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Nutritional ecology of a marine teleost: maternal maturation diets affect egg and larval composition of Southern Flounder (*Paralichthys lethostigma*)

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Nutritional ecology of a marine teleost: maternal maturation diets affect egg and larval composition of Southern Flounder (*Paralichthys lethostigma*)

by

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Abstract

Nutritional ecology of a marine teleost: maternal maturation diets affect egg and larval composition of Southern Flounder (*Paralichthys lethostigma*)

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Maternally derived nutrients in marine fish yolk play important roles in early larval development. A principal subclass of these nutrients is fatty acids (FAs), which are involved in a variety of key physiological processes related to gene regulation, cellular membrane structure, and energy production. FAs residing in yolk are acquired from maternal sources either immediately prior to spawning (income breeders) or through somatic reserves established from dietary intake well-before spawning (capital breeders). The FA composition of eggs has been previously linked to measures of egg quality and subsequent larval quality. A study was conducted to determine how changes in maturation diets fed to Southern Flounder affect egg composition and egg quality. Differences in egg composition were assessed from broodstock populations fed one of four different diets using gas chromatography. It was found that levels of 20 of 27 FAs measured in eggs had direct positive relationships with amounts of the same FA in the maternal diet when the maternal diet changed at least 16 weeks before spawning. Among

the egg quality metrics measured, hatching rate and larval hatching length were most sensitive to differences in maternal diet that produced compositional changes in eggs.

A second study had two objectives, to: (1) investigate the relationships among egg and larval composition and maternal diet and egg composition for effects of metabolic programming; and (2) determine how FA composition of the larval body affects larval performance in ecologically relevant survival skills (e.g., routine swimming and predator evasion). Eggs and larvae from the first study were reared until two distinct developmental stages (15- and 35-days post-hatching, dph) and larvae were used in performance assays at each stage. Several FAs found to have direct diet-egg relationships in the first study were found to affect larval body composition at 15-dph (16:1ω7, 20:4ω6, 20:1ω9) and 35-dph (18:1ω7, 20:1ω9, 22:5ω3, 22:6ω3:20:4ω6). Since larvae were all fed the same high-quality diet, the presence of differences in body FA composition in 15- and 35-dph larvae suggests that maternal diet alters egg composition which, in turn, affects lipid metabolism in the larvae, a process known as nutritional programming. Further, several significant relationships were observed between these FAs and larval performance metrics. These studies suggest that maternal diet can have important consequences for egg and larval quality in hatchery settings even when larval diet is of high-quality and proposes several candidate FAs for future studies investigating nutritional programming in Southern Flounder.

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Chapter 1: Background

Maternally derived nutrients contained in yolk play critical roles during the early development of marine fishes. As the only source of nutrition during embryogenesis, yolk contains a variety of macro- and micro-nutrients that support proper development and growth before exogenous feeding begins (Rainuzzo et al. 1997). Yolk is highly concentrated in a class of diverse biomolecules known as fatty acids (FAs), which serve key roles in metabolism, cellular structure, and gene regulation (Rustan and Drevon, 2005). FAs residing in the yolk of marine fish eggs have two immediate sources: recent maternal diet or maternal body stores (some of which derive from the earlier maternal diet), although both sources can be used simultaneously. Thus, egg FA composition is intricately linked to maternal diet and the timing of dietary changes that may occur as a result of seasonal reproductive migrations or scheduled feeding regime changes in captive settings. Prior work has demonstrated that egg composition can have long term consequences for physiological and ecologically relevant performance of offspring, even when the offspring are fed a high-quality diet (Fuiman and Perez 2015; Perez and Fuiman 2015; Burns and Fuiman 2019). Therefore, understanding the relationships among maternal diet, egg composition, and ultimately larval performance may be important for increasing the efficacy of captive rearing programs used for stock enhancement and placing species-specific, maternal nutritional requirements into working ecological frameworks.

Essential fatty acids (EFAs) are necessary in species-specific quantities for the survival of vertebrates (Sargent et al. 1995). These EFAs are deemed essential, because they cannot be synthesized *de novo* in physiologically necessary quantities and must be acquired through dietary intake (Parrish 2009). An organism's ability to elongate and desaturate FA precursors into long

chain FAs relies on the presence and activity levels of enzymes that catalyze these processes, such as FA elongases (ELOVLs) and desaturases (FADs) (Hastings et al. 2001). FAs are characterized by the number of double bonds in their carbon chain, and at which carbon atom the first double bond occurs, beginning from the carboxyl end (e.g., 16:1ω7 indicates 16 carbons, 1 double bond at carbon 7 from carboxyl end) (Davidson and Cantrill, 1985). Based on their structure, FAs fall into several categories, including saturated FAs (SFAs, without double bonds), mono-unsaturated FAs (MUFAs, one double bond), polyunsaturated FAs (PUFAs, at least two double bonds) and highly unsaturated FAs (HUFAs, at least three double bonds). The dominant EFAs for marine fishes are docosahexaenoic acid (22:6ω3, DHA), arachidonic acid (20:4ω6, ARA), and eicosapentaenoic acid (20:5ω3, EPA), all of which have been well studied (Watanabe 1993; Sargent 1997; Zhang et al. 2019).

Mortality is exceptionally high during the early life of fishes, and the primary causes of this morality are predation and starvation (Bailey and Houde 1989; Houde 1997). Larval survival depends upon having certain survival skills, in particular the ability to detect and evade predators and to find food (Fuiman and Magurran, 1994). Performance of these survival skills is related to size and developmental stage, but also nutritional status. For example, the quality of the diet fed to larvae can significantly affect their growth, swimming performance, vision, and hearing (Bell et al. 1995; Sargent et al. 1999; Perez and Fuiman 2015). Additionally, previous investigations of Red Drum (*Sciaenops ocellatus*) and Southern Flounder (*Paralichthys lethostigma*) revealed that the composition of the maternal diet affects egg quality, specifically, the nutrients that embryos and early larvae use for development and growth (Fuiman and Faulk 2013, 2014; Burns and Fuiman 2019, 2020). Further, several measures of larval survival skills (i.e., traits related to

predator evasion and foraging) are also correlated with egg quality and broodstock diet even when larvae are reared under uniform conditions and fed the same high-quality diet (Perez and Fuiman 2015; Fuiman and Perez 2015; Burns and Fuiman 2019). Thus, the choice of broodstock diet can affect egg quality and have a beneficial or detrimental effect on the survival skills of their offspring when they are released into natural habitats.

Southern Flounder is a popular target for commercial and recreational anglers off the Texas coast, annually contributing $2.2 billion in total economic activity to the region (Vega et al. 2011). Flounder populations have seen marked declines since the mid 1980s, with commercial catch decreasing from 500,000 fish per year to less than 100,000 fish per year by 2007, and recreational catch declining from 200,000 fish per year to less than 50,000 fish per year by 2007 (Froeschke et al. 2010). More recent analyses suggest this population trend is geographically widespread, as the recreational catch in Louisiana decreased by 80% in weight from 2013 to 2017 (Erickson et al. 2021), and commercial catch in the South Atlantic also decreased by 80% from 1989 to 2017 (Flowers et al. 2019). To address the declining population trend, the Texas Parks and Wildlife Department (TPWD) implemented efforts to improve Southern Flounder populations beginning in 2006. These efforts included limiting gigging in 2009, shortening of the gigging season, and in 2021, reducing the harvestable slot length. Another tool being used by TPWD to manage Southern Flounder populations is a stock enhancement program (Miller et al. 2010). Every year since 2006, TPWD coastal hatcheries produced approximately 25,000 Southern Flounder fingerlings and stocked them into Texas estuaries with the goal of bolstering natural populations. Significant new investment has gone into the flounder stocking program, as new facilities have been constructed at the Marine Development Center in Corpus Christi, TX,

USA, and at Sea Center Texas in Lake Jackson, TX, USA, specifically to increase production of Southern Flounder fingerlings for stock enhancement.

Knowledge of the contribution of broodstock nutrition to egg and larval quality of Southern Flounder is relatively poor, but there have been recent advances. To begin, several EFAs in larval tissues have been shown to be important to ecologically-relevant performance of Southern Flounder larvae. Oberg and Fuiman (2015) fed Southern Flounder larvae diets of live rotifers (*Brachionus plicatilis*) enriched with different EFA profiles and found that concentrations of DHA in the larval diet were positively correlated with the amount of DHA in the body of larvae and with their responsiveness to a simulated predator. The importance of DHA in larval Southern Flounder to responsiveness was confirmed in subsequent experiments (Burns and Fuiman 2019). Since responsiveness is the primary determinant of survival for larval fishes under predatory attack (Fuiman et al. 2006), these findings clearly demonstrated the importance of larval diet to larval survival skills and identified levels of DHA in the larval body that are biomarkers of larval quality. These studies provide the basis for investigating whether egg composition affects subsequent larval composition, even when the larvae are reared under uniform conditions with a high-quality diet, as has been shown for Red Drum (Fuiman and Perez 2015).

Burns and Fuiman (2019) showed that DHA levels in larval tissues are negatively correlated with the levels of a DHA precursor (ω3 DPA, docosapentaenoic acid) in eggs, even when larval diet was the same. Since ω3 DPA and other EFAs cannot be synthesized by Southern Flounder, the amount of EFAs present in eggs are determined by maternal dietary intake of those EFAs. Because broodstock diets fed to captive Southern Flounder are different

from the natural diet, eggs produced by captive Southern Flounder have a very different biochemical composition from naturally-produced eggs (Figure 1.1), including the important biomarkers of larval survival skills. Taken together, these pieces of evidence suggest that broodstock diet could be an important factor in larval performance and, ultimately, the survival of stocked larvae.

The overarching goal of this thesis was to develop a better understanding of the dynamics of diet-egg transfer of FAs and their importance to viability of larvae in hatcheries and after release into the environment. Two studies were designed to address two principal questions concerning Southern Flounder: (1) Do different broodstock maturation diets (those fed well in advance of spawning) result in eggs of different FA composition and; (2) Is larval performance of survival skills related to broodstock diet when larvae are reared under identical conditions using a high-quality diet. In the first study, four groups of Southern Flounder broodstock were conditioned for spawning with different maturation diets. Two groups were placed on a common diet and then switched to two different diets well-before, and throughout, their reproductive period. The other two groups of Southern Flounder broodstock were fed a common diet plus a nutritional supplement. One of those groups received the supplement year-round, while the other received it just one month prior to, and throughout, their reproductive period. All adults were strip-spawned and egg quality and composition were assessed. In the second study, larvae from the spawns obtained in the first study were reared under uniform conditions and predator evasion and foraging traits were measured at two developmental stages.

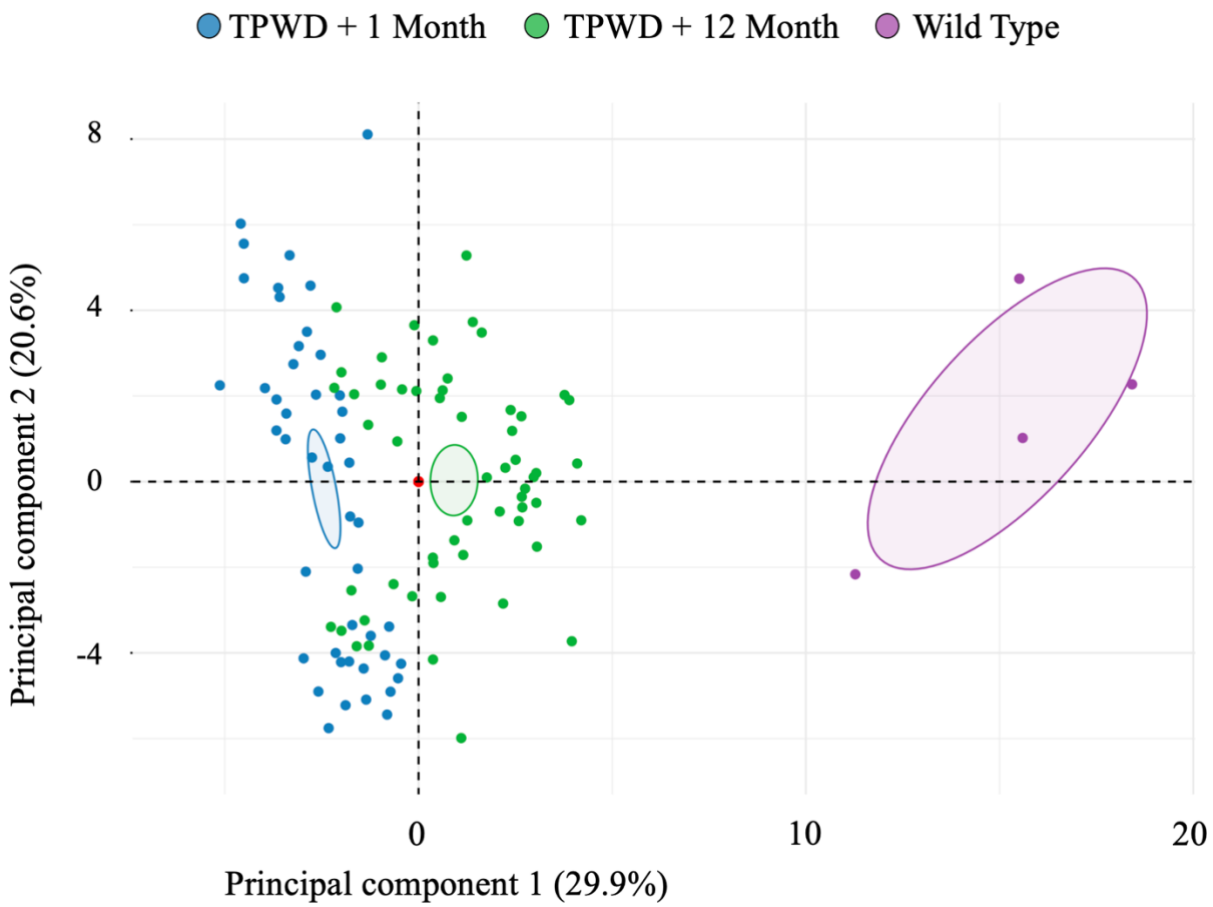

Figure 1.1. Principal component scores for Southern Flounder egg FA composition (% total FA). 95% confidence ellipses for the mean are shown for eggs produced from two Texas Parks and Wildlife Department (TPWD) diets (blue = 1 month supplementation; green = 12 month supplementation) and eggs produced from wild caught Southern Flounder in Texas before receiving a captive diet (Wild Type).

Chapter 2: Maternal maturation diets affect egg composition and quality in Southern Flounder, *Paralichthys lethostigma*

INTRODUCTION

The yolk of marine fish eggs contains an array of macro- and micro-nutrients that support proper growth and development from fertilization until the onset of exogenous feeding (Sargent 1995; Rainuzzo et al. 1997). One particularly important class of nutrients in yolk that has received considerable research attention are fatty acids (FAs). FAs serve diverse cellular roles across taxa including membrane structure, energy metabolism, and gene regulation (Rustan and Drevon, 2005). The immediate source of FAs in marine fish yolk depends on the resource allocation strategy used by the mother. Capital breeding strategists rely on somatic nutrient stores to build eggs, while income breeders allocate recent dietary nutrition to eggs (Jönsson 1997). Therefore, FAs in eggs can come from maternal body stores (capital breeders) or recent maternal diet (income breeders). Although the capital—income breeding dichotomy of energy allocation to reproduction appears simple, some animals utilize an intermediate strategy which draws on stored resources when incoming nutrition is deficient (Wheatley et al. 2008; Burns and Fuiman 2020). Prior research has shown that the FA composition of eggs of some subtropical marine fishes depends on the availability and composition of FAs in the recent maternal diet (i.e., income breeding; McBride et al. 2015). Further, differences in egg composition created by a change in the maternal diet can affect egg quality metrics such as fecundity, fertilization rate, and hatching success (Watanabe et al. 1984; Fernández-Palacios et al. 1994; Yanes-Roca et al. 2009).

Southern Flounder (*Paralichthys lethostigma*) is a highly prized target of commercial and recreational anglers off the Texas Coast, contributing to billions of dollars in regional economic activity each year (Vega et al. 2011). However, Southern Flounder populations have experienced consistent and geographically widespread declines over the last 40 years across the Gulf of Mexico (Froeschke et al. 2010; Erickson et al. 2021) and other South Atlantic waters (Flowers et al. 2019). In response to this declining population trend, the Texas Parks and Wildlife Department (TPWD) implemented efforts to improve Southern Flounder populations beginning in 2006. These efforts included limiting gigging in 2009, shortening the gigging season, and reducing the harvestable slot length in 2021. In addition to these efforts, TPWD created a stock enhancement program in 2006 (Miller et al. 2010). Each year, captive broodstock are conditioned for spawning over many months, eggs are obtained by hormone injection and strip-spawning, and the larvae are reared until they are released into coastal waters to supplement wild Southern Flounder populations. A better understanding of the relationships between maternal diet, egg composition, and egg quality could improve broodstock management procedures for stock enhancement efforts and assist in predicting the consequences of variations in prey availability for reproductive success of natural populations.

Knowledge of the relationships between broodstock diet, egg composition, and egg quality is important for maximizing production, but this knowledge for Southern Flounder is limited to manipulations of the spawning diet (i.e., near the time of spawning), rather than the maturation diet. When docosahexaenoic acid (DHA, 22:6ω3) content of the spawning diet was increased or decreased, there was a detectable change in DHA content of their eggs (in the same direction) after just three weeks (Burns and Fuiman 2020). Eggs had not equilibrated to the increase in dietary

intake of DHA after 8 weeks. Interestingly, 5 weeks after a reduction in DHA content of the spawning diet, DHA content of eggs began to increase, apparently because somatic reserves of DHA obtained from the maturation diet were used to supplement egg composition (Burns and Fuiman 2020). This suggests that Southern Flounder have the capacity to compensate, at least partially, for dietary deficiencies around the time of spawning by using nutrients accumulated in their tissues. Thus, the timing of a change in diet has important consequences for egg composition.

The first objective of this research was to understand the relationship between Southern Flounder maturation diets and egg composition. Specifically, which FAs in eggs are influenced by dietary intake? The second objective was to assess whether maternal diet or egg composition affects egg quality metrics, such as fecundity, egg viability, hatching length, hatching rate, and energy density of eggs.

METHODS

A broodstock population comprised of 99 adults was established at the University of Texas Marine Science Institute's Fisheries and Mariculture Laboratory (FAML) in Port Aransas, TX, USA, in 2019 and 2020. Males (M) and females (F) were distributed evenly (39F:10M and 40F:10M) into 2, 36-kL recirculating raceways and were fed a conditioning diet of equal parts (1:1 by wet weight) Brown Shrimp (*Farfantepenaeus aztecus*) and Spanish Sardine (*Sardinella aurita*) *ad libitum* on Mondays, Wednesdays, and Fridays from May through September (16 weeks). The conditioning diet was intended to equilibrate FA reserves before changing to experimental diets. One of two experimental diets (Brown Shrimp only, Spanish Sardine only) was implemented *ad libitum* on Mondays, Wednesdays, and Fridays for each raceway beginning the second week of September. Spawning took place between January and April of the first year,

after which all fish were placed on the conditioning diet until mid-September when each raceway was given the opposite experimental diet. Water temperature and photoperiod were adjusted to mimic the natural seasonal progression Southern Flounder experience, resulting in ripe adults by mid-January each year. Flounder were maintained at 18.0°C, 32 ppt salinity and 10h:14h (light:dark) photoperiod between January and April.

Gravid females were anesthetized by placing them in a bath of 15 ppm Eugenol (clove oil) in seawater and then injected with Ovaprim SGnRHa + Domperidone (0.5 mL kg^{-1} wet weight; Chemical, Inc., Ferndale, WA, USA) to induce ovulation. Forty-eight hours after injection, females were manually strip-spawned, and subsamples of unfertilized eggs were rinsed three times in deionized water and stored at -80°C for analysis of FA composition using flame ionization detector gas chromatography (FID-GC). Milt from two to four anesthetized males from the same dietary treatment as the stripped female was activated with sea water and mixed with eggs from a single female, and fertilization was promoted by actively mixing the gametes with a feather for at least 3 min. Eggs were allowed to rest for at least 15 min before being transferred to containers of clean sea water supplied with gentle aeration.

In addition to the experiments conducted at FAML, Southern Flounder eggs were received from two broodstock populations maintained by the Coastal Fisheries Division at TPWD. One population, located at the Marine Development Center, Corpus Christi, TX, USA, was fed a diet of shrimp, squid, and mackerel (2:1:1 wet weight) Mondays, Wednesdays, and Fridays *ad libitum* year-round (TPWD base diet). These broodstock fish were provided with a nutritional supplement (Mazuri Aquatic Gel, PMI Nutrition International, MN, USA) Tuesdays and Thursdays *ad libitum,* one month prior to, and throughout the spawning season. A second

population, located at Sea Center Texas, Lake Jackson, TX, USA, was fed the TPWD base diet, but the nutritional supplement was fed year-round. Strip-spawning procedures at the TPWD facilities were conducted in a similar manner to the populations at FAML, except that Tricaine Methanesulfonate (MS-222, manufacturer recommended concentration) was used as the anesthetic. Also, the number of males used to fertilize a spawn depended upon the number of ripe males available on a given day, with some spawns using as few as one male for fertilizations.

Analyses

A suite of egg quality metrics were assessed for each of the four dietary treatments, including fecundity, egg viability, hatching rate, hatching length, and energy density. Fecundity was measured as the volume of eggs stripped at the time of spawning. Egg viability was measured as the volume of eggs floating 24 h after fertilization divided by the volume measured fecundity for the same spawn. Hatching rate was determined by distributing a subsample of 100-150 eggs from a fertilized spawn equally among five, 250-ml containers holding seawater from the broodstock tank. Forty-eight hours after fertilization, newly hatched larvae were removed from the containers, enumerated, and photographed using a stereo microscope for subsequent measurement of hatching length. Energy density of freeze-dried eggs (cal g^{-1} dry weight) was measured by researchers at the Brigham's Women's Hospital (BWH Metabolic Core) at Harvard Medical School using a Parr 6725 semi-microbomb oxygen calorimeter.

Samples of diet items and eggs were frozen at -80°C until they were prepared for FA analysis. FA profiles (27 individual FAs) were measured for the separate broodstock diet items and eggs using flame ionization detector - gas chromatography (FID-GC, Shimadzu GC-2014)

following methods described by Faulk and Holt (2005). Samples of diet items and eggs were freeze dried for at least 24 h and homogenized in a chloroform/methanol solution (2:1, v/v), which included an internal standard (tricosanoic acid, 23:0). FA methyl esters were obtained after transesterification of total lipids by adding boron trifluoride. All FAs are reported as a percentage of total FAs identified. See appendix for mg g^{-1} dry weight values.

Statistical Analyses

Initial relationships between broodstock diet and FA composition of eggs were explored using principal component analyses (PCA, using the correlation matrix on 27 fatty acid variables, each rescaled to mean = 0). One-way analysis of variance (ANOVA) tested whether multivariate egg composition (principal component scores) varied significantly among diet groups. One-way ANOVA was also used to test differences in egg quality metrics among dietary treatments. FA variables that were strongly correlated (Pearson correlation coefficient, $r > 0.65$) with a principal component, were examined for correlation with egg quality metrics. To assess diet-egg relationships, one-way ANOVA tests were performed on individual FAs of diet treatments and eggs. When ANOVA produced significant results, post-hoc pairwise comparisons of FA composition of diet items and eggs were used (Tukey's HSD with Bonferroni-Holm, family-wise, error rate correction). Only direct diet-egg relationships (i.e., an increase in dietary intake of a FA results in an increase in that same fatty acid in the eggs) were considered relevant. All statistical analyses were conducted in R (R Studio 1.3.1073) using FactoMineR, ggplot2, and dplyr packages.

RESULTS

Diet composition

There were substantial differences in FA composition among the three main broodstock diets (FAML shrimp, FAML sardine, TPWD; Table 2.1). The TPWD base diet had significantly higher amounts of 7 FA than the FAML sardine diet (18:1ω9, 18:4ω3, 20:1ω9, 20:3ω3, 20:4ω3, 20:5ω3, and 22:1ω11) and the FAML shrimp diet (14:0, 18:1ω9, 18:4ω3, 20:1ω9, 20:4ω6, 20:4ω3, and 22:1ω11). The FAML shrimp diet had significantly higher amounts of 10 FAs than the TPWD base diet (15:0, 17:0, 16:3ω4, 18:0, 18:1ω7, 20:2ω6, 20:4ω6, 20:5ω3, 22:5ω6, and 22:5ω3), and the FAML sardine diet had significantly higher amounts of eight FAs than the TPWD base diet (15:0, 16:0, 16:1ω7, 16:2ω4, 17:0, 18:0, 18:3ω6 and 22:5ω6). Comparing the two FAML diets, the shrimp diet had significantly higher amounts of nine FAs than the sardine diet (16:3ω4, 18:1ω7, 20:1ω9, 20:2ω6, 20:4ω6, 20:3ω3, 20:4ω3, 20:5ω3, and 22:5ω3); whereas the sardine diet had significantly higher levels of eight FAs than the shrimp diet (14:0, 16:0, 16:1ω7, 16:2ω4, 18:3ω6, 18:4ω3, 22:5ω6, 22:6ω3). Eight FAs comprised 76% of the nutritional supplement (14:0, 16:0, 16:1ω7, 18:0, 18:1ω9, 18:2ω6, 20:5ω3, 22:6ω3, Table 2.1) and two of these FAs represented 29% of the nutritional supplement (16:0, 22:6ω3).

Egg composition

Spawns were obtained from 10 to 28 females from each of the four diet treatments each year, resulting in 22-40 spawns for each diet overall. Principal component analysis summarized the variation in FA composition of eggs in six components (eigenvalue > 1.0), with 80.4% of variance explained (Figure 2.1). Each diet produced eggs with a distinctive FA profile (Table

2.2). All pairs of diets were significantly different on PC1 (40.1% of total variance explained), all but two pairs (FAML shrimp vs TPWD + 12 month; FAML sardine vs TPWD + 1 Month) were significantly different on PC2, and all pairs but one (FAML shrimp vs TPWD + 12 month) were significantly different on PC3 (Figure 2.1 B).

Pairwise comparisons revealed that FAML sardine eggs had significantly higher amounts of eight FAs than eggs from both TPWD diets (15:0, 16:0, 16:1ω7, 16:2ω4, 17:0, 18:0, 18:3ω6, 22:5ω6) and FAML shrimp eggs had significantly higher amounts of eight FAs than eggs from both TPWD diets (15:0, 17:0, 16:3ω4, 18:0, 18:1ω7, 20:4ω6, 22:5ω6, and 22:5ω3). Eggs from both TPWD diets had significantly higher amounts of seven FAs than eggs from the FAML sardine diet (18:1ω9, 18:4ω3, 20:1ω9, 20:3ω3, 20:4ω3, 20:5ω3, and 22:1ω11) and significantly higher amounts of eight FAs than eggs from the FAML shrimp diet (14:0, 18:1ω9, 18:4ω3, 20:1ω9, 20:2ω6, 20:4ω3, 20:5ω3, and 22:1ω11). Eggs from the FAML sardine diet had significantly higher amounts of seven FAs than FAML shrimp eggs (16:0, 16:2ω4, 18:3ω6, 18:4ω3, 20:4ω3, 22:5ω6, and 22:6ω3) and FAML shrimp eggs had significantly higher amounts of ten FAs than FAML sardine eggs (14:0, 16:1ω7, 16:3ω4, 18:1ω7, 20:1ω9, 20:2ω6, 20:4ω6, 20:3ω3, 20:5ω3, and 22:5ω3).

Diet-egg relationships

Twenty FAs that had significantly higher levels in one diet than another diet (Diet composition, above) also had significantly greater levels in the eggs produced by the same diet (Egg composition, above). This indicates that these 20 FAs (14:0, 15:0, 16:0, 17:0, 18:0, 16:1ω7,

16:2ω4, 16:3ω4, 18:1ω7, 18:3ω6, 18:1ω9, 20:1ω9, 20:4ω6, 20:3ω3, 20:4ω3, 22:5ω3, 22:5ω6, 22:1ω11, 20:5ω3, 22:6ω3) had direct diet-egg relationships (Figure 2.2).

For the TPWD diets, in which the supplement was given year-round or only 1 month before spawning, eggs from the TPWD + 12 month diet had higher PC2 loadings for four FAs. All four of these FAs were in higher amounts in the supplement than the TPWD base diet and three of them (14:0, 16:1ω7, 18:2ω6) were among the top four FAs in the supplement relative to the TPWD base diet (2.7 to 7.9% higher in the supplement than the TPWD base diet). The fourth (20:5ω3) was 0.7% higher in the supplement than the base diet.

Egg quality

Egg quality metrics were highly variable from spawn to spawn, so few of the egg metrics differed significantly among parental dietary treatments. Parental dietary treatment had a significant effect on hatching length ($P < 0.001$). All pairwise differences among dietary treatments were significant except between the FAML sardine and the TPWD + 1 month diets (Table 2.3). The FAML shrimp diet produced the largest larvae at hatching (2.71 mm) and the TPWD + 12 month diet produced the smallest (2.30 mm), a difference of 18%.

Overall mean hatching rate was 42.9% ($\pm$ 36.9%) and differences among diets were marginally significant ($P = 0.06$). The continuously supplemented TPWD diet produced eggs with the greatest hatching rate (70.2%), while the FAML shrimp diet produced eggs with less than half that hatching rate (32.2%). Batch fecundity was highly variable from one female to another (averaging 85.9 ± 58.2 ml or approximately $124{,}000 \pm 84{,}000$ eggs). Generally, TPWD diet groups produced 25% fewer eggs per spawn than the FAML diet groups (Table 2.3), but there were no differences among dietary treatments ($P = 0.08$). Egg viability averaged 20.5% $\pm$ 30.6%) and did

not differ among diet groups (P = 0.63). Egg energy density was remarkably consistent (5657.9 ± 449.5 cal g^{-1} dry weight) with no differences among diet treatments (P = 0.88).

Egg composition – egg quality relationships

Egg FA composition for the subset of spawns for which hatching length was measured was summarized by a separate PCA. Principal component 1 (43.3% of total variance) was negatively correlated with hatching length (Figure 2.3; P = 0.006). Two individual FAs were negatively correlated (r < -0.60) with PC1 and with hatching length (18:2ω6 and 18:3ω3; Figure 2.3 B-C, P < 0.001).

Principal components 1-5 were not correlated with hatching rate (P > 0.05). However, two FAs that were highly correlated with PC1 had positive (20:4ω3, r = 0.94, P = 0.049) and weakly negative relationships (22:5ω6, r = -0.88, 0.06) with hatching rate.

DISCUSSION

The differences in FA profiles measured in eggs from different parental diets mostly corresponded to differences in the FA profiles of their respective diets, resulting in numerous direct diet-egg relationships. This result suggests that many FAs in the diet are incorporated into oocytes with little modification, which is consistent with a previous study of Southern Flounder (Burns and Fuiman 2020) and a study of another subtropical marine teleost, Red Drum (*Sciaenops ocellatus*) (Hou et al. 2020). In Red Drum, 11 FAs had significant diet-egg correlations (Hou and Fuiman 2020). In the present study, 20 FAs had significant diet-egg correlations. The absence of such a relationship for 7 out of 27 of the measured FAs may be due to transformations (elongation, desaturation, oxidation) of dietary FAs by the adults between absorption by the intestine and

vitellogenesis (Dalsgaard et al. 2003; Hou and Fuiman 2020). Alternatively, the lack of relationships may be due to incorporation of FAs into eggs from non-dietary sources (somatic stores) when dietary intake of lipids or certain FAs is low (Budge et al. 2006), which was previously observed in Southern Flounder (Burns and Fuiman 2020). Further, for some FAs, very small amounts in the diet may not present a constraint for egg composition and thus, those FAs would not be highly variable in eggs regardless of diet.

Interestingly, hatching rate was negatively correlated with levels of 18:2ω6 (α-linoleic acid) and 18:3ω3 (linolenic acid) in eggs. Recent research has demonstrated that 18:2ω6 in chicken yolk was negatively correlated with body length and weight at hatching (Fu et al. 2020). Linolenic acid was not implicated in the study of chicken yolk, but it is a product of α-linoleic acid desaturation via Δ15FAD; however, this pathway is thought to be inactive in marine fishes (Hou and Fuiman 2020). Hatching length is an important trait in larviculture, as it reflects the state of development at hatching. Larvae that are larger at hatching are more morphologically developed than smaller larvae. Larger larvae may start feeding sooner and have a larger gape and greater swimming abilities to locate and capture prey (Hunter 1981; Laurel et al. 2018). Since successful initiation of first feeding after yolk absorption is critical to stave off irreversible starvation (Houde 1974; Hunter 1981), captively reared larvae may be more successful at first feeding when length at hatching is greater. The negative correlations between hatching length and two FAs in eggs suggest that lower levels of one or both FAs in the parental diet may be beneficial for larval feeding and survival. Interestingly, 18:2ω6 and 18:3ω3 were present in sizeable amounts in the nutritional supplement (9.2% and 2.1%) of the TPWD diet, while they were minor contributors to total FAs in the FAML shrimp diet (1.73% and 0.63%). Overall, eggs produced from diets at FAML (shrimp,

sardines) had reduced levels of these FAs and larger mean hatching lengths relative to eggs produced from either supplemented diet. However, it is possible that other constituents of eggs that were not measured in this study (e.g., amino acids, hormones) may have influenced length at hatching. Additionally, although larvae from the TPWD + 12 month diet produced the smallest larvae at hatching, their eggs had the greatest hatching rates (70%) while eggs produced from the FAML shrimp diet had the lowest (32%). The much lower mean hatching rate for the shrimp diet may offset the potential benefits to production of the larger hatching size by producing much lower quantities of viable yolk-sac larvae, and thus decreasing the number of larvae that survive the critical transition to exogenous feeding.

A comparison of results from the two TPWD diets, in which one group received the nutritional supplement 1 month before spawning and the other group received it year-round, provides insight into the temporal window during which egg composition can be influenced by parental diet. The observation that the 12-month supplementation produced eggs with more of some FAs than the 1-month supplementation (Figure 2.1 A, B) suggests that it takes more than 1-month for Southern Flounder egg composition to equilibrate to the parental diet. Further clarity about the temporal window during which egg composition can be influenced by parental diet can be obtained by combining results from this study with results from an earlier study of Southern Flounder that were fed the same diets as were used here but starting closer to spawning (0-8 weeks, first-time spawners of Burns and Fuiman 2020). Analysis of the combined dataset showed that egg FA composition responded quickly when the parental diet was changed from shrimp plus sardines (1:1) to shrimp only. Changes took longer to become apparent when the parental diet was changed

from shrimp plus sardines to sardines only. Equilibration of egg composition to the shrimp or sardine diet took 9-16 weeks (Figure 2.4 A-C).

This study revealed the extent to which broodstock diet affects egg FA composition and the effects on egg quality. Knowing which FAs in eggs can be manipulated through broodstock diet, when to alter the diet, and the subsequent effects on egg quality is information that can be applied in captive rearing settings to enhance the quantity and quality of eggs produced. Separately, this same knowledge may be useful in an ecological context. In Texas, Southern Flounder migrate from estuaries to offshore spawning habitats during the late fall. This migration is close enough in time to spawning that egg FA composition is likely dominated by the FA composition of estuarine prey. Since estuarine habitats are especially susceptible to natural and anthropogenic factors that can alter prey availability or abundance (Fuiman 2018), egg composition and the corresponding egg quality are vulnerable when environmental perturbations arise.

Fatty acid	FAML Shrimp	FAML Sardine	TPWD Base	Supplement
14:0	1.67 ± 0.42	4.72 ± 0.96	3.89 ± 1.92	6.46 ± 0.82
15:0	1.03 ± 0.12	1.11 ± 0.10	0.48 ± 0.10	0.55 ± 0.03
16:0	12.73 ± 0.69	24.26 ± 1.14	13.70 ± 2.65	18.24 ± 0.08
16:1ω7	4.48 ± 0.41	5.97 ± 1.06	3.74 ± 0.99	8.30 ± 0.49
16:2ω4	0.51 ± 0.11	1.02 ± 0.16	0.56 ± 0.16	1.42 ± 0.09
17:0	1.70 ± 0.31	1.47 ± 0.13	0.66 ± 0.10	0.43 ± 0.06
16:3ω4	1.86 ± 0.41	0.52 ± 0.17	0.59 ± 0.13	1.15 ± 0.08
18:0	7.53 ± 1.26	7.67 ± 0.43	3.86 ± 0.79	3.93 ± 0.05
18:1ω9	6.72 ± 0.58	6.83 ± 1.00	10.11 ± 6.89	9.45 ± 0.20
18:1ω7	3.90 ± 0.57	2.76 ± 0.28	1.87 ± 1.27	2.82 ± 0.10
18:2ω6	1.73 ± 0.36	1.65 ± 0.25	1.32 ± 0.27	9.17 ± 0.35
18:3ω6	0.40 ± 0.08	0.70 ± 0.21	0.37 ± 0.08	0.30 ± 0.03
18:3ω4	0.21 ± 0.15	0.20 ± 0.23	0.11 ± 0.04	0.31 ± 0.02
18:3ω3	0.63 ± 0.21	0.75 ± 0.24	0.91 ± 0.33	2.11 ± 0.10
18:4ω3	0.39 ± 0.15	0.73 ± 0.18	2.20 ± 1.21	1.69 ± 0.12
20:1ω9	1.22 ± 0.55	0.47 ± 0.09	7.80 ± 1.74	2.04 ± 0.08
20:2ω6	1.13 ± 0.23	0.29 ± 0.05	0.44 ± 0.05	0.20 ± 0.01
20:3ω6	0.30 ± 0.20	0.45 ± 0.09	0.16 ± 0.05	0.28 ± 0.01
20:4ω6	6.13 ± 1.12	2.60 ± 0.16	1.95 ± 0.18	1.19 ± 0.02
20:3ω3	0.26 ± 0.08	0.10 ± 0.03	0.26 ± 0.04	0.17 ± 0.03
20:4ω3	0.49 ± 0.07	0.33 ± 0.04	0.74 ± 0.21	0.99 ± 0.02
20:5ω3	13.93 ± 1.42	6.36 ± 0.81	9.18 ± 1.35	9.97 ± 0.40
22:1ω11	0.33 ± 0.16	0.10 ± 0.04	9.35 ± 5.12	1.45 ± 0.52
22:4ω6	0.80 ± 0.38	0.48 ± 0.18	0.36 ± 0.08	0.17 ± 0.01
22:5ω6	0.98 ± 0.11	1.51 ± 0.33	0.41 ± 0.08	0.37 ± 0.03
22:5ω3	1.69 ± 0.24	0.90 ± 0.13	1.11 ± 0.24	1.96 ± 0.10
22:6ω3	15.31 ± 1.90	20.78 ± 4.45	16.14 ± 2.09	10.88 ± 0.89

Table 2.1 Fatty acid composition (% total fatty acids) of Southern Flounder broodstock diets. TPWD represents the base diet of shrimp, squid, and mackerel (2:1:1 by wet weight). Supplement is Mazuri Gel which was provided with the TPWD diet on two different schedules. Values are mean + S.D.

Fatty acid	FAML sardine	FAML Shrimp	TPWD + 1 Month	TPWD + 12 Month
14:0	2.71 ± 0.33	2.9 ± 0.51	3.98 ± 1.16	3.71 ± 0.76
15:0	0.86 ± 0.10	0.88 ± 0.25	0.44 ± 0.13	0.48 ± 0.18
16:0	22.64 ± 1.30	21.03 ± 4.31	18.40 ± 3.32	19.7 ± 1.43
16:1ω7	5.61 ± 0.86	6.47 ± 1.11	3.77 ± 0.54	4.91 ± 0.81
16:2ω4	1.00 ± 0.13	0.66 ± 0.17	0.87 ± 0.09	0.76 ± 0.18
17:0	0.86 ± 0.13	0.78 ± 0.15	0.36 ± 0.07	0.58 ± 0.22
16:3ω4	0.69 ± 0.11	1.21 ± 0.19	0.43 ± 0.07	0.56 ± 0.12
18:0	3.75 ± 0.48	3.30 ± 0.42	2.72 ± 0.40	2.81 ± 0.32
18:1ω9	10.13 ± 1.48	12.45 ± 1.26	14.59 ± 3.25	13.45 ± 2.22
18:1ω7	3.58 ± 0.64	4.24 ± 0.72	3.09 ± 0.65	3.44 ± 0.51
18:2ω6	1.35 ± 0.12	1.21 ± 0.18	1.85 ± 0.69	3.50 ± 1.53
18:3ω6	0.29 ± 0.05	0.25 ± 0.05	0.17 ± 0.06	0.20 ± 0.06
18:3ω4	0.08 ± 0.02	0.16 ± 0.06	0.07 ± 0.04	0.15 ± 0.08
18:3ω3	0.37 ± 0.06	0.26 ± 0.12	0.73 ± 0.24	0.86 ± 0.21
18:4ω3	0.39 ± 0.08	0.21 ± 0.10	1.70 ± 0.84	1.50 ± 0.45
20:1ω9	0.41 ± 0.11	0.51 ± 0.12	4.93 ± 1.40	2.71 ± 1.18
20:2ω6	0.28 ± 0.15	0.41 ± 0.21	0.49 ± 0.52	0.52 ± 0.54
20:3ω6	0.22 ± 0.07	0.26 ± 0.06	0.30 ± 0.23	0.29 ± 0.22
20:4ω6	3.01 ± 0.49	4.38 ± 0.64	1.18 ± 0.20	1.51 ± 0.24
20:3ω3	0.19 ± 0.04	0.20 ± 0.06	0.29 ± 0.06	0.28 ± 0.06
20:4ω3	0.41 ± 0.07	0.38 ± 0.06	1.07 ± 0.27	0.90 ± 0.18
20:5ω3	3.42 ± 0.73	3.68 ± 0.56	4.47 ± 1.59	5.43 ± 0.95
22:1ω11	0.04 ± 0.03	0.10 ± 0.04	1.89 ± 1.09	0.68 ± 0.70
22:4ω6	1.04 ± 0.40	1.63 ± 0.67	0.34 ± 0.39	0.42 ± 0.35
22:5ω6	1.41 ± 0.21	1.06 ± 0.15	0.37 ± 0.07	0.37 ± 0.08
22:5ω3	4.09 ± 0.64	5.83 ± 0.63	3.66 ± 0.33	4.05 ± 0.44
22:6ω3	27.43 ± 2.74	19.97 ± 3.40	22.22 ± 2.29	20.75 ± 2.60

Table 2.2. Fatty acid composition (% total fatty acids) of Southern Flounder eggs produced under four dietary regimes. Values are mean + S.D.

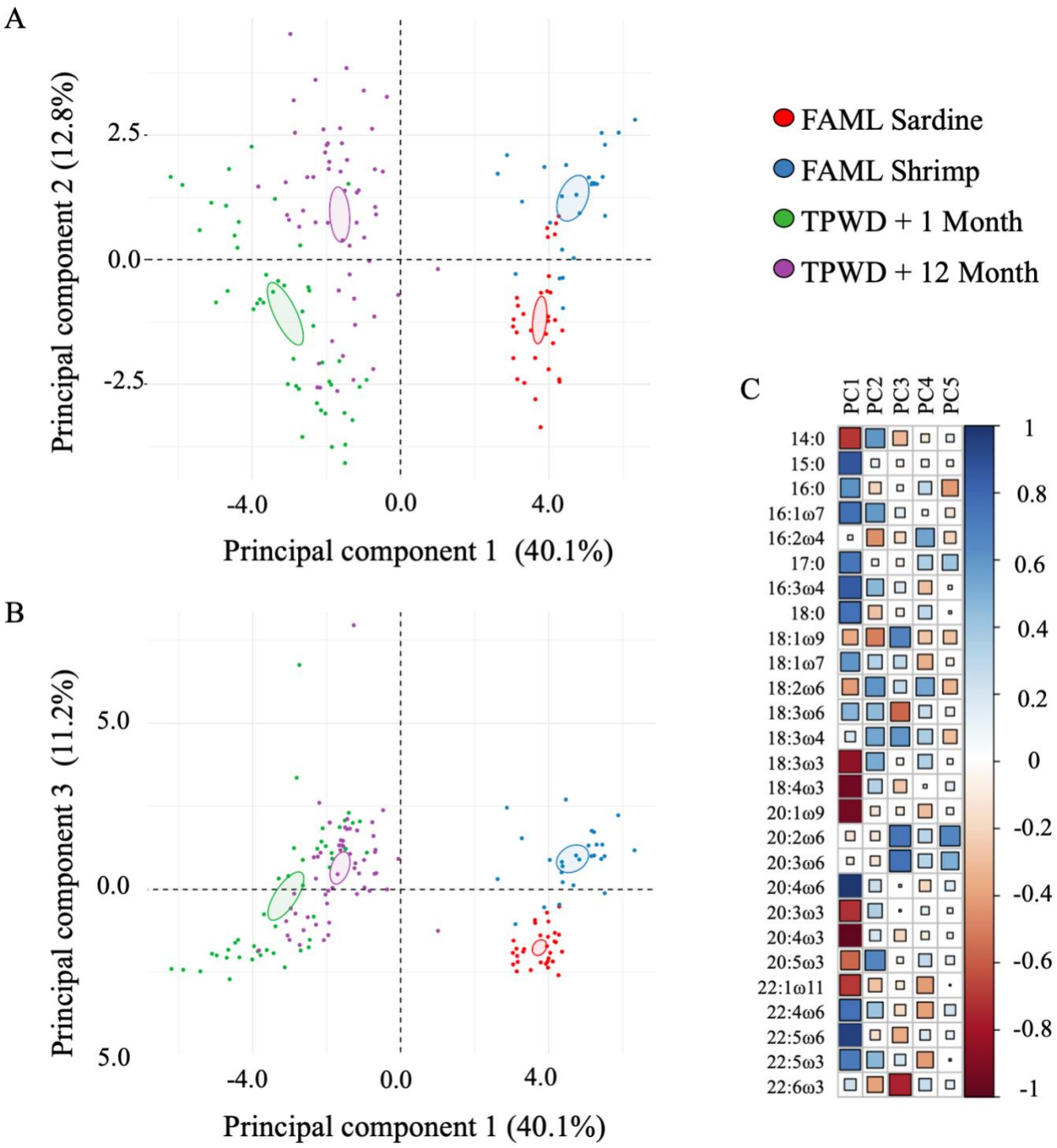

Figure 2.1. (A, B) Principal component scores for Southern Flounder egg fatty acid composition (% total FA). 95% confidence ellipses for the mean are shown for each dietary treatment. (C) Correlation matrix describing correlation of each fatty acid with scores on the first five principal components (PC1-PC5).

Egg quality metric	FAML Shrimp	FAML Sardine	TPWD + 1 month	TPWD + 12 month
Fecundity (ml)	98.80 ± 62.42 (n = 25)	100.28 ± 53.25 (n = 40)	76.49 ± 65.30 (n = 35)	74.20 ± 51.96 (n = 49)
Egg viability (%)	24.35 ± 33.19 (n = 17)	13.69 ± 14.34 (n = 15)	26.56 ± 46.0 (n = 5)	17.44 ± 28.90 (n = 18)
Hatching rate (%)	32.16 ± 36.71 (n = 13)	36.68 ± 37.85 (n = 23)	45.13 ± 35.36 (n = 4)	70.20 ± 24.83 (n = 10)
Hatching length (mm)	2.71 ± 0.27 (n = 145)	2.61 ± 0.27 (n = 256)	2.56 ± 0.19 (n = 65)	2.30 ± 0.19 (n = 144)
Energy density (cal g^{-1} dry weight)	5671.34 ± 328 (n = 17)	5664.58 ± 481 (n = 20)	5287.88 ± 444 (n = 18)	5687.91 ± 585 (n = 14)

Table 2.3. Egg quality measurements (mean + s.d.) for four parental dietary treatments. Sample size, n, is the number of spawns.

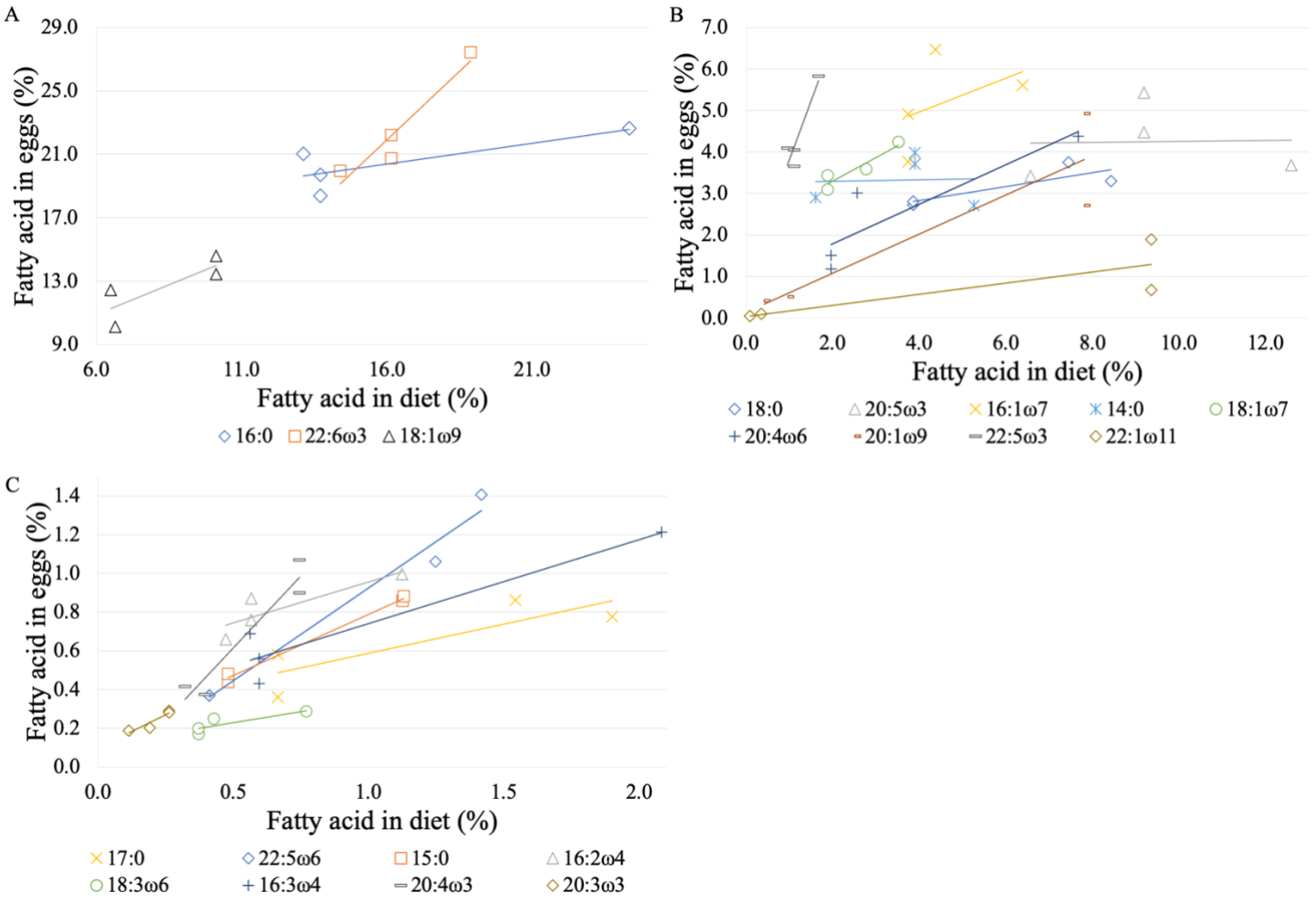

Figure 2.2. Direct diet-egg relationships. Points represent mean values (% of total fatty acids) for individual fatty acids in the diet and the eggs produced from each diet (Tables 2.1 and 2.2).

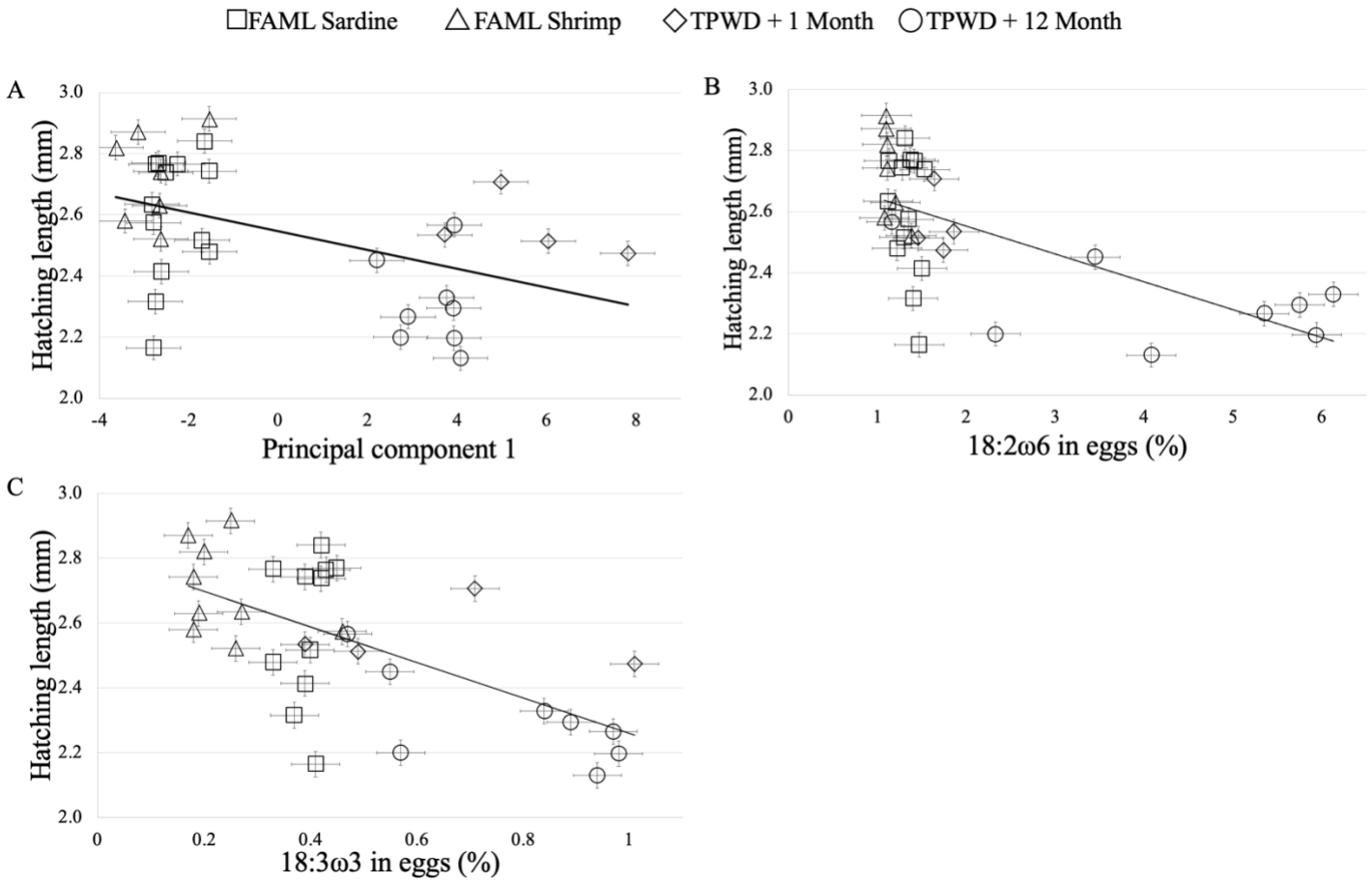

Figure 2.3. Relationships between hatching length and (A) egg principal component 1 score (r = 0.47), (B) 18:2ω6 in eggs (r = 0.64), and (C) 18:3ω3 in eggs (r = 0.63).

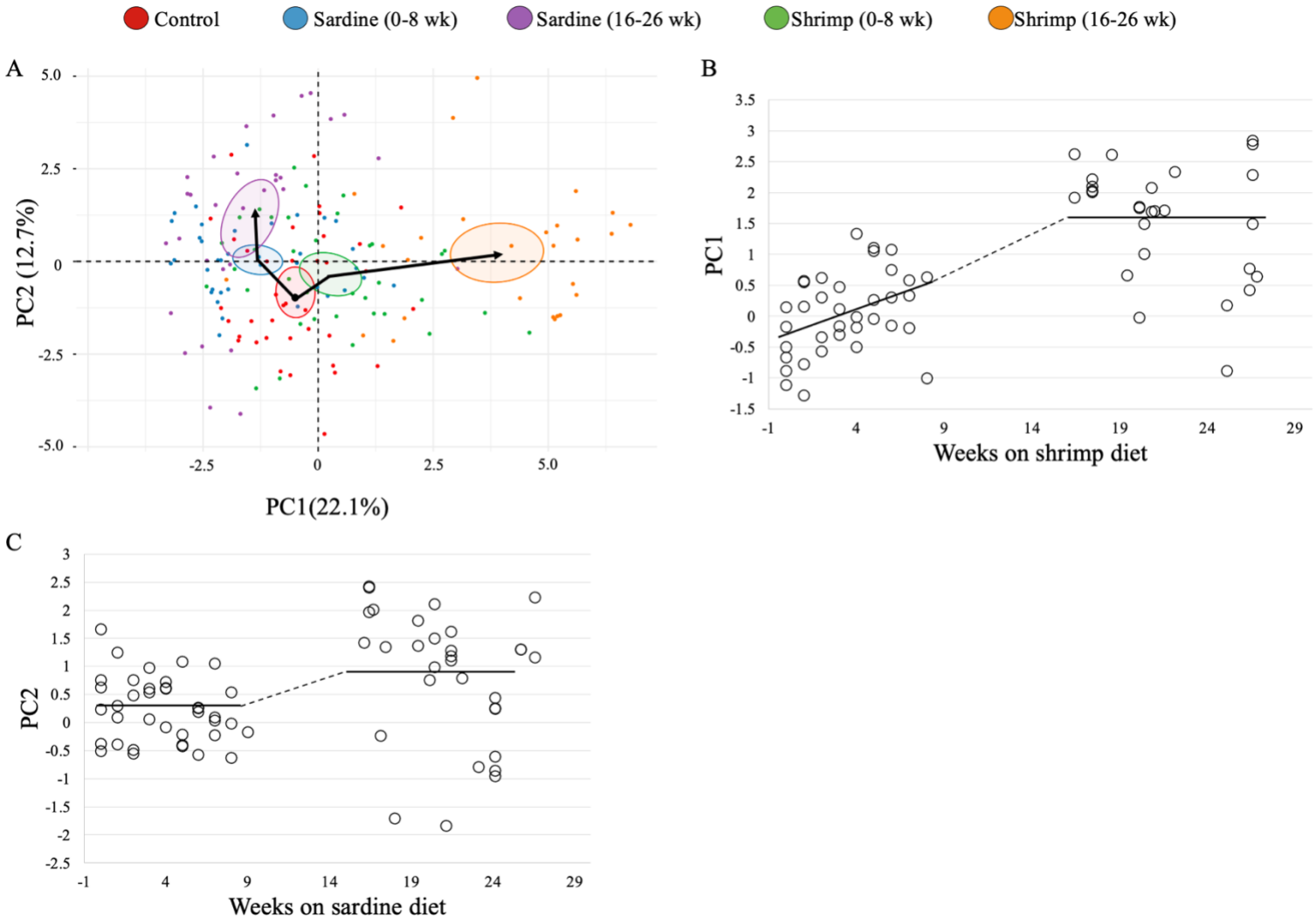

Figure 2.4. Temporal changes in fatty acid profiles of Southern Flounder eggs after a parental diet change. (A) Fatty acid profiles of eggs are defined by two principal components (PC1: shrimp profile; PC2: sardine profile). (B, C) Timeline for equilibration of eggs to parental diet of (B) shrimp and (C) sardines. Control diet was shrimp and sardines (1:1). 95% confidence ellipses for the mean are shown for each dietary treatment. Arrows connect centers of each ellipse. Increasing values of PC1 represent progress toward equilibration of eggs to the shrimp diet; increasing values of PC2 represent equilibration to the sardine diet. Data for Sardine (0-8 wk) and Shrimp (0-8 wk) from Burns and Fuiman (2020).

Chapter 2: Appendix

Fatty acid	FAML Shrimp	FAML Sardine	TPWD Shrimp	Squid	Mackerel	Supplement
14:0	0.54 ± 0.24	5.76 ± 1.42	1.04 ± 0.99	3.14 ± 1.08	33.27 ± 12.56	10.01 ± 1.33
15:0	0.40 ± 0.16	1.33 ± 0.34	0.40 ± 0.15	0.40 ± 0.10	2.18 ± 0.66	0.85 ± 0.06
16:0	3.95 ± 1.47	28.48 ± 6.09	5.43 ± 2.56	22.25 ± 5.25	77.89 ± 16.46	28.28 ± 1.04
16:1ω7	1.41 ± 0.52	7.28 ± 1.64	2.19 ± 1.34	2.95 ± 1.39	26.88 ± 6.02	12.87 ± 0.89
16:2ω4	0.19 ± 0.18	1.24 ± 0.39	0.24 ± 0.21	0.64 ± 0.46	3.96 ± 0.99	2.19 ± 0.14
17:0	0.63 ± 0.16	1.74 ± 0.47	0.75 ± 0.21	0.58 ± 0.12	1.49 ± 0.55	0.67 ± 0.07
16:3ω4	0.73 ± 0.26	0.56 ± 0.19	0.68 ± 0.22	0.32 ± 0.15	1.91 ± 0.79	1.78 ± 0.12
18:0	2.60 ± 0.86	8.82 ± 1.72	3.24 ± 0.91	4.59 ± 1.41	14.31 ± 4.87	6.09 ± 0.26
18:1ω9	2.03 ± 0.75	8.09 ± 1.82	2.41 ± 0.90	13.06 ± 7.87	85.54 ± 45.11	14.64 ± 0.65
18:1ω7	1.13 ± 0.51	3.30 ± 0.70	1.38 ± 0.74	2.19 ± 0.83	10.53 ± 8.32	4.37 ± 0.26
18:2ω6	0.58 ± 0.16	1.93 ± 0.44	0.62 ± 0.20	1.21 ± 0.45	9.33 ± 1.74	14.21 ± 0.76
18:3ω6	0.18 ± 0.18	0.81 ± 0.31	0.17 ± 0.08	0.63 ± 0.38	1.75 ± 0.48	0.46 ± 0.02
18:3ω4	0.13 ± 0.19	0.22 ± 0.29	0.10 ± 0.07	0.12 ± 0.04	0.48 ± 0.25	0.48 ± 0.03
18:3ω3	0.22 ± 0.18	0.92 ± 0.33	0.20 ± 0.08	0.91 ± 0.45	7.76 ± 2.20	3.27 ± 0.18
18:4ω3	0.16 ± 0.19	0.91 ± 0.28	0.11 ± 0.09	1.08 ± 0.62	23.84 ± 8.01	2.61 ± 0.22
20:1ω9	0.39 ± 0.25	0.53 ± 0.13	0.67 ± 0.54	11.11 ± 3.13	63.23 ± 11.23	3.16 ± 0.17
20:2ω6	0.38 ± 0.19	0.33 ± 0.10	0.37 ± 0.15	0.53 ± 0.13	1.57 ± 0.23	0.31 ± 0.01
20:3ω6	0.15 ± 0.19	0.55 ± 0.16	0.16 ± 0.10	0.13 ± 0.07	0.67 ± 0.31	0.43 ± 0.03
20:4ω6	2.42 ± 1.03	2.96 ± 0.52	2.49 ± 0.53	1.55 ± 0.30	2.70 ± 0.69	1.84 ± 0.08
20:3ω3	0.12 ± 0.19	0.13 ± 0.05	0.07 ± 0.03	0.64 ± 0.11	1.00 ± 0.27	0.26 ± 0.05
20:4ω3	0.19 ± 0.18	0.40 ± 0.10	0.19 ± 0.12	0.87 ± 0.39	5.84 ± 1.32	1.54 ± 0.07
20:5ω3	3.98 ± 1.41	7.63 ± 1.82	5.03 ± 2.26	14.72 ± 2.89	43.14 ± 7.67	15.46 ± 0.88
22:1ω11	0.17 ± 0.18	0.12 ± 0.06	0.15 ± 0.07	8.31 ± 3.58	91.54 ± 33.76	2.24 ± 0.81
22:4ω6	0.42 ± 0.15	0.56 ± 0.13	0.46 ± 0.28	0.21 ± 0.14	0.71 ± 0.22	0.27 ± 0.02
22:5ω6	0.43 ± 0.17	1.61 ± 0.28	0.40 ± 0.10	0.36 ± 0.11	1.25 ± 0.49	0.58 ± 0.05
22:5ω3	0.55 ± 0.19	1.08 ± 0.22	0.64 ± 0.29	0.87 ± 0.40	7.69 ± 1.46	3.04 ± 0.18
22:6ω3	4.35 ± 1.95	22.27 ± 3.93	4.09 ± 0.99	36.61 ± 6.10	72.41 ± 12.94	16.86 ± 1.41

Table 2.4. Fatty acid composition (mean mg g^{-1} ± s.d.) of Southern Flounder broodstock diet items. Supplement is Mazuri Gel which was provided with the TPWD diet on two different schedules.

Fatty acid	FAML Shrimp	FAML Sardine	TPWD + 1 Month	TPWD + 12 Month
14:0	4.0 ± 1.14	4.46 ± 1.07	6.58 ± 2.74	5.92 ± 1.94
15:0	1.23 ± 0.41	1.42 ± 0.33	0.72 ± 0.29	0.75 ± 0.35
16:0	29.36 ± 7.07	37.53 ± 6.6	30.86 ± 9.15	31.6 ± 7.66
16:1ω7	8.87 ± 1.98	9.35 ± 2.68	6.33 ± 1.86	7.61 ± 1.92
16:2ω4	0.93 ± 0.29	1.63 ± 0.39	1.41 ± 0.5	1.18 ± 0.35
17:0	1.11 ± 0.29	1.41 ± 0.31	0.61 ± 0.19	0.99 ± 0.52
16:3ω4	1.67 ± 0.39	1.15 ± 0.3	0.76 ± 0.24	0.89 ± 0.26
18:0	4.65 ± 0.82	6.17 ± 1.04	4.9 ± 2.00	4.56 ± 1.3
18:1ω9	17.55 ± 2.65	17.21 ± 3.63	24.94 ± 8.03	21.48 ± 6.91
18:1ω7	5.84 ± 1.42	5.92 ± 1.89	5.63 ± 2.74	5.49 ± 1.58
18:2ω6	1.69 ± 0.38	2.21 ± 0.53	3.50 ± 2.11	5.38 ± 2.62
18:3ω6	0.34 ± 0.09	0.45 ± 0.14	0.32 ± 0.18	0.31 ± 0.13
18:3nω4	0.21 ± 0.11	0.12 ± 0.04	0.13 ± 0.09	0.22 ± 0.13
18:3ω3	0.36 ± 0.21	0.58 ± 0.2	2.28 ± 4.95	1.36 ± 0.48
18:4ω3	0.3 ± 0.19	0.59 ± 0.24	2.94 ± 1.86	2.42 ± 1.01
20:1ω9	0.73 ± 0.20	0.70 ± 0.2	7.95 ± 3.47	4.54 ± 2.61
20:2ω6	0.58 ± 0.34	0.44 ± 0.21	0.79 ± 0.77	0.85 ± 1.06
20:3ω6	0.35 ± 0.11	0.35 ± 0.16	0.51 ± 0.38	0.43 ± 0.4
20:4ω6	5.89 ± 1.64	4.55 ± 1.77	2.13 ± 0.87	2.42 ± 0.64
20:3ω3	0.28 ± 0.11	0.29 ± 0.1	0.78 ± 1.39	0.45 ± 0.14
20:4ω3	0.51 ± 0.16	0.64 ± 0.21	1.83 ± 0.69	1.48 ± 0.51
20:5ω3	4.98 ± 1.82	5.09 ± 2.33	7.62 ± 3.41	8.70 ± 2.70
22:1ω11	0.19 ± 0.21	0.15 ± 0.23	3.05 ± 2.07	1.21 ± 1.24
22:4ω6	2.17 ± 1.05	1.66 ± 0.82	0.66 ± 0.84	0.83 ± 0.72
22:5ω6	1.40 ± 0.40	2.07 ± 0.83	0.65 ± 0.23	0.59 ± 0.2
22:5ω3	7.78 ± 2.15	6.17 ± 2.52	6.02 ± 1.85	6.59 ± 1.78
22:6ω3	26.52 ± 9.18	40.07 ± 17.35	35.42 ± 11.57	33.32 ± 10.05

Table 2.5. Fatty acid composition (mean mg g^{-1} ± s.d.) of Southern Flounder eggs from four dietary treatments.

Chapter 3: Maternal maturation diets influence larval performance in the marine teleost, Southern Flounder, *Paralichthys lethostigma*

INTRODUCTION

Marine teleost embryos rely on maternally derived nutrition for growth and development in the form of yolk (Sargent 1995). Fatty acids (FAs) comprise an important class of nutrients in marine fish yolk and are known to play critical roles in energy production, cellular membrane structure and fluidity, and gene regulation (Rustan and Drevon, 2005). The FA composition of yolk has been demonstrated in several species to affect metabolic processes or phenotype later in life (Morais et al. 2014; Perez and Fuiman 2015; Burns and Fuiman 2019). This phenomenon, known as metabolic or nutritional programming, alters important metabolic pathways based on the nutritional status during critical developmental windows even after the initial stimulus (i.e., embryonic nutritional status) has subsided (Hou and Fuiman 2020).

Maternal FA intake largely determines egg FA composition for several marine fishes (Watanabe et al. 1984; Izquierdo et al. 2001; Burns and Fuiman 2020). In the marine flatfish Southern Flounder (*Paralichthys lethostigma*), the proportions of 20 (of 27) FAs in eggs were directly related to the proportions of the same FAs in the maternal diet (Chapter 2). For some species, the FA composition of eggs affects larval body FA composition. In Red Drum (*Scianeops ocellatus*), for example, eggs with low levels of the long-chain FA docosahexaenoic acid (22:6ω3, DHA) resulted in lower DHA levels in late-stage larvae compared to offspring produced from eggs with higher DHA levels, even after consuming the same exogenous diet for several weeks (Fuiman and Perez, 2015). Additionally, a study of Senegalese sole (*Solea senegalensis*) determined that maternal diet affected transcription in enzymatic pathways

important in long-chain FA desaturation (FAD) and elongation (ELOVL) (Morais et al. 2014). Evidence of nutritional programming also exists for Southern Flounder, as the levels of omega-3 docosapentaenoic acid (22:5ω3) in eggs were negatively correlated with levels of DHA in the larval body at 15-dph and 35-dph larvae (Burns and Fuiman, 2019). Together, these studies provide evidence of nutritional programming in marine fishes and indicate that maternal diet alters the nutritional composition of eggs, which in turn, affects the ability for larvae to accumulate and utilize FAs later in life.

Alterations to metabolic pathways via nutritional programming may have implications for larval survival, as survival skills (foraging capability and predator evasion) have been linked to larval nutritional status. For example, in Southern Flounder larvae, two FAs that are concentrated in the head and are important for neural and retinal function (DHA 22:6ω3, ARA 20:4ω6; Horrocks and Yeo, 1999) were positively correlated with responsiveness to a visual predatory stimulus (Oberg and Fuiman 2015). These findings were later confirmed in subsequent experiments, in which a positive relationship between responsiveness and larval whole-body DHA:ARA was observed (Burns and Fuiman, 2019). These findings demonstrate the important link between larval body FA composition and a critical survival skill for larvae. Thus, if egg composition affects larval FA composition by means of nutritional programming, then adult diet could be an important factor in determining larval performance of ecologically relevant survival skills.

The possibility that adult diet could affect larval performance of survival skills has important practical implications, because Southern Flounder populations have experienced

consistent and geographically widespread declines over the last 40 years (Froeschke et al. 2010; Flowers et al. 2019; Erickson et al. 2021). In response to this trend, the Texas Parks and Wildlife Department (TPWD) created a stock enhancement program for Southern Flounder in 2006 (Miller et al. 2010). Each year, captive broodstock are conditioned to spawn, and the eggs and larvae they produce are reared and ultimately released into coastal waters to augment natural Southern Flounder populations. Broodstock diets fed to captive flounder are different from their natural diet and so eggs produced by captive flounder have a very different biochemical composition than naturally-produced eggs (Figure 1.1).

This study of Southern Flounder examined the relationships between egg composition, larval body composition, and larval performance of ecologically relevant survival skills. Specifically, the research sought to answer two questions: (1) does parental diet and subsequent egg FA composition affect larval body FA composition when larvae are reared on a common diet? and (2) does larval body FA composition have consequences for foraging and anti-predatory skills when larvae are reared on a common diet? Assessing these relationships and whether they have ecologically important consequences for larval survival can increase the usefulness and efficiency of stock enhancement programs by providing information to hatchery managers about which FAs can be manipulated through broodstock diet and how the resultant egg and larval compositions affect offspring performance.

METHODS

A Southern Founder broodstock population was established at the University of Texas Marine Science Institute's Fisheries and Mariculture Laboratory (FAML) in Port Aransas, TX, USA. Water temperature and photoperiod were maintained at 18.0°C, 32 ppt salinity and

10h:14h (light:dark) photoperiod January – April. Broodstock were evenly distributed into two 36-kL recirculating raceways and were fed a conditioning diet of equal parts (1:1 by wet weight) Brown Shrimp (*Farfantepenaeus aztecus*) and Spanish Sardine (*Sardinella aurita*) *ad libitum* on Mondays, Wednesdays, and Fridays from May through September (16 weeks). Beginning the second week of September, the broodstock in one raceway were fed brown shrimp only and the broodstock in the other raceway were fed Spanish Sardines only in equal amounts on Mondays, Wednesdays, and Fridays.

During the spawning season, gravid female Southern Flounder were anesthetized by adding 15 ppm Eugenol (clove oil) to sea water prior to injection with Ovaprim SGnRHa + Domperidone (0.5 mL kg^{-1} wet weight; Chemical, Inc., Ferndale, WA, USA) to induce ovulation. Forty-eight hours after injection, eggs were obtained via manual strip-spawning and fertilized with milt from two to four anesthetized males from the same dietary treatment as the stripped female.

In addition to the experiments conducted at FAML, Southern Flounder eggs were received from two broodstock populations maintained by the TPWD Coastal Fisheries Division. The first population, located at the Marine Development Center, Corpus Christi, TX, USA, was fed a diet of shrimp, squid, and mackerel (2:1:1 wet weight) Mondays, Wednesdays, and Fridays *ad libitum* year-round. These broodstock were provided with a nutritional supplement (Mazuri Aquatic Gel, PMI Nutrition International, MN, USA) Tuesdays and Thursdays *ad libitum*, one month prior to, and throughout the spawning season. The second population, located at Sea Center Texas, Lake Jackson, TX, USA, was fed the same diet as those at the Marine Development Center except that the nutritional supplement was fed year-round. Strip-spawning

procedures at the TPWD facilities were conducted in a similar manner to the populations at FAML except that Tricaine Methanesulfonate (MS-222, manufacturer recommended concentration) was used as the anesthetic and the number of males used to fertilize a spawn depended upon the number of ripe males available on a given day, with some spawns using as few as one male for fertilization.

Larval rearing methods

Up to 10 ml of eggs from a single spawn were placed into a 160-L conical tank of seawater with gentle aeration. Larvae were reared under constant temperature (18.5°C), salinity (32 ppt), and photoperiod (10 h light: 14 h dark) for 35 days. Seawater was processed through a sand filter, ozone filter, and UV filter before use in larval rearing tanks. Ammonia (NH_3) levels in larval rearing tanks were measured weekly. When NH_3 levels were > 1 ppm, fresh seawater or Cloram-X (Reed Mariculture, Campbell, CA, USA) was added to reduce ammonia levels to < 1 ppm. Larvae were fed enriched rotifers (*Brachionus* sp., 5 ml^{-1}) from first feeding (4 dph) through 23 dph, newly hatched *Artemia* nauplii from 19-23 dph (0.05 ml^{-1}), and *Artemia* (0.05-0.1 ml^{-1}) enriched with Algamac 3050 DHA 10 (Aqua-fauna Bio-Marine, Hawthorn, CA, USA) according to the manufacturer's recommendations from 24-34 dph. Larvae from all four parental diet groups were reared at FAML using this same protocol.

Behavioral Assays

Two assays were used to derive larval performance measurements at two developmental stages (15 and 35 dph): routine swimming and predator evasion. At least five larvae were selected from each spawn and at each developmental stage to be used in both assays. Each fish was tested in both assays individually, although different fish were used for each developmental

stage. The foraging assay measured the routine behavior of an unfed fish under constant, unstimulated conditions for a period of 30 s. The assay produced two measures of performance: routine swimming speed (total distance traveled divided by 30 s) and linearity of the swimming path (expressed as net:gross displacement ratio or NGDR). The predator evasion assay presented an individual fish with a visual stimulus that simulated an attacking predator as a small black ellipse (long axis vertical) in the middle of the white background. The ellipse rapidly increased in size over 1 s, simulating the cross section of a looming predatory fish. This assay measured the fish's responsiveness (present or absent) and the latency (time between onset of the stimulus and start of the response). Responses of larvae were either very subtle movements (Type 1) or full escape responses (Type 2). Distance, duration, and speed of the escape response were measured for Type 2 responses only.

These assays followed methods described by Fuiman and Ojanguren (2011) with improvements as described below. Briefly, at each developmental stage (15-dph or 35-dph), larvae were removed from rearing tanks before the morning feeding and placed in individual acclimation chambers (4.1 x 4.1 x 5.6 cm, 35 mm water depth for 15-dph larvae; 10.0 x 10.0 x 10.0, 50 mm water depth for 35-dph larvae) for 45 min. After this initial acclimation period, one chamber was transferred to the middle of an arena adjacent to an LCD monitor, which showed a blank white screen. A mirror, tilted at 45° from the horizontal plane was placed on one side of the arena. A video camera was positioned above the arena with a view of the arena and the mirror. Video recording (at 30 frames per second, fps) was started 3 min after moving the chamber to the arena to record the larva's unstimulated routine swimming behavior for 30 s. After concluding the routine swimming assay and when a larva had moved near the LCD

monitor, the simulated predatory stimulus was triggered. Responses to the predatory stimulus were recorded on video at 240 fps.

Analyses of movements of larvae in both assays were conducted using ImageJ and the MTrackJ add-on (Meijering et al. 2012). The position of the tip of the larva's snout was recorded in the horizontal plane (X, Y coordinates) and, using the mirror, in the vertical plane (Z). These values were calibrated to millimeters and distances between points were calculated using the Pythagorean theorem for three dimensions. Time was measured as the number of frames divided by the framing rate used for video recording (30 or 240 fps). After both behavioral assays were completed larvae were pooled by spawn and frozen at -80°C for subsequent biochemical analysis.

Biochemical Analysis

FA profiles (27 individual FAs measured) of eggs and 15-dph and 35-dph larvae were measured using gas chromatography (FID-GC, Shimadzu GC-2014 Flame Ionization Detector – Gas Chromatograph) following methods described by Faulk and Holt (2005). Each sample analyzed included at least five whole larvae from the same spawn which had been freeze dried for at least 24 h and homogenized in a chloroform/methanol solution (2:1, v/v), which included an internal standard (tricosanoic acid, 23:0). FA methyl esters were obtained after transesterification of total lipids by adding boron trifluoride. All FAs are reported as a percentage of total FAs identified (FA profiles expressed in mg g^{-1} dry weight are provided in the appendix).

Statistical Analysis

Principal component analysis (PCA, using the correlation matrix on 27 fatty acid variables, each rescaled to mean = 0) was used to summarize the major trends in larval FA

composition at each developmental stage. To determine if significant differences in larval composition exist, PC scores from each parental diet group were tested by analysis of variance (ANOVA). Correlations were then computed between eggs and larvae for only those individual FAs that were highly correlated ($|r| \geq 0.65$) with a principal component on which diet groups differed significantly. Relationships between larval performance and larval FA composition were examined for individual FAs that had significant egg-larva correlations. Statistical analyses were conducted in R (R Studio 1.3.1073) using FactoMineR, ggplot2, dplyr packages.

RESULTS

Larval fatty acid composition

At 15-dph, FA composition was measured on larvae from 21 spawns (7 - 24 larvae pooled in each sample) from 3 - 8 spawns per diet treatment (Table 3.1). PCA revealed differences in larval FA composition among parental diet groups. Six PCs were retained (eigenvalue > 1.0, 79% of variance explained), but ANOVA showed that only PC1 (32% of total variance explained) separated any of the treatment groups ($P = 0.029$). Generally, larvae from the FAML shrimp and FAML sardine diet groups had lower PC1 scores than larvae from the two TPWD diets (Figure 3.1). The greatest pairwise difference was between larvae from the FAML shrimp group and larvae from the TPWD + 1 month group ($P = 0.07$). The difference between larvae from the FAML shrimp diet group and the TPWD + 12 month group was weaker ($P = 0.10$). Five FAs had strong negative correlations ($r \leq -0.65$) with PC1 scores (14:0, 16:1ω7, 20:4ω6, 15:0 and 22:6ω3), indicating higher levels of these FAs in larvae from FAML (shrimp and sardine diets) than in larvae from TPWD diets. Four FAs had strong positive correlations (r

≥ 0.65) with PC1 (20:1ω9, 20:2ω6, 18:0, and 20:3ω6), indicating higher levels in larvae from TPWD diets than the FAML diets.

At 35-dph, FA composition was measured on larvae from 24 spawns (5 - 12 larvae pooled in each sample) from 4 - 8 spawns per diet treatment (Table 3.2). PCA revealed subtle differences in larval FA composition among parental diet groups. Six PCs were retained, explaining 81.4% of the variance in larval FA composition at 35 dph. The primary patterns of variation in larval FA composition at 35-dph (PC1 and PC2) accounted for 47.5% of the total variance (25.8% and 21.6%, respectively). PC1 distinguished larvae from the TPWD +1 month diet from all other groups, although the differences were not significant (Figure 3.2A, $P > 0.05$). Four FAs had strong positive correlations ($r \geq 0.65$) with PC1 scores, indicating higher levels of 18:0, 16:0, 22:5ω3, 20:3ω6 in larvae from the TPWD + 1 month diet compared to the other diets. Three FAs had strong negative correlations ($r \leq -0.65$) with PC1, indicating lower levels of 18:3ω3, 18:4ω3, and 18:3ω6 in larvae from the TPWD + 1 month diet.

PC2 scores tended to separate larvae from FAML diets (shrimp, sardine) from larvae from TPWD diets, although the difference was not significant (Figure 3.2B, $P = 0.5$). Two FAs were moderately correlated ($r \geq 0.60$) with PC2 (22:6ω3 and 20:4ω6) and two FAs were strongly negatively correlated ($r \leq -0.65$) with PC2 (18:1ω9 and 20:1ω9).

Analysis of variance showed that only PC5 (6.5% of total variance explained) separated any of the treatment groups ($P = 0.02$). Generally, larvae from the FAML shrimp and FAML sardine diet groups had higher scores on PC5 than the other two diet groups (Figure 3.2C). PC5 scores for larvae from the FAML sardine diet were different from larvae produced by the TPWD + 12 month diet ($P = 0.02$). The difference between larvae from the FAML shrimp and TPWD +

1 month diets was weaker (P = 0.10). One FA (16:2ω4) had a strong positive correlation (r ≥ 0.65) with PC5 scores, indicating higher levels of this FA in larvae from the FAML diets at 35-dph than the other diet treatments.

Larval composition – egg composition relationships

At 15-dph, three FAs in larvae (16:1ω7, 20:4ω6, 20:1ω9) were positively correlated with the same FA in eggs (Figure 3.3; P ≤ 0.05). Because previous research on 15-dph Southern Flounder identified a correlation between larval responsiveness and larval DHA:ARA (Burns and Fuiman 2019), the ratio of these two essential FAs was examined. Here, no meaningful correlation was found between egg and larval DHA:ARA ratio (P > 0.05). However, DHA (22:6ω3) was highly correlated (r = -0.78) with PC1, and although there was no correlation between DHA in larvae and DHA in eggs, there were positive correlations between DHA in larvae and ARA in eggs (Figure 3.5D; P = 0.03) and between larval body ARA and ARA in eggs (P = 0.01).

At 35-dph, three FAs in larvae (18:1ω7, 20:1ω9, 22:5ω3) were correlated with the amount of the same FA in eggs (Figure 3.4; P ≤ 0.05). Two essential FAs, EPA (20:5ω3) and ARA (20:4ω6), were strongly correlated with PC2 (r = 0.59, 0.64). Their ratio, EPA:ARA, in larvae was correlated EPA:ARA in eggs (Figure 3.4D; P = 0.0008).

Larval performance – larval composition relationships

At 15 dph, behavioral performance traits of 215 larvae were tested from 21 spawns (7 - 24 larvae pooled in each sample) with 3 - 8 spawns per diet treatment (Table 3.3). Considering only FAs that had direct egg-larva correlations (i.e., those for which yolk can be altered through

adult diet), there were three significant relationships between routine swimming metrics and larval FAs at 15 dph. Routine swimming NGDR was positively correlated with larval 16:1ω7 ($P = 0.02$), and routine swimming speed was marginally correlated with larval 20:1ω9 and with larval DHA:ARA ($P = 0.008$) (Figure 3.5).

In the predator evasion assay, mean responsiveness of 15-dph larvae was 40%. Although the proportion of fish responding to the simulated predator was comparable to previous studies utilizing this assay (Oberg and Fuiman 2015; Burns and Fuiman 2019), many responses were of Type 1 so that response distance, duration, and speed could not be measured, reducing the sample sizes for these traits (Table 3.3). In fact, no larvae from the FAML Shrimp or TWPD + 1 month diet groups gave a normal (Type 2) escape response. There were no relationships between response distance, duration, or speed and larval FA levels (ANOVA, $P > 0.05$).

At 35 dph, behavioral traits of 216 larvae were tested from 24 spawns, with 4 - 8 spawns per diet treatment and 5 - 12 larvae from each spawn. Routine swimming NGDR was positively correlated with EPA:ARA in larvae ($P = 0.015$) and marginally negatively correlated with 22:5ω3 in larvae ($P = 0.07$) (Figure 3.6A, D). Mean responsiveness to the predatory stimulus was slightly greater than for 15-dph larvae, averaging 42% across all diet treatments. Responsiveness was negatively correlated with three FAs in larvae (16:1ω7, 18:1ω7, 20:1ω9) and positively correlated with 22:5ω3 in larvae (ω3DPA, $P = 0.06$) (Figure 3.7). Response duration was negatively correlated with 20:1ω9 larvae ($P = 0.06$) (Figure 3.6C).

DISCUSSION

FA composition of 15- and 35-dph larvae varied significantly among parental diet treatments even though all larvae were reared in the same way. Specifically, four FAs that varied

in eggs in response to their level in the parental diet also varied in the larvae (16:1ω7, 18:1ω7, 20:1ω9, 22:5ω3). These were not simply carryover of higher or lower levels of these fatty acids from eggs to larvae, as 15-dph larvae had 2.3 times more total FA than eggs on a mg g^{-1} basis. Thus, since larval FA content at 15-dph is more than double that of an egg, and even more for 35-dph larvae, most of their body composition originates from the exogenous larval diet. Further, the endogenous FA signal that were characteristic of eggs in Senegalese sole (*Solea senegalensis*) larvae disappeared 7-dph after consuming a common exogenous diet (Morais et al. 2015). Since all larvae in the present study were fed the same exogenous diet, differences in body composition associated with parental diet were likely due to differences in how larvae absorbed, metabolized, and/or incorporated the fatty acids from that diet, which is consistent with nutritional programming. The four fatty acids that had direct diet-egg-larva relationships (16:1ω7, 18:1ω7, 20:1ω9, 22:5ω3) are reasonable candidates for nutritional programming stimuli because of the significant differences observed in 35-dph larvae even when fed identical high-quality diets.

The principal findings of this study, that parental diet can alter the fatty acid composition of larvae and some aspects of their performance, may have practical application for improving larval culture and the efficacy of a stock enhancement program for this species. Current practice is to provide enriched rotifers (*Brachionus plicatilis*) as the first feed and to wean flounder larvae onto *Artemia* nauplii beginning around 17-dph. Larvae that have faster routine swimming speeds or more linear routine swimming paths (high NGDR), may have a higher success rate of transitioning from rotifers to *Artemia* and ultimately surviving this dietary transition as these two traits increase the rate of encounter with prey (Coughlin et al. 1992). Notably, both of these

routine swimming metrics were positively related to a mono-unsaturated FA (MUFA) (20:1ω9 and 16:1ω7, respectively) in 15-dph larvae. Therefore, using a parental diet that increases the levels of these MUFAs in eggs could improve larval survival through this diet change. The levels of three MUFAs (16:1ω7, 18:1ω7, and 20:1ω9) in 35-dph larvae were negatively correlated with responsiveness to the simulated predator, a trait determined to be of special importance to survival in nature (Fuiman et al 2006). This finding corroborates prior research that also observed a negative correlation between responsiveness and the amount of 20:1ω9 in the larval body of 35-dph Southern Flounder (Burns and Fuiman 2019). However, these opposing responses to MUFA levels in larvae at two developmental stages present trade-offs between performance in the hatchery and performance after release into natural habitats, which need to be evaluated.

Future work should assess how the four FA identified here as possible nutritional programming stimuli affect larval performance and FA metabolism in larval Southern Flounder. Since these FA appear to be important in determining larval performance and their levels in eggs can be manipulated through broodstock diet, designing an experiment that uses both broodstock and larval dietary manipulations would prove useful for further defining the interactions between egg and larval composition and mechanisms of nutritional programming.

Fatty acid	FAML Shrimp	FAML Sardine	TPWD + 1 month	TPWD + 12 month
14:0	3.59 ± 0.65	3.40 ± 0.84	1.59 ± 0.32	2.27 ± 1.53
15:0	0.61 ± 0.21	0.63 ± 0.11	0.40 ± 0.03	0.44 ± 0.18
16:0	18.10 ± 1.295	18.03 ± 1.52	18.55 ± 2.21	18.29 ± 2.32
16:1ω7	5.59 ± 0.35	4.35 ± 1.425	2.57 ± 0.32	3.25 ± 0.96
16:2ω4	0.85 ± 0.24	0.95 ± 0.40	0.60 ± 0.10	0.52 ± 0.14
17:0	0.35 ± 0.16	0.46 ± 0.19	0.46 ± 0.04	0.47 ± 0.06
16:3ω4	1.28 ± 0.42	1.17 ± 0.31	1.13 ± 0.81	1.45 ± 0.61
18:0	4.86 ± 1.67	7.72 ± 2.93	10.51 ± 0.74	8.90 ± 2.59
18:1ω9	5.63 ± 2.44	5.66 ± 0.61	7.06 ± 2.40	6.02 ± 1.39
18:1ω7	3.40 ± 0.72	3.93 ± 0.68	3.82 ± 0.13	4.33 ± 0.897
18:2ω6	3.63 ± 0.35	3.27 ± 0.68	2.99 ± 0.36	3.42 ± 0.66
18:3ω6	0.34 ± 0.12	0.36 ± 0.07	0.26 ± 0.04	0.30 ± 0.06
18:3nω4	0.04 ± 0.03	0.05 ± 0.03	0.21 ± 0.17	0.09 ± 0.09
18:3ω3	1.16 ± 0.35	2.19 ± 1.76	1.25 ± 0.81	1.67 ± 1.29
18:4ω3	1.06 ± 0.81	0.93 ± 0.35	0.53 ± 0.21	2.82 ± 4.60
20:1ω9	0.70 ± 0.52	0.59 ± 0.26	1.46 ± 0.54	1.10 ± 0.46
20:2ω6	0.28 ± 0.06	0.37 ± 0.13	0.46 ± 0.06	0.50 ± 0.23
20:3ω6	0.52 ± 0.25	0.58 ± 0.19	0.88 ± 0.19	0.81 ± 0.23
20:4ω6	4.60 ± 1.16	4.79 ± 0.61	3.82 ± 0.33	4.10 ± 0.53
20:3ω3	0.20 ± 0.09	0.39 ± 0.18	0.28 ± 0.16	0.33 ± 0.23
20:4ω3	0.89 ± 0.17	0.81 ± 0.28	0.90 ± 0.09	0.84 ± 0.144
20:5ω3	8.37 ± 1.27	6.23 ± 1.71	5.33 ± 0.31	6.43 ± 0.87
22:1ω11	0.14 ± 0.03	0.12 ± 0.06	0.19 ± 0.19	0.10 ± 0.06
22:4ω6	0.19 ± 0.14	0.49 ± 0.64	0.43 ± 0.66	0.28 ± 0.31
22:5ω6	1.92 ± 1.16	2.17 ± 0.88	1.98 ± 0.85	1.71± 0.38
22:5ω3	5.36 ± 1.26	4.62 ± 1.25	7.22 ± 0.99	7.31 ± 1.61
22:6ω3	18.05 ± 4.15	16.30 ± 2.65	12.84 ± 3.51	12.84 ± 4.83
n	4	6	3	8

Table 3.1. Fatty acid composition (% total fatty acids) of 15-dph Southern Flounder larvae produced under four parental dietary regimes. Values are mean ± S.D. n = number of spawns sampled.

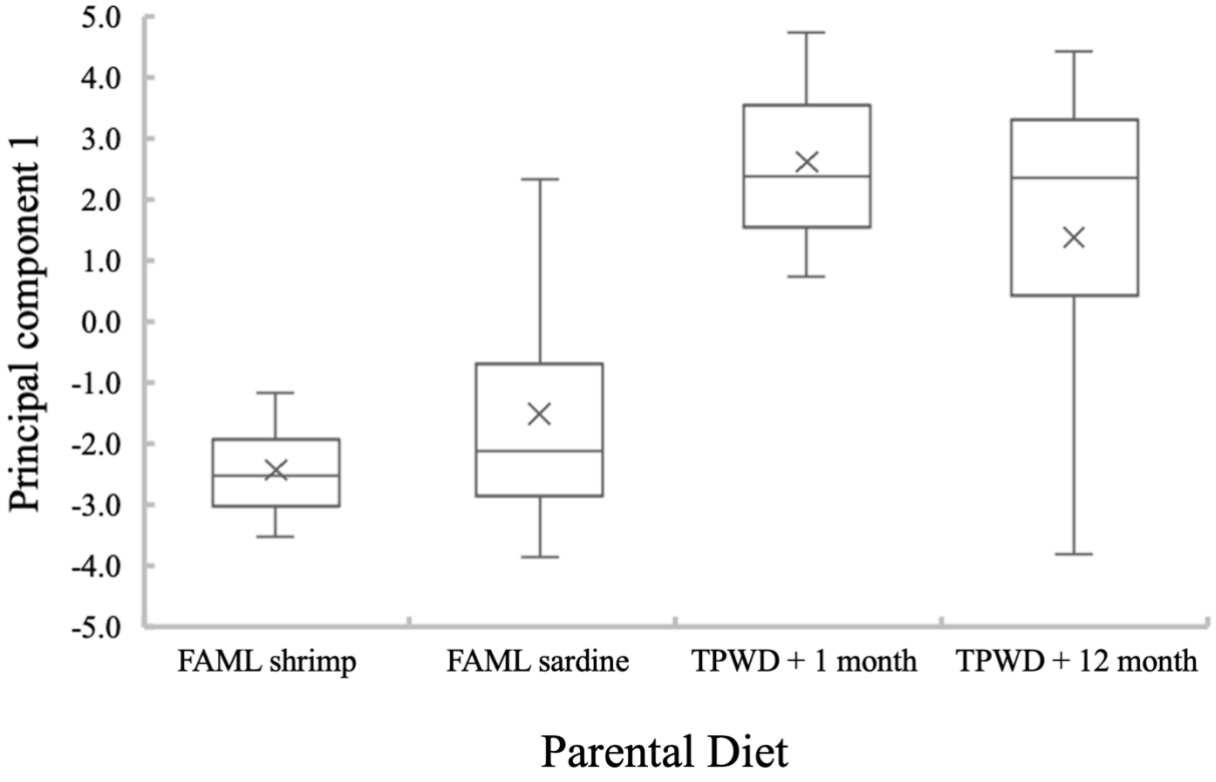

Figure 3.1. Scores on principal component 1 for 15-dph larval body FA composition (% total FA). Rectangle = interquartile range, horizontal line within rectangle = median, X = mean, vertical line = minimum and maximum value.

Fatty acid	FAML Shrimp	FAML Sardine	TPWD + 1 month	TPWD + 12 month
14:0	1.82 ± 0.25	1.61 ± 0.25	1.57± 0.31	1.65 ± 0.25
15:0	0.174 ± 0.03	0.16 ± 0.05	0.18 ± 0.06	0.19 ± 0.02
16:0	15.66 ± 1.24	15.44 ± 1.83	15.83 ± 1.83	15.03 ± 1.24
16:1ω7	2.43 ± 0.19	2.21 ± 0.21	2.34 ± 0.33	2.49 ± 0.19
16:2ω4	0.15 ± 0.04	0.22 ± 0.12	0.16 ± 0.05	0.18 ± 0.04
17:0	0.49 ± 0.009	0.48 ± 0.14	0.57 ± 0.10	0.65 ± 0.009
16:3ω4	0.78 ± 0.06	0.95 ± 0.35	1.06 ± 0.37	0.87 ± 0.06
18:0	6.15 ± 0.21	6.81 ± 0.91	6.63 ± 0.83	6.05 ± 0.21
18:1ω9	15.09 ± 0.71	15.33± 1.09	16.29 ± 3.45	14.18 ± 0.71
18:1ω7	9.04 ± 0.86	8.30 ± 1.01	6.64 ± 1.81	7.59 ± 0.86
18:2ω6	4.45 ± 0.38	4.24 ± 0.37	4.22 ± 0.53	4.22 ± 0.38
18:3ω6	0.41 ± 0.03	0.40 ± 0.03	0.41 ± 0.05	0.44 ± 0.03
18:3nω4	0.03 ± 0.02	0.08 ± 0.06	0.08 ± 0.06	0.09 ± 0.02
18:3ω3	13.47 ± 1.13	12.45 ± 2.50	13.00 ± 0.96	13.99 ± 1.13
18:4ω3	2.24 ± 0.24	2.06 ± 0.48	2.02 ± 0.31	2.43 ± 0.24
20:1ω9	1.14 ± 0.13	1.07 ± 0.25	0.83 ± 0.22	1.00 ± 0.13
20:2ω6	0.39 ± 0.02	0.40 ± 0.03	0.38 ± 0.06	0.36 ± 0.02
20:3ω6	0.34 ± 0.01	0.34 ± 0.06	0.38 ± 0.04	0.32 ± 0.01
20:4ω6	2.80 ± 0.23	2.86 ± 0.46	2.71 ± 0.30	2.69 ± 0.23
20:3ω3	3.47 ± 0.37	3.46 ± 0.53	3.30 ± 0.16	3.40 ± 0.37
20:4ω3	1.16 ± 0.10	1.18 ± 0.16	1.17 ± 0.04	1.13 ± 0.10
20:5ω3	5.31 ± 0.43	5.45 ± 0.71	5.20 ± 0.74	5.57 ± 0.43
22:1ω11	0.28 ± 0.05	0.27 ± 0.05	0.33 ± 0.11	0.27 ± 0.05
22:4ω6	0.44 ± 0.11	0.61 ± 0.17	0.52 ± 0.27	0.51 ± 0.11
22:5ω6	0.58 ± 0.08	0.78 ± 0.31	0.71 ± 0.31	0.83 ± 0.08
22:5ω3	1.41 ± 0.13	1.74 ± 0.58	2.01 ± 0.42	1.74 ± 0.13
22:6ω3	2.83 ± 0.34	3.31 ± 1.03	3.14 ± 0.80	3.97 ± 0.34
n	4	7	4	8

Table 3.2. Fatty acid composition (% total fatty acids) of 35-dph Southern Flounder larvae produced under four parental dietary regimes. Values are mean ± S.D. n = number of spawns sampled.

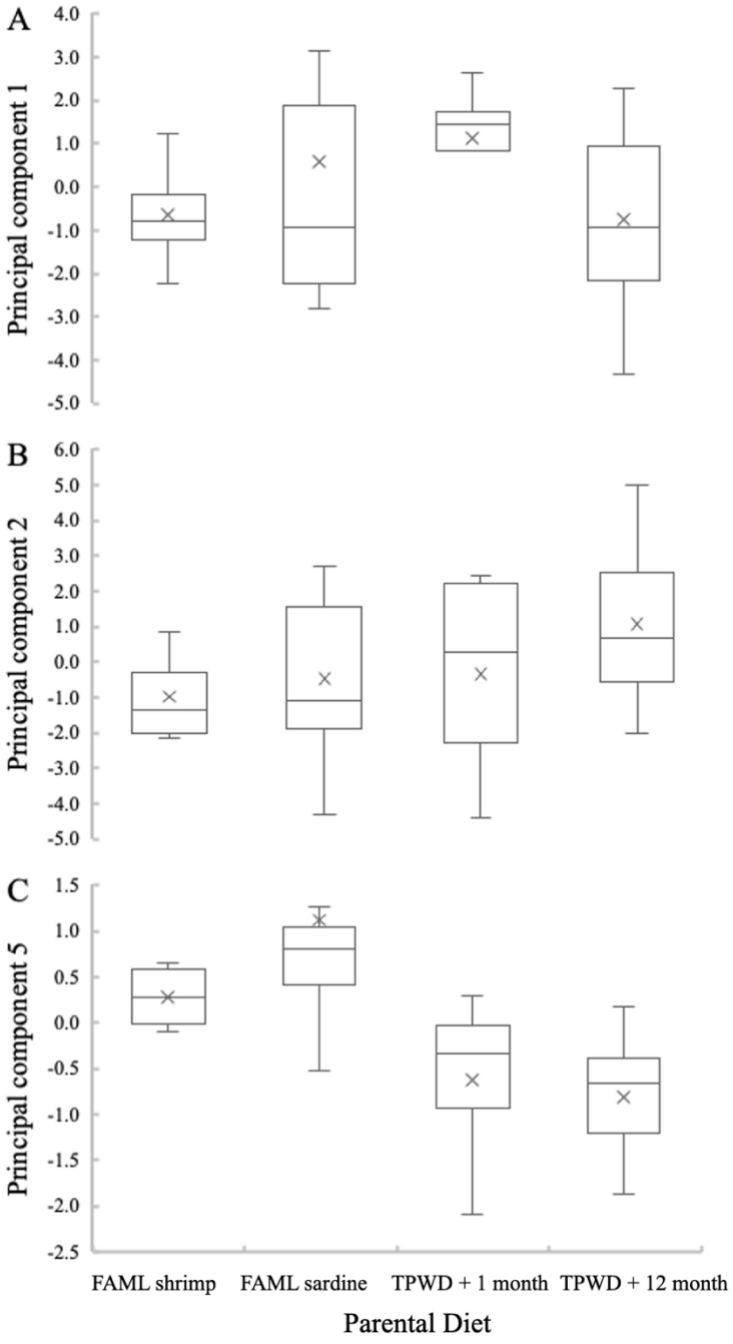

Figure 3.2. (A) Scores on principal component 1, (B) principal component 2, (C) and principal component 5 for 35-dph Southern Flounder larval body FA composition (% total FA). Rectangle = interquartile range, horizontal line within rectangle = median, X = mean, vertical line = minimum and maximum value, excluding outliers.

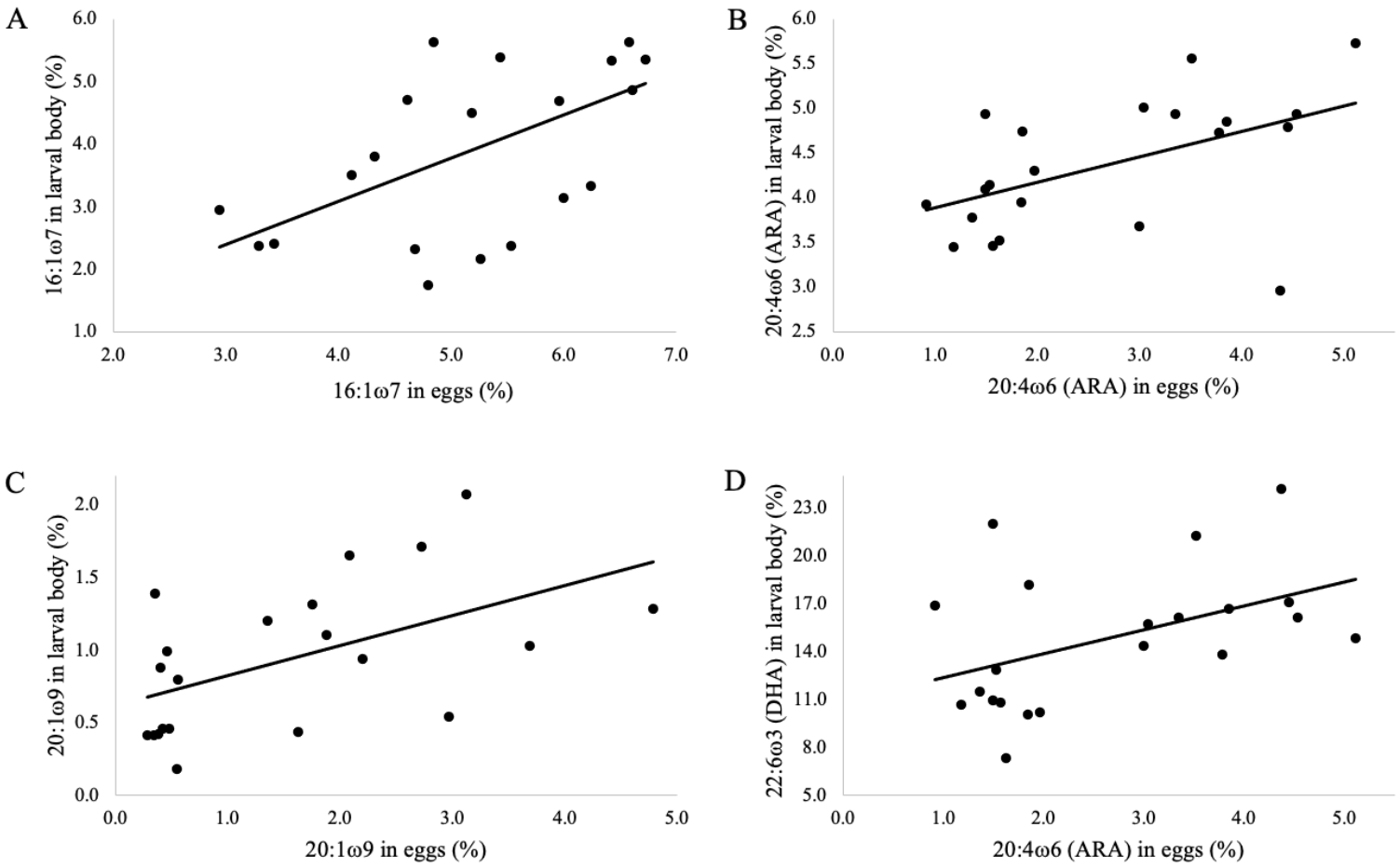

Figure 3.3. Significant relationships between larval and egg fatty acid composition (% total fatty acids) for 15-dph Southern Flounder. (A) 16:1ω7 (r = 0.57, P = 0.01), (B) 20:4ω6 (ARA; r = 0.51, P = 0.01), (C) 20:1ω9 (r = 0.53, P = 0.01), (D) 22:6ω3 (DHA) and egg 20:4ω6 (ARA; r = 0.46, P = 0.03).

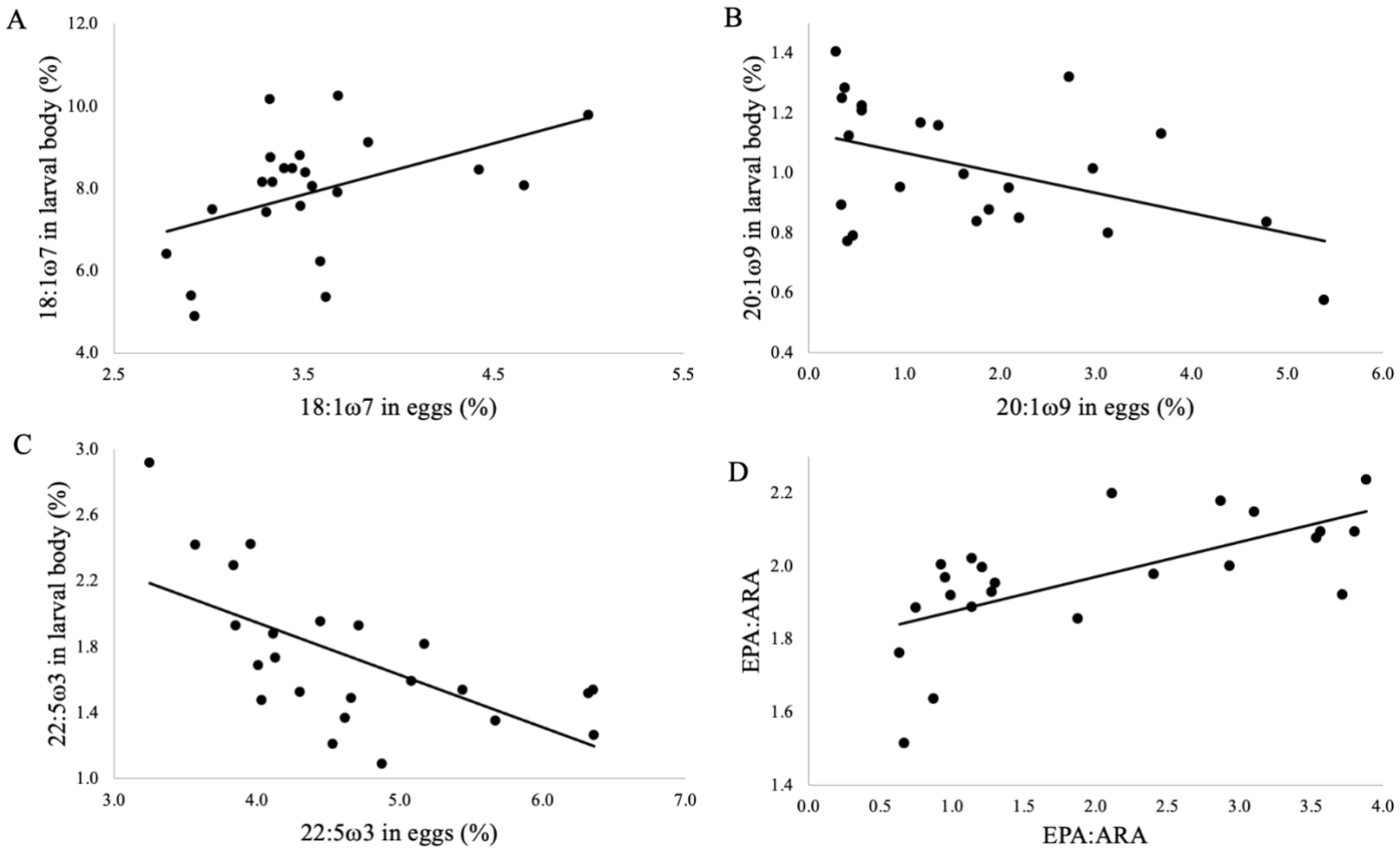

Figure 3.4. Significant relationships between larval and egg fatty acid composition (% total fatty acids) for 35-dph Southern Flounder. (A) 18:1ω7 (r = 0.46, P = 0.02), (B) 20:1ω9 (r = 0.46, P = 0.02), (C) 22:5ω3 (ω3 DPA; r = 0.63, P < 0.01), (D) EPA:ARA (r = 0.65, P < 0.01).

	Parental diet			
Performance Metric	FAML Shrimp	FAML Sardine	TPWD + 1 Month	TPWD + 12 Month
Larval stage: 15 dph				
n	24	80	27	84
Routine speed (mm s^{-1})	2.23 ± 1.62	2.54 ± 1.91	3.32 ± 1.95	2.98 ± 2.15
Routine NGDR	0.30 ± 0.17	0.28 ± 0.21	0.25 ± 0.17	0.27 ± 0.23
Responsiveness	0.38	0.38	0.56	0.31
n_r	9	30	15	26
Response speed (mm s^{-1})	NA	88.53 ± 109.18	NA	55.35 ± 32.07
Response distance (mm)	NA	22.28 ± 31.54	NA	9.19 ± 3.48
Response latency (s)	0.65 ± 0.10	0.67 ± 0.17	0.60 ± 0.28	0.75 ± 0.42
Response duration (s)	NA	0.25 ± 0.05	NA	0.29 ± 0.11
Larval stage: 35 dph				
n	38	81	30	67
Routine speed (mm s^{-1})	4.56 ± 2.44	3.95 ± 1.87	3.38 ± 2.26	4.31 ± 2.51
Routine NGDR	0.26 ± 0.16	0.26 ± 0.20	0.28 ± 0.22	0.33 ± 0.24
Responsiveness	0.32	0.41	0.50	0.48
n_r	14	33	15	32
Response speed (mm s^{-1})	62.69 ± 15.87	92.92 ± 101.44	123.81 ± 120.21	119.74 ± 55.68
Response distance (mm)	12.35 ± 4.59	18.59 ± 15.43	39.26 ± 61.47	34.60 ± 33.40
Response latency (s)	0.70 ± 0.13	0.64 ± 0.07	0.66 ± 0.10	0.62 ± 0.08
Response duration (s)	0.21 ± 0.03	0.26 ± 0.10	0.51 ± 0.30	0.33 ± 0.23

Table 3.3. Routine swimming and predator evasion metrics for 15- and 35-dph Southern Flounder larvae from different parental diet treatments. n is the number of larvae tested for routine swimming metrics and responsiveness; nr is the number of larvae responding to the simulated predatory stimulus. NA indicated traits not measured because no type 2 responses occurred.

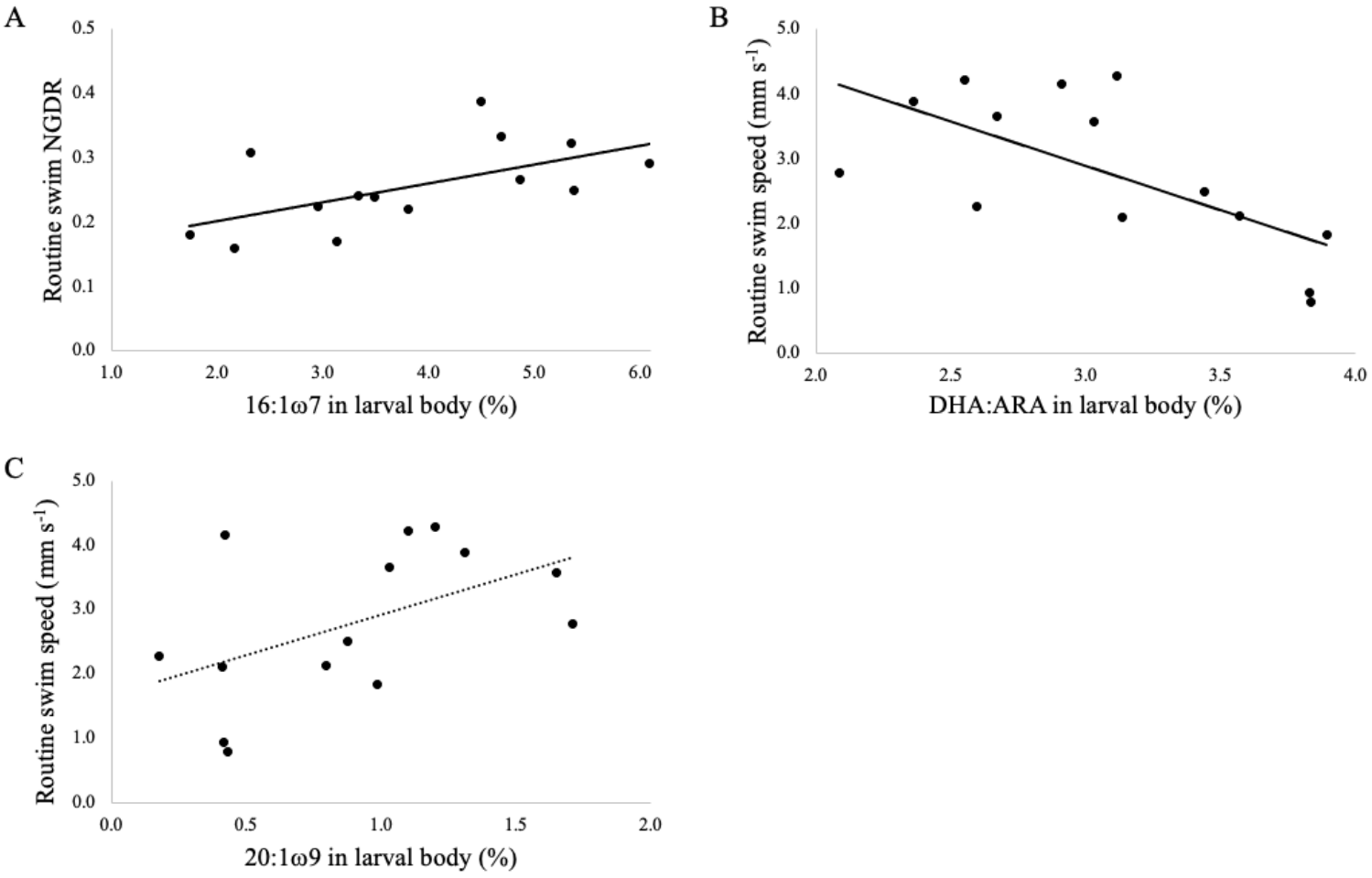

Figure 3.5. Significant relationships between larval routine swimming performance and larval fatty acid composition at 15 dph (% total fatty acids). (A) Routine swimming NGDR and larval 16:1ω7 ($r = 0.59$, $P = 0.02$), (B) Routine swimming speed (mm s^{-1}) and larval DHA:ARA ($r = 0.67$, $P = 0.008$). (C) Routine swimming speed (mm s^{-1}) and larval 20:1ω9 ($P = 0.06$). Solid line denotes significant correlation ($P < 0.05$), dotted line denotes marginally significant correlation ($r = 0.51$, $P < 0.10$).

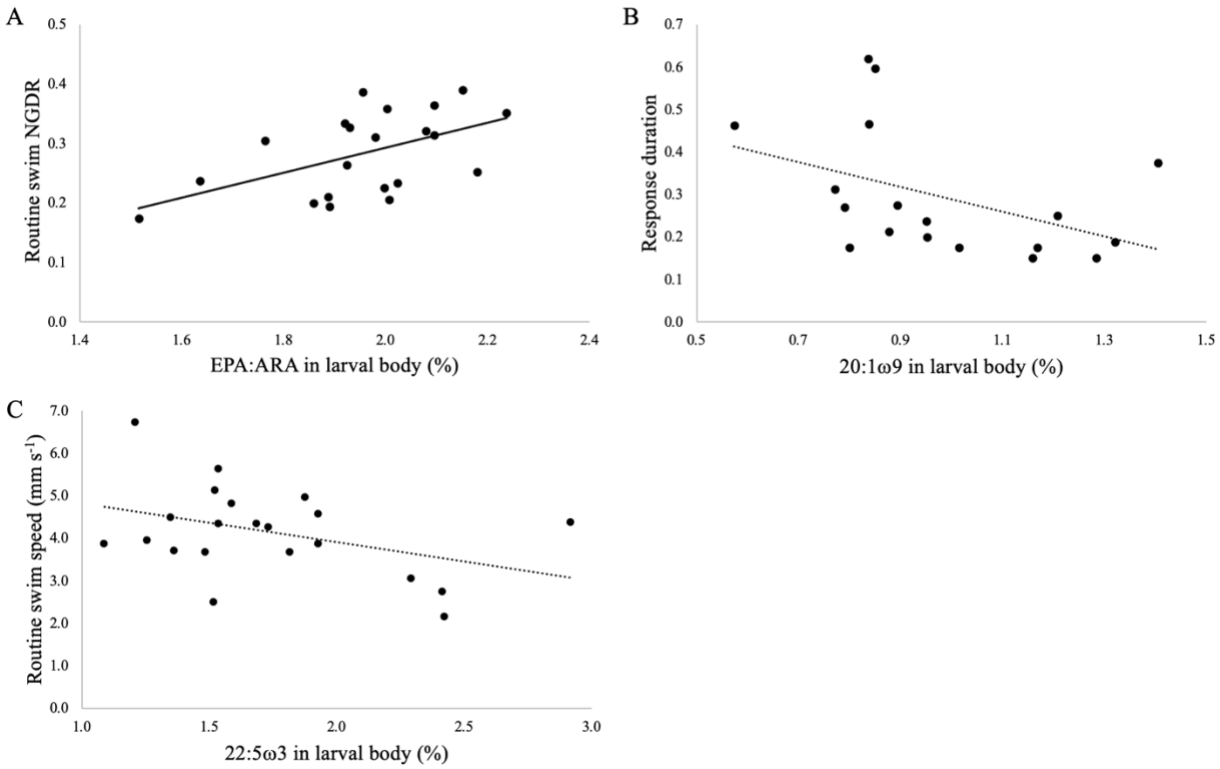

Figure 3.6. Significant relationships between larval performance and larval fatty acids at 35-dph (% total fatty acids). (A) Routine swimming NGDR and EPA:ARA ($r = 0.52$, $P = 0.01$), (B) Response duration and 20:1ω9 ($r = 0.44$, $P = 0.01$), and (C) Routine swimming speed (mm s^{-1}) and 22:5ω3 ($r = 0.40$, $P = 0.07$). Solid line denotes significant correlation ($P < 0.05$), dotted line denotes marginally significant correlation ($P < 0.10$).

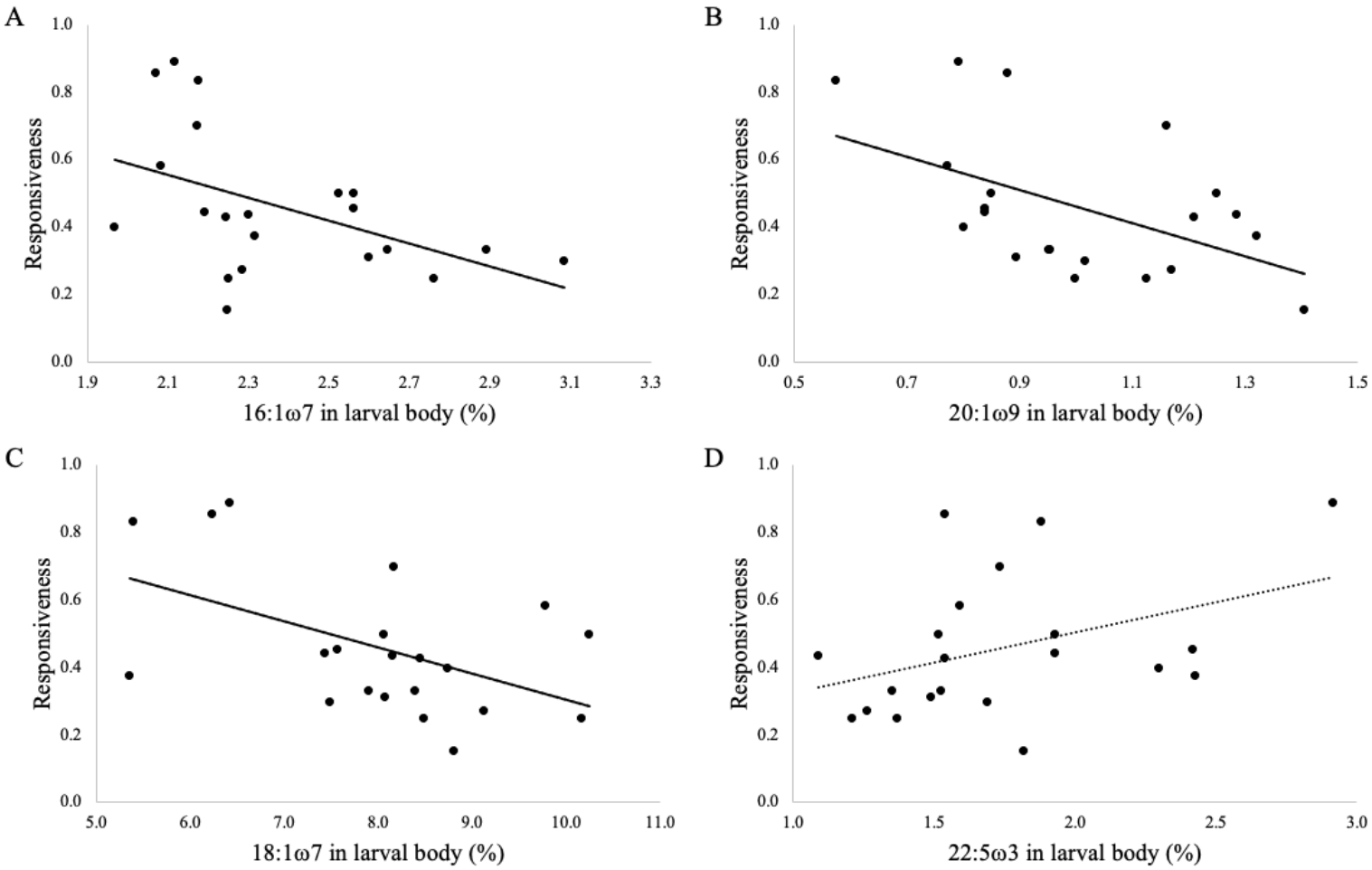

Figure 3.7. Significant relationships between larval performance and larval fatty acids at 35-dph (% total fatty acids). (A) Responsiveness and 16:1ω7 (r = 0.48, P = 0.02), (B) Responsiveness and 20:1ω9 (r = 0.51, P = 0.01), (C) Responsiveness and 18:1ω7 (r = 0.50, P = 0.02), (D) Responsiveness and 22:5ω3 (r = 0.40, P = 0.07).

Chapter 3: Appendix

Fatty Acid	FAML Shrimp	FAML Sardine	TPWD Base	Supplement
14:0	1.67 ± 0.42	4.72 ± 0.96	3.79 ± 1.92	6.46 ± 0.82
15:0	1.03 ± 0.12	1.11 ± 0.1	0.47 ± 0.10	0.55 ± 0.03
16:0	12.73 ± 0.69	24.26 ± 1.14	13.85 ± 2.65	18.24 ± 0.08
16:1ω7	4.48 ± 0.41	5.97 ± 1.06	3.88 ± 0.99	8.30 ± 0.49
16:2ω4	0.51 ± 0.11	1.02 ± 0.16	0.57 ± 0.16	1.42 ± 0.09
17:0	1.70 ± 0.31	1.47 ± 0.13	0.65 ± 0.10	0.43 ± 0.06
16:3ω4	1.86 ± 0.41	0.52 ± 0.17	0.58 ± 0.13	1.15 ± 0.08
18:0	7.53 ± 1.26	7.67 ± 0.43	3.87 ± 0.79	3.93 ± 0.05
18:1ω9	6.72 ± 0.58	6.83 ± 1.00	10.71 ± 6.89	9.45 ± 0.20
18:1ω7	3.90 ± 0.57	2.76 ± 0.28	2.03 ± 1.27	2.82 ± 0.10
18:2ω6	1.73 ± 0.36	1.65 ± 0.25	1.31 ± 0.27	9.17 ± 0.35
18:3ω6	0.40 ± 0.08	0.70 ± 0.21	0.36 ± 0.08	0.30 ± 0.03
18:3ω4	0.21 ± 0.15	0.20 ± 0.23	0.12 ± 0.04	0.31 ± 0.02
18:3ω3	0.63 ± 0.21	0.75 ± 0.24	0.90 ± 0.33	2.11 ± 0.10
18:4ω3	0.39 ± 0.15	0.73 ± 0.18	2.11 ± 1.21	1.69 ± 0.12
20:1ω9	1.22 ± 0.55	0.47 ± 0.09	7.65 ± 1.74	2.04 ± 0.08
20:2ω6	1.13 ± 0.23	0.29 ± 0.05	0.44 ± 0.05	0.20 ± 0.01
20:3ω6	0.30 ± 0.20	0.45 ± 0.09	0.16 ± 0.05	0.28 ± 0.01
20:4ω6	6.13 ± 1.12	2.60 ± 0.16	1.91 ± 0.18	1.19 ± 0.02
20:3ω3	0.26 ± 0.08	0.10 ± 0.03	0.26 ± 0.04	0.17 ± 0.03
20:4ω3	0.49 ± 0.07	0.33 ± 0.04	0.74 ± 0.21	0.99 ± 0.02
20:5ω3	13.93 ± 1.42	6.36 ± 0.81	9.31 ± 1.35	9.97 ± 0.40
22:1ω11	0.33 ± 0.16	0.10 ± 0.04	8.88 ± 5.12	1.45 ± 0.52
22:4ω6	0.80 ± 0.38	0.48 ± 0.18	0.35 ± 0.08	0.17 ± 0.01
22:5ω6	0.98 ± 0.11	1.51 ± 0.33	0.39 ± 0.08	0.37 ± 0.03
22:5ω3	1.69 ± 0.24	0.90 ± 0.13	1.12 ± 0.24	1.96 ± 0.10
22:6ω3	15.31 ± 1.90	20.78 ± 4.45	15.95 ± 2.09	10.88 ± 0.89

Table 3.4. Fatty acid composition (% total fatty acids) of Southern Flounder broodstock diet items. TPWD represents the base diet of shrimp, squid, and mackerel (2:1:1 by wet weight). Mazuri is the diet supplement that was applied with the TPWD diet on two different schedules.

Fatty Acid	Rotifers	*Artemia*
14:0	6.06 ± 0.74	1.33 ± 0.42
15:0	0.53 ± 0.04	0.21 ± 0.02
16:0	20.93 ± 1.27	11.88 ± 1.55
16:1ω7	5.51 ± 1.11	2.90 ± 0.21
16:2ω4	0.80 ± 0.24	0.17 ± 0.02
17:0	0.34 ± 0.02	0.72 ± 0.08
16:3ω4	0.25 ± 0.06	0.73 ± 0.05
18:0	1.69 ± 0.24	4.86 ± 0.47
18:1ω9	1.88 ± 0.37	13.1 ± 1.61
18:1ω7	2.20 ± 0.32	9.76 ± 1.30
18:2ω6	3.07 ± 0.64	4.87 ± 0.43
18:3ω6	0.46 ± 0.07	0.44 ± 0.06
18:3ω4	0.01 ± 0.01	0.03 ± 0.03
18:3ω3	1.00 ± 0.20	25.93 ± 2.89
18:4ω3	0.31 ± 0.11	4.09 ± 0.82
20:1ω9	0.33 ± 0.10	0.57 ± 0.12
20:2ω6	0.18 ± 0.06	0.19 ± 0.02
20:3ω6	0.71 ± 0.10	0.15 ± 0.02
20:4ω6	2.33 ± 0.18	1.51 ± 0.58
20:3ω3	0.09 ± 0.02	0.60 ± 0.12
20:4ω3	0.75 ± 0.05	0.58 ± 0.05
20:5ω3	8.26 ± 2.09	4.28 ± 1.12
22:1ω11	0.11 ± 0.03	0.03 ± 0.03
22:4ω6	0.18 ± 0.05	0.01 ± 0.03
22:5ω6	8.96 ± 1.45	1.03 ± 0.61
22:5ω3	2.15 ± 0.44	0.04 ± 0.04
22:6ω3	24.95 ± 3.24	2.76 ± 1.51

Table 3.5. Fatty acid composition (% total fatty acids) of Southern Flounder larval diet items after being enriched Algamac 3050 DHA 10 (> 45 min for rotifers, overnight for *Artemia*).

Chapter 4: Conclusions

The studies described in this thesis were focused on understanding the nutritional ecology of Southern Flounder (*Paralichthys lethostigma*) and the role fatty acids (FAs) play in constraining egg and larval quality. The first study revealed that FA composition of the diet fed to Southern Flounder 16-weeks before spawning largely determined the FA composition of their eggs. Impressively, for 20 of the 27 FAs measured, higher amounts of these FAs in the maternal diet resulted in eggs also having higher amounts of those same FAs. Further, it was found that two measures of egg quality (hatching length and hatching rate) were sensitive to egg FA composition. This indicates that a collection of FAs can be manipulated in the maternal diet to influence egg composition, and some of these FAs can affect subsequent egg quality. This study also resolved the temporal window that is necessary for diet-egg equilibration in Southern Flounder. Previous research demonstrated that a change in the amount of Docosahexaenoic acid (22:6ω3) in the maternal diet could be detected in egg composition 3-weeks after a diet change (Burns and Fuiman 2020). By combining data from that prior study with data from the present study, it was revealed that diet-egg equilibration takes between 8 and 16 weeks to occur. Knowing when to implement broodstock dietary changes is important for hatcheries and can ultimately provide cost-savings by not providing supplemental feeds year-round, and rather, only providing them during the temporal window that is necessary for diet-egg FA equilibration.

Separately, these findings provide a framework for understanding the ecological consequences of dietary regime change in estuarine habitats of Southern Flounder. Previous work revealed Southern Flounder have high estuarine fidelity and do not move far outside of an estuary (< 1 km) until it is time to spawn in the fall, at which point they make long migrations offshore (> 50 km) (Craig et al. 2015). Estuarine habitats are prone to environmental disturbances (droughts, floods, freezes, harmful algal blooms) which may alter food web dynamics (Fuiman 2018), and since FA composition of Southern Flounder eggs is determined by the maternal diet 8 to 16 weeks

before spawning, food web perturbations may alter egg composition in a significant manner. Future research efforts could focus on characterizing the FA profiles of wild Southern Flounder eggs and both estuarine and offshore prey items. This approach would provide deeper insight into the differences between eggs from captively reared Southern Flounder and those from the wild.

The second study in this thesis explored the relationships among egg and larval composition and subsequent larval performance of ecologically-relevant survival skills at two discrete developmental stages (15 and 35 days post hatch, dph). It was found that three FAs in 15-dph larvae (16:1ω7, 20:1ω9, 20:4ω6) and three FAs and one FA ratio in 35-dph larvae (18:1ω9, 20:1ω9, 22:5ω3, ARA:EPA) had direct relationships with their respective levels found in the eggs from which they were derived. Since all larvae were fed the same high-quality diet, this indicates that a larva's ability to accumulate or synthesize these FA's during these life stages is influenced by egg FA composition, probably a result of altered lipid metabolism in larvae, a phenomenon known as nutritional programming. Further, the levels of these FAs in larvae were associated with certain larval performance metrics related to foraging and predator evasion. For example, routine swimming speed was correlated with the ratio of two important essential fatty acids (EFAs), docosahexaenoic acid (DHA) and arachidonic acid (ARA), and the mono-unsaturated FA (MUFA) 20:1ω9 in 15-dph larvae. In 35-dph larvae, routine swimming speed was negatively correlated with 22:5ω3, an important FA that is a precursor to DHA. Overall, the relationships between larval body composition and larval performance were not congruent at the two developmental stages. This suggests the effects of metabolic programming in Southern Flounder may change as growth and development alter physiological processes related to FA absorption, synthesis, conversion, utilization, and storage. Future research of Southern Flounder should determine expression of key enzymes involved in FA metabolic processes to examine if a causal link exists between egg and larval FA composition. With this information, it would be possible to manipulate FAs in maternal diets to influence egg FA composition, resulting in altered expression pathways in Southern

Flounder larvae. This approach could be used to direct larval phenotype based on the objectives of a production facility. Together, these studies demonstrate several ways that Southern Flounder maturation diets can affect the eggs and larvae they produce and places the results into captive rearing and ecological contexts.

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