

ARTICLE

# Ontogenetic differences in muscle fatty acid profile of white sharks *Carcharodon carcharias* off Guadalupe Island, México

Diferencias ontogenéticas de la composición de ácidos grasos del músculo del tiburón blanco *Carcharodon carcharias* en Isla Guadalupe, México

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**Resumen.-** *Carcharodon carcharias* es una especie protegida que ocupa el tope de la mayoría de las redes tróficas en las que ocurre. Áreas de agregación que han sido relacionadas a eventos de alimentación son de especial interés para la conservación de la especie, siendo Isla Guadalupe una de ellas. El propósito de este estudio fue describir el perfil de ácidos grasos del músculo de esta especie por primera vez en Isla Guadalupe, México, a partir de biopsias obtenidas mediante un método no letal, con el fin de probar la existencia de diferencias ontogenéticas y sexuales en relación a la dieta y al uso de hábitat. Se exploran los perfiles de ácidos grasos y de biomarcadores de diferentes individuos como fuentes de información integrada de su dieta. El análisis de los perfiles de ácidos grasos de individuos de diferentes tallas (2,3-5,0 m) sugiere la existencia de un cambio de dieta entre juveniles y adultos que ocurre alrededor de los 3 m. Los biomarcadores de ácidos grasos indicaron un mayor grado de carnivorismo en adultos que en juveniles. Asimismo, sirvieron para inferir información acerca del uso del hábitat, sugiriendo que los juveniles se alimentan preferentemente en aguas poco profundas cerca de la costa, mientras que los adultos se alimentan en aguas profundas de zonas oceánicas y costeras. Este estudio representa una primera aproximación para considerar el uso de ácidos grasos como una herramienta relevante para elucidar cambios ontogenéticos de dieta y uso de hábitat a lo largo de la ontogenia de *C. carcharias*. Sin embargo, para corroborarlo, son necesarios estudios con un mayor número de muestras.

**Palabras clave:** *Carcharodon carcharias*, ácidos grasos, músculo, biomarcadores, Isla Guadalupe

**Abstract.-** *Carcharodon carcharias* is a protected species occupying the apex of most marine foodwebs where they are present. Aggregation areas, such as Guadalupe Island, México, that have been related to feeding events, are of special interest for this species conservation. The aim of this study was to describe the fatty acid profile of *C. carcharias*' muscle for the first time in Guadalupe Island, using non-lethal biopsy methods to determine ontogenetic and sex differences in relation to diet and habitat use. Fatty acid profiles and biomarkers from different individuals are explored as a source of integrated information of their diet. Analysis of the fatty acid composition of individuals with varying total lengths (2.3-5.0 m) suggested a dietary shift between juveniles and adults occurring at approximately 3 m. Fatty acid biomarkers indicated a higher degree of carnivorousness in adults than in juveniles. Additionally, these ecological tracers suggested that juveniles feed in shallow waters close to the coast, while adults feed in deep waters along inshore and offshore areas. This study represents a first step towards using fatty acid composition as a relevant tool for further understanding dietary shifts and habitat use throughout the ontogeny of *C. carcharias*. However, to corroborate this, further studies with larger sample sizes are required.

**Key words:** *Carcharodon carcharias*, fatty acids, muscle, biomarkers, Guadalupe Island

## INTRODUCTION

*Carcharodon carcharias* (Linnaeus, 1758) occupies the apex of most marine foodwebs where present (Bonfil *et al.* 2005). Although this species is listed on Appendix II of The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and is classified as "Vulnerable" on the Red List of Threatened Species (International Union for Conservation of Nature) (Rigby *et al.* 2019), our current understanding of the life-history

and foraging ecology of this species has been consistently improving (Kerr *et al.* 2006, Carlisle *et al.* 2012, Fallows *et al.* 2013, French *et al.* 2014, Jaime-Rivera *et al.* 2014, Pethybridge *et al.* 2014, Meyer *et al.* 2017, Tamburin *et al.* 2019). Given the conservation status of this species, methods relying on nonlethal sampling techniques are required to study its trophic ecology (Pethybridge *et al.* 2014). However, studies based on biochemical methods, such as fatty acid (FA) analyses, on foraging ecology in the

Northeast Pacific are scarce (Kerr *et al.* 2006, Carlisle *et al.* 2012, Jaime-Rivera *et al.* 2014, Tamburin *et al.* 2019). FA analysis has been shown to be a highly informative nonlethal method to study foraging ecology and food webs (Iverson *et al.* 2004), and is typically employed in three main lines of study: examining predator dietary shifts through the identification of intraspecific differences in FA distributions or “signatures” (Iverson 1993); using individual or ratios of FAs as biomarkers of trophic pathways and habitat (Dalsgaard *et al.* 2003, Meyer *et al.* 2019); and finally, estimating the diet from the FA signatures of predator and prey (Budge *et al.* 2006).

The FA signatures of marine species can be traced through several trophic levels up to the top predators (Ramos & González-Solis 2012). For example, several studies have suggested elasmobranchs cannot synthesize long chain polyunsaturated fatty acids (LC-PUFA) considered as essential nutrients for several physiological processes (Brett & Müller-Navarra 1997, Tocher 2010, Sardenne *et al.* 2017). Instead, these FAs must be transferred across food webs through diet (Dalsgaard *et al.* 2003, Iverson *et al.* 2004), likely influencing trophodynamics (Caraveo-Patiño *et al.* 2009, Sardenne *et al.* 2017) and can thus be used to track dietary items (*e.g.*, diatoms and/or dinoflagellate origin) the predator is feeding on (Couturier *et al.* 2013a).

In elasmobranchs, adipose tissue is absent (Ballantyne 1997), and members of this group have a limited capacity for lipid oxidation in extrahepatic tissues (Zammit & Newsholme 1979, Ballantyne 1997, Speers-Roesch & Treberg 2010). Their muscle FA profiles can provide an integrated diet signal over time scales ranging from weeks to months (Beckmann *et al.* 2013, Meyer *et al.* 2019). Additionally, this signal may be representative for LC-PUFA rich prey items (Schauffler *et al.* 2005, Pethybridge *et al.* 2010, 2011) and habitat (Meyer *et al.* 2019). Hence, it is likely that the muscle FA profile of *C. carcharias* (especially their LC-PUFA content) would be influenced by its prey as suggested for other elasmobranchs (Couturier *et al.* 2013a); and may be used as an indicator of ontogenetic dietary shifts and habitat use (Wai *et al.* 2012, Beckmann *et al.* 2013, Meyer *et al.* 2017, 2019).

Knowledge on the muscle FA composition, compared to other tracers such as stable isotopes, could provide information on how this predator uses this foraging ground at a finer scale (Meyer *et al.* 2019). Previous findings of *C. carcharias* in this area would indicate the existence of ontogenetic differences in diet and habitat use (Carlisle *et al.* 2012, Jorgensen *et al.* 2012, Jaime-Rivera *et al.* 2014, Hoyos-Padilla *et al.* 2016). Therefore, intraspecific differences in FA composition would be expected to reflect

ontogenetic dietary changes and sexual differences related to physiological needs. However, there are no studies that describe the FA profile for this population or its intraspecific differences.

Guadalupe Island is an important aggregation area for *C. carcharias*, which has been related to feeding events, and is currently listed as a Protected Natural Area by the Mexican government (Jaime-Rivera *et al.* 2014, Skomal *et al.* 2015, Hoyos-Padilla *et al.* 2016). Due to the importance of the island for the species, and the conservation status of this population, the aim of this study was to describe for the first time the profile of muscle FA in specimens of *C. carcharias* from this area and to evaluate its composition as a feasible method to demonstrate ontogenetic and sexual differences in relation to diet and habitat use.

## MATERIALS AND METHODS

Nine samples of muscle tissue from sharks with total lengths (TL) ranging 2.5–4.8 m were obtained at Guadalupe Island (29°00'N, 118°26'W), 240 km off Baja California Peninsula (Hoyos-Padilla *et al.* 2016) during August–November 2015. Sharks were lured from a 6.4 m boat with bait and biopsied at the base of the first dorsal fin. A pole spear with a steel, rectangular shaped dart (RB), measuring 10.5, 0.8 and 0.4 cm in length, breadth and height, respectively (Reeb & Best 2006), with a stopper at the end to halt penetration (4.6 and 0.02 cm in diameter and thickness, respectively) as suggested by Jaime-Rivera *et al.* (2013). For each examined specimen, sex was recorded, and total length (TL) was estimated relative to vessel length. Samples were frozen and stored at -80 °C at Centro de Investigaciones Biológicas del Noroeste (CIBNOR) in La Paz, B.C.S.

### LIPID EXTRACTION AND FATTY ACID ANALYSIS

Muscle samples were freeze-dried and homogenized before lipid extraction. Total lipids were extracted with an adaptation of the method of Bligh & Dyer (1959). Samples were kept in solvent at -20 °C for 24 h (Christie 2003). An aliquot of 0.5 ml was used for direct transesterification. Total lipids were then quantified through gravimetric analysis.

Fatty acid methyl esters (FAMES) were obtained from the retained aliquot. Direct methanolic-HCl transesterification was undertaken (90 °C for 2 h). Subsequently, FAMES were extracted with heptane and distilled water and quantified using gas chromatography (Agilent Technologies 7820A, Santa Clara, Ca, USA). Fatty acids (FAs) ranging from 14:0 to 22:6n3 in the samples were identified by comparing the retention time against the Supelco standard (CRM47885). FA content was expressed as the mean percentage contributing to the overall FA profile (mean ± S.D.).

## STATISTICAL ANALYSES

Sharks were separated into two ontogenetic classifications (I and II). Classification I comprised of three size classes (juveniles, subadults, and adults) using TL of the individuals according to Bruce & Bradford (2012). Classification II comprised two size classes (juveniles-II and adults-II) with individuals classified in accordance to a dietary shift reported to occur at around 3 m (Tricas & McCosker 1984, Carlisle *et al.* 2012).

Due to the small sample size, non-parametric Wilcoxon rank sums-test was used to detect total lipid content differences between sex and size classes, which were performed with R 3.4.2 software (The Comprehensive R Archive Network)<sup>1</sup>.

Of the 40 FAs detected, 16 were used to carry out multivariate analyses (with mean  $\geq 0.1$  %) in order to identify significant differences among size classes (Table 1). To test for overlap between groups of Classification I, an Analysis of Similarities (ANOSIM) was performed based on the Bray Curtis distances calculated from the square root transformed data with a significance level of  $P < 0.05$ . The ANOSIM-R values proposed by Pethybridge *et al.* (2010); indicated to what extent the groups overlapped ( $R > 0.75$ : well separated groups;  $R = 0.50-0.75$ : separated but overlapping groups;  $R = 0.25-0.50$ : separated but strongly overlapping groups;  $R < 0.25$ : barely separated groups). A non-metric multidimensional scaling (MDS) was performed to visualize the grouping of individual sharks and a similarity percentage analysis (SIMPER) to quantify the contribution of the individual FAs to the separation between the designated groups.

**Table 1. Overall fatty acid composition (%) in muscle of *Carcharodon carcharias* relative to sex and size categories / Composición de ácidos grasos (%) con respecto al sexo y clases de talla**

	Total	Females	Males	Classification I			Classification II	
				Juveniles	Subadults	Adults	Juveniles-II	Adults-II
n	9	5	4	3	3	3	5	4
Fatty acid								
14:0	0.9 $\pm$ 0.4	1.0 $\pm$ 0.1	0.9 $\pm$ 0.6	1.1 $\pm$ 0.05	1.0 $\pm$ 0.1	0.7 $\pm$ 0.5	1.0 $\pm$ 0.0	0.7 $\pm$ 0.4
16:0	20.2 $\pm$ 2.8	19.8 $\pm$ 3.0	20.6 $\pm$ 3.0	21.1 $\pm$ 4.1	21.5 $\pm$ 1.7	17.9 $\pm$ 0.9	21.6 $\pm$ 2.7	18.3 $\pm$ 1.1
18:0	14.1 $\pm$ 3.1	15.0 $\pm$ 3.5	13.0 $\pm$ 2.5	17.5 $\pm$ 3.01	13.6 $\pm$ 0.4	11.3 $\pm$ 0.4	16.0 $\pm$ 2.6	11.7 $\pm$ 1.02
$\Sigma$ SFA	35.2 $\pm$ 5.3	35.8 $\pm$ 6.0	34.5 $\pm$ 5.9	39.7 $\pm$ 6.6	36.1 $\pm$ 2.1	29.9 $\pm$ 1.5	38.7 $\pm$ 4.3	30.8 $\pm$ 2.2
16:1n-9	0.4 $\pm$ 0.2	0.5 $\pm$ 0.2	0.3 $\pm$ 0.1	0.5 $\pm$ 0.3	0.4 $\pm$ 0.0	0.4 $\pm$ 0.0	0.4 $\pm$ 0.2	0.4 $\pm$ 0.0
16:1n-7	1.5 $\pm$ 0.8	1.8 $\pm$ 0.8	1.2 $\pm$ 0.7	1.7 $\pm$ 1.4	1.5 $\pm$ 0.3	1.3 $\pm$ 0.1	1.7 $\pm$ 0.8	1.2 $\pm$ 0.1
18:1n-9t	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.2	0.3 $\pm$ 0.1	0.5 $\pm$ 0.1	0.6 $\pm$ 0.0	0.4 $\pm$ 0.0	0.5 $\pm$ 0.0
18:1n-9	10.9 $\pm$ 1.9	11.7 $\pm$ 1.9	10.0 $\pm$ 1.6	11.2 $\pm$ 3.4	11.5 $\pm$ 0.0	10.1 $\pm$ 0.9	11.3 $\pm$ 2.2	10.4 $\pm$ 1.0
18:1n-7	6.1 $\pm$ 1.5	6.9 $\pm$ 1.4	5.2 $\pm$ 1.2	5.9 $\pm$ 2.8	6.4 $\pm$ 0.6	6.0 $\pm$ 0.3	6.1 $\pm$ 1.8	6.1 $\pm$ 1.3
20:1n-9	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.03 $\pm$ 0.06	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.0
22:1n-9	0.3 $\pm$ 0.1	0.3 $\pm$ 0.2	0.4 $\pm$ 0.1	0.2 $\pm$ 0.2	0.4 $\pm$ 0.1	0.4 $\pm$ 0.0	0.3 $\pm$ 0.1	0.3 $\pm$ 0.0
$\Sigma$ MUFA	19.5 $\pm$ 3.9	21.4 $\pm$ 3.9	17.2 $\pm$ 3.5	19.8 $\pm$ 8.0	20.3 $\pm$ 0.6	18.4 $\pm$ 0.9	20.4 $\pm$ 4.9	19.4 $\pm$ 1.1
18:2n-6t	0.3 $\pm$ 0.6	0.1 $\pm$ 0.1	0.5 $\pm$ 0.8	0.6 $\pm$ 1.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.4 $\pm$ 0.6	0.1 $\pm$ 0.0
18:2n-6	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.08	0.5 $\pm$ 0.0	0.5 $\pm$ 0.0	0.5 $\pm$ 0.0	0.4 $\pm$ 0.0
18:3n-3	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.09 $\pm$ 0.08	0.3 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.1	0.2 $\pm$ 0.0
20:4n-6 (ARA)	7.8 $\pm$ 2.1	8.1 $\pm$ 2.2	7.4 $\pm$ 2.2	7.11 $\pm$ 3.8	7.6 $\pm$ 0.5	8.7 $\pm$ 0.7	7.2 $\pm$ 2.4	8.4 $\pm$ 0.7
20:5n-3 (EPA)	0.9 $\pm$ 0.4	1.0 $\pm$ 0.4	0.7 $\pm$ 0.3	0.8 $\pm$ 0.6	0.8 $\pm$ 0.0	1.0 $\pm$ 0.2	0.8 $\pm$ 0.4	0.9 $\pm$ 0.1
22:6n-3 (DHA)	12.1 $\pm$ 5.1	12.1 $\pm$ 5.1	12.2 $\pm$ 5.9	6.5 $\pm$ 4.6	13.2 $\pm$ 1.4	16.7 $\pm$ 0.6	8.8 $\pm$ 4.1	16.1 $\pm$ 1.0
$\Sigma$ PUFA	21.8 $\pm$ 6.3	21.9 $\pm$ 6.8	21.6 $\pm$ 7.5	15.7 $\pm$ 8.6	22.5 $\pm$ 1.5	27.0 $\pm$ 0.3	18.1 $\pm$ 6.2	26.3 $\pm$ 1.5
$\Sigma$ n-3	13.2 $\pm$ 5.0	13.2 $\pm$ 5.3	13.1 $\pm$ 6.2	7.4 $\pm$ 5.3	14.2 $\pm$ 1.3	17.8 $\pm$ 0.7	9.8 $\pm$ 4.5	17.3 $\pm$ 1.2
$\Sigma$ n-6	8.6 $\pm$ 1.7	8.7 $\pm$ 2.2	8.5 $\pm$ 1.4	8.2 $\pm$ 3.3	8.3 $\pm$ 0.4	9.2 $\pm$ 0.7	8.2 $\pm$ 2.1	9.0 $\pm$ 0.7
Trophic markers								
DHA/EPA	14.6 $\pm$ 4.9	12.9 $\pm$ 5.9	16.8 $\pm$ 2.7	9.1 $\pm$ 3.8	17.4 $\pm$ 2.6	17.4 $\pm$ 2.5	11.8 $\pm$ 4.6	18.2 $\pm$ 2.5
ARA/EPA	9.9 $\pm$ 2.6	8.7 $\pm$ 1.7	11.4 $\pm$ 2.9	10.6 $\pm$ 4.4	10.0 $\pm$ 0.6	9.2 $\pm$ 2.2	10.2 $\pm$ 2.8	9.5 $\pm$ 1.9

Each value in the table represents the mean  $\pm$  standard deviation

SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids

<sup>1</sup>The Comprehensive R Archive Network, CRAN. © The R Foundation. <<https://cran.r-project.org>>

Subsequently, sharks were rearranged into Classification II. Juveniles-II included sharks with TL  $\leq 3$  m; and adults-II included sharks with TL  $> 3$  m. To test the differences between the groups of Classification II, the Permutational multivariate analysis of variance (PERMANOVA) was used with a significance level of  $P < 0.05$  and SIMPER to quantify the contribution of individual FAs to the separation between the designated groups. Finally, 2 biomarkers were compared using non-parametric analysis of variance (Wilcoxon ranked sum test). The first biomarker was docosahexaenoic acid/eicosapentaenoic acid (DHA/EPA) ratio, known to be an indicator of the degree of carnivorism (Dalsgaard *et al.* 2003, Milisenda *et al.* 2018); and the arachidonic acid/eicosapentaenoic acid (ARA/EPA) ratio which can provide information on benthic/coastal inputs (Pethybridge *et al.* 2014). All multivariate analyses were performed with the software PAST 3 (Hammer *et al.* 2001).

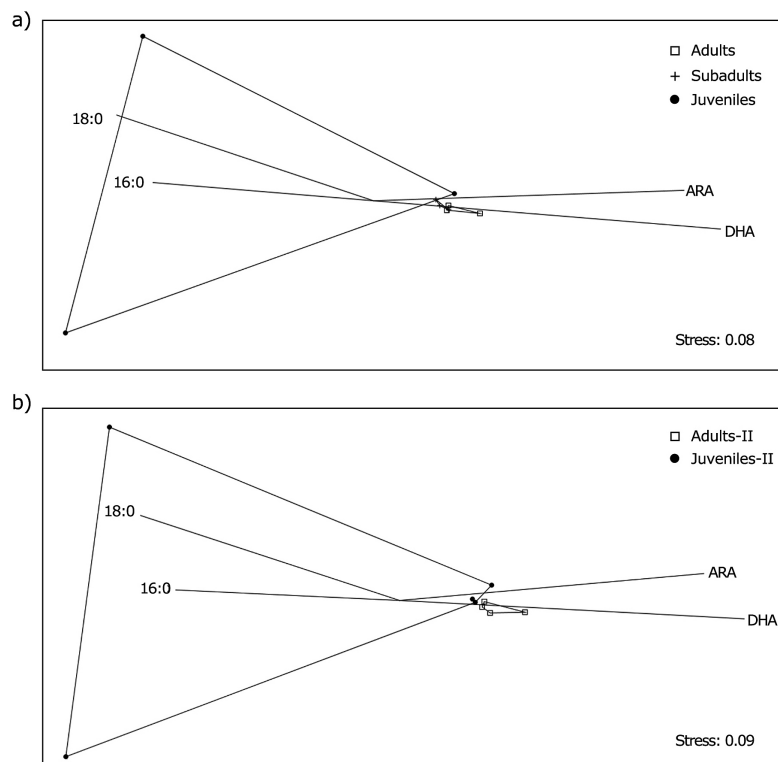
## RESULTS

Nine biopsies were performed: 5 corresponding to females, and 4 to males. In addition, the same individuals were arranged according to Classification I, comprising 3 juveniles (TL  $> 1.75$ -3.0 m); 3 subadults (TL  $> 3.0$ -3.6 m for males and TL  $> 3.0$ -4.8 m for females); and 3 adults (TL  $> 3.6$  m for males and TL  $> 4.8$  m for females); and Classification II comprised of 5 juveniles-II (TL  $> 1.75$ -3.0 m) and 4 adults-II (TL  $> 3.0$  m).

Mean total lipid content in muscle of *C. carcharias* was  $1.6 \pm 0.3\%$ . No differences were found relative to sex (Wilcoxon  $W = 13$ ;  $P > 0.05$ ), Classification I (Kruskal-Wallis  $H = 2.5$ ; d.f. = 2;  $P > 0.05$ ) or Classification II (Wilcoxon  $W = 11$ ;  $P > 0.05$ ).

Table 1 describes overall FA content data by sex, and Classification I and II. Total saturated fatty acids (SFA) accounted for  $35.2 \pm 5.3\%$ , polyunsaturated fatty acids accounted for  $21.8 \pm 6.3\%$  and monounsaturated fatty acids accounted for  $19.5 \pm 3.9\%$ . Predominant FAs were 16:0, 18:1n-9, DHA and ARA.

No significant differences were found when comparing overall FA composition relative to sex (ANOSIM  $R = -0.068$ ;  $P > 0.05$ ). ANOSIM test of Classification I suggested size classes were separated but overlapping (ANOSIM  $R = 0.5144$ ;  $P < 0.05$ ). According to pairwise-R values, juveniles and subadults were barely separated groups ( $R = 0.07$ ). On the other hand, subadults and adults, were separated but overlapping ( $R = 0.7$ ). Also, values indicated separation with overlapping between juveniles and adults ( $R = 0.63$ ). Overlapping by subadults was corroborated by MDS analysis, suggesting the existence of two size classes instead of three (Fig. 1). Therefore, subadult sharks were rearranged into either juveniles-II or adults-II (Classification II), which were significantly different (PERMANOVA  $F = 3.31$ ;  $P < 0.05$ ).



**Figure 1. Multi-Dimensional Scaling (MDS) of overall fatty acid composition of *Carcharodon carcharias* relative to size class: a) Classification I; b) Classification II / Análisis de Escalamiento Multidimensional (MDS) de la composición de ácidos grasos de *Carcharodon carcharias* respecto a su clase de talla: a) Clasificación I; b) Clasificación II**

Comparisons of DHA/EPA and ARA/EPA between juveniles-II and adults-II resulted in significant differences which were corroborated by MDS analysis (Fig. 2). The ratio DHA/EPA was significantly different (Wilcoxon  $W=45$ ;  $P<0.05$ ) with higher values in adults-II (18.12%) than in juveniles-II (11.8%). ARA/EPA ratio was significantly different (Wilcoxon  $W=45$ ;  $P<0.05$ ), with higher values in juveniles-II (10.2%) than in adults-II (9.5%).

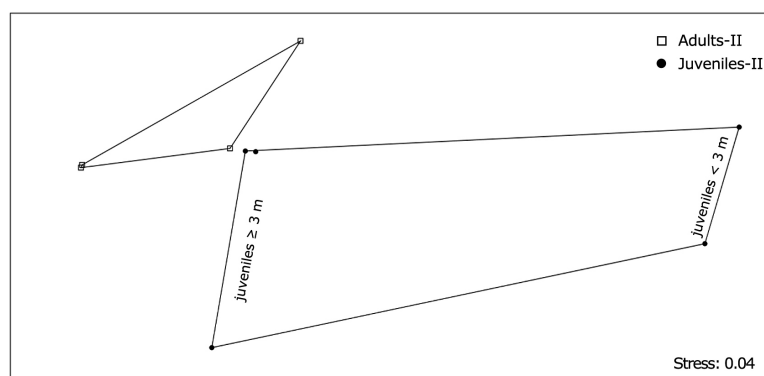
Overall average dissimilarity determined by SIMPER between size classes was 9.9% for both Classifications. The greatest sources of dissimilarity between groups for both classifications were DHA, 18:0, 16:0 and ARA. Adults were characterized by high contents of LC-PUFAs (ARA, DHA) while saturated 16:0 and 18:0 were characteristic of juveniles. The contribution of the FAs to the separation of the groups were: DHA (20.7%), 18:0 (10.25%), ARA (8.8%) and 16:0 (7.6%) for Classification I; and DHA (21.69%), 18:0 (10.56%), 16:0 (8.6%) and ARA (8.44%) for Classification II.

## DISCUSSION

### LIPID CONTENT AND FATTY ACID COMPOSITION

The results of this study suggest intraspecific differences in FA composition of the muscle of *Carcharodon carcharias*, which might be related to factors such as diet, habitat use, and to selective conservation of FAs necessary for physiological processes such as reproduction (Bell *et al.* 1985, 1992; Pethybridge *et al.* 2010, Rodriguez-Barreto *et al.* 2012, Beckmann *et al.* 2013, Couturier *et al.* 2013b, Davidson *et al.* 2014, Pethybridge *et al.* 2014, Meyer *et al.* 2017, 2019).

The low lipid content, and fatty acid composition of *C. carcharias* in Guadalupe Island was similar to what has been reported in Australia,  $2.9 \pm 0.6\%$  (Pethybridge *et al.* 2014),  $0.6 \pm 0.1\%$  (Meyer *et al.* 2017), and South Africa 0.61 and 1.85% (Davidson *et al.* 2011). As in other elasmobranch species, its muscle was high in LC-PUFAs, especially DHA and ARA as in accordance to previous studies (Pethybridge *et al.* 2010, 2014; Davidson *et al.* 2011, McMeans *et al.* 2012, Davidson & Cliff 2014, Meyer *et al.* 2017). Contents of LC-PUFAs could be explained by dietary intake due to reduced production capacity of these particular FAs by elasmobranchs (Beckmann *et al.* 2013, Couturier *et al.* 2013b, Pethybridge *et al.* 2014). Some level of enzyme-induced biomodification of FAs into LC-PUFA has been previously proposed for teleosts (Tocher 2003). However, a recent study of 106 distinct records of muscle profiles from different species showed that both low and high trophic level populations contained high levels of LC-PUFA, suggesting that increases of these FAs content may be driven by prey availability rather than by biomodification (Meyer *et al.* 2019). High contents of DHA were expected because the marine food web provides high levels of n-3 LC-PUFA (EPA and DHA) and relatively low levels of n-6 LC-PUFA (ARA) (Davidson *et al.* 2014). Moreover, elasmobranchs have been reported to present high contents of n-3 LC-PUFA, likely due to the prevalence of this family of FAs in marine environments (Nelson *et al.* 2000, 2001; Davidson *et al.* 2011).



**Figure 2. Multi-Dimensional Scaling (MDS) of biomarkers of *Carcharodon carcharias* relative to size class (Classification II) / Análisis de Escalamiento Multidimensional (MDS) de los biomarcadores de *Carcharodon carcharias* respecto a su clase de talla (Clasificación II)**



Since fish and other sharks have the ability to selectively deposit lipid and FAs in different tissues (Pethybridge *et al.* 2010), probably *C. carcharias* could be using extrahepatic tissues, such as muscle, as a reservoir for ARA in the form of phospholipids. Moreover, despite their low availability throughout marine prey, several extrahepatic tissues of elasmobranchs have been reported to show high contents of this FA, including brain, muscle and skin (Ballantyne 1997, Davidson & Cliff 2002, Stoknes *et al.* 2004, Økland *et al.* 2005, Davidson *et al.* 2011, Couturier *et al.* 2013a, b; Rohner *et al.* 2013, Pethybridge *et al.* 2014). Therefore, while relatively abundant, n-3 LC-PUFA are potentially stored in both muscle and liver because they are more readily available from diet, limited amounts of ARA would tend to be stored in extrahepatic tissues such as muscle, and even higher amounts in sub-dermal tissue (Bell *et al.* 1985, 1992; Davidson *et al.* 2014, Meyer *et al.* 2017).

It is hypothesized that the conservation of ARA in muscle can occur based on previous findings that have demonstrated that this FA is one of the main nutrients necessary to ensure reproductive success in many fish species (Tocher 2010). In fish, ARA is mobilized from cell membranes for the production of the prostaglandins PGE2 and PGF2, which are involved in sexual maturity and ovulation (Sargent *et al.* 1999, Tocher *et al.* 2008, Rodriguez-Barreto *et al.* 2012). Thereby, having a reserve of a FA necessary for reproduction but not widely available in the environment might be an advantage. However, because of the small sample size, it was not possible for us to corroborate whether ARA is conserved in the muscle for later use in *C. carcharias* reproduction. Studies using a wider range of size classes and tissues would be necessary to confidently determine this. Moreover, because FA composition of different tissues may be associated to divergent functions and underlying physiology (Meyer *et al.* 2017), it would be appropriate to compare changes on FA composition between tissues considered as possible ARA reservoirs such as muscle, and sub-dermal tissue to its possible destination (*e.g.*, gonads).

#### SIZE CLASS FATTY ACID COMPOSITION

Fatty acids have been found to serve as tracers for multiple factors (Meyer *et al.* 2019). Therefore, it is considered that when interpreting the FA biomarkers, the life history of the specific taxa examined must be taken into account to better understand the role of the factors driving the intraspecific differences. An ecophysiological approach is recommended because the physiology of all species interacts with the physical and biological environment, and might relate to ecological factors such as habitat use (Ferry-Graham & Gibb 2008, Meyer *et al.* 2019).

Previous studies suggested that the need to acquire and conserve ARA might influence trophodynamics by triggering species migration to foraging grounds to hunt on prey items rich in this FA (Caraveo-Patiño *et al.* 2009, Sardenne *et al.* 2017). For example, contents of ARA in muscle of marine mammals have been proven to be high (Davidson & Cliff 2014). A previous study in which the FA profiles of three species of marine mammals were compared with the profiles of muscle and liver of *C. carcharias*, suggested the marine mammals with higher contents of ARA in their muscle (bottlenose dolphin and common dolphin) were the most similar to the ones in the predator muscle (Davidson & Cliff 2014). Therefore, it is hypothesized that marine mammals could be an important source of this FA for *C. carcharias* as previously suggested for South Africa (Davidson & Cliff 2014). Additionally, it is inferred that to meet their physiological requirement for ARA, individuals of *C. carcharias* migrate to Guadalupe Island, where they have shown fidelity in that site, with juveniles that remain throughout the year and adults that arrive as soon as July and they leave as late as March (Domeier & Nasby-Lucas 2006, Hoyos-Padilla *et al.* 2016). This foraging ground serves as a haul-out and pupping site for the Northern elephant seal, *Mirounga angustirostris*, the Guadalupe fur seal, *Arctocephalus townsendi*, and the California sea lion, *Zalophus californianus* (Domeier & Nasby-Lucas 2006). Stable isotope analyses of adult white sharks off Guadalupe Island showed that individuals feed on these marine mammals (Jaime-Rivera *et al.* 2014). Hence, it is possible that *C. carcharias* adults assimilate an important portion of ARA from local marine mammals into muscle cell membranes. On the other hand, previous telemetry data (Hoyos-Padilla *et al.* 2016) showed juveniles undertake nocturnal shallow excursions possibly related to feeding on various prey including squid (Gallo-Reynoso 2005, Jaime-Rivera *et al.* 2014, Hoyos-Padilla *et al.* 2016); which has been reported to have high contents of ARA (2.4–11.8%) (Saito *et al.* 2014). Therefore, it is possible that this prey could be an alternative source of FA for juveniles that cannot yet feed on marine mammals.

Differences found for overall FA composition and biomarkers between juveniles-II and adults-II are probably a reflection of an ontogenetic dietary shift at approximately 3 m, when sharks have been reported to begin consuming marine mammals (Tricas & McCosker 1984, Carlisle *et al.* 2012). Our results suggest biomarkers may be used to identify ontogenetic dietary shifts even within groups by highlighting structural modifications of the FAs constituents of the muscle phospholipids (Couturier *et al.* 2013a, Murzina *et al.* 2016). For example, a significant increase ( $P = 0.003$ ) on DHA/EPA (from 11.8 in juveniles-

II to 18.1% in adults-II) associated to increases of DHA contents, indicate that *C. carcharias* adults are carnivores that feed on prey with a higher trophic level than the prey of juveniles (Dalsgaard *et al.* 2003, Meyer *et al.* 2019). Additionally, values obtained for this biomarker (DHA/EPA > 1) indicate that *C. carcharias* uses a trophic pathway dominated by dinoflagellates. Furthermore, high contents of DHA have previously been associated to the deep-sea environment and low temperatures for several chondrichthyan species (Dalsgaard *et al.* 2003, Meyer *et al.* 2019). For *C. carcharias*, conservation of high contents of this FA are probably related to vertical migrations to hunt in deep waters with low temperatures (Hoyos-Padilla *et al.* 2016), as well as their high trophic position (Sardenne *et al.* 2017). Two different studies support this hypothesis: 1) tagging of adults in Guadalupe Island indicate that adults reach depths of 200 m in waters with temperatures of less than 12 °C (Hoyos-Padilla *et al.* 2016); 2) a study using an autonomous underwater vehicle (AUV) to follow juveniles and adults, reported a subsurface predatory behaviour through a rapid vertical approach from depths around 150 m. *C. carcharias* was observed bumping and biting the AUV at depths ranging from 53 to 90 m (Skomal *et al.* 2015).

A biomarker proposed to provide information on benthic/coastal inputs is ARA/EPA, because the trophic webs of these environments (*e.g.*, reefs) are known to have higher contents of ARA (Sardenne *et al.* 2016). A decrease of this ratio as size increases suggests that juveniles have a higher benthic/coastal diet input than adults (Pethybridge *et al.* 2014, Sardenne *et al.* 2016, Meyer *et al.* 2019). Along with ARA/EPA ratio, isotopic analysis (Carlisle *et al.* 2012, Jaime-Rivera *et al.* 2014) and tracking (Weng *et al.* 2007, Hoyos-Padilla *et al.* 2016) have proved juveniles are coastal residents that utilize neritic habitats of the Southern California Bight, Baja California, and Guadalupe Island. Adults on the other hand, are known to undertake long offshore migrations in addition to their coastal feeding (Weng *et al.* 2007, Nasby-Lucas *et al.* 2009, Jorgensen *et al.* 2010, 2012; Carlisle *et al.* 2012, Domeier & Nasby-Lucas 2013, Jaime-Rivera *et al.* 2014) in which foraging has also been suggested (Carlisle *et al.* 2012, Jorgensen *et al.* 2012, Jaime-Rivera *et al.* 2014).

The present study represents a first step to consider the FA profile as a relevant tool for the corroboration of ontogenetic dietary shifts and habitat use of *C. carcharias*. If dietary shifts are related to the need of the species to obtain specific nutrients necessary to undertake physiological processes such as reproduction (Murzina *et al.* 2013, 2016); it is possible that different ontogenetic stages of *C. carcharias* could also be identified by associating their biochemical composition to their physiological state

instead of only considering their TL. Classification of individuals according to ontogenetic groups by their FA composition is important for this species, because sampling and recording morphometric/biologic traits such as TL or sexual maturity of individuals is difficult due to logistic restrictions. Further studies with larger sample sizes are needed to elucidate the FA ecophysiological features along the ontogeny of *C. carcharias*.

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