



## 3D chemoecology and chemotaxonomy of corals using fatty acid biomarkers: Latitude, longitude and depth



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### ABSTRACT

With the objective of uncovering differences in the fatty acid (FA) composition of hexa- and octocorals from different climatic zones (equatorial, subtropical and tropical) and distinct habitats (e.g. rock and coral reefs; intertidal to deep-sea environments), the FA composition of 36 hexa- and octocoral species (132 specimens) was analysed (including the first characterization of organisms from the order Scleractinia and deep-sea gorgonians). PCA was applied in a FA matrix of the ten major PUFAs to detect differences among coral groups. Fatty acid profile analysis confirmed that C24 polyunsaturated FAs are suitable chemotaxonomic biomarkers to separate hexa- and octocorals. The polyunsaturated FA 22:6n-3 was identified as a useful biomarker to distinguish between zoantharians and scleractinians. Also, we discuss the role of food availability (type of phytoplankton assemblage) in relation to autotrophic carbon significance and in the establishment of FA profiles of octocorals from the West and East coasts of the Atlantic Ocean. Furthermore, we show that the occurrence of high levels of primary productivity hinder the use of FA profiles to distinguish between zooxanthellate and azooxanthellate octocorals. Finally, we present and discuss the particular traits of the FA profile of deep-sea gorgonians while comparing it with that of shallow species.

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## 1. Introduction

Hexacorals and octocorals (phylum Cnidaria) are marine invertebrates that typically live in colonies (composed by several individual polyps) and inhabit a wide range of environments (Lopes et al., 2012; Pires et al., 2014; Roth, 2014).

In these organisms lipids can make up to 40% of their dry weight and thus play a key role as the main source of stored energy (Bachar et al., 2007; Houlbreque and Ferrier-Pagès, 2009). Fatty acids, the “building blocks” of lipids, are known to be involved in the majority of biochemical and physiological processes of these cnidarians (Ibarguren et al., 2014) and are obtained through heterotrophic and autotrophic pathways (Dalsgaard et al., 2003). These compounds may be acquired through feeding on planktonic organisms and/or microorganisms associated with detritus and mucus (Leal et al., 2014b), as well as by uptake of particulate and organic matter dissolved in the sediment and water column (Leal et al., 2014a). Several hexa- and octocoral species live in symbiosis with unicellular dinoflagellates of the genus *Symbiodinium* [commonly known as zooxanthellae; Baker, 2003]. These photosynthetic endosymbionts use light as the energy source to fix inorganic carbon (in the form of bicarbonate) and produce organic compounds that provide alternative metabolic pathways for the host (Muscattine and Porter, 1977). Photoautotrophic carbon may notably contribute to the energy reserves of the cnidarian host (Rodrigues and Grottoli, 2006; Leal et al., 2013; Baumann et al., 2014) as it is quickly converted into lipids. Lipids are carried into ‘host’ tissues in the form of ‘fat droplets’, consisting of triglycerides, wax esters and free fatty acids (Patton et al., 1983).

Due to the specificity in FA biosynthesis ability of certain organisms, FAs have been widely used as chemotaxonomic and chemoecological biomarkers (e.g. Latyshev et al., 1991; Shin et al., 2008; Imbs et al., 2009). For example, the presence of highly specific polyunsaturated fatty acids (PUFAs) such as 20:4n-6 and 20:5n-3 is generally indicative of external food sources such as zoo- and phytoplankton, respectively (Kellogg and Patton, 1983; Latyshev et al., 1991). Similarly, several FAs have been identified as biomarkers for the presence of zooxanthellae including the PUFAs 16:2n-7, 18:3n-6 and 18:4n-3 (Imbs et al., 2009). Accordingly, differences in trophic ecology and relationship with symbionts are known to contribute to differences in the FA profiles of corals.

Besides diet and symbiont presence, the FA profile of individuals may be influenced by environmental factors. Indeed, organisms have the ability to change the balance between saturated and unsaturated fatty acids of polar lipids in response to new environmental conditions, in order to maintain a suitable membrane fluidity [homeoviscous adaptation; Sinensky, 1974]. In general, lower temperatures lead to an increase in the unsaturation of FA while higher temperatures are responsible for an increase in the levels of saturated fatty acids (Hall et al., 2002). As such, differences in the PUFA profile of hexa- and octocorals may occur in relation to different water temperatures and concurrently, depths.

The present study strongly increases the existing knowledge on the chemoecology and chemotaxonomy of hexa- and octocorals by expanding the previously spatially-limited knowledge on these topics [the large majority of species studied so far are originated from Vietnam (94%)]. Here, we uncovered differences in FA composition of hexa- and octocorals from different climatic zones (equatorial, subtropical and tropical) and distinct habitats (e.g. rock and coral reefs; intertidal to deep-sea environments).

## 2. Material and methods

### 2.1. Sampling

A detailed overview of all coral species used in the present study is provided in [Supplemental Table 1](#).

### 2.2. Shallow-living corals

Specimens of shallow-living hexacorals (n = 19 species) and octocorals (n = 14) were collected by scuba-divers between June 12th and July 8th, 2013 in Mexico [(La Gallega Reef (n = 2); Madagascar Reef (n = 10); Mahahual Reef (n = 9); Puerto Morelos Reef (n = 6)] at depths ranging 1–6 m and mainland Portugal [(Setúbal (n = 3)] ([Supplemental Table 1](#)) at depths ranging 0.5–2 m, in a total of 117 specimens ([Fig. 1](#); [Supplemental Table 1](#)). Colonies of the same species were collected at different locations. Samples were immediately placed in liquid nitrogen and stored in the laboratory at –80 °C for posterior analyses.

### 2.3. Deep-sea corals

Throughout 2009, during the EMEPC/LUSO/2009 ROV campaign made in the scope of the Portuguese Task Group for the Extension of the Continental Shelf (EMEPC) project, fifteen deep-sea gorgonians (n = 3 species) were collected off the Azores archipelago (Portugal) ([Fig. 1](#); [Supplemental Table 1](#)), namely in Furnas de Fora, Dom João de Castro Seamount and São Jorge Channel at depths ranging between 313 and 1077 m. Following collection, samples were immediately stored in an on-board –80 °C freezer. Species belonging to the suborder Holaxonia, commonly designated as gorgonians, were analysed as one group (i.e. gorgonians) for the sake of clarity.

### 2.4. Fatty acid analysis

Samples [145–301 mg for hexacorals and 300–301 mg for octocorals (dry mass)] were dissolved in 5 mL of acetyl chloride/methanol (1:19 v/v; Merck), shaken for 30 s, and heated (80 °C; 1 h) according to [Baptista et al. \(2012\)](#). After cooling in room

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