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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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 carnivorism 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.

Keywords: 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
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 extrapleptic tissues (Zammit & Newsholme 1979, Ballantyne 1997, Speers-Roesch & Treger 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 (Schaufler 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 Supeleco standard (CRM47885). FA content was expressed as the mean percentage contributing to the overall FA profile (mean ± S.D.).
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).

Of the 40 FAs detected, 16 were used to carry out multivariate analyses (with mean ≥ 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

| Fatty acid | Classification I | Classification II |
|-----------|------------------|------------------|
|           | Total Females | Males | Juveniles | Subadults | Adults | Juveniles-II | Adults-II |
| n         | 9 | 5 | 4 | 3 | 3 | 3 | 5 | 4 |
| SFA       | 35.2 ± 5.3 | 35.8 ± 6.0 | 34.5 ± 5.9 | 39.7 ± 6.6 | 36.1 ± 2.1 | 30.9 ± 4.5 | 38.7 ± 4.3 | 30.8 ± 2.2 |
| 16:0      | 14 ± 5.9 | 10.0 ± 1.0 | 0.9 ± 0.6 | 1.1 ± 0.5 | 1.0 ± 0.1 | 0.7 ± 0.5 | 1.0 ± 0.0 | 0.7 ± 0.4 |
| 16:1-19   | 20.2 ± 2.8 | 19.8 ± 3.0 | 20.6 ± 3.0 | 21.1 ± 4.1 | 21.5 ± 1.7 | 17.9 ± 0.9 | 21.6 ± 2.7 | 18.3 ± 1.1 |
| 18:0      | 14.1 ± 3.1 | 15.0 ± 3.5 | 13.0 ± 2.5 | 17.5 ± 3.0 | 13.6 ± 0.4 | 11.3 ± 0.4 | 16.0 ± 2.6 | 11.7 ± 0.2 |
| ∑SFA      | 35.2 ± 5.3 | 35.8 ± 6.0 | 34.5 ± 5.9 | 39.7 ± 6.6 | 36.1 ± 2.1 | 30.9 ± 4.5 | 38.7 ± 4.3 | 30.8 ± 2.2 |
| 16:1-19   | 0.4 ± 0.2 | 0.5 ± 0.2 | 0.3 ± 0.1 | 0.5 ± 0.3 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.2 | 0.4 ± 0.0 |
| 16:1-7    | 1.5 ± 0.8 | 1.8 ± 0.8 | 1.2 ± 0.7 | 1.7 ± 1.4 | 1.5 ± 0.3 | 1.3 ± 0.1 | 1.7 ± 0.8 | 1.2 ± 0.1 |
| 18:1-9    | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.2 | 0.3 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.5 ± 0.0 | 0.5 ± 0.0 |
| 18:1-11   | 10.9 ± 1.9 | 11.7 ± 1.9 | 10.8 ± 1.6 | 11.2 ± 3.4 | 11.5 ± 0.0 | 10.1 ± 0.9 | 11.3 ± 2.2 | 10.4 ± 1.0 |
| 18:1-18   | 6.5 ± 1.5 | 6.9 ± 1.4 | 5.2 ± 1.2 | 5.9 ± 2.8 | 6.4 ± 0.6 | 6.0 ± 0.3 | 6.1 ± 1.8 | 6.1 ± 1.3 |
| 20:1-11   | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.03 ± 0.06 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.0 |
| 22:1-11   | 0.3 ± 0.1 | 0.3 ± 0.2 | 0.4 ± 0.1 | 0.2 ± 0.2 | 0.4 ± 0.1 | 0.4 ± 0.0 | 0.3 ± 0.1 | 0.3 ± 0.0 |
| ∑MUFSA    | 19.5 ± 3.9 | 21.4 ± 3.9 | 17.2 ± 3.5 | 19.8 ± 8.0 | 20.3 ± 0.6 | 18.4 ± 0.9 | 20.4 ± 4.9 | 19.4 ± 1.1 |
| 18.2-6n    | 0.3 ± 0.6 | 0.1 ± 0.1 | 0.5 ± 0.8 | 0.6 ± 1.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.4 ± 0.2 | 0.1 ± 0.0 |
| 18.2-6n    | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.08 | 0.5 ± 0.0 | 0.5 ± 0.0 | 0.5 ± 0.0 | 0.5 ± 0.0 |
| 18.3-3     | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.09 ± 0.08 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.1 | 0.2 ± 0.0 |
| 20.4-6n-ARA| 7.8 ± 2.1 | 8.1 ± 2.2 | 7.4 ± 2.2 | 7.1 ± 3.8 | 7.6 ± 0.5 | 8.7 ± 0.7 | 7.2 ± 2.4 | 8.4 ± 0.7 |
| 20.5-3 EPA  | 0.9 ± 0.4 | 1.0 ± 0.4 | 0.7 ± 0.3 | 0.8 ± 0.6 | 0.8 ± 0.0 | 1.0 ± 0.2 | 0.8 ± 0.4 | 0.9 ± 0.1 |
| 22.6n-3 DHA | 12.1 ± 5.1 | 12.1 ± 5.1 | 12.2 ± 5.9 | 6.5 ± 4.6 | 13.2 ± 1.4 | 16.7 ± 0.6 | 8.8 ± 4.1 | 16.1 ± 1.0 |
| ∑P UFA    | 21.8 ± 6.3 | 21.9 ± 6.8 | 21.6 ± 7.5 | 15.7 ± 8.6 | 22.5 ± 1.5 | 27.0 ± 0.3 | 18.1 ± 6.2 | 26.3 ± 1.5 |
| ∑n-3      | 13.2 ± 5.0 | 13.2 ± 5.3 | 13.1 ± 6.2 | 7.4 ± 5.3 | 14.2 ± 1.3 | 17.8 ± 0.7 | 9.8 ± 4.5 | 17.3 ± 1.2 |
| ∑n-6      | 8.6 ± 1.7 | 8.7 ± 2.2 | 8.5 ± 1.4 | 8.2 ± 3.3 | 8.3 ± 0.4 | 9.2 ± 0.7 | 8.2 ± 2.1 | 9.0 ± 0.7 |
| Trophic markers |            |            |            |            |            |            |            |            |
| DHA/EPA   | 14.6 ± 4.0 | 12.9 ± 5.9 | 16.8 ± 2.7 | 9.1 ± 3.8 | 17.4 ± 2.6 | 17.4 ± 2.5 | 11.8 ± 4.6 | 18.2 ± 2.5 |
| ARA/EPA   | 9.9 ± 2.6 | 8.7 ± 1.7 | 11.4 ± 2.9 | 10.6 ± 4.4 | 10.0 ± 0.6 | 9.2 ± 2.2 | 10.2 ± 2.8 | 9.5 ± 1.9 |

Each value in the table represents the mean ± standard deviation

SFA: Saturated Fatty Acids; MUFSA: Monounsaturated Fatty Acids; P UFA: Polyunsaturated Fatty Acids

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1 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 ≤ 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 ± 0.3%. No differences were found relative to sex (Wilcoxon \( W= 13; P > 0.05 \)), Classification I (Kruskal-Wallis \( H= 2.5; \text{d.f.}= 2; P > 0.05 \)) 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 ± 5.3%, polyunsaturated fatty acids accounted for 21.8 ± 6.3% and monounsaturated fatty acids accounted for 19.5 ± 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](image)
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 where 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 ± 0.6% (Pethybridge et al. 2014), 0.6 ± 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)](image-url)
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, Ókland 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, Rodríguez-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 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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LITERATURE CITED

Arts MT & CC Kohler. 2009. Health and condition in fish: the influence of lipids on membrane competency and immune response. In: Arts MT, MT Brett & MJ Kainz (eds). Lipids in aquatic ecosystems, pp. 237-255. Springe Science + Business Media, Seattle.

Ballantyne JS. 1997. Jaws: The inside story. The metabolism of elasmobranch fishes, Comparative Biochemistry and Physiology, Part B. Biochemistry and Molecular Biology 118(4): 703-742.

Beckmann CL, JG Mitchell, L Seuront, DJ Stone & C Huveneers. 2013. Experimental evaluation of fatty acid profiles as a technique to determine dietary composition in benthic elasmobranchs. Physiological and Biochemical Zoology 86: 266-278.

Bell JG, JR Dick, JR Sargent & AH Mvicar. 1992. Dietary linoleic acid affects phospholipid fatty acid composition in heart and eicosanoid production by cardiomyocytes from Atlantic salmon (Salmo salar). Comparative Biochemistry and Physiology, Part A: Physiology 103(4): 337-342.

Bell MV, RJ Henderson & JR Sargent. 1985. Changes in the fatty acid composition of phospholipids from turbot (Scophthalmus maximus) in relation to dietary polyunsaturated fatty acid deficiencies. Comparative Biochemistry and Physiology, B, Comparative Biochemistry 81(1): 193-198.

Bligh EG & WJ Dyer. 1959. A rapid method of total lipid extraction and purification Canadian Journal of Biochemistry and Physiology 37(8): 911-917.
Bonfil R, M Meyer, MC Scholl, R Johnson, S O’Brien, H Oosthuizen, S Swanson, D Kotze & M Paterson. 2005. Transoceanic migration, spatial dynamics, and population linkages of white sharks. Science 310(5745): 100-103.

Brett M & D Müller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic food web processes. Freshwater Biology 38: 483-499.

Bruce BD & RW Bradford. 2012. Habitat use and spatial dynamics of juvenile white sharks, *Carcharodon carcharias*, in Eastern Australia. In: Michael L (ed). Global perspectives on the biology and life history of the white shark, pp. 225-254. CRC Press, Boca Raton.

Budge SM, SJ Iverson & HN Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. Marine Mammal Science 22(4): 759-801.

Caraveo-Patiño J, Y Wang, LA Soto, K Ghebremeskel, C Lehane & MA Crawford. 2009. Eco-physiological repercussions of dietary arachidonic acid in cell membranes of active tissues of the Gray whale. Marine Ecology 30(4): 437-447.

Carlisle AB, SL Kim, BX Semmens, DJ Madigan, SJ Jorgensen, CR Perle, SD Anderson, TK Chapple, PE Kanive & BA Block. 2012. Using stable isotope analysis to understand the migration and trophic ecology of Northeastern Pacific White Sharks (*Carcharodon carcharias*) PLoS ONE 7(2): e30492. <doi:10.1371/journal.pone.0030492>

Chapman DD, DL Abercombie, CJ Douady, EK Pikitch, MJ Stanhope & SS Mahmood. 2003. A streamlined, bi-organelle, multiplex PCR approach to species identification: Application to global conservation and trade monitoring of the great white shark, *Carcharodon carcharias*. Conservation Genetics 4(4): 415-425.

Christie WW. 2003. Lipid analysis: Isolation, separation, identification and structural analysis of lipids, 338 pp. Oily Press, Bridgewater.

Couturier LIE, CA Rohner, AJ Richardson, AD Marshall, FRA Jaine, MB Bennett, KA Townsend, SJ Weeks & PD Nichols. 2013a. Stable isotope and signature fatty acid analyses suggest reef manta rays feed on demersal zooplankton. PLoS One 8(10): e77152. <doi:10.1371/journal.pone.0077152>

Couturier LIE, CA Rohner, AJ Richardson, SJ Pierce, AD Marshall, FRA Jaine, KA Townsend, MB Bennett, SJ Weeks & PD Nichols. 2013b. Unusually high levels of n-6 polyunsaturated fatty acids in whale sharks and reef manta rays. Lipids 48: 1029-1034.

Dalsgaard J, MS John, G Kattner, D Muller-Navarra & W Hagen. 2003. Fatty acids as trophic markers in the pelagic marine environment. Advances in Marine Biology 46: 225-340.

Davidson B, J Sidell, J Rhodes & G Cliff. 2011. A comparison of the heart and muscle total lipid and fatty acid profiles of nine large shark species from the east coast of South Africa. Fish Physiology and Biochemistry 37(1): 105-112.

Davidson BC, W Nel, A Rais, V Namdarizandi, S Vizarra & G Cliff. 2014. Comparison of total lipids and fatty acids from liver, heart and abdominal muscle of scalloped (*Sphyra lewini*) and smooth (*Sphyra zygaena*) hammerhead sharks. SpringerPlus 3: 521. <doi:10.1186/2193-1801-3-521>

Domeier ML & N Nasby-Lucas. 2006. Annual re-sightings of photographically identified white sharks (*Carcharodon carcharias*) at an eastern Pacific aggregation site (Guadalupe Island, Mexico). Marine Biology 150(5): 977-984.

Domeier ML & N Nasby-Lucas. 2013. Two-year migration of adult female white sharks (*Carcharodon carcharias*) reveals widely separated nursery areas and conservation concerns. Animal Biotelemetry 1(2). <doi.org/10.1186/2050-3385-1-2>

Fallows C, AJ Gallagher & N Hammerschlag. 2013. White Sharks (*Carcharodon carcharias*) scavenging on whales and its potential role in further shaping the ecology of an apex predator. PLoS ONE 8(4): e60797. <doi.org/10.1371/journal.pone.0060797>

Ferry-Graham LA & AC Gibb. 2008. Ecophysiology. In: Jorgensen SE & BD Fath (eds). Encyclopedia of ecology, pp. 1121-1125. Newnes, Spain.

French GCA, M Stürup, S Rizzuto, JH Van Wyk, D Edwards, RW Dolan & WOH Hughes. 2017. The tooth, the whole tooth and nothing but the tooth: tooth shape and ontogenetic shift dynamics in the white shark *Carcharodon carcharias*. Journal of Fish Biology 91(4): 1032-1047. <https://doi.org/10.1111/jfb.13396>

Gallo-Reynoso JP. 2005. Los cetáceos de Isla Guadalupe. En: Santos del Prado E & K Peters (eds). Isla Guadalupe restauración y conservación, pp. 203-217. CONANP, México.

Hammer Ø, DAT Harper & PD Ryan. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeoontology Electronica 4(1): 1-9. <http://palaeo-electronica.org/2001_1/past/issue1_01.htm>

Hoyos-Padilla EM, AP Klimley, F Galván-Magaña & A Antoniou. 2016. Contrasts in the movements and habitat use of juvenile and adult white sharks (*Carcharodon carcharias*) at Guadalupe Island, Mexico. Animal Biotelemetry 4(1). <doi.org/10.1186/s40317-016-0106-7>

Iverson SJ. 1993. Milk secretion in marine mammals in relation to foraging: Can milk fatty acids predict diet? Symposia of the Zoological Society of London 66: 263-291.

Iverson SJ, C Field, WD Bowen & W Blanchard. 2004. Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecological Monographs 2: 211-235.

Iverson SJ. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Arts MT, MT Brett & MJ Kainz (eds). Lipids in aquatic ecosystems, pp. 281-307. Springer Science + Business Media. <doi:10.1007/978-0-387-89566-2>
Jaime-Rivera M, J Caraveo-Patiño, EM Hoyos-Padilla & F Galván-Magaña. 2013. Evaluation of biopsy systems for sampling white shark *Carcharodon carcharias* (Lamniformes: Lamnidae) muscle for stable isotope analysis. Revista de Biología Marina y Oceanografía 48(2): 345-351.

Jaime-Rivera M, J Caraveo-Patiño, M Hoyos-Padilla & F Galván-Magaña. 2014. Feeding and migration habits of white shark *Carcharodon carcharias* (Lamniformes: Lamnidae) from Isla Guadalupe inferred by analysis of stable isotopes δ 15 N and δ 13 C. Revista de Biología Tropical 62(2): 637-647.

Jayasinghe C, N Gotoh, S Tokairin, H Ehara & S Wada. 2003. Inter species changes of lipid compositions in liver of shallow-water sharks from the Indian Ocean. Fisheries Science 69(3): 644-653.

Jayasinghe C, N Gotoh & S Wada. 2012. Regiospecific analysis of shark liver triacylglycerols. JAOCs, Journal of the American Oil Chemists’ Society 89(10): 1873-1884.

Jorgensen SJ, CA Reeb, TK Chapple, S Anderson, C Perle, SR Van Sommeran, C Fritz-Cope, AC Brown, AP Klimley & BA Block. 2010. Philopatry and migration of Pacific white sharks. Proceedings of the Royal Society B, Biological Sciences 277: 679-688.

Jorgensen SJ, NS Arnoldi, EE Estess, TK Chapple, M Rückert, SD Anderson & BA Block. 2012. Eating or meeting? Cluster analysis reveals intraspecies of white shark (*Carcharodon carcharias*) migration and offshore behavior. PLoS ONE 7(10): e47819. <doi:10.1371/journal.pone.0047819>

Kerr LA, AH Andrews, GM Cailliet, TA Brown & KH Coale. 2006. Investigations of δ14C, δ13C, and δ15N in vertebrate of white shark (*Carcharodon carcharias*) from the eastern North Pacific Ocean. Environmental Biology of Fishes 77: 337-353.

McMeans BC, MT Arts & AT Fisk. 2012. Similarity between predator and prey fatty acid profiles is tissue dependent in Greenland sharks (*Somniosus microcephalus*): Implications for diet reconstruction. Journal of Experimental Marine Biology and Ecology 429: 55-63.

Meyer L, H Pethybridge, PD Nichols, C Beckmann, BD Bruce, JM Werry & C Huveneers. 2017. Assessing the functional limitations of lipids and fatty acids for diet determination: The importance of tissue type, quantity, and quality. Frontiers in Marine Science 4: 1-12. <doi.org/10.3389/fmars.2017.00369>

Meyer L, H Pethybridge, PD Nichols, C Beckmann & C Huveneers. 2019. Abiotic and biotic drivers of fatty acid tracers in ecology: A global analysis of chondrichthyan profiles. Functional Ecology 33(7): 1243-1255.

Milisenda G, S Rossi, S Vizzini, VL Fuentes, JE Purcell, U Tilves & S Piraino. 2018. Seasonal variability of diet and trophic level of the gelatinous predator *Pelagia noctiluca* (Scyphozoa). Scientific Reports 8(12140): 1-13. <doi.org/10.1038/s41598-018-30474-x>

Murzina SA, ZA Nefedova, S Falk-Petersen, PO Ripatti, TR Ruokolainen, SN Pekkoeva & NN Nemova. 2013. Lipid status of the two high latitude fish species, *Leptoctinus maculatus* and *Lumpenus fabricii*. International Journal of Molecular Sciences 14(4): 7048-7060.

Murzina SA, ZA Nefedova, SN Pekkoeva, AE Veselov, DA Efremov & NN Nemova. 2016. Age-specific lipid and fatty acid profiles of Atlantic salmon juveniles in the Varzuga River. International Journal of Molecular Sciences 17(7): 1050. <doi.org/10.3390/ijms17071050>

Nashy-Lucas N, H Dewar, CH Lam, KJ Goldman & ML Domeier. 2009. Environmental characterization of the Eastern Pacific shared offshore foraging area. PLoS ONE 4(12): e8163. <doi:10.1371/journal.pone.0008163>

Nelson MM, CF Pfieger, BD Mooney & PD Nichols. 2000. Lipids of gelatinous Antarctic zooplankton: Cnidaria and Ctenophora. Lipids 35(5): 551-559.

Nelson MM, BD Mooney, PD Nichols & CF Pfieger. 2001. Lipids of Antarctic ocean amphipods: Food chain interactions and the occurrence of novel biomarkers. Marine Chemistry 73(1): 53-64.

Ökland HMW, IS Stoknes, JF Remme, M Kjerstad & M Synnes. 2005. Proximate composition, fatty acid and lipid class composition of the muscle from deep-sea teleosts and elasmobranchs. Comparative Biochemistry and Physiology, Part B. Biochemistry and Molecular Biology 140(3): 437-443.

Pethybridge H, R Daley, P Virtue & P Nichols. 2010. Lipid composition and partitioning of deepwater chondrichthyan: Inferences of feeding ecology and distribution. Marine Biology 157(6): 1367-1384.

Pethybridge H, RK Daley & PD Nichols. 2011. Diet of demersal sharks and chimaeras inferred by fatty acid profiles and stomach content analysis. Journal of Experimental Marine Biology and Ecology 409(1-2): 290-299.

Pethybridge HR, CC Parrish, BD Bruce, JW Young & PD Nichols. 2014. Lipid, fatty acid and energy density profiles of white sharks: Insights into the feeding ecology and ecophysiology of a complex top predator. PLoS One 9(5): e97877. <doi:10.1371/journal.pone.0097877>

Ramos R & J González-Solís. 2012. Trace me if you can: The use of intrinsic biogeochemical markers in marine top predators. Frontiers in Ecology and the Environment 10: 258-266.

Reeb D & PB Best. 2006. A biopsy system for deep-core sampling of the blubber of southern right whales. Marine Mammal Science 22(1): 206-213.

Ribgy CL, R Barreto, J Carlson, D Fernando, S Fordham, MP Francis, K Herman, RW-Jabado, KM Lin, CG Lowe, A Marshall, N Pacoureau, E Romanov, RB Sherley & H Winker. 2019. *Carcharodon carcharias*. The IUCN Red List of Threatened Species 2019. <doi.org/10.2305/IUCN.UK.2019-3.RLTS.T3855A2878674.en>

Rodriguez-Barreto D, S Jerez, JR Cejas, MV Martin, NG Acosta, A Bolaños & A Lorenzo. 2012. Comparative study of lipid and fatty acid composition in different tissues of wild and cultured female broodstock of greater amberjack (*Seriola dumerili*). Aquaculture 360/361: 1-9.

Rohner CA, LIE Couturier, AJ Richardson, SJ Pierce, CEM Prebble, MJ Gibbons & PD Nichols. 2013. Diet of white sharks *Rhincodon typus* inferred from stomach content and signature fatty acid analyses. Marine Ecology Progress Series 493: 219-235.
Saito H, M Sakai & T Wakabayashi. 2014. Characteristics of the lipid and fatty acid compositions of the Humboldt squid, Dosidicus gigas: The trophic relationship between the squid and its prey. European Journal of Lipid Science and Technology 116(3): 360-366.

Sardenne F, S Hollanda, S Lawrence, R Albert-Arrisol, M Degroote & N Bodin. 2017. Trophic structures in tropical marine ecosystems: a comparative investigation using three different ecological tracers. Ecological Indicators 81: 315-324.

Sargent JR, L McEvoy, A Estevez, JG Bell, M Bell, J Henderson & DR Tocher. 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179: 217-230.

Schaufler L, R Heintz, M Sigler & L Hulbert. 2005. Fatty acid composition of sleeper shark (Somniosus pacificus) liver and muscle reveals nutritional dependence on planktivores. ICES CM Documents: 1-19.

Skomal GB, EM Hoyos-Padilla, A Kukulya & R Stokey. 2015. Subsurface observations of white shark Carcharodon carcharias predatory behaviour using an autonomous underwater vehicle. Journal of Fish Biology 87(6): 1293-1312.

Speers-Roesch B & JR Treberg. 2010. The unusual energy metabolism of elasmobranch fishes. Comparative Biochemistry and Physiology, Part A. Molecular and Integrative Physiology 155(4): 417-434.

Stoknes IS, HMW Økland, E Falch & M Synnes. 2004. Fatty acid and lipid class composition in eyes and brain from teleosts and elasmobranchs. Comparative Biochemistry and Physiology, Part B. Biochemistry and Molecular Biology 138(2): 183-191.

Tamburin E, SL Kim, FR Elorriaga-Verplancken, DJ Madigan, M Hoyos-Padilla, A Sánchez-González & F Galván-Magaña. 2019. Isotopic niche and resource sharing among young sharks (Carcharodon carcharias and Isurus oxyrinchus) in Baja California, Mexico. Marine Ecology Progress Series 613: 107-124.

Tocher DR. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 11(2): 107-184.

Tocher DR. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquaculture Research 41(5): 717-732.

Tocher DR, EÅ Bendiksen, PJ Campbell & JG Bell. 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture 280(1-4): 21-34.

Tricas TC & JE McCosker. 1984. Predatory behavior of the white shark (Carcharodon carcharias), with notes on its biology. Proceedings of California Academy Science 43: 221-238.

Wai TK, JWT Young, VYY Lam, KM Leung, D Dudgeon & GA Williams. 2012. Monsoons and habitat influence trophic pathways and the importance of terrestrial-marine linkages for estuary sharks. Ecosphere 3(1). <doi.org/10.1890/es11-00276.1>

Watson RR & KA Dickson. 2001. Enzyme activities support the use of liver lipid-derived ketone bodies as aerobic fuels in muscle tissues of active sharks. Physiological and Biochemical Zoology 74(2): 273-282.

Weng KC, AM Boustany, P Pyle, SD Anderson, A Brown & BA Block. 2007. Migration and habitat of white sharks (Carcharodon carcharias) in the eastern Pacific Ocean. Marine Biology 152(4): 877-894.

Zammit VA & EA Newsholme. 1979. Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobranch fish. The Biochemical Journal 184(2): 313-322.