Supplementary Material

Dynamics of diet-egg transfer of fatty acids in the teleost fish, red drum (*Sciaenops ocellatus*)

Zhenxin Hou, Cynthia K. Faulk and Lee A. Fuiman

University of Texas Marine Science Institute, 750 Channel View Drive, Port Aransas, Texas 78373, USA

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Supplementary Materials and Methods

Broodstock care

Adult red drum (length: 90 – 100 cm, weight: 9 – 15 kg) were maintained at the Fisheries and Mariculture Laboratory (FAML) of the University of Texas Marine Science Institute in Port Aransas, TX, and the Texas Parks and Wildlife (TPWD) CCA Marine Development Center in Corpus Christi, TX. Adults were collected from nearby waters at least 1 year prior to each diet change and presumed to be genetically unrelated. Fish were held in 12,000-16,000 L recirculating tanks and were induced to spawn naturally under a controlled temperature (24-26°C) and photoperiod (10:14 L:D) regime at a salinity of 30-38 ppt and 6.15-7.40 mg L^{-1} dissolved oxygen.

Diet components were previously frozen shrimp (*Farfantepenaeus aztecus* or *Litopenaeus setiferus*), Spanish sardine (*Sardinella aurita*), Atlantic mackerel (*Scomber scombrus*), and squid (*Loligo opalescens*). In some experiments, diets were supplemented with capsules containing Algamac ARA (Alg ARA) and Algamac 3050 (Alg 3050), which have high levels of ARA (20:4n-6) and DHA (22:6n-3), respectively. These products are commercial live prey enrichments derived from spray-dried cells of *Cyrothecodinium* and heterotrophically grown *Schizochytrium* sp. algae, respectively (Aquafauna Bio-Marine Inc, Hawthorne, CA, USA). Soy lecithin (phosphatidylcholine) was purchased from MilliporeSigma (St. Louis, MO, USA), and was high in 18:2n-6. Algamac ARA, Algamac 3050, and soy lecithin were fed to the fish in gelatin capsules that were placed inside whole shrimp or squid. Vitalis CAL is a pelleted marine fish feed (Skretting, St. Andrews, NB, CA).

Fish were hand fed several pieces of one prey type at a time that were spread around the tank so that, as much as possible, all broodstock received similar amounts of food. Feeding was immediately stopped when the designed ration was reached or when fish stopped eating to make sure there were no uneaten food items in the tanks. The weight of unconsumed food, if any, was taken into account when recording the ingested amount for that day. A daily feeding record is provided as a separate file (Supplementary Document D1).

Table S1: Summary of diet-shift experiments for lag analysis. Each row indicates the sequence and duration (days) of each diet.

Tank	Date of		Number of spawns	Diet change	
	first spawn	last spawn		Diets	Duration
MT1	11 Mar 2018	12 Sept 2018	34	shrimp, liver	34
				shrimp, squid	35
				shrimp, sardine	57
				mackerel	78
				shrimp, squid, sardine	6
MT6	24 Mar 2012	19 Jun 2012	23	shrimp, squid, sardine	33
				squid, shrimp, sardine, 4.2 g Alg ARA	58
				shrimp	26
MT7	11 Mar 2018	8 Jun 2018	17	sardine, squid	34
				sardine	35
				squid	45
MT8	27 Oct 2011	5 Jan 2012	27	shrimp, squid	13
				shrimp, squid, mackerel	36
				shrimp	52
MT9	24 Feb 2012	19 Sept 2012	32	shrimp	31
				shrimp, squid	56
				shrimp	112
				shrimp, squid, sardine	41

Lag analysis

Lag analyses were conducted on data obtained from five broodstock tanks that were given multiple diet shifts in sequence. Details of the multiple-diet-shift experiments are shown in Table S1. Egg samples were collected whenever fish spawned, approximately every 2-6 days. Spawns sampled for these experiments are shown in a separate file (Supplementary Document D2).

The recorded amounts of food ingested at each meal were used to calculate a time series of daily intake for each tank, by calculating a moving average of dietary FA intake per fish for the seven preceding days. The time series of daily intake started 1 month before the first spawn sampled (24 days for MT1 and MT7) and ended on the date of the last spawn sampled.

For each FA in each experiment, the Pearson correlation coefficient, r, was calculated between the amount of that FA in the eggs and the mean daily intake of that FA on the days of the spawns sampled (lag = 0). Correlation coefficients were also calculated between the FA in eggs and mean daily intake on each day prior to the spawn dates up to 30 days (lag = 1-30). A correlogram was constructed for each FA in each experiment to ensure there was a progressive trend in correlations (i.e., maximum r was not random) (Figure S1) and the maximum r was used to estimate the lag between ingestion and incorporation into eggs. When the maximum r did not fall along the trendline in its correlogram (i.e., random error), the lag was designated as the next highest r on the trendline. This occurred only once: the second highest r (r = 0.75, at a lag of 7 days) was designated for 16:1n-7/16:0 in MT7 because the maximum r (r = 0.79) at a lag of 20 days was not on the trendline.

Incorporation rate analysis

Table S2 summarizes details of the 21 single-diet-shift experiments. Each diet shift represented a different amount of change in dietary intake for each FA. The simple dilution model predicts that turnover (or incorporation) rate of a FA is proportional to the magnitude of change in dietary intake. The magnitude of change for each FA (ΔFA; mg d^{-1} $fish^{-1}$) was calculated as the difference between the 28-day mean of dietary intake after and 28-day mean of dietary intake before the diet shift.

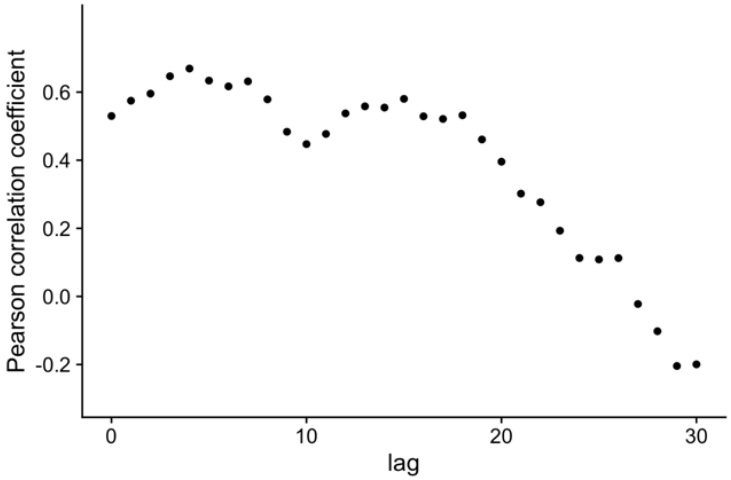

Figure S1. Sample correlogram showing the strength of the relationship (expressed as *r*) between egg DHA content and daily DHA dietary intake for lags of 0-30 days in tank MT1. Here, the maximum *r* (0.67) corresponds to a lag of 4 days.

Since spawning frequency could not be controlled, the number of and interval between egg samples obtained for each experiment varied (approximately every 2-8 days), resulting in inconsistent accuracy and precision of estimates of incorporation rate.

For three FA in experiment 17 (18:2n-6, 18:3n-6, and 18:3-4), the slopes of the linear regression between egg FA level and the time since a diet change were considered inaccurate estimates of incorporation rates and were excluded from the analysis because the levels of these FA in eggs stabilized after the first spawn following the shift. Other estimates were deemed inaccurate because the standard error of those slopes (I_{FA}) were large (> 4 standard deviations above the mean of all standard errors in the data set for that FA; Table S3).

Linear regressions between I_{FA} and ΔFA were used to test the prediction of the simple dilution model (Figure 4).

Biochemical analysis

The amount of FA in the eggs and dietary items was measured by gas chromatography using established methods (Faulk and Holt 2005). Briefly, lyophilized samples were homogenized and lipids were extracted with 2:1 chloroform: methanol (v/v). A known amount of tricosanoic acid (23:0) (Supelco, Inc., Bellefonte, PA, USA) was added before homogenization as an internal standard. Fatty acid methyl esters (FAME) were prepared by saponification in potassium hydroxide in methanol and transesterification with 14% boron trifluoride in methanol. FAME were dissolved in hexane before analysis by gas chromatography. Samples collected in 2010 (exp 1) were analyzed on a Hewlett-Packard 5890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and a Supelcowax 10 fused silica capillary column (30 m long, 0.53 mm internal diameter, 1.0 μm thickness; Supelco, Inc.). All other samples were analyzed on a Shimadzu GC-2014 gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with an FID and a Phenomenex ZB-WAX plus capillary column (30 m long, 0.53 mm internal diameter, 1.0 μm thickness; Phenomenx,

Table S2: Summary of single-diet-shift experiments. An arrow separates the diet before the diet shift from the diet after the shift. Experiments using VB and MT tanks were conducted at TPWD and FAML, respectively. Commercial products included in some diets were Algamac ARA (Alg ARA), Algamac 3050 (Alg 3050), soy lecithin, and Vitalis CAL.

Expt.	Diet change	Date of diet shift	Tank	Number of		
				fish	females	spawns
1	shrimp, mackerel, squid → shrimp, squid, 1.8 g Alg ARA	9 Jun 2010	VB07	2	1	13
2	shrimp, squid → shrimp, squid, 1.3 g Alg ARA	20 Jun 2011	VB07	2	1	8
3	shrimp, squid, 1.3 g Alg ARA → shrimp, squid, 3.8 Alg ARA	5 Aug 2011	VB07	2	1	4
4	squid, shrimp, sardine → squid, shrimp, sardine, 4.2 g Alg ARA	28 Mar 2012	MT6	4	1	12
5	squid, shrimp, sardine, 4.2 g Alg ARA → shrimp	25 May 2012	MT6	4	1	7
6	shrimp, squid → shrimp, squid, mackerel	10 Oct 2011	MT8	2	1	5
7	shrimp, squid, mackerel → shrimp	15 Nov 2011	MT8	2	1	15
8	shrimp → shrimp, squid	24 Feb 2012	MT9	2	1	8
9	shrimp, squid → shrimp	20 Apr 2012	MT9	2	1	7
10	shrimp → shrimp, squid, sardine	10 Aug 2012	MT9	2	1	10
11	shrimp, squid, liver, mackerel → shrimp, liver	19 Jul 2012	VB3-1	5	3	9
12	shrimp, squid, liver, mackerel → shrimp, squid, mackerel	3 Sept 2012	VB4-1	5	3	7
13	shrimp, squid, sardine → shrimp	15 Aug 2012	MT7	4	2	7
14	shrimp, 4.9 g Alg 3050 → shrimp, squid	23 Jan 2012	MT8	2	1	9
15	shrimp → Vitalis CAL	8 Jul 2013	VB3-3	5	3	14
16	shrimp, sardine, squid, 3.1 g soy lecithin → shrimp, sardine, squid	25 Aug 2014	MT9	3	1	11
17	shrimp, liver → shrimp, squid	21 Mar 2018	MT1	4	2	10
18	shrimp, squid → shrimp, sardine	25 Apr 2018	MT1	4	2	4
19	shrimp, sardine → mackerel	21 Jun 2018	MT1	4	2	4
20	sardine, squid → sardine	21 Mar 2018	MT7	4	2	4
21	sardine → squid	25 Apr 2018	MT7	4	2	5

Torrance, CA, USA) or a Supelcowax 10 column. FAME were identified by comparison with commercial standards.

Supplementary results

Prey composition

Feed items varied greatly in FA profile. FA profile for the same feed item sometimes varied over the years. A full FA profile for prey items can be found in a separate file Supplementary Document D3.

Supplementary References

Faulk CK, Holt GJ (2005) Advances in rearing cobia *Rachycentron canadum* larvae in recirculating aquaculture systems: Live prey enrichment and greenwater culture. *Aquaculture* 249:231–243. doi: 10.1016/j.aquaculture.2005.03.033

Table S3: Inaccurate estimates of I_{FA}, as determined by large standard errors for the slope (> 4 standard deviations above the mean of all standard errors).

Fatty acid	Experiment
14:0	19
15:0	6
16:0	3, 6, 9, 12
ΣSAT	6, 9
18:1n-7	5
18:1n-9	12
20:1n-9	19
22:1n-11	19
ΣMUFA	12
16:2n-4	6
16:3n-4	9
18:3n-4	6, 17
18:2n-6	17
18:3n-6	6, 17
20:2n-6	6
20:3n-6	11
20:4n-6	5
Σ(n-6)PUFA	11
18:4n-3	19
20:4n-3	19
22:5n-3	6
22:6n-3	9
ΣPUFA	9
ΣHUFA	9
Σ(n-3) PUFA	9
Σ(n-3) HUFA	9
ΣC16/ΣC18	9
Σ(n-3)/Σ(n-6) PUFA	9
Σ(n-3)/Σ(n-6) HUFA	14, 21