9

spawns per diet group) were collected and larvae were reared under common conditions (described below) until 21 dph.

Based on the results obtained from Experiment 1, we conducted Experiment 2, a paired-design diet-shift experiment. Three red drum broodstock tanks (H3, H4, MT7) were fed a diet of only shrimp, and after five spawns were collected and reared, the diet was shifted to an oily fish (Table 2; Spanish sardine or thread herring *Opisthonema oglinum*[1]). Spawns were collected and larvae were reared until 21 dph using the same method as Experiment 1.

Larval rearing and sampling

For each spawn, 5 ml of floating eggs (approximately 5000 eggs) were placed into a 150-L cone-shaped tank equipped with internal biofilters and gently aerated. Two additional samples were rinsed twice in deionized water and preserved at −80 °C until biochemical analysis.

Larvae were fed enriched rotifers (*Brachionus plicatilis* L-strain) twice daily at a concentration of 5 ml^{-1} on 3 to 11 dph. Newly hatched *Artemia* sp. nauplii were added to the diet on 10 and 11 dph. Starting 12 dph, larvae were fed enriched *Artemia* sp. nauplii twice daily at a concentration of 250–400 L^{-1}. Rotifers were enriched with Algamac 3050 (0.2 g of enrichment per one million rotifers; Aqua-fauna Bio-Marine; www.aquafauna.com) for 45–60 min prior to feeding. *Artemia* sp. were enriched overnight with Algamac 3050 (0.3 g of enrichment per 100,000 *Artemia* sp.). Temperature, salinity, and photoperiod were maintained at 27.5±0.5 °C, 31±2 ppt, and 12:12 L:D, respectively. At 21 dph, at least 40 larvae were sampled, euthanized by an overdose of MS-222 (0.3 mg ml^{-1}), rinsed twice in deionized water and frozen at −80 °C until analysis. Images of approximately 20 larvae were taken under a microscope for measurement of standard length. Egg and larval samples from Experiment 1 were used for fatty acid and untargeted lipidomic analyses. Egg and larval samples from Experiment 2 were used for fatty acid and lipid class analyses.

Biochemical analysis

Fatty acid analysis

Fatty acid compositions of eggs and larvae were measured by gas chromatography following established methods (Faulk and Holt 2005; see Supplementary Materials). Fatty acids (in total lipids) were quantified as concentrations (mg g^{-1} dw) and as compositions (% total fatty acids).

Untargeted lipidomic analysis

In Experiment 1, untargeted lipidomics analysis was used to characterize the full lipid profiles of the eggs and larvae using a high-resolution Hybrid Quadrupole-Orbitrap mass spectrometer (Q Exactive, Thermo Scientific, Waltham, MA, USA) equipped with an automated chip-based nanoelectrospray ionization (nESI) source (TriVersa NanoMate, Advion, Ithaca, NY). Details on the sample preparation and instrument parameters can be found in the Supplementary Materials. Identified lipid metabolite peak intensities were standardized by sample dry weight. The concentration of each lipid class (mg g^{-1} dw) was the sum of concentrations of lipid species from the respective lipid class, which were computed based on the internal standard peak intensity.

Lipid class analysis

In Experiment 2, lipid class composition of the eggs and larvae was analyzed using UHPLC following previous methods (Hou and Fuiman 2021; details see Supplementary Materials). Lipid class concentrations were quantified as concentrations (mg g^{-1} dw). Fatty acid composition within major lipid classes were quantified as compositions (% total fatty acids).

Statistical analysis

Principal components analysis (PCA) with varimax rotation based on scaled (mean=0 and standard deviation [SD]=1) data was used to summarize egg and larval compositional profiles and to detect differences among diet groups. Probabilistic quotient

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[1] Types of fish feeds were switched due to a supply shortage during the 2020 COVID pandemic. The two fish feeds showed similar lipid class and fatty acid profiles, which are provided as supplementary files.

Table 1 Summary of broodstock diet treatments and spawns in Experiment 1

Spawning tank	Broodstock diet	No. of spawns	Spawn date	Days on diet
MT1	Shrimp	4	1/21/18–1/31/18	> 1 yr
MT7	Sardine	4	4/2/18–4/26/18	12–36
MT7	Squid	6	4/29/18–6/8/18	5–45
MT7	Liver & squid	9	8/9/18–9/9/18	29–60
MT1	Mackerel	5	7/5/18–8/26/18	14–66

normalization (PQN; Dieterle et al. 2006) was performed before scaling larval fatty acid concentration and lipidomic data. PQN has been used in metabolomics studies to account for variations in the overall concentrations of samples caused by dilutions. In our dataset, PQN removed the effect of concurrent changes in fatty acid concentrations (i.e., overall increase in lipid constituent concentrations in some samples) to enable comparison of relative changes in the concentration of each lipid constituent.

Permutational multivariate analysis of variance (PERMANOVA; with 9999 permutations) was used to test for significant differences in lipid class composition of eggs and larvae from different maternal diet groups. Multivariate homogeneity of group dispersions was verified. When the PERMANOVA result was significant, analysis of variance (ANOVA), followed by Tukey's tests for multiple pairwise comparisons, was performed on each lipid class. One-way ANOVA and post-hoc Tukey's tests were also used to test for differences in multivariate composition (PCA scores) and standard length among diet groups. Kruskal–Wallis tests and Welch one-way ANOVA were used when normality and homogeneity of variance assumptions were violated, respectively. Log-transformed data were used when both assumptions were violated. The non-parametric Mann–Whitney

U-test (MWU) was used for pairwise comparisons following Kruskal–Wallis or Welch one-way ANOVA, and to test for differences in the concentrations of fatty acid and lipid species in larvae from the shrimp and non-shrimp diet groups (all non-shrimp diet groups were combined as a single group for comparison). False detection rate (FDR, $\alpha = 0.05$) was employed for multiple comparisons (Benjamini and Hochberg 1995).

Given the apparent inter-broodstock tank variability in Experiment 2, two-way PERMANOVA and ANOVA were used to test for the effect of maternal diet (shrimp, fish) and female (H3, H4, MT7) on larval standard length, or egg and larval lipid class and fatty acid profiles. When the interaction term for two-way ANOVA was significant, simple main effects were analyzed (i.e., separate one-way ANOVA of one variable for each level of the second variable). The Pearson correlation coefficients (r) was used to assess the relationship between the amount of lipid (mg larva^{-1}) and standard length of larvae from the same broodstock tank and maternal diet group. To test whether the rate of the lipid accumulation with standard length differed due to maternal diet (shrimp, fish) or female (H3, H4, MT7), larvae were arranged into five groups based on these two factors (group H3-fish was excluded because there were only two spawns).

Table 2 Summary of broodstock diet treatments and spawns in Experiment 2

Spawning tank	Broodstock diet	No. of spawns	Spawn date	Days on diet
H3	Shrimp	5	1/29/20–2/12/20	73–86
	Sardine	2[1]	3/9/20, 4/30/20	14, 66
H4	Shrimp	5	1/15/20–1/26/20	71–82
	Sardine	5	4/5/20–5/1/20	41–67
MT7	Shrimp	5	2/29/20–4/26/20	25–82
	Herring	5	5/14/20–5/27/20	18–31

[1]One broodstock tank (H3) stopped spawning after producing two spawns on the sardine diet

Analysis of covariance (ANCOVA) was used to compare the slopes of the regression of amount of lipid (mg larva^{-1}) on standard length among diet groups (interaction term). When the ANCOVA interaction term was significant (i.e., slopes significantly different among treatments), Tukey's multiple comparison of regression slopes was performed.

Statistical analyses were conducted using the R 4.0.3 (R Core Team 2020), Rcpm package (v 1.0.2; Derks 2020), exactRankTests package (v 0.8–32; Hothorn and Hornik 2021), and rstatix package (v 0.6.0; Kassambara 2020). All figures were produced using ggplot2 (Wickham 2016) and cowplot packages (v 1.1.0; Wilke 2020).

Results

Experiment 1 — egg lipid compositional profile

Untargeted lipidomics measured 275 lipid species in 10 lipid classes in red drum eggs, with 134 lipid fatty acid chains identified. There were significant differences in the lipid class profiles of the eggs from different diet groups (Table 3; PERMANOVA

$pseudo\text{-}F_{(4, 23)} = 8.3$; $P < 0.001$). Concentrations of all measured lipid classes differed significantly among diet groups except triglycerides (TG) and diglycerides (DG).

Different adult diets produced distinctive egg fatty acid profiles, as indicated by the significantly different PC1 and PC2 scores for the five groups (Fig. 1a; ANOVA, $F_{(4, 23)} = 114.6$, and $F_{(4, 23)} = 138.3$, respectively, $P < 0.001$). Lipidomic profiles were equally distinctive and their patterns of variability among diet groups as expressed by PCA were very similar to those for fatty acid profiles (Fig. 1a, b). PC1 and PC2 scores for five groups were significantly different (PC1: Welch ANOVA, $F_{(4, 10.9)} = 103.1$, $P < 0.001$; PC2: Kruskal–Wallis $X^2_{(4)} = 22.6$, $P < 0.001$).

Experiment 1 — larval lipid compositional profile

The mean standard length for larvae at 21 dph was 7.9 ± 0.8, 8.4 ± 1.4, 8.8 ± 1.0, 9.3 ± 1.7, and 10.5 ± 1.3 mm for the liver & squid, mackerel, squid, shrimp, and sardine diet groups, respectively. The only significant difference in larval length among diet groups was between the liver &

Table 3 Lipid class concentrations (mean $\pm$ 1 SD, mg lipid g^{-1} dw, n is number of spawns) in red drum eggs from different maternal diets in Experiment 1. Boldface type indicates lipid class concentrations that differed significantly among diet groups (Kruskal–Wallis or Welch ANOVA, FDR = 0.05). For each lipid class, values that share the same superscript letter are not significantly different (Pairwise MWU, adjusted $P < 0.05$). Lipid classes: *WE*, wax ester; *CE*, cholesteryl ester; *TG*, triglyceride; *DG*, diglyceride; *MG*, monoglyceride; *PA*, phosphatidic acid; *PE*: phosphatidylethanolamine; *PI*, phosphatidylinositol; *PC*, phosphatidylcholine; *SM*, sphingomyelin; *NL*, sum of neutral lipids; *PL*, sum of polar lipids; *TL*, sum of total lipids

| Lipid class	Shrimp (n = 4)	Mackerel (n = 5)	Sardine (n = 4)	Squid (n = 6)	Liver & squid (n = 9)
WE	80.9 ± 12.9^b	84.2 ± 14.9^b	114.1 ± 15.6^{ab}	134.7 ± 7.0^a	115.3 ± 23.2^{ab}
CE	9.5 ± 1.0^b	10.6 ± 3.0^{ab}	10.8 ± 0.9^b	13.9 ± 0.9^a	11.7 ± 1.5^b
TG	34.8 ± 5.4	34.6 ± 9.4	31.1 ± 2.7	37.1 ± 3.1	31.1 ± 6.6
DG	4.0 ± 0.1	3.5 ± 0.3	3.3 ± 0.2	3.6 ± 0.6	3.4 ± 0.2
MG	0.02 ± 0.00^b	0.03 ± 0.01^{ab}	0.03 ± 0.01^{ab}	0.03 ± 0.01^a	0.02 ± 0.01^b
PA	19.5 ± 0.3^b	19.2 ± 0.8^b	16.6 ± 0.8^c	19.0 ± 2.1^{bc}	25.2 ± 2.4^a
PE	2.4 ± 0.2^{ab}	2.7 ± 0.2^a	2.7 ± 0.1^a	2.4 ± 0.1^b	2.0 ± 0.1^c
PI	0.1 ± 0.0^a	0.0 ± 0.1^{ab}	0.1 ± 0.0^a	0.1 ± 0.1^a	0.0 ± 0.0^b
PC	30.0 ± 0.4^b	33.5 ± 1.8^{ab}	33.5 ± 1.0^{ab}	32.6 ± 2.7^{ab}	35.3 ± 2.1^a
SM	1.0 ± 0.1^b	1.3 ± 0.2^{ab}	1.1 ± 0.1^{ab}	1.4 ± 0.1^a	1.1 ± 0.1^b
NL	129.2 ± 14.7^b	133 ± 24.4^b	159.3 ± 15.1^b	189.3 ± 8.2^a	161.5 ± 29.4^b
PL	53.0 ± 0.5^c	56.7 ± 2.6^b	54.0 ± 1.8^{bc}	55.5 ± 4.7^{bc}	63.6 ± 4.3^a
TL	182.2 ± 15.0^{bc}	189.8 ± 22.4^c	213.3 ± 14.0^{bc}	244.8 ± 8.3^a	225.2 ± 29.0^{ab}

(a)

(b)
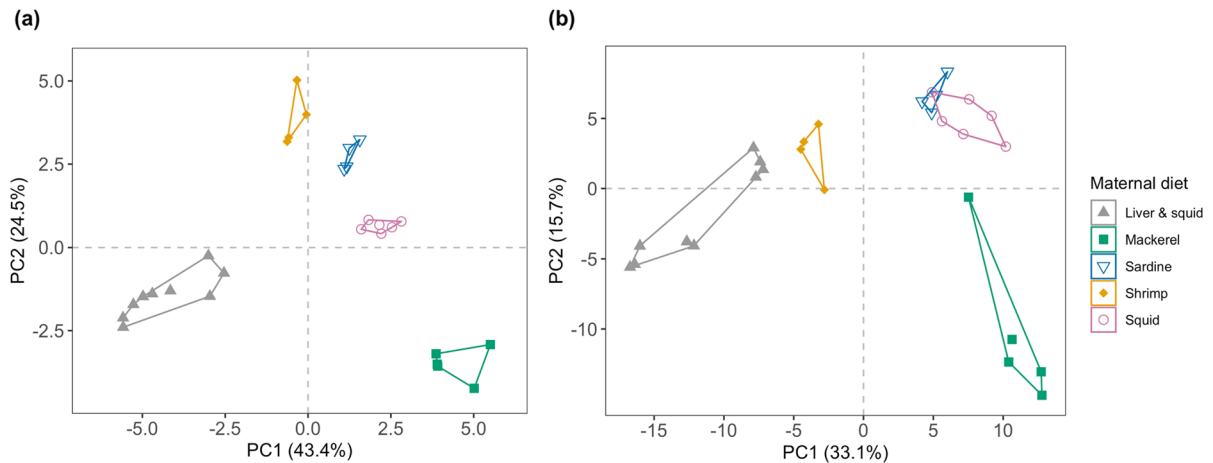
Fig. 1 Principal component analysis of (**a**) fatty acid concentrations (mg g^{-1} dw) and (**b**) lipidomic profiles (peak intensities) of red drum eggs produced by adult fish fed different diets in Experiment 1. Principal component loadings are in Table S3 and Fig. S1, respectively. Each point represents a spawn.

Colors and symbols indicate diet groups (gray filled triangle: liver & squid diet; green square: mackerel diet; blue open triangle: sardine; orange diamond: shrimp diet; pink circle: squid diet)

Table 4 Lipid class concentrations (mean ± 1 SD, mg lipid g^{-1} dw, n is number of spawns) in red drum larvae from different maternal diets at 21 dph in Experiment 1. Boldface type indicates lipid class concentrations that differed significantly among diet groups (ANOVA, FDR = 0.05). For each lipid class, values that share the same superscript letter are not significantly different (Tukey, adjusted $P < 0.05$). Abbreviations for lipid classes can be found in Table 1 caption

Lipid class	Shrimp ($n=4$)	Mackerel ($n=5$)	Sardine ($n=4$)	Squid ($n=6$)	Liver & squid ($n=9$)
WE	5.2 ± 1.7^{ab}	5.8 ± 1.6^{ab}	3.8 ± 0.8^{b}	3.9 ± 0.3^{b}	6.0 ± 0.7^{a}
CE	5.5 ± 0.2	5.4 ± 1.1	5.1 ± 2.4	5.7 ± 0.6	6.1 ± 1.4
TG	28.3 ± 8.6^{a}	15.7 ± 2.5^{b}	20.9 ± 6.6^{ab}	17.7 ± 3.9^{b}	15.3 ± 2.6^{b}
DG	4.0 ± 0.2	4.3 ± 0.3	4.1 ± 0.3	4.1 ± 0.2	4.2 ± 0.3
MG	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
PA	0.8 ± 0.0^{a}	0.7 ± 0.0^{b}	0.7 ± 0.0^{b}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}
PE	15.7 ± 0.3	15.2 ± 0.7	15.4 ± 0.4	15.5 ± 0.5	15.4 ± 0.4
PI	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.0
PS	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
PC	29.5 ± 0.8	29.9 ± 1.4	30.2 ± 0.7	30.5 ± 0.5	30.1 ± 1.0
SM	1.6 ± 0.2	1.5 ± 0.2	1.6 ± 0.1	1.4 ± 0.0	1.5 ± 0.1
NL	43.2 ± 8.9^{a}	31.3 ± 4.1^{ab}	33.9 ± 9.5^{ab}	31.5 ± 4.0^{b}	31.8 ± 1.5^{b}
PL	47.8 ± 1.0	47.5 ± 2.1	48.1 ± 0.9	48.2 ± 0.8	47.9 ± 1.4
TL	91.0 ± 9.0^{a}	78.8 ± 3.1^{b}	82.0 ± 9.6^{ab}	79.7 ± 4.5^{b}	79.7 ± 2.0^{b}

squid and the sardine groups (ANOVA, $F_{(4, 23)} = 3.9$, $P = 0.01$; Tukey, adjusted $P < 0.05$).

Untargeted lipidomics measured 304 lipid species in 11 lipid classes in larvae at 21 dph, with 120 lipid fatty acid chains identified. There were significant differences in lipid class profiles of the larvae from different diet groups, mostly due to differences in concentrations of wax esters (WE) and TG (Table 4; PERMANOVA, *pseudo-$F_{(4, 23)} = 5.5$; $P = 0.003$*).

Larvae from the shrimp diet group differed from larvae from all other diet groups in both fatty acid profiles and lipidomic profiles despite receiving the same larval diet, as indicated by their significantly lower PC1 scores (Fig. 2a, b; ANOVA, $F_{(4, 23)} = 7.9$, and $F_{(4,23)} = 6.5$, respectively, $P < 0.001$; Tukey, adjusted $P < 0.05$). Strongest loadings on PC1 based on fatty
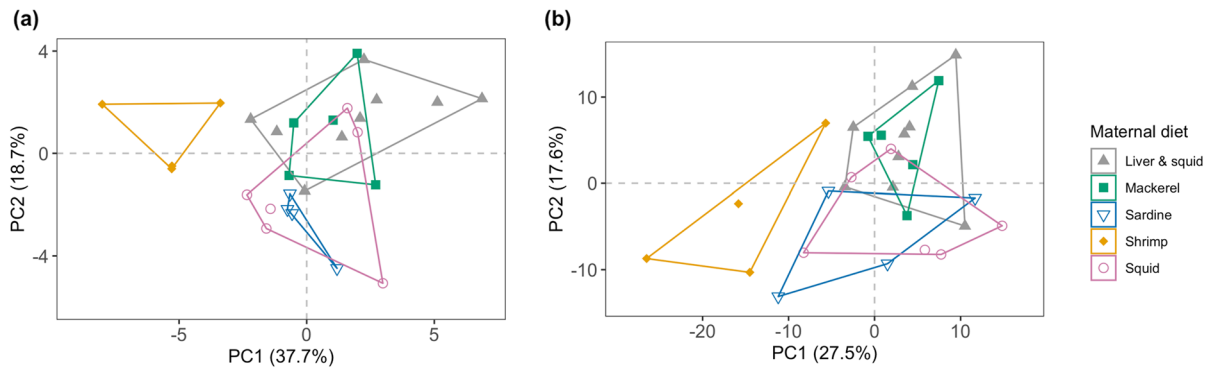
Fig. 2 Principal component scores (with varimax rotation) of (**a**) fatty acid profiles (mg g^{-1} dw) and (**b**) lipidomic profiles (peak intensities) of red drum larvae at 21 dph reared from eggs produced by adult fish fed different diets in Experiment 1 (data were PQN and scaled). Principal component loadings are in Table 5 and Fig. S2, respectively. Each point represents larvae reared from a spawn. Colors and symbols indicate diet groups (gray filled triangle: liver & squid diet; green square: mackerel diet; blue open triangle: sardine; orange diamond: shrimp diet; pink circle: squid diet)

acid concentrations were for the fatty acids 18:4n-3, 18:3n-3, 14:0, 20:4n-3, 18:3n-6 (loadings < -0.7), and 18:0, 22:4n-6, 16:0, ARA, 17:0 (loadings > 0.7; Table 5). The lipid species with strong, negative loadings on PC1 based on the lipidomic profile (loadings < −0.7) included 41 TG, 2 DG, and 1 PA species (Fig. S2).

Based on the PCA results (Fig. 2), larvae were arranged into two groups for further analysis: shrimp diet and non-shrimp diet. Mann–Whitney U-tests identified 13 fatty acids (shrimp > non-shrimp: 18:4n-3, 14:0, 18:3n-3, 20:3n-3, 20:4n-3; shrimp < non-shrimp: 18:1n-7, 17:0, 22:4n-6, 18:0, 22:5n-3, 18:1n-9, ARA, 16:0), and 109 lipid species (of 304) that were significantly different between the shrimp and non-shrimp diet groups (FDR = 0.05). Among the 109 lipid species, 53, 8, 4, and 3 were TG, DG, PA, and sphingomyelin (SM) species, respectively, and all of these were significantly higher larvae from the shrimp diet group than the non-shrimp group (Figs. 3 and S3). An additional 32 phosphatidylcholine (PC) species and 9 phosphatidylethanolamine (PE) species, respectively, were significantly different, with some higher in the shrimp diet group and some lower (Figs. 3 and S3).

Experiment 2 — egg lipid compositional profile

Results from Experiment 1 suggested the maternal diet of shrimp may have caused differences in offspring fatty acid and lipidomic (in particular, TG) profiles (Fig. 2). Experiment 2 used a paired-design diet shift to verify the programming effect of the shrimp diet. When individual brookstock tanks were given the shrimp diet, then shifted to the fish diet, there were significant changes in the lipid class composition of the eggs (Table 6; 2-way PERMANOVA, $pseudo\text{-}F_{(1, 21)} = 3.8$; $P = 0.02$). There was also a significant female effect on egg lipid class composition (Table 6; 2-way PERMANOVA $pseudo\text{-}F_{(2, 21)} = 2.8$; $P = 0.02$).

PCA results showed changes in egg fatty acid profiles when the adult diet changed from shrimp to fish (primarily an increase in scores on PC1, Fig. 4). PC1 scores were significantly affected by both maternal diet ($F_{(1,21)} = 75.4$, $P < 0.001$) and female ($F_{(2,21)} = 5.4$, $P = 0.01$). There was a statistically significant interaction between maternal diet and female on PC2 scores ($F_{(2,21)} = 7.2$, $P < 0.01$). In addition, fatty acid profiles of the major lipid classes (wax esters/steryl esters (WE/SE), TG, PC) in eggs all changed significantly after the diet shift (Fig. S4; see Supplementary Materials).

Experiment 2 — larval lipid compositional profile

The mean standard length for larvae at 21 dph did not differ among broodstock tanks or between diet groups ($P > 0.05$). The standard length for larvae from the shrimp and fish diet groups averaged 8.6 ± 0.5, 7.9 ± 2.1 mm for H3, 9.6 ± 1.6, 8.8 ± 1.2 mm for H4, and 8.8 ± 1.9, 8.8 ± 2.1 mm for MT7.

Table 5 Principal component loadings (with varimax rotation) for fatty acid concentrations (mg g^{-1} dw) of red drum larvae at 21 dph reared from eggs produced by adult fish fed different diets in Experiment 1 shown in Fig. 2a. Boldface type highlights strong loadings

Fatty acid	PC1	PC2
14:0	**−0.84**	0.07
15:0	0.38	0.05
16:0	**0.85**	−0.16
16:1n-7	−0.56	−0.29
16:2n-4	−0.23	0.43
17:0	**0.80**	−0.18
16:3n-4	0.55	0.19
18:0	**0.92**	−0.15
18:1n-9	0.48	**−0.73**
18:1n-7	0.68	−0.48
18:2n-6	−0.44	**−0.84**
18:3n-6	**−0.77**	0.19
18:3n-4	0.24	0.64
18:3n-3	**−0.90**	−0.21
18:4n-3	**−0.92**	−0.04
20:1n-9	−0.08	0.49
20:2n-6	−0.34	−0.33
20:3n-6	−0.45	−0.58
20:4n-6	**0.82**	0.26
20:3n-3	−0.59	−0.39
20:4n-3	**−0.83**	−0.43
20:5n-3	−0.09	0.32
22:1n-11	−0.16	0.36
22:4n-6	**0.89**	−0.22
22:5n-6	0.19	**0.80**
22:5n-3	0.59	−0.34
22:6n-3	0.19	**0.77**

Concentrations of total lipid and each lipid class did not vary significantly between larvae from the shrimp and fish diet groups at 21 dph ($P > 0.05$), but there was a significant female effect on TG, NL, TL concentrations of larvae (Table 7; $P < 0.05$). Within broodstock tanks, the amounts of individual lipid classes per larva (mg larva^{-1}) were strongly correlated with larval standard length ($r = 0.78$ to 0.998; Fig. S5). Neutral lipid accumulation (particularly TG) with standard length differed due to female, but not maternal diet (ANCOVA interaction $P < 0.05$; Fig. S5; see Supplementary Materials).

Concentrations of fatty acids (mg g^{-1} dw) in total lipids of 21-dph larvae were summarized by PCA (Fig. 5). Despite receiving the same larval diet, there were significant effects of both maternal diet and female on the fatty acid profiles in total lipids of 21-dph larvae. The interaction between diet and female on PC1 scores was significant ($F_{(2, 21)} = 27.9$, $P = 0.02$). Strongest loadings on PC1 were for 18:2n-6, 20:4n-3, 20:3n-3, 18:3n-3 (loadings < -0.7), and ARA, 16:0, DHA, 22:5n-6, 15:0 (loadings > 0.7; Table 8). PC2 primarily represented the female effect ($F_{(2, 21)} = 5.3$, $P < 0.05$), with significantly different PC2 scores for larvae from H4 compared to the larvae from the other two broodstock tanks (Kruskal–Wallis $X^2_{(2)} = 11.0$, $P < 0.005$; MWU, adjusted $P < 0.05$). The most influential fatty acids on PC2 were 22:5n-3, 16:2n-4, 20:1n-9, 22:4n-6 (loadings < -0.7), and 18:4n-3 (loadings > 0.7; Table 8).

The effect of maternal diet on overall fatty acid concentrations in 21-dph larvae differed among females. For larvae from H3 and H4, the maternal diet of fish (sardine) was associated with overall higher concentrations of fatty acids compared to larvae from the shrimp diet groups; whereas for larvae from MT1 in Experiment 1 (equivalent to the conditions of Experiment 2) and MT7, the maternal diet of fish (mackerel and herring, respectively) was associated with overall lower concentrations of fatty acids than larvae from the shrimp diet (Fig. 6).

Larval fatty acid profiles in each of the three major lipid classes (PC, PE, TG) changed in response to the parental diet shift (Fig. S6). The effect was strongest in larval TG (Fig. S7), where the shrimp diet was associated with higher levels of most MUFA and PUFA, including 18:2n-6, 18:3n-6, 20:2n-6, 18:3n-3, 18:4n-3, 20:4n3, and lower levels of long-chain HUFA, including ARA, 22:4n-6, 22:5n-6, EPA, 22:5n-3, and DHA (Fig. 7, Table S5). Similar but weaker patterns for these n-3 and n-6 PUFA were noted in PE, PC, and TL (Fig. S7).

Discussion

This study provided evidence that maternal nutrition can program lipid metabolism of red drum larvae. Specifically, (1) egg compositions were altered by changes in maternal diets; (2) larvae reared from those eggs exhibited differential accumulation of total fatty acids and differing fatty acid composition despite receiving the same larval diet for almost
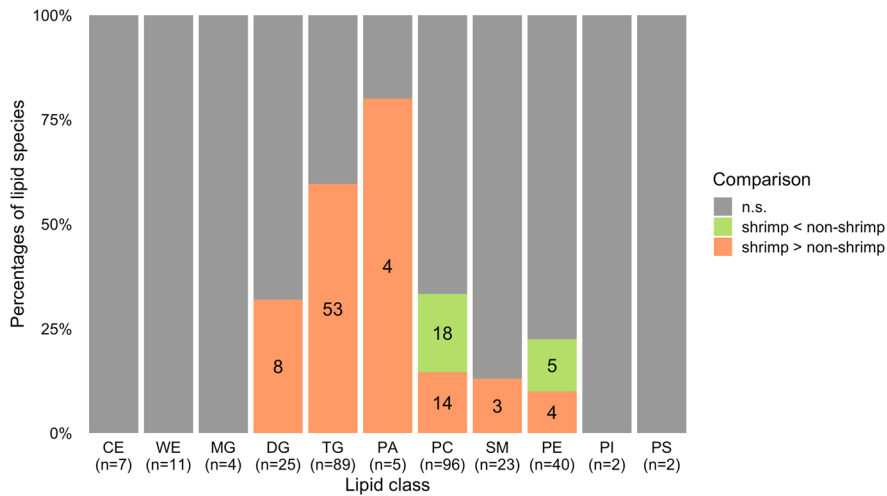
Fig. 3 Percentages of lipid species that were significantly different between larvae from the shrimp diet and non-shrimp diet groups, by lipid class in Experiment 1. Colors inidicate direction of difference (green: shrimp < non-shrimp; orange: shrimp > non-shrimp; gray: not significantly different (n.s.)). Total number of identified lipid species (*n*) is indicated in the parentheses. Total number of significant lipid species is indicated on the bars. Lipid classes: CE, cholesteryl ester; WE, wax ester; MG, monoglyceride; DG, diglyceride; TG, triglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; SM, sphingomyelin; PE: phosphatidylethanolamine; PI, phosphatidylinositol; PS: phosphatidylserine

Table 6 Lipid class concentrations (mg lipid g^{-1} dw, mean ± 1 SD) of red drum eggs from different broodstock tanks and maternal diets in Experiment 2. Boldface type indicates lipid class concentrations that differ significantly among diet groups.

Asterisks indicate significant differences between larvae from different broodstock tanks (two-way ANOVA, $P < 0.05$). There was no significant interaction between tank and diet. Abbreviations for lipid classes can be found in Table 1 caption

Lipid class	H3		H4		MT7	
	Shrimp	Fish	Shrimp	Fish	Shrimp	Fish
WE/SE	69.3 ± 8.9	66.2 ± 9.4	63.6 ± 6.7	54.7 ± 15.0	76.7 ± 2.6	58.9 ± 4.5
TG	63.2 ± 13.6	72.7 ± 11.2	62.4 ± 4.5	62.1 ± 3.5	69.8 ± 4.9	66.0 ± 8.0
ST	8.3 ± 1.4	8.6 ± 0.5	10.3 ± 1.2	8.7 ± 1.3	9.2 ± 1.2	9.5 ± 1.1
PE[*]	15.1 ± 0.8	14.7 ± 1.5	9.7 ± 4.8	12.0 ± 1.8	15.0 ± 2.1	13.4 ± 2.0
PI/PA[*]	3.9 ± 0.3	4.3 ± 0.2	2.4 ± 1.3	3.6 ± 0.6	4.2 ± 0.4	4.1 ± 0.5
PS[*]	2.7 ± 0.2	2.8 ± 0.1	1.6 ± 0.6	2.2 ± 0.6	2.8 ± 0.5	2.8 ± 0.3
PC[*]	55.9 ± 4.9	58.4 ± 2.8	44.0 ± 17.7	46.9 ± 5.4	53.5 ± 7.9	55.1 ± 4.5
NL	140.7 ± 22.2	147.5 ± 20.2	136.2 ± 10.4	125.4 ± 18.8	155.7 ± 4.3	134.4 ± 10.3
PL[*]	77.6 ± 5.8	80.3 ± 4.6	57.6 ± 24.1	64.7 ± 8.0	75.4 ± 10.0	75.3 ± 7.0
TL[*]	218.3 ± 27.1	227.8 ± 15.6	193.8 ± 18.0	190.1 ± 20.8	231.1 ± 10.0	209.8 ± 15.5

3 weeks; (3) fatty acid profiles of TG were the most susceptible to modification by the maternal diet and showed reduced levels of HUFA in larvae from the maternal diet of shrimp only; and (4) effects of maternal diets on fatty acid accumulation by larvae varied among mothers.

Differential accumulation of fatty acids and lipids

Body fatty acid levels and composition are the results of a sequence of interdependent and cross-regulated molecular events and reflect the dynamics of various lipid homeostatic pathways, including ingestion,

Fig. 4 Principal component analysis (with varimax rotation) of fatty acid concentrations (mg g^{-1} dw) of red drum eggs produced by adult fish fed different diets in Experiment 2. Principal component loadings are in Table S4. Each point represents a spawn. Colors indicate diet groups (blue: fish diet; orange: shrimp diet). Symbols indicate broodstock tanks (circle: H3; triangle: H4; square: MT7)
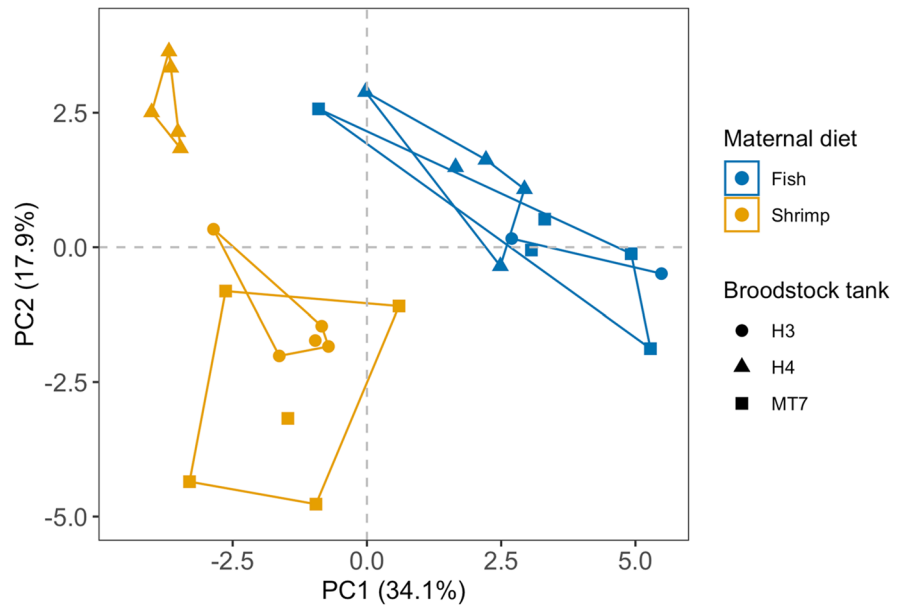

Table 7 Lipid class concentrations (mg lipid g^{-1} dw; mean ± 1 SD) of red drum larvae from different broodstock tanks and maternal diets at 21 dph in Experimenet 2. Asterisks indicate significant differences between larvae from different broodstock tanks (two-way ANOVA, $P < 0.05$). There was no significant interaction between tank and diet. Abbreviations for lipid classes can be found in Table 1 caption

Lipid class	H3		H4		MT7	
	Shrimp	Fish	Shrimp	Fish	Shrimp	Fish
TG*	20.2 ± 6.4	17.8 ± 11.4	34.9 ± 15.4	31.6 ± 9.0	19.1 ± 9.4	18.5 ± 8.3
ST	12.7 ± 0.8	12.4 ± 1.3	12.9 ± 1.2	12.3 ± 0.8	11.4 ± 1.3	11.9 ± 2.7
PE	20.4 ± 1.8	23.9 ± 3.8	18.9 ± 2.9	21.9 ± 3.0	23.4 ± 3.0	20.9 ± 3.3
PI/PA	3.3 ± 0.4	3.7 ± 0.6	3.2 ± 0.4	4.0 ± 0.7	3.5 ± 0.6	3.2 ± 0.3
PS	10.3 ± 1.2	10.9 ± 1.6	8.6 ± 1.9	10.6 ± 1.5	10.2 ± 1.9	9.9 ± 2.0
PC	46.4 ± 2.4	52.2 ± 14.8	48.0 ± 4.3	53.8 ± 7.4	49.1 ± 5.2	47.2 ± 6.6
NL*	32.9 ± 7.0	30.2 ± 12.7	47.8 ± 14.3	44.0 ± 8.4	30.5 ± 9.6	30.4 ± 9.0
PL	80.3 ± 5.3	90.6 ± 20.8	78.6 ± 9.3	90.2 ± 12.3	86.2 ± 9.4	81.3 ± 10.8
TL*	113.2 ± 7.0	120.8 ± 33.4	126.4 ± 6.8	134.2 ± 11.9	116.7 ± 13.7	111.7 ± 16.9

digestion and absorption, lipoprotein transport and tissue uptake, oxidation, and lipid acylation and remodeling (Tocher 2003; Ferramosca and Zara 2014). In the current study, there are several ways that nutritional programming could increase the accumulation of fatty acids in red drum larvae: increased appetite/intake, increased efficiency of digestion and/or absorption in the gut, or decreased rate of fatty acid catabolism. Appetite and food intake respond to gut-brain signals generated by energy balance, and are regulated by interactions of the sensory, endocrine,

and nervous systems (Rønnestad et al. 2013; Bonacic et al. 2016). Early nutrition has been shown to alter food intake through changes in the structure of the hypothalamus (cell number, innervation) and expression of hypothalamic appetite-regulating neuropeptides in mammals (Plagemann et al. 2000; Cripps et al. 2005). In fishes, a study of rainbow trout *Oncorhynchus mykiss* reported changes in the expression of an anorexigenic peptide (POMC, proopiomelanocortin) and an orexigenic peptide (NPY, neuropeptide Y) after hatching as a result of different levels
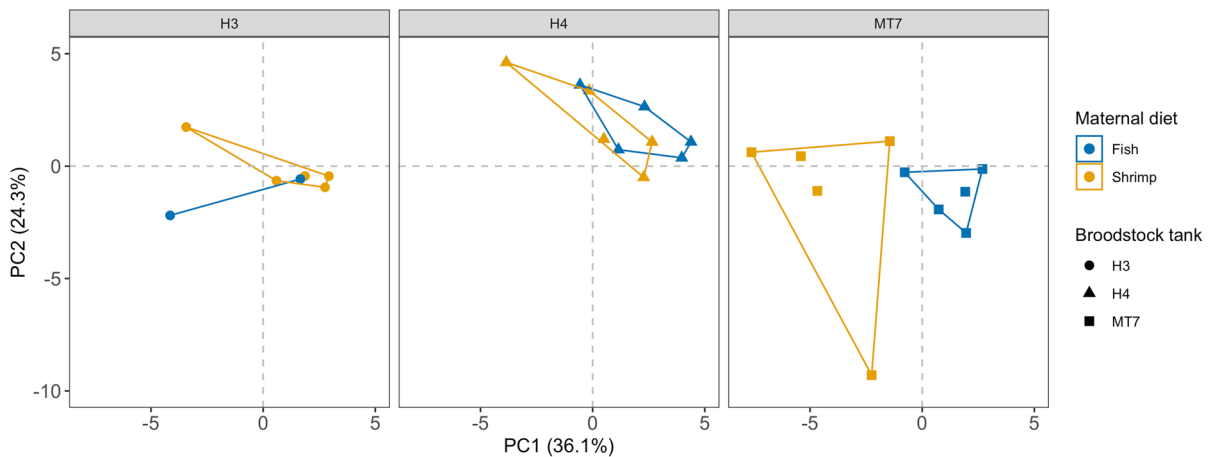
Fig. 5 Principal component analysis of fatty acid profiles (mg g^{-1} dw; PQN and scaled) in total lipids of 21-dph red drum larvae produced by adult fish fed different diets from Experiment 2. Principal component loadings are in Table 8. Each point represents larvae reared from a spawn. Colors indicate diet groups (blue: fish diet; orange: shrimp diet). Symbols indicate broodstock tanks (circle: H3; triangle: H4; square: MT7)

of methionine in maternal diets, with POMC lasting until approximately 3 weeks after first feeding (Fontagné-Dicharry et al. 2017). Another study of rainbow trout found that a plant-based diet given at first feeding for 3 weeks increased food intake when fed the same diet again at 7 months of age, likely due to an altered flavor and feed preference (Geurden et al. 2013; Balasubramanian et al. 2016).

While evidence for a maternal dietary effect on nutrient retention (ingestion, digestion, absorption) in young fishes is scarce, several studies have found that the first-feeding diet programs nutrient retention. Gilthead seabream *Sparus aurata* larvae that were fed a soybean meal (SBM) for 2 weeks at first feeding exhibited decreased pancreatic enzyme activities. Two weeks after removal of the SBM diet, most enzyme activities returned but growth remained reduced (Perera and Yúfera 2016a), pointing to the possibility that even temporary modification of some physiological pathways can lead to permanent phenotypic changes. Juvenile zebrafish showed altered peptide absorption and fatty acid transport when fed a SBM diet for 3 days at first feeding, suggesting that the intestine may be susceptible to programming by the early diet (Perera and Yúfera 2016b). In the intestinal mucosal cells, hydrolyzed dietary lipids (e.g., free fatty acids) are mostly re-esterified and incorporated into chylomicrons or very low density lipoproteins (VLDL). Enhanced gut clearance (absorption

and transport of chylomicrons and VLDL) can increase ingestion and assimilation of dietary fatty acids (Hadas et al. 2003), whereas reduced fatty acid absorption and transport efficiency (e.g., bottleneck in chylomicron synthesis) could cause accumulation of lipid droplets within the gut enterocytes, and reduce further absorption and assimilation (Morais et al. 2005). TG in chylomicrons and VLDL are then hydrolyzed by lipoprotein lipase at peripheral tissue sites and absorbed for catabolism or storage (Sheridan 1988; Tocher 2003). Turkmen et al. (2017) found that a maternal diet of linseed oil (low HUFA) caused downregulation of hepatic lipoprotein lipase (*lpl*) expression in adult gilthead seabream offspring. The reduced lipid deposition into the liver is likely the cause of the observed reduction in liver lipid content (Turkmen et al. 2017). Furthermore, reduced lipoprotein synthesis in the liver could result in reduced transport of lipids to extrahepatic tissues and accumulation of lipid vacuoles in hepatocytes, and therefore, increase storage of lipids (Salhi et al. 1999). Therefore, it is possible that early nutrition programs offspring nutrient retention (absorption, transport) via actions on lipoprotein metabolism.

Nutritional programming might increase the metabolic rate of larvae which would increase energy expenditure and decrease retention of dietary fatty acids. Studies of gene expression reported downregulation of *cptI* expression in the liver in gilthead

Table 8 Principal component loadings for fatty acid profiles (mg g^{-1} dw; PQN and scaled) in total lipids of red drum larvae at 21 dph reared from eggs produced by adult fish fed different diets in Experiment 2 shown in Fig. 5. Boldface type highlights strong loadings

Fatty acid	PC1	PC2
14:0	0.42	0.69
15:0	**0.73**	0.16
16:0	**0.90**	0.12
16:1n-7	−0.47	0.58
16:2n-4	−0.30	**−0.80**
17:0	0.58	0.23
16:3n-4	−0.05	−0.50
18:0	0.53	−0.57
18:1n-9	−0.53	0.51
18:1n-7	−0.45	0.54
18:2n-6	**−0.98**	0.03
18:3n-6	0.35	0.22
18:3n-4	0.47	−0.20
18:3n-3	**−0.72**	0.67
18:4n-3	−0.36	**0.85**
20:1n-9	−0.41	**−0.76**
20:2n-6	−0.23	−0.24
20:3n-6	0.03	−0.63
20:4n-6	**0.95**	0.02
20:3n-3	**−0.89**	0.15
20:4n-3	**−0.92**	−0.04
20:5n-3	−0.09	0.23
22:1n-11	0.63	0.00
22:4n-6	0.31	**−0.73**
22:5n-6	**0.87**	0.43
22:5n-3	−0.32	**−0.80**
22:6n-3	**0.88**	0.40

seabream as a result of a maternal linseed oil diet (Turkmen et al. 2017), and in juvenile rainbow trout when given a vitamin-supplemented first-feeding diet (Panserat et al. 2017), although corresponding phenotypic changes were absent in both studies.

The elevated retention of dietary fatty acids in larvae may have been used for TG synthesis, as fish larvae are generally thought to be unable to synthesize phospholipids de novo (Tocher 2003). However, fish larvae may have limited capacity to increase TG synthesis, because lipids are mainly catabolized to meet energetic demands or incorporated into the cellular membranes in the form of cholesterol and phospholipids (10% and 70% of total lipids, respectively,

of red drum larvae at 21 dph). The linear increases in the amount of different lipid classes with standard length (Fig. S5) suggest isometric lipid accumulation and that a major lipid depot has not yet developed in 21-dph red drum larvae. Therefore, larvae may accumulate more TG in lipid depots, such as liver, muscle, or adipose tissues, as they shift their energy allocation strategy from growth to storage after the larval period (Norton et al. 2001; Litvin et al. 2011; Giraldo et al. 2017). If so, differences in TG synthesis induced by maternal diet, if present, would become more significant at later stages. In addition, it is likely that the previously established positive correlation between egg and larval DHA content (Fuiman and Perez 2015) was because eggs with low DHA content (similar to eggs from a shrimp diet) induced greater TG synthesis/accumulation, "diluting" the overall DHA (and other HUFA) pool, which is normally more concentrated in the phospholipids.

Results from Experiment 2 revealed different responses among females in the changes in offspring lipid accumulation induced by maternal diet. Turkmen et al. (2019b) suggested that genetic variability may interfere with the programming effect of maternal diet on offspring fatty acid metabolism. Adult gilthead seabream showed varying levels of upregulation of *fads2* expression in response to a plant-based diet. Their offspring showed variations in gene expression levels (*cpt1*, *elovl6*) that were related to parental *fads2* expression levels. Furthermore, these juvenile fishes also showed growth and methylation levels in the promoter regions of *fads2* that were correlated with the parental *fads2* expression level in addition to the maternal diet (Turkmen et al. 2019b).

In addition to genetic variability, it has been suggested that the discrepancies in phenotypic outcomes of early nutrition could be accounted for by the timing of the nutritional stimulus relative to organogenesis (Cripps et al. 2005; Symonds et al. 2009), and by environmental and behavioral factors (Trujillo et al. 2006; Panserat et al. 2019). In our study, the duration of the maternal diet differed among diet groups. In Experiment 1, the shrimp diet was provided to the broodstock tank for more than a year before the onset of spawning. In contrast, the females in Experiment 2 had only been on the shrimp diet for about 2 months and may have had body stores to draw upon for lipid incorporation in eggs, leading to the somewhat different egg fatty acid profiles from different tanks

Fig. 6 Heat map showing concentrations of fatty acids (mg g^{-1} dw) in total lipids of red drum larvae at 21 dph produced by adult fish (MT1 from Experiment 1, and H3, H4, MT7 from Experiment 2) fed shrimp or fish diets. Color of each cell indicates fatty acid concentrations, which were scaled (mean = 0, SD = 1) separately for each broodstock tank to highlight maternal dietary effect. Columns represent diet groups from each broodstock tank. Rows represent an individual fatty acid or their group sums
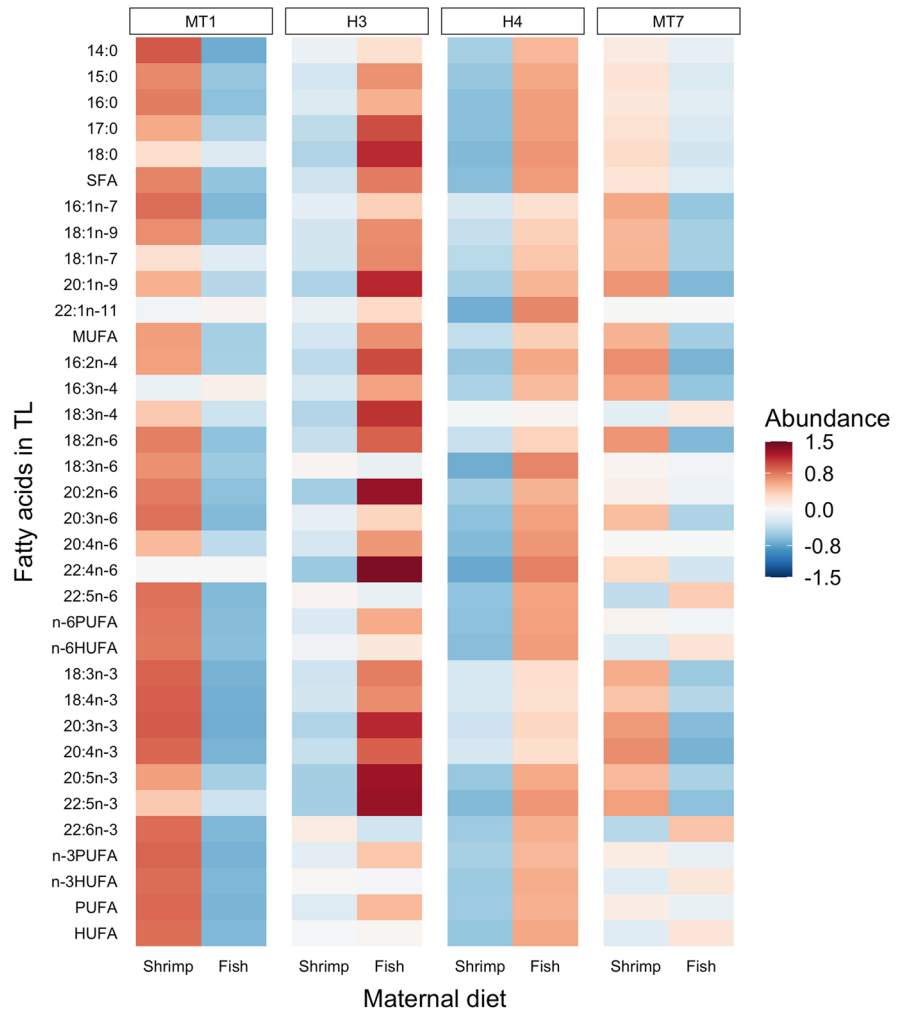
(Fig. 4). Furthermore, it is possible that the different types of oily fish used (Atlantic mackerel, Spanish sardine, or thread herring) contributed to the incongruent observations among broodstock tanks.

Different fatty acid compositions

There were consistent differences in the larval PUFA composition (expressed as % total fatty acids) as a result of changes in maternal diets, although the differences sometimes were not statistically significant. Larvae from the shrimp group generally showed elevated levels of n-3 and n-6 PUFA (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 18:3n-3, 18;4n-3, 20:3n-3, 20:4n-3) and reduced long-chain HUFA (ARA, 22:4n-6, 22:5n-6, EPA, 22:5n-3, DHA) in both experiments. This pattern was especially apparent in the TG

fraction in Experiment 2. Variations in PUFA composition of larvae fed the same larval diet could arise from (1) HUFA biosynthesis or (2) selective retention and incorporation of dietary fatty acids into tissues.

The fatty acids that were elevated in larvae from the shrimp diet group are in the initial steps of the n-3 and n-6 HUFA biosynthetic pathways ("precursor PUFA"), whereas the fatty acids that were reduced in those larvae were products of the same pathways ("product HUFA"). The opposing responses of these groups of fatty acids suggests that the n-3 and n-6 biosynthetic pathways were downregulated in larvae from the shrimp diet group. The key biosynthetic step between these precursor PUFA and product HUFA is mediated by Δ5 desaturase activity, which converts 20:4n-3 and 20:3n-6 into EPA and ARA, respectively. This is a rate-limiting step in HUFA biosynthesis.

Fig. 7 Heat map showing abundance of fatty acids (% total fatty acids) in TG of red drum larvae at 21 dph produced by adult fish from Experiment 2. Color of each cell indicates fatty acid percentages, which were scaled (mean = 0, SD = 1) separately for each broodstock tank to highlight maternal dietary effect. Columns represent diet groups from each broodstock tank. Rows represent individual Rows represent individual fatty acids or their group sums
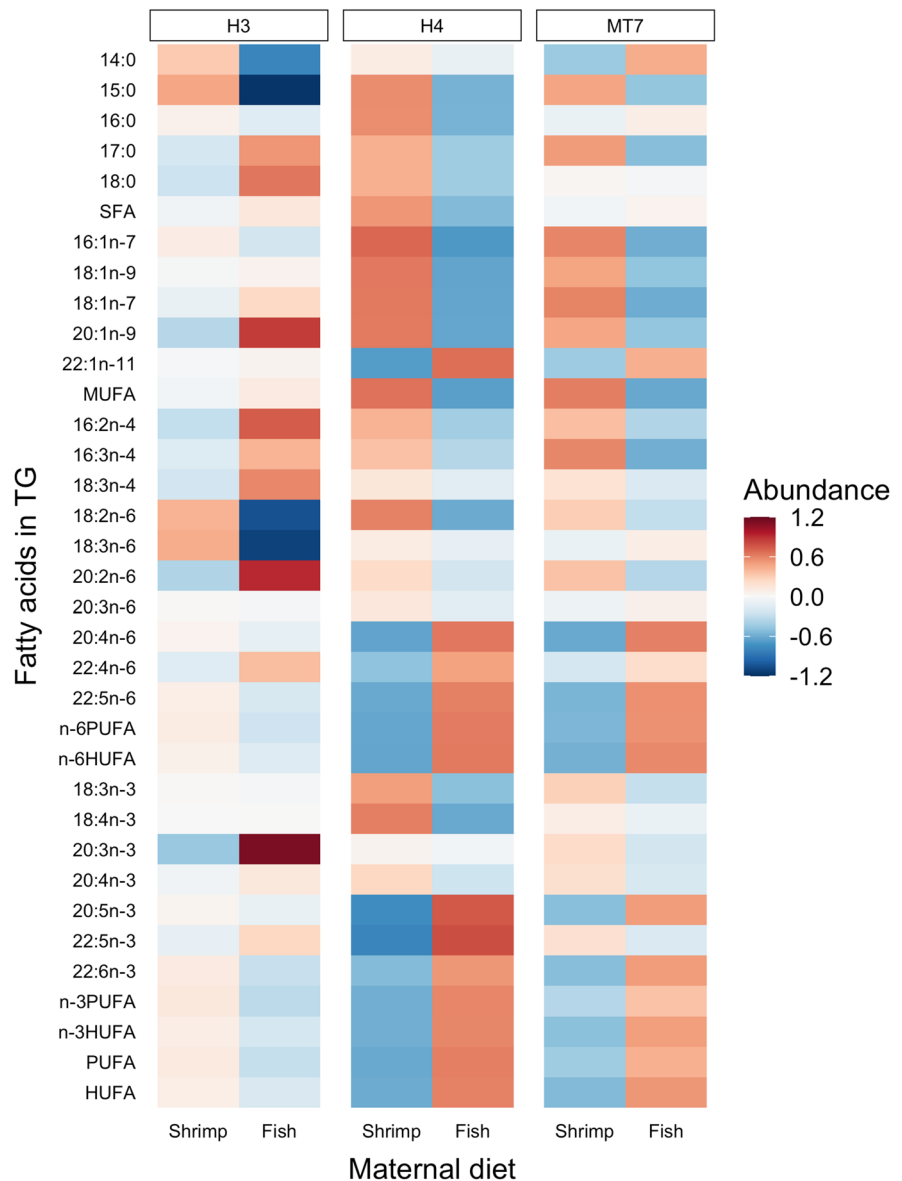
The $\Delta 5$ desaturation step is relatively active in several freshwater and marine teleosts (references in Kabeya et al. 2015; Monroig et al. 2018), but inactive in nibe croaker *Nibea mitsukurii*, which is in the same family as red drum (Kabeya et al. 2015). However, a separate study of red drum larvae reared on a larval diet with high levels of 18:3n-3 (precursor) and low levels of DHA (product) showed that 21-dph larvae from the shrimp maternal diet group had elevated levels of n-3 PUFA precursors (i.e., 18:3n-3, 18:4n-3; 20:3n-3; 20:4n-3) compared to larvae from the non-shrimp maternal diet group (Faulk and Fuiman, unpublished

data). This suggests the maternal diet of shrimp altered $\Delta 5$ desaturase activity in larvae, similar to the present study. A direct measurement of $\Delta 5$ desaturase activity in future studies may help clarify the effects of maternal diet on larval fatty acid metabolism.

Several studies have reported effects on the biosynthetic pathway of marine fish larvae as a result of differences in maternal nutrition. For example, when adult gilthead seabream were provided a linseed oil diet (high in 18:2n-6 and 18:3n-3), larvae showed increased *fads2* expression (Izquierdo et al. 2015; Turkmen et al. 2019a), increased levels

of ARA, EPA, DHA, and reduction in their precursors in the liver (Turkmen et al. 2019a). Interestingly, Senegalese sole embryos and newly-hatched larvae showed an increased capacity for HUFA synthesis (elevated *elovl 5* and Δ4 desaturase expression) in response to a lower egg DHA level originating from the maternal diet (Morais et al. 2014). However, those changes reversed in older larvae. Similarly, HUFA deficiency in the larval diet increased Δ6 desaturase expression and DHA content in polar lipids in European seabass juveniles, when challenged with a HUFA-deficient diet (Vagner et al. 2007, 2009).

Digestive enzymes differ in substrate and positional specificities and their functional development in fish larvae is nutrient sensitive (Lazo et al. 2011). Differences in the retention of long-chain HUFA may occur if early nutrition alters the development or relative expression of those enzymes. For example, given that PUFA are generally esterified to the sn-2 position of TG and phospholipids, the different actions of sn-1,3-specific lipase (Small 1991; Sargent et al. 2002; Tocher 2003), sn-2-specific pancreatic enzyme phospholipase A2 (Rønnestad et al. 2013), and non-specific lipase (Tocher 2003) may cause different levels of PUFA hydrolysis and absorption. Furthermore, during TG synthesis in intestinal mucosal cells and at the storage site, it has been suggested that the re-esterified fatty acids are often different from the original ones (Small 1991). It is possible that nutritional programming enhances selection for HUFA during re-esterification. Further studies could investigate the enzymes involved in the de- and re-acylation processes (e.g., substrate-specific lipases and acyltransferases).

Another mechanism to explain reduced levels of HUFA in the shrimp diet group could be elevated peroxisomal β-oxidation of HUFA. Initial chain shortening of long-chain HUFA (> C20) during oxidation occurs in peroxisomes, after which further oxidation occurs in the mitochondria (Reddy and Mannaerts 1994). Peroxisome proliferator-activated receptors (PPARs) regulate the activities of several peroxisomal enzymes involved in fatty acid β-oxidation and have been implicated in mediating nutritional programming in mammals and fishes (e.g., Vagner et al. 2009; references in Cripps et al. 2005 and Hou and Fuiman 2020). PPARs also mediate lipid homeostatic processes, including biosynthesis (Δ6 and Δ5 desaturases, stearoyl CoA Δ9 desaturase), fatty acid transport (fatty acid transport protein), and tissue uptake (lipoprotein lipase) in response to dietary lipids (Reddy and Mannaerts 1994; Tocher 2003; Patsouris et al. 2006). Future studies should examine the response of PPAR genes to nutritional programming, given their key role in the regulation of HUFA synthesis and oxidation.

Nutritional programming stimulus

There are several constituents of the maternal diets (shrimp vs. fish) that could have stimulated programming effects. First, shrimp contain much less lipid than the other diets. Various studies have demonstrated the effects of dietary lipid level on lipid metabolic pathways in mammals and fishes, including transport, tissue uptake, de novo lipogenesis, and TG synthesis (Lu et al. 2013; Duarte et al. 2014; He et al. 2015). Shrimp also contain relatively low amounts of PUFA. Dietary PUFA can influence lipid metabolism by regulating the transcription of critical genes involved in certain metabolic pathways (Tocher 2003; Ferramosca and Zara 2014). For example, dietary PUFA (especially n-3 HUFA) have been shown to suppress the expression of Δ6 and Δ5 desaturase via regulation by PPAR, and lipogenesis mediated via sterol regulatory element binding protein-1 (SREBP-1), but increase lipid oxidation and storage (Nakamura and Nara 2003; Tang et al. 2003; Ferramosca and Zara 2014; Duarte et al. 2014). Third, shrimp is characterized by a relatively low n-3:n-6 ratio. A diet with low n-3:n-6 ratio during gestation and lactation in rats was associated with permanently reduced DHA levels the brains of offspring (Li et al. 2006). Some studies also attributed the hypotriacylglycerolaemic (TG-reducing) effect of fish oil (relative to vegetable oil) to a high dietary n-3 HUFA content (Morais et al. 2011a, b). These effects are consistent with the changes we observed in red drum from a maternal diet shrimp (lower DHA, higher TG). Fourth, there are comparatively higher levels of sterols in shrimp than in fish. Dietary cholesterol can act as a ligand for SREBP to regulate the biosynthesis of long-chain HUFA from C18 precursors (Leaver et al 2011), and it can be metabolized into bile salts (via bile acids) to facilitate intestinal lipid absorption (Parish et al. 2008). Lastly, shrimp contain a higher amount of astaxanthin, an antioxidant, which could reduce peroxidative stress that is usually caused by high PUFA. In particular, astaxanthin has been shown

to decrease plasma TG concentrations in mice (Yang et al. 2014). These dietary components of shrimp may have synergistic effects on offspring lipid metabolic pathways via altered egg compositions or via epigenetic modifications to adult genomes that are passed on to the next generation.

Implications

Given the ever-increasing demand for food resources from aquaculture, nutritional programming may be a viable approach to improve production and food quality at a lower cost and with a lower environmental impact (Engrola et al. 2018; Panserat et al. 2019). Many studies are aiming to improve utilization of more sustainable, plant-based feeds as replacements for fish-based diets through nutritional interventions during the larval period (Geurden et al. 2007, 2014; Gong et al. 2015; Rocha et al. 2016a, b; Perera and Yúfera 2016a, b; Zambonino-Infante et al. 2019). Our study suggests an alternative strategy of altering maternal diet, which introduces nutritional stimuli at the start of the embryonic period. But more work is needed to identify the critical window for nutritional programming, and how long the programming effects created in this manner persist.

The present study has also demonstrated that nutritional programming can influence larval utilization of exogenous nutrients, which leads to altered body fatty acid composition. Previous studies have found correlations between larval body fatty acid composition and performance of behaviors that are crucial to survival (e.g., predator evasion response; Fuiman and Perez 2015; Oberg and Fuiman 2015; Burns and Fuiman 2019). In addition, a field study documented a close correlation between interannual variations in egg composition and climate-driven changes in prey fields of adult red drum (Fuiman 2018). Therefore, maternal diets and egg composition vary in nature, and it is possible that nutritional programming may occur naturally, leading to consequences for larval survival and possibly recruitment success.

Future directions

As described above, the observed changes in larval lipid and fatty acid accumulation and composition could derive from altered ingestion, digestion, absorption, biosynthesis, or oxidation of fatty acids by larvae. In particular, a programming effect on HUFA biosynthesis (Δ5 desaturase activity) seems the most likely explanation for the differential fatty acid composition of larvae. Examination of the key genes and/or enzymes involved in these lipid homeostatic pathways, especially on the tissue level, could clarify the physiological mechanisms responsible for the differences in larval composition. Tracer studies (e.g., radioactive label) could determine retention and the metabolic fate of dietary fatty acids. Furthermore, the variation among females revealed in this study could be explained by genetics (e.g., gene polymorphism), epigenetic modifications, or non-genetic traits that include, but are not limited to, female size, age, condition, and physiological state (e.g., hormone levels). Future studies should control or address these factors by incorporating them into the experimental design and the statistical models used for data analysis. Molecular approaches, such as transcriptomics, may provide insight into the role played by female genotype. In addition, more studies are needed to identify the nutrient component(s), present or absent in the shrimp diet, that are the stimuli for nutritional programming. It is possible that non-lipid nutritional constituents (e.g., amino acids, vitamins) in the eggs are also influenced by changes in maternal diet and act as programming stimuli. Controlling the dietary intervention by formulated diets or microinjection into fertilized eggs could significantly advance our understanding of nutritional programming in fishes.

Data availability The associated data are available at Texas Data Repository https://doi.org/10.18738/T8/BM2XBS.

Code availability Not applicable.

Declarations

Ethics approval All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin (AUP-2016–00011 and AUP-2018–00302).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest The authors declare no competing interests.

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