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Temporal dynamics of lipid and fatty acid characteristics of Gulf Menhaden, *Brevoortia patronus*, in the northern Gulf of Mexico

Robert T. Leaf [a,*], Jesse Trushenski [b,1], Nancy J. Brown-Peterson [c], Michael J. Andres [a]

[a] Division of Coastal Sciences, The University of Southern Mississippi, 703 East Beach Dr., Ocean Springs, MS 39564, United States
[b] Center for Fisheries, Aquaculture, and Aquatic Sciences, Southern Illinois University Carbondale, 1125 Lincoln Dr., Life Science II Room 251, Mail Code 6511, Carbondale, IL 62901, United States
[c] Center for Fisheries Research and Development, School of Ocean Science and Technology, The University of Southern Mississippi, 703 East Beach Dr., Ocean Springs, MS 39564, United States

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ABSTRACT

Gulf Menhaden, *Brevoortia patronus*, in the northern Gulf of Mexico support a large commercial fishery and are thought to play an important trophic role in the coastal ecosystem. The temporal dynamics of both fatty acid and oil content have a direct impact on the value of Gulf Menhaden to predators and to the fishery. In this work, we describe how oil content of Gulf Menhaden varies with season, sex, age, condition, and tissue and investigate how fatty acid composition of mature ($\geq$137.5 mm FL) female tissues varies with season, month, and tissue type. We found pronounced temporal (January to October) variation in mean oil content ranging from 0.062 to 0.579 mg g^{-1} that exhibited a significant ($p < 0.001$) seasonal pattern. We observed significant differences in oil content between tissue (muscle vs. ovary) of mature females and these exhibited a significant seasonal contrast, indicating that females were provisioning eggs in the fall. PERMANOVA analysis indicated the existence of significant differences ($p < 0.001$) in the composition of fatty acids of muscle tissue collected in different months. Mean eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and long chain polyunsaturated fatty acid (LC-PUFA) levels exhibited significant seasonal differences ($p < 0.05$), and in the case of DHA and LC-PUFA, both exhibited mean tissue-specific differences ($p < 0.05$). This work indicates that the value of Gulf Menhaden as prey and a fishery resource in the region varies during the year and we propose that trophic models of the Gulf of Mexico ecosystem should account for this variation.

1. Introduction

The ecosystem impacts of removing forage fishes (schooling, high biomass finfish and invertebrate stocks that occupy low trophic levels and are prey to a variety of avian, fish, and mammal predators) has garnered much attention in recent years (Hilborn et al., 2017; Pikitch et al., 2018). An outstanding issue is to determine to what extent the abundance of a single forage fish species in an ecosystem mediates the population growth rates of predator taxa. Hilborn et al. (2017) analyzed 50 forage fish stocks and concluded that predator population growth rates are not correlated to forage fish abundance, highlighting the role of the environment in influencing forage fish population dynamics which has been reported by others (Szuwalski and Hilborn, 2015). In the current paper, we evaluate the intra-annual dynamics of lipid content and fatty acid composition of Gulf Menhaden, *Brevoortia patronus*, in the northern Gulf of Mexico (nGOM). Additionally, we seek to understand the intra-annual dynamics of these components, which will improve understanding of the importance of this taxa to the diets of predator species.

Gulf Menhaden supports a large commercial fishery in the nGOM and is an important trophic component of the ecosystem (Geers et al., 2014; Sagarese et al., 2016). In the last ten years, approximately 400 to 450 $\times$ 10^5 metric tons (mt) of biomass in the coastal waters of the nGOM have been harvested each year, and this level of extraction qualifies the Gulf Menhaden stock as the second largest fishery, by weight, in the United States (Vaughan et al., 2007). The primary products of the reduction processing of the harvested fish are fish meal, which is used for animal feed in agriculture and aquaculture, and fish oil, which is used as a feed additive in these industries and for human consumption (Smith, 1991). Menhaden (*Brevoortia* spp.) oil is a valued human and feed supplement because of its constituent fatty acids. These include long-chain polyunsaturated fatty acids (LC-PUFAs, fatty acids comprising 20 or more carbon atoms and 3 or more double bonds) including the "omega-3" (first double bond is located at the 3rd

* Corresponding author.
E-mail address: robert.leaf@usm.edu (R.T. Leaf).
[1] Current address: Evaqua Farms LLC, 4074 N 2000 E Rd, Filer, ID 83328.

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carbon from the methyl end of the fatty acid molecule; n−3), eicosapentaenoic acid (20:5n−3,[2] EPA), and docosahexaenoic acid (22:6n−3, DHA), all of which are known to be involved in a number of physiological processes related to the cardiovascular and neurological systems, immunity, and reproduction (DeMeester, 2013; Glencross, 2009).

Beyond the value of the commercial fishery, Gulf Menhaden play an important role as consumers and prey in the trophic ecology of the nearshore and coastal pelagic ecosystem. Gulf Menhaden are considered a critical conduit of energy to higher trophic levels (Geers et al., 2014; Sagarese et al., 2016). Olsen et al. (2014) documented, using stable isotope analysis, that Gulf Menhaden <125 mm TL have a mixed diet but feed primarily on phytoplankton, and as they get larger, transition to feeding on zooplankton. Similar feeding patterns have been described for its congener Atlantic Menhaden, *B. tyrannus* (Friedland et al., 2006). The seasonal movement pattern has implications for energy transfer. Fish move inshore to the coastal zone in the spring and reside in nearshore habitats throughout the summer. In the fall, the stock moves offshore for reproduction. Gulf Menhaden have an extended (October through March) reproductive season (Brown-Peterson et al., 2017). Spawned larvae and adults move into the nearshore coastal zone in the nGOM during the late spring where they are prey for a variety of birds (Short et al., 2017) and fishes (Goodyear, 1967; Knight and Hastings, 1987; Scharf and Schlicht, 2000; Sekavec, 1974; Strelcheck et al., 2004).

The importance of fatty acid composition in fish diets has received increased attention in recent years and is generally well documented in the field of aquaculture (e.g., see reviews by Trushenski and Bowzer, 2013; Turchini et al., 2009). Gulf Menhaden provide fatty acids to consumers that aid in their growth and development; for this reason, menhaden and other fish oils are often used to supplement aquaculture feeds (Tocher, 2010). There is an increasing interest in understanding the species-specific and temporal dynamics of fatty acids (Pethybridge et al., 2014). In particular, "essential fatty acids" are critical to organismal growth and development (Litzow et al., 2006) and, because they cannot be synthesized *de novo*, are hypothesized to be a potentially limiting factor to ecosystem productivity at higher trophic levels (Fuiman et al., 2015). Fatty acids play an important role in growth, reproduction, and swimming abilities of fishes (Tocher, 2010). LC-PUFAs like EPA and DHA have recognized physiological functions, but many fishes are able to satisfy physiological demand for EPA and DHA by consuming their biosynthetic precursor, alpha-linolenic acid (18:3n−3, ALA) and synthesizing the n−3 LC-PUFA *de novo* through a series of elongation and desaturation steps (Sargent et al., 1999). For these fishes, ALA is considered "essential"; even though EPA and DHA are the molecules in physiological demand. Other fishes are not able to make these biosynthetic transformations and must consume EPA and DHA directly to satisfy physiological demand for n−3 LC-PUFAs. Some species lack the ability to transform ALA into EPA and DHA altogether, whereas others are incapable of making such transformations during early life history or in sufficient amounts to keep up with physiological demand. Thus, LC-PUFAs including EPA and DHA are critical during endogenous and early exogenous feeding, and maternal reserves of LC-PUFAs and deposition of these nutrients in the ova are an important predictor of reproductive and larval success (Izquierdo et al., 2001; Lane and Kohler, 2006; Lewis et al., 2010; Rainuzzo et al., 1997). Poor growth and survival may occur without sufficient levels of essential fatty acids as a result of overt deficiency or due to the re-allocation and utilization of proteins as energy sources for processes other than muscle development (Glencross, 2009). Provisioning of LC-PUFA is especially important for larval and juvenile fish when fast growth rates are necessary for predator evasion and survival (Anderson, 1988; Rice et al., 1993).

The role Gulf Menhaden play as a prey species has gained more attention recently (Geers et al., 2014; Sagarese et al., 2016), in part because of the estimated annual number of Gulf Menhaden (107.67 and 58.1 billion age 0 and age 1+, respectively, in 2013; SEDAR, 2013). An emerging field in ecology is the use of the multivariate composition of fatty acid profiles of consumers to inform understanding of their trophic niche breadth (Dalsgaard et al., 2003; Graeve et al., 1997; Kelly and Scheibling, 2012), and fatty acids have been shown to be useful "biomarkers" for understanding prey composition for some groups (Reuss and Poulsen, 2002). The use of fatty acids as an alternative to stomach content analysis overcomes the biases of inferring diet composition from differentially preserved food items in stomachs (Michener and Kaufman, 2007), and is particularly applicable for Gulf Menhaden prey since they are seemingly digested at a faster rate than other finfish species. In this work, we describe general and specific characteristics of oil content and fatty acid composition of Gulf Menhaden collected from August 2014 to February 2016. We examine the seasonal-, sex-, age-, condition-, and tissue-specific oil content of Gulf Menhaden individuals and investigate how fatty acid composition of mature (≥137.5 mm FL) females varies with month, season, and tissue type. We discuss our results in the context of the ecosystem services provided by Gulf Menhaden within the nGOM.

2. Material and methods

Gulf Menhaden were obtained from the commercial fishery and fishery-independent sampling from August 2014 to February 2016, but herein we focus our seasonal analysis on those fish collected in August to October (fall) and January to April (spring). These seasonal designations coincide with the onset of spawning (fall) and post-spawning (spring) periods of Gulf Menhaden. Additional fish were collected in May and June to expand the monthly analysis. Fish were obtained from Omega Protein Corporation's Moss Point, Mississippi facility, the Louisiana Department of Fisheries and Wildlife, and the University of Southern Mississippi's Center for Fisheries Research and Development. All fish were taken in the coastal region (< 30 m depth) in the nGOM (Louisiana and Mississippi, USA coastal waters). Upon collection, fish were immediately placed on ice and processed within 24 h of capture when possible; otherwise fish were frozen and remained fully or partially frozen during processing. Fish were measured (FL, mm), weighed (W, g), and Fulton's condition factor (K) was determined (Nash et al., 2006):

$$K = 100 \times \frac{\mathrm{W}}{\mathrm{FL}^3}.$$

Each fish was assigned to an age class using established methods (SEDAR, 2013); (VanderKooy, 2009): Scales (n = 10) above the left pectoral fin were removed from each individual by abrading them with a dull scalpel, rinsed in warm tap water, dried, and mounted on glass microscope slides. Age determination was performed by counting scale annuli on images projected using an Eberbach macro-projector at 48× magnification. All fish were assumed to have a birthdate of January 1 (Ahrenholz, 1991). Fish captured in January and February, whose scales had a large margin exterior to the last annulus, were assigned the next greater integer age.

Sex (male, female, or immature) and maturity status of females (mature or immature) was determined by processing gonadal tissue histologically following standard techniques; females

[2] Numeric fatty acid nomenclature follows the format of A:Bn-C, where A is the number of carbon atoms in the molecule, B is the total number of double bonds, and C is the position of the first double bond, counting carbon atoms from the methyl end of the molecule. Thus, 20:5n−3 indicates the fatty acid has 20 carbon atoms and 5 double bonds, with the first of those at the 3rd carbon from the methyl end.

were considered sexually mature if cortical alveolar oocytes were present (Brown-Peterson et al., 2017). Briefly, gonadal tissue was fixed in neutral buffered 10% formalin solution for a minimum of one week, rinsed in running tap water for a minimum of eight hours, dehydrated in a series of graded ethanol solutions, and then embedded in paraffin wax. Tissue was sectioned to a thickness of 4 μm and stained with hematoxylin and eosin following standard procedures. Gonadal tissue was evaluated microscopically following established methods (Brown-Peterson et al., 2011; Lowerre-Barbieri et al., 2011) and classified as reproductively active or reproductively inactive.

Fresh and frozen muscle and ovary tissues were evaluated for oil content (mg oil/g freeze-dried tissue) and processed for the determination of fatty acid composition. Tissue samples were collected by dissection with care given to avoid subcutaneous fat, skin, bone, and viscera. Each tissue sample was weighed (1 ± 0.1 g), lyophilized, reweighed to determine moisture/dry matter content, and pulverized using mortar and pestle. Total lipid content was determined gravimetrically following chloroform–methanol extraction methods (Folch et al., 1957): 12 mL of 2:1 (v/v) chloroform: methanol was added to the pulverized sample, mixed with a vortex mixer, and placed in a −20 °C freezer for 1 h. Following freezing, 3 mL of aqueous KCl (0.88% w/v) was added to the sample. The extract was further processed using a vortex mixer and centrifuged at 5,000 rpm (4,472 g) for a minimum of 5 min to allow for the recovery of total lipids from the organic phase. The organic phase was passed through a sodium sulfite filter to remove aqueous and particulate material. The solvent was evaporated under nitrogen gas at 45 °C, and desiccated *in vacuo* overnight. The total lipid extract was weighed, purged with nitrogen gas, and placed into a −80 °C freezer prior to shipping (overnight on dry ice) to the Center for Fisheries, Aquaculture, and Aquatic Sciences (CFAAS) at Southern Illinois University Carbondale (Carbondale, IL) for fatty acid analysis. Upon receipt at the CFAAS, lipid samples were returned to −80 °C prior to further processing and analysis. Total lipid extracts were subjected to acid-catalyzed transmethylation performed overnight (Christie, 1982) to generate fatty acid methyl esters (FAMEs). FAMEs were separated and identified by gas chromatography and compared with external standards (Laporte and Trushenski, 2011).

To evaluate contrasts in mean oil content as a function of sex, age, and season of collection (fall and spring) we used a series of two-way ANOVAs. Homogeneity of variance among treatments and normal distribution of treatment-specific responses were verified using Levene's and Bartlett tests, respectively, for all parametric analyses. Simple linear regression was used to describe the relationship of individual condition (K) and oil content. Finally, we evaluated the mean response of LC-PUFAs, EPA, and DHA from mature female muscle and ovary tissue using ANOVA to understand how the mean values of these fatty acids varied with season, month, and tissue type.

The multivariate fatty acid profile was determined for each individual and we focused on those fatty acids that comprised at least 1% of total FAMEs. Non-parametric, permutational multivariate analysis of variance (PERMANOVA) was performed to evaluate contrasts in the multivariate composition of the fatty acid profiles of females using the independent categorical variables season, spawning phase, and tissue (muscle or ovary). The "vegan" package (Oksanen et al., 2017) in the R statistical programming language (R Core Team, 2016) was used to perform all multivariate analyses. An analysis of similarity percentages "SIMPER" was used to identify individual fatty acids that contributed to dissimilarity among or between treatment levels. Additionally, we performed a qualitative analysis to determine contrast in seasonal and tissue-specific coefficient of distance (D_{jh}) values (Turchini et al., 2006). Coefficient of distance is an index that compares the difference in fatty acid profiles of muscle and ovarian tissue between the spring and fall groups, calculated as:

$$D_{jh} = \left[\sum_{i=1}^{n} \left(p_{ij} - p_{ih}\right)^2\right]^{\frac{1}{2}}.$$

P_{ij} is the percent content of the ith fatty acid in the spring seasonal group (j) and P_{ih} is the percent content of the ith fatty acid in the fall seasonal group (h). Only individual fatty acids species were used in the calculation of D_{jh}. The D_{jh} values were calculated from mean compositional data and a single value was calculated for each tissue type and season.

3. Results

Muscle oil content of the 171 individual tissue samples were taken from male, female, and unsexed individual Gulf Menhaden. Oil content exhibited seasonal and condition-specific contrasts. There was no significant difference ($F_{2,130} = 0.14$, $p = 0.869$) in mean oil content between males (mean 0.362 mg g^{-1}) and females (mean 0.357 mg g^{-1}), but there was a significant difference in mean muscle oil content between the fall and spring periods ($F_{1,130} = 150.66$, $p < 0.001$). No interaction (season × sex) effect was observed ($F_{1,130} = 0.26$, $p = 0.614$). Mean oil content was 0.369 mg g^{-1} in the fall and 0.174 mg g^{-1} in the spring for pooled males and females. Immature/unsexed Gulf Menhaden exhibited similar mean spring oil content to males and females sampled during that season, 0.168 mg g^{-1} (Fig. 1); no immature Gulf Menhaden were sampled in the fall period. We did not observe significant differences in pooled male and female mean muscle tissue oil content among age classes one, two, and three ($F_{2,80} = 0.68$, $p = 0.508$, Fig. 2) nor was there a significant interaction effect (season × sex, $F_{2,80} = 1.96$, $p = 0.147$). However, there was a significant seasonal effect ($F_{2,80} = 52.59$, $p < 0.001$). We observed significant differences in mean oil content between seasons in mature female tissue ($F_{1,90} = 8.55$, $p < 0.001$, Fig. 3) and significant differences in mean oil content between tissue types (muscle and ovary, $F_{1,90} = 35.25$, $p < 0.001$). No interaction effect was observed (season × tissue, $F_{1,90} = 1.69$, $p = 0.196$).

We evaluated the mean responses of season and individual condition on oil content (pooled male, female, and unsexed individuals) to understand their respective impacts. We found a significant ($p < 0.01$) and positive ($\beta_1 = 5.42$, 95% confidence interval 2.91 to 7.92) relationship of the scaled value of muscle oil content to Fulton's condition factor (K; Fig. 4a). Fulton's condition exhibited monthly contrasts, increasing during the calendar year (Fig. 4b). Our evaluation of the mean response of mature female's tissue EPA, LC-PUFA, and DHA, using ANOVA, indicated variable mean response of these fatty acid species to season and tissue type (Fig. 5). We found significant differences ($p < 0.001$) in mean EPA levels in ovary and muscle tissue but did not observe seasonal differences in mean EPA level. Mean DHA and mean LC-PUFA levels both exhibited differences in response to its season of collection ($p < 0.05$) and tissue ($p < 0.05$).

Our analysis indicated seasonal changes in the multivariate composition of fatty acid profiles. We restricted our analysis to fatty acids ($n = 14$) that represented at least 1% of total FAMEs: 16:2n−4, 16:3n−4, 18:2n−6 (linoleic acid), 18:4n−3 (stearidonic acid), 20:4n−6 (arachidonic acid), 22:6n−3 (docosahexaenoic acid), 16:0 (hexadecaenoic acid), 18:0 (octadecanoic acid), 18:1n−9 (oleic or cis−9-octadecanoic acid), 18:1n−7, 14:0 (tetradecanoic acid), 16:1, 20:5n−3 (eicosapentaenoic acid), and 22:5n−3 (docosapentaenoic acid). The PERMANOVA analysis indicated the existence of significant differences ($p < 0.001$) in the multivariate composition of the major fatty acid constituents of

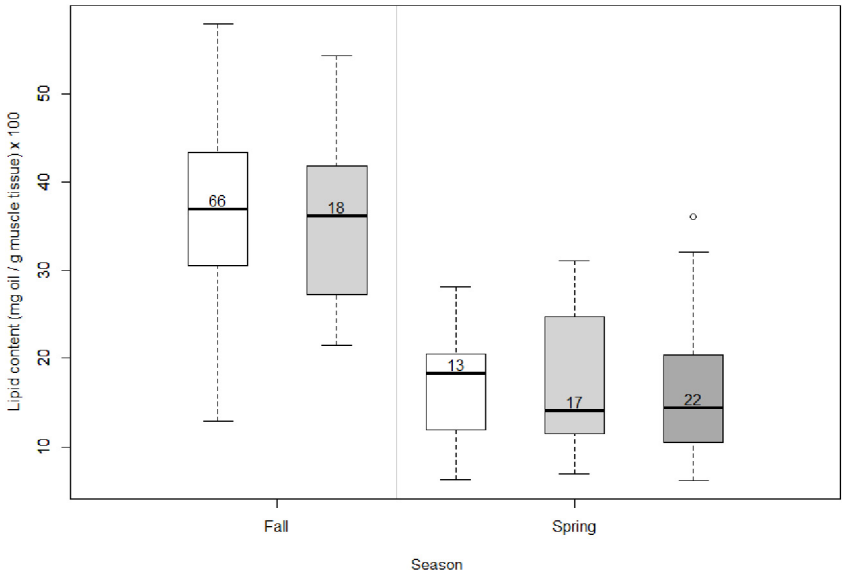

Fig. 1. Lipid content (mg oil per gram of muscle tissue × 100) of female (open box), male (light gray box), and unsexed (dark gray) Gulf Menhaden for the fall and spring seasonal periods. No unsexed fish were analyzed in the fall. Dark lines in each box represent median values, the box is the interquartile range (IQR, 25th to 75th percentile values), and whiskers are range of the data, up to 1.5 X the lower and upper IQR. Those data outside the range of the whiskers are plotted as points. The number of samples are displayed in each box.

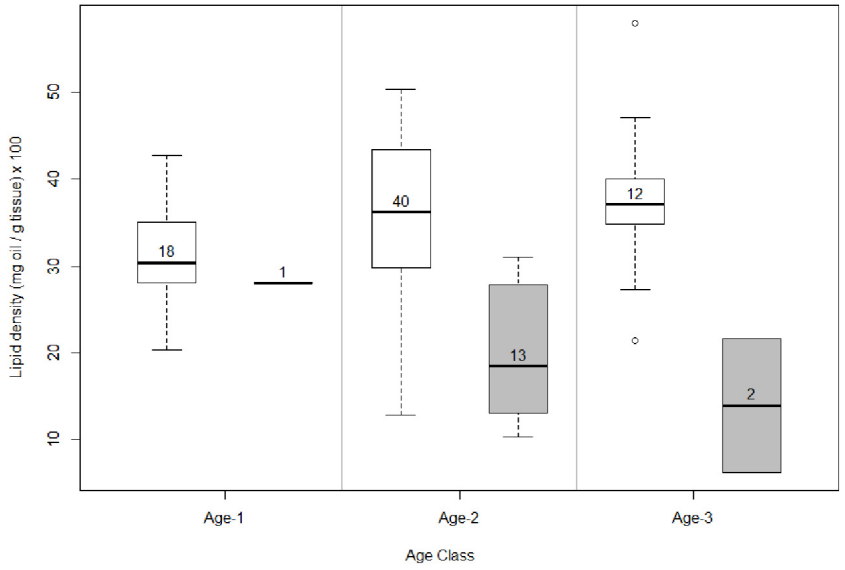

Fig. 2. Age-specific and seasonal, fall (open boxes) and spring (gray boxes), contrasts in Gulf Menhaden lipid content (mg oil per gram of muscle tissue × 100, sexes pooled). Dark lines in each box represent median values, the box is the interquartile range (IQR, 25th to 75th percentile values), and whiskers are range of the data, up to 1.5 X the lower and upper IQR. Those data outside the range of the whiskers are plotted as points. The number of samples are displayed in each box.

Table 1
PERMANOVA model to evaluate the impact of season of collection on the composition of the nine fatty acid species that make up at least 1% of total fatty acid composition of mature female Gulf Menhaden muscle tissue.

Factor	d.f.	Sum of squares	Mean squares	*F* Model	R^2	*p*-value
Season	1	119.99	119.99	9.58	0.09	0.001
Tissue	1	37.33	37.33	2.98	0.03	0.027
Season X tissue	1	26.22	26.22	2.09	0.02	0.06
Residuals	96	1202.47	12.53		0.87	
Total	99	1386			1	

mature, female Gulf Menhaden collected in different seasons (fall vs. spring, $p = 0.001$, Table 1) and from different tissue (muscle vs. ovary, $p = 0.027$). We note the variation in sampling intensity: our analysis included 65 muscle and 10 ovary samples collected in the fall and 19 muscle and 6 ovary samples collected in the spring.

The similarity percentages analysis indicated that a common suite of fatty acids were responsible for the observed differences in fatty acid composition of mature females evaluated in the spring and fall and from muscle vs. ovary tissue. The fatty acids 16:0, 20:5n−3, 22:6n−3, 14:0, 16:1, and 18:1n−9 contributed to 83.3%

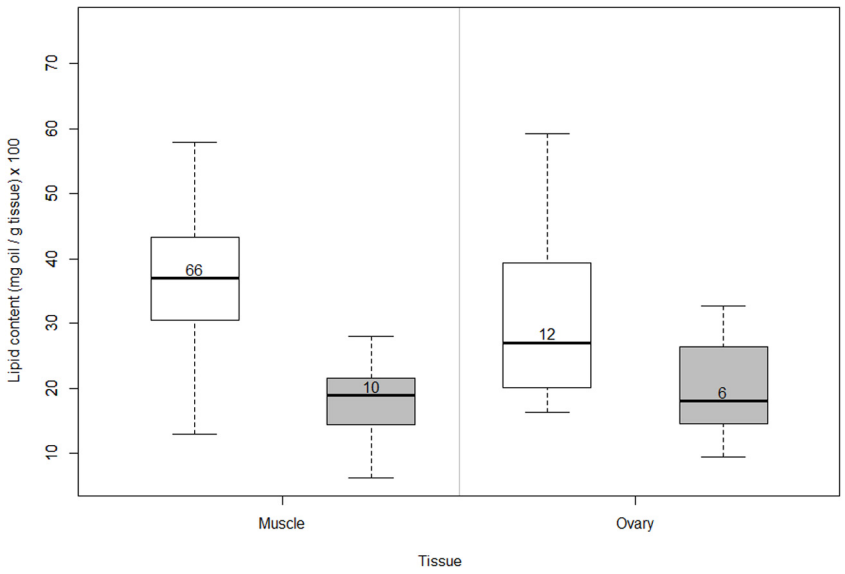

Fig. 3. Tissue specific (muscle and ovary) and seasonal (fall, open boxes; spring, gray boxes), contrasts in mature female Gulf Menhaden lipid content (mg oil per gram of muscle tissue × 100). Dark lines in each box represent median values, the box is the interquartile range (IQR, 25th to 75th percentile values), and whiskers are range of the data, up to 1.5 X the lower and upper IQR. The number of samples are displayed in each box.

Table 2a
Similarity percentages analysis "SIMPER" of the contrast in the multivariate fatty acid composition measured in the spring and fall of the 14 constituents that comprise at least 1% of total fatty acid composition of mature female Gulf Menhaden muscle tissue.

Spring vs. Fall	Average contribution to overall dissimilarity	Standard deviation of contribution	Average (Spring)	Average (Fall)	Cumulative contribution
16:0	0.033	0.023	30.54	25.51	21.3%
20:5n-3	0.031	0.022	6.35	11.50	41.1%
22:6n-3	0.030	0.026	6.20	10.11	60.1%
14:0	0.014	0.011	11.06	10.12	68.8%
16:1	0.013	0.011	12.77	11.11	77.2%
18:1n-9	0.010	0.007	6.83	7.08	83.3%
18:0	0.005	0.004	5.32	4.97	86.8%
22:5n-3	0.004	0.003	1.69	2.16	89.6%
18:4n-3	0.004	0.003	1.79	1.77	92.2%
16:3n-4	0.003	0.002	1.53	1.34	94.1%
16:2n-4	0.003	0.002	1.61	1.26	95.8%
20:4n-6	0.002	0.002	0.97	1.24	97.3%
18:1n-7	0.002	0.002	3.60	3.48	98.8%
18:2n-6	0.002	0.001	1.29	1.20	100.0%

Table 2b
Similarity percentages analysis "SIMPER" of the contrast in the composition measured in the muscle and ovary tissue of the 14 constituents that comprise at least 1% of total fatty acid composition of mature female Gulf Menhaden muscle tissue.

Muscle vs. Ovary	Average contribution to overall dissimilarity	Standard deviation of contribution	Average (Muscle)	Average (Ovary)	Cumulative contribution
22:6n-3	0.028	0.023	9.05	9.54	19.8%
20:5n-3	0.026	0.019	10.12	10.71	38.3%
16:0	0.026	0.020	26.91	26.00	56.6%
14:0	0.012	0.010	10.29	10.71	65.4%
16:1	0.012	0.010	11.46	11.87	73.9%
18:1n-9	0.011	0.009	7.22	5.97	82.0%
18:0	0.006	0.005	5.04	5.12	86.3%
22:5n-3	0.004	0.003	2.00	2.28	89.0%
18:4n-3	0.004	0.003	1.81	1.63	91.6%
20:4n-6	0.003	0.002	1.14	1.34	93.5%
16:3n-4	0.003	0.002	1.42	1.23	95.3%
16:2n-4	0.002	0.002	1.38	1.19	96.9%
18:1n-7	0.002	0.002	3.53	3.43	98.5%
18:2n-6	0.002	0.001	1.20	1.34	100.0%

of the observed difference in the seasonal comparison (Table 2a). Mean values of 16:0, 14:0, and 16:1 were elevated in the spring relative to those in the fall, and mean values of 20:5n−3, 22:6n−3, and 18:1n−9 were reduced in the spring relative to the fall. The same constituent fatty acids in the seasonal analysis (16:0, 20:5n−3, 22:6n−3, 14:0, 16:1, and 18:1n−9) were responsible for 82% of the significant difference in the muscle and ovary tissue contrast (Table 2b). The fatty acids 22:6n−3, 20:5n−3, and 14:0 were elevated in the ovary relative to those measured in the muscle tissue. The results of the similarity percentages are evaluated graphically (Fig. 6) and illustrate the relative reduction and enhancement of constituent fatty acids. Muscle samples collected in the fall did tend to have lower mean proportions of 20:5n−3 and 22:6n−3 and higher proportions of 14:0, 16:0, 16:2n−4, 16:3n−4,

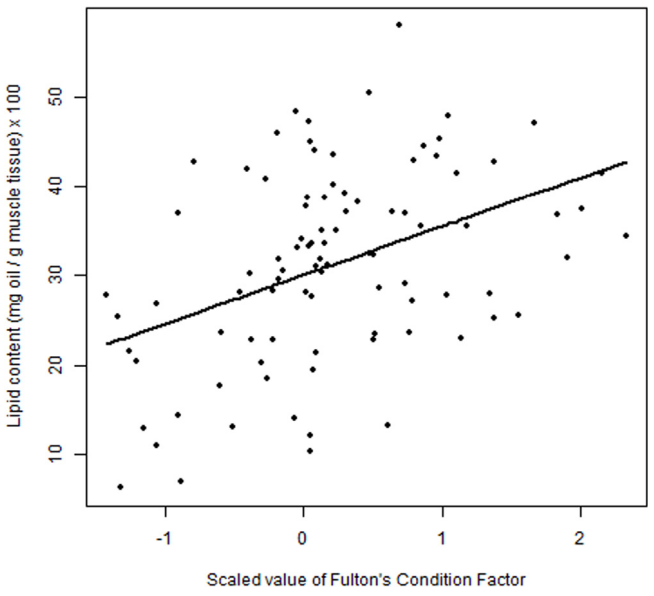

Fig. 4a. Linear relationship of the scaled (number of standard deviations) and centered (to the mean) value of Fulton's condition factor and Gulf Menhaden lipid content (mg oil per gram of muscle tissue × 100) for n = 171 individuals.

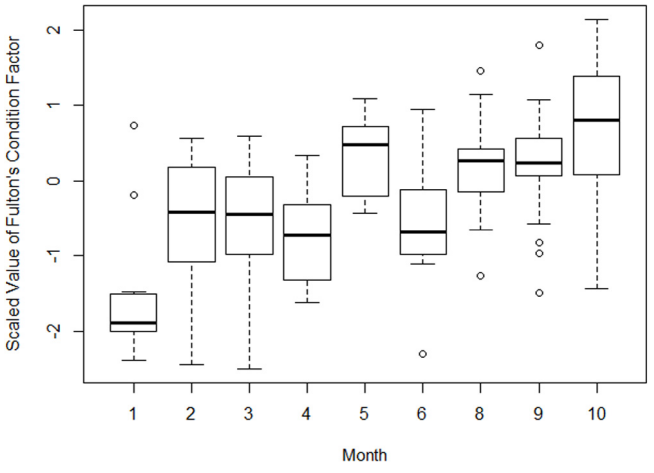

Fig. 4b. Relationship of the scaled (number of standard deviations) and centered (to the mean) value of Fulton's condition factor and month of collection for Gulf Menhaden. Data were not available for July, November, and December. Dark lines in each box represent median values, the box is the interquartile range (IQR, 25th to 75th percentile values), and whiskers are range of the data, up to 1.5 X the lower and upper IQR. Outliers are indicated by open circles. The number of individuals in each month range from n = 12 to n = 61.

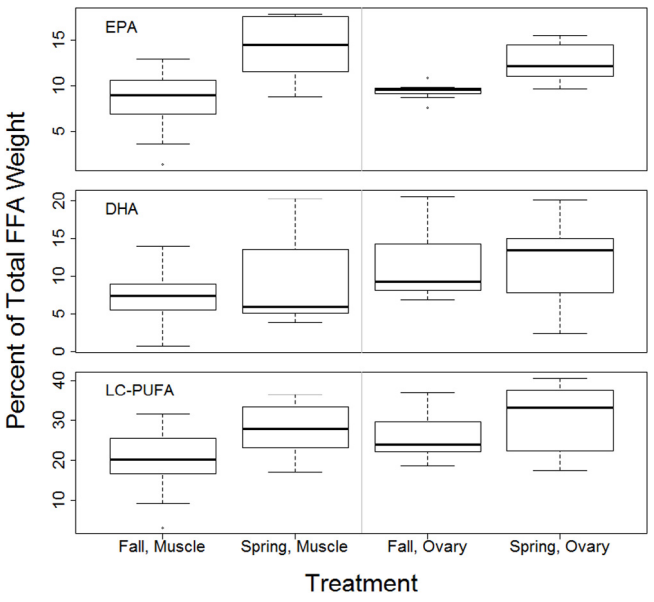

Fig. 5. Levels (% total fatty acid methyl esters) of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and long-chain polyunsaturated fatty acids (LC-PUFA) in mature female Gulf Menhaden muscle and ovary tissues collected in the spring (muscle, n = 10 and ovary, n = 6) and fall (muscle, n = 66 and ovary, n = 12).

and 18:2n−6 relative to muscle samples collected in the spring. With respect to ovarian tissues, samples collected in the fall had lower levels of 16:2n−4, various monounsaturated fatty acids, and 20:5n−3 and higher proportions of 18:4n−3, 20:4n−6, 22:6n−3, and, especially, 18:2n−6 relative to samples collected in the spring. Coefficient of distance values indicate seasonal differences in composition were more pronounced in ovarian tissue ($D_{jh} = 1.11$) than muscle tissue ($D_{jh} = 0.65$).

4. Discussion

In this work, we report the presence of significant seasonal variation in the temporal dynamics of oil content and fatty acid composition of Gulf Menhaden collected in the nGOM. There is significant temporal variation in oil content and fatty acid composition and evidence of partitioning of fatty acids between muscle and ovarian tissues, but we did not find detectable differences in these metrics based on age or sex within the same season. The seasonal contrasts we observe are likely an effect of the interaction of the stock with the temporally and spatially dynamic variation of phytoplankton productivity in the northern Gulf of Mexico, the seasonal movement of the stock between the shelf and the coastal zone, and metabolic processes associated with provisioning for spawning. The observed variations in seasonal oil content and fatty acid composition have consequences for understanding the magnitude of ecosystem services that Gulf Menhaden provide and for the utility of using fatty acids as biomarkers to detect the extent of predation on Gulf Menhaden.

Our analysis of the variation in muscle tissue oil collected in the fall and spring indicates that Gulf Menhaden respond to changes in seasonal productivity in the northern Gulf of Mexico. Abiotic forcing mechanisms may serve to promote the development of primary and secondary production by delivering nutrients and promoting mixing (Grimes, 2001). Additionally, gonadal recrudescence occurs between August and November, with the greatest Gonadosomatic Index (GSI) values seen in November for both male and female Gulf Menhaden (Brown-Peterson et al., 2017), which likely also contributes to these differences.

Temporal changes in fatty acid composition of fish tissue has been documented in a variety of fish stocks (Pethybridge et al., 2014). The observed temporal contrasts in fatty acid composition we document here are likely due to variations in the regional phytoplankton community. In the nGOM, the community composition of surface-level phytoplankton taxa is controlled by variations in salinity, temperature, and light conditions (Qian et al., 2003) all of which exhibit intra- and inter-annual variability. Because fatty acid composition of autotrophic plankton varies depending on taxonomy (Budge et al., 2014; Dalsgaard et al., 2003), the relative abundance of fatty acids available to planktivores similarly varies as a reflection of the composition of microalgae in the food web. Further, the enrichment and depletion of LC-PUFA among consumers follows seasonal patterns: individuals feeding on diets rich in

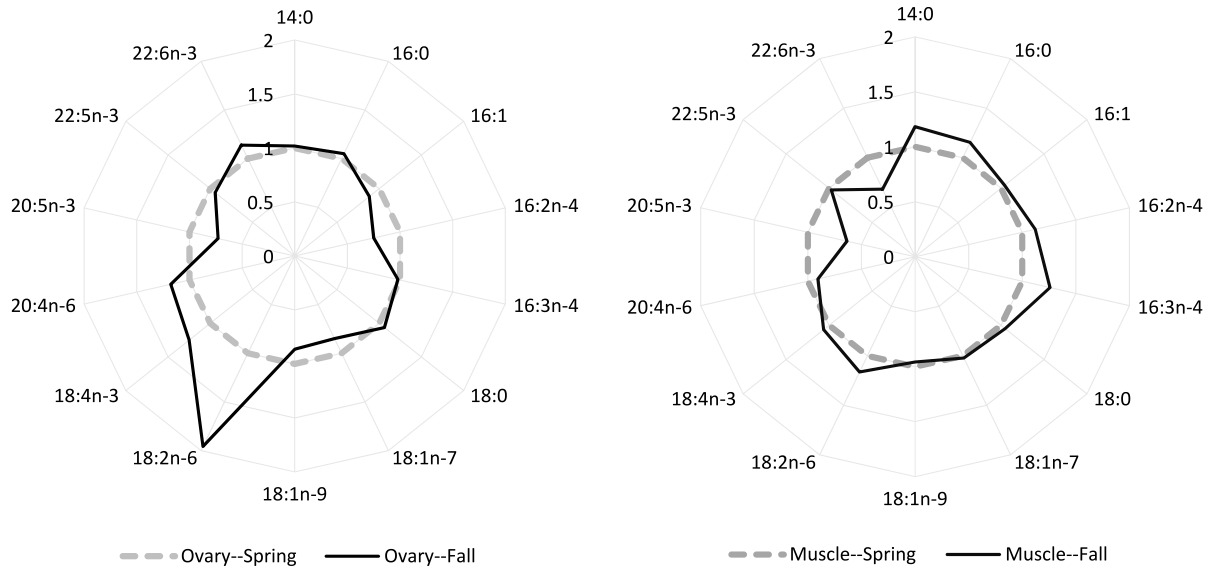

Fig. 6. Fatty acid profiles of ovary tissue (left panel) and muscle (right panel) from mature female Gulf Menhaden in the spring (gray line) and fall (black line) seasons. Note that all values have been standardized relative to the spring samples by dividing the mean % fatty acid methyl esters by the appropriate mean observed for the spring samples. Consequently, all spring values are equal to 1 (standardized to themselves); fall values greater than 1 indicate higher levels and less than 1 indicate lower levels compared to the spring values.

diatoms and dinoflagellates are enriched with respect to LC-PUFA during cooler months and depleted with respect to LC-PUFA when cyanobacteria are more abundant during the warmer summer months when the water column is more stratified (Chakraborty and Lohrenz, 2015). In a two-year (1982 and 1983) analysis of Gulf Menhaden fatty acid composition, Joseph (1985) reported significant ($\alpha = 0.05$) changes in the annual mean values of 14:0, 16:0, 16:1n−7, 18:1n−7, 18:1n−9, 22:6n−3, and 22:5n−3. When fatty acid composition was evaluated at smaller temporal scales (June, July, August, September, and October), differences in the mean values of all ten fatty acids evaluated (14:0, 16:0, 18:0, 16:1n−7, n−718:1n−7, 18:1n−9, 18:4n−3, 20:5n−3, 22:6n−3, and 22:5n−3) were apparent. Joseph's (1985) work and this study document significant intra-annual variation in the composition of fatty acids in the nGOM food web. Such seasonal variation complicates the use of fatty acids as biomarkers for predators of Gulf Menhaden, and this work is a necessary step to establishing baseline conditions of fatty acid composition in the nGOM.

In addition to intra-annual productivity dynamics, the observed contrast in seasonal oil content and monthly fatty acid composition may be a result, in part, of the movement dynamics of the Gulf Menhaden stock. Gulf Menhaden exhibit seasonal migration, occupying the coastal zone in the mid-spring to early fall and then moving to the inner- and mid-shelf in the late fall, residing and spawning in offshore waters until the following spring. Chakraborty and Lohrenz (2015) have characterized the taxonomic composition of plankton in the nGOM into "estuarine", "inner shelf", "mid-shelf", and "slope" regions. These authors report that the relative productivity and taxonomic characteristics of the autotrophic plankton vary in each of these regions, and in an analysis of pigments report that estuarine and inner shelf regimes are dominated by diatoms (>50% of chl *a*), cryptophytes and cyanobacteria. The mid-shelf region displays a mixed taxonomic assemblage that is influenced temporally by the extent of stratification and the magnitude of freshwater influence. Comprehensive spatial sampling of Gulf Menhaden and their associated prey in the coastal and shelf habitats that they inhabit would hasten understanding which of the interacting factors promotes variability in tissue-specific fatty acid composition.

The results of the temporal analysis of condition, oil content, and fatty acid composition indicate that Gulf Menhaden exhibit pronounced intra-annual metabolic changes. Spawning of Gulf Menhaden occurs from October to March (Brown-Peterson et al., 2017), with peak spawning in December (Lewis and Roithmayr, 1980). Our observation of seasonal variations in oil content are consistent with enhanced lipid storage following coastal residence in preparation for migration to relatively oligotrophic offshore waters for spawning. Samples of the oil content of fish muscle tissue taken from the spring exhibit reduced levels indicating the metabolic costs of overwintering and spawning. Because oil content is a primary determinant of energy density (Anthony et al., 2000), the metabolic costs of spawning can be determined from the difference in energy storage observed between the two seasons. Our observations that 18:2n−6 is elevated in ovary tissue in the fall indicates that mature females are provisioning eggs with this fatty acid. In Pacific white shrimp, *Penaeus vannamei*, elevated 18:2n−6 levels were associated with improved survival in some larval stages (Palacios et al., 2001). Similarly, the growth performance (weight gain and feed efficiency) of juvenile Grass Carp, *Ctenopharyngodon idellus*, is enhanced in a diet with elevated levels of 18:2n−6 (Zeng et al., 2016), although ovum enrichment with this fatty acid has proven problematic in terms of White Bass, *Morone chrysops*, reproductive success (Lane and Kohler, 2006; Lewis et al., 2010).

5. Conclusion

At the ecosystem level, the contrast in the energy density of Gulf Menhaden observed between seasons indicates that the value of Gulf Menhaden as a prey item and to the fishery in the northern Gulf of Mexico varies substantially during the year. Recent work evaluating annual oil content patterns of Gulf Menhaden (Leaf, 2017) indicates that annual variation in oil content also exists and that variations in oil content are positively related to the magnitude of spring Mississippi River discharge and negatively related to El Niño phase. The intra-annual variation in oil content we report in this work among age classes and between sexes has implications in our understanding of the temporal importance of Gulf Menhaden as a prey item. A variety of trophic models have described the role of Gulf Menhaden in the diets of fishes of higher trophic levels (Geers et al., 2014; Sagarese et al., 2016). These models do not incorporate the pronounced intra-annual variation in oil content and

may serve to overestimate the importance of Gulf Menhaden as a prey item during some parts of the year when it is a relatively lower value prey. There continues to be a critical need to understand predator diets (Oshima and Leaf, 2018) and to develop seasonal models of food web dynamics in the nGOM. The oil content of the harvested stock varies inter-annually (Leaf, 2017) and here we show that fish oil characteristics and quantity varies seasonally as well. An aspect not investigated in this work is the spatial variability in the fatty acid composition of Gulf Menhaden that has been previously reported, specifically significant differences in mean values of 16:0, 18:0, 18:1n−7, 18:1n−7, 18:1n−9, 18:4n−3, 22:6n−3, and 22:5n−3 in the oil of Gulf Menhaden sampled from the commercial reduction facilities at Cameron, LA, and Moss Point, MS (sampled in this study) (Joseph, 1985). Further research on oil content and fatty acid composition of Gulf Menhaden may help the fishery target Gulf Menhaden stocks with preferred oil content and composition.

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