Biochemical Systematics and Ecology 66 (2016) 297–306

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

"Gone with the wind": Fatty acid biomarkers and chemotaxonomy of stranded pleustonic hydrozoans (*Velella velella* and *Physalia physalis*)

Ana Rita Lopes [a], Miguel Baptista [a, *], Inês C. Rosa [a], Gisela Dionísio [a, b], José Gomes-Pereira [c], José Ricardo Paula [a], Cátia Figueiredo [a], Narcisa Bandarra [d], Ricardo Calado [b], Rui Rosa [a]

[a] *MARE – Marine and Environmental Sciences Centre, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*
[b] *Departamento de Biologia & CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*
[c] *Centre of IMAR and Department of Oceanography and Fisheries, University of the Azores, 9901-862 Horta, Portugal*
[d] *Departamento de Inovação Tecnológica e Valorização dos Produtos da Pesca, IPIMAR/IPMA, Avenida de Basília, 1449-006 Lisboa, Portugal*

ARTICLE INFO

Article history:
Received 10 August 2015
Received in revised form 25 March 2016
Accepted 27 March 2016
Available online 19 May 2016

Keywords:
Fatty acids
Velella velella
Physalia physalis
Pleustonic hydrozoans
Cnidarians
Chemotaxonomy

ABSTRACT

Marine pleustonic species such as the hydrozoans *Velella velella* and *Physalia physalis*, are known to drift in the world's oceans driven by winds, currents and tides. Here we present the first chemotaxonomic characterization, based on the fatty acid (FA) profile, of these two charismatic oceanic species that thrive in the interface layer between air and the water column in adult stages. Moreover, we compared their FA profiles with those from other representative cnidarian orders (Rhizostomeae, Anthomedusae, Siphonophorae, Alcyonacea, Scleractinia, Helioporacea and Pennatulacea). *Velella velella* and *P. physalis* mainly differed in the presence of symbiotic dinoflagellates markers (18:3*n*-6, 18:4*n*-3 and 20:5*n*-3 polyunsaturated FAs), present in higher percentage in the former, and bacterial markers (odd-numbered, branched and 18:1*n*-7 FAs), which were more representative in the latter. When comparing these species' FA profiles with the ones of other cnidarians orders, the presence/absence of endosymbionts and of specific FAs (tetracosapentaenoic and tetracosahexaenoic acids) as well as the latitudinal habitats were the main drivers for the distinction between groups.

1. Introduction

Lipids are important constituents of all marine organisms. They play a major role in energy storage and cell structuring (Harland et al., 1993; Ward, 1995) and are deeply involved in several biochemical and physiological processes (Ward, 1995; Rodrigues et al., 2008). Given the importance of lipids in organisms' functioning, the study of their main components – fatty acids (FAs) - is imperative when attempting to uncover species' ecological traits (Imbs et al., 2010; Baptista et al., 2012, 2014).

The FA profile of an organism is determined by environmental and biotic factors, including its synthesis ability which is genetically inherited (Sargent and Whittle, 1981; Napolitano et al., 1997; Dalsgaard et al., 2003), feeding regime (Arts et al.,

* Corresponding author.
E-mail address: msbaptista@fc.ul.pt (M. Baptista).

http://dx.doi.org/10.1016/j.bse.2016.03.016
0305-1978/

2001; Dalsgaard et al., 2003; Sara, 2009), environmental parameters (e.g. temperature (Oku et al., 2003; Imbs and Yakovleva, 2012)) and associated microbiome (e.g. zooxanthellae (Imbs et al., 2009, 2014)). Organisms are usually able to biosynthesize saturated FAs (SFAs) and monounsaturated FAs (MUFAs) in levels that meet their requirements (Kaneda, 1991; Mortillaro et al., 2009; Parrish, 2013). Nevertheless, photosynthetic symbionts such as zooxanthellae may also be an important source of SFAs (Patton et al., 1983), while bacteria may be an important source of certain MUFAs, odd numbered and branched FAs (Dalsgaard et al., 2003). On the other hand, biosynthesis of polyunsaturated FAs (PUFAs) is generally limited to a restricted group of organisms (e.g. phytoplankton) (Drazen et al., 2008; Mortillaro et al., 2009; Sara, 2009) and the majority of animals have to obtain them through dietary intake (Patton et al., 1983). This way, differences in environmental conditions could lead to changes in the FA profile of an organism once distant locations could diverge in abiotic parameters such as temperature, as well as in food sources availability (Freites et al., 2002, 2010).

By knowing the origin of specific FAs, these can be used as chemotaxonomic markers (Imbs et al., 2010). In fact, FAs have been successfully used for the chemosystematics of different taxonomic groups (e.g. Volkman et al., 1998; Bergé and Barnathan, 2005; Imbs et al., 2007b) and are widely used as biomarkers in marine food-web studies (Dalsgaard et al., 2003; Imbs and Dautova, 2008; Colaço et al., 2009).

Numerous studies have already described the FA profile of several cnidarian groups (e.g. Stillway, 1976; Imbs et al., 2007b; Morais et al., 2009). However, the large majority only focus in the class Anthozoa (e.g. Imbs et al., 2007b, 2010; Imbs and Dautova, 2008). With only a few studies regarding the FA composition of hydrozoans (e.g. Stillway, 1976; Morais et al., 2009; Mortillaro et al., 2009), and any of these addressing the chemotaxonomy of these organisms.

The Portuguese man-of-war [*Physalia physalis* (Linnaeus, 1758), family Siphonophora] and the wind sailor [*Velella velella* (Linnaeus, 1758), family Anthoathecata] are two pleustonic siphonophoran hydrozoan species. *Physalia physalis* is an ubiquitous species that inhabits tropical and subtropical waters (Purcell, 1984), while *V. velella* occurs in warm and temperate waters (Purcell et al., 2012). Both species are carnivorous and important members of a specialized ocean surface community (pleuston), being predated by several invertebrate and vertebrate species (Stillway, 1976). Following this, the knowledge on the biochemistry of such ecologically relevant species is crucial. The present work describes the FA profiles of *P. physalis* and *V. velella* and establishes, for the first, time the chemotaxonomic discrimination: i) between these two hydrozoans, and ii) between these hydrozoans and other cnidarian groups.

2. Materials and methods

2.1. Biological sampling

Pleustonic hydrozoan colonies of *Velella velella* and *Physalia physalis* were hand collected in Cabo Raso, Cascais, mainland Portugal (approx. 38.709882°N, −9.486837°W) and Praia de Porto Pim, Faial Island, Azores (approx. 38.523453°N, −28.626355°W), respectively. Sampling collection took place in April 2013, with the specimens reaching the shore driven by onshore winds. Water temperature at sampling sites ranged between 15–17 °C in Cascais and 17–18 °C in the Azores (max 22.8 °C; source: AVHRR SST averages for 8 day period, IMAR-DOP/UAç). Following collection, three pooled samples (six whole colonies per pool) were vacuum packed and frozen at −80 °C. For biochemical analyses, frozen samples were freeze-dried for 72 h at −50 °C under low pressure (approximately 10^{-1} atm), powdered using a grinder (Retsch Grindomix GM200, Düsseldorf, Germany), and stored at −80 °C.

2.2. Fatty acid analyses

The determination of the FA profile was based on the experimental procedure previously described by Rosa et al. (2007) and Baptista et al. (2012). Triplicate samples (300–330 mg of dry mass per sample) were dissolved in 5 mL of acetyl chloride/methanol (1:19 v/v; Merck), shaken, and heated at 80 °C for 1 h. After cooling, 1 mL of Milli-Q distilled water and 2 mL of n-heptane pro-analysis (Merck) were added and samples were shaken and centrifuged (2300*g*, 5 min) until phase separation. The moisture content of the upper phase was removed using anhydrous sodium sulfate (Panreac). An aliquot (2 μl) of the upper phase was injected onto a gas chromatograph (Varian Star 3800 Cp, Walnut Creek, CA, USA) equipped with an autosampler and fitted with a flame ionization detector at 250°C for fatty acid methyl ester (FAME) analysis. The separation was carried out with helium as carrier gas at a flow rate of 1 mL min^{-1} in a capillary column DB-WAX (30 m length x 0.32 mm internal diameter; 0.25 μm film thickness; Hewlett-Packard, Albertville, MN) programmed at 180 °C for 5 min, raised to 220 at 4 °C min^{-1}, and maintained at 220 °C for 5 min with the injector at 250 °C. FAME identification (% total FA) was accomplished through comparison of retention times with those of Sigma, Nu Check Preap and Larodan Fine Chemicals standards.

2.3. Statistical Analysis

The percentage of individual FAs obtained for *V. velella* and *P. physalis* was tested for normality and homoscedasticity (Kolmogorov-Smirnov and Levene's tests, respectively) and subsequently compared using *t*-test analyses.

In order to perform an intra-phylum analysis, the FA profiles of 27 cnidarian species belonging to seven different orders: Rhizostomeae (n = 1), Anthomedusae (n = 2), Siphonophorae (n = 1), Alcyonacea (n = 11), Scleractinia (n = 10), Helioporacea (n = 1) and Pennatulacea (n = 1), were compiled from available literature. Details on the species used in the intra-phylum

analysis, including information on the presence of symbionts and number of specimens, are available in Table SI. Provided that most authors solely focus on FAs representing ≥0.1% of total FA, the FAs exhibiting lower concentrations were not considered in this analysis. A total of 26 FAs (14:0, 15:0, 16:0, 17:0, 18:0, Anteiso 16:0, Iso 17:0, 16:1*n*-9, 16:1*n*-7, 18:1*n*-9, 18:1*n*-7, 20:1*n*-9, 20:1*n*-7, 22:1*n*-11, 16:2*n*-4, 16:3*n*-3, 18:2*n*-6, 18:4*n*-3, 20:3*n*-3, 20:4*n*-3, 20:5*n*-3, 22:5*n*-6, 22:5*n*-3, 22:6*n*-3, 24:5*n*-6, 24:6*n*-3) were used in a principal component analysis (PCA). PCA reduces the number of dimensions produced by the large number of variables and uses linear correlations (components) to identify those FAs that contributed most to the separation between species (Quinn and Keough, 2002).

PCA was complemented with a multivariate analysis of variance (MANOVA) in order to identify significant differences in the variation of individual FAs percentage of colonies belonging to different taxonomical groups. The Wilks' lambda was considered in this analysis. Organisms belonging to Helioporaceae, Rhizostomae and Scleractinia (symbiotic) were excluded from the analysis of variance (n = 1 for each specimen). As significant differences between groups were found, one-way ANOVA followed by multiple comparisons tests (Unequal N HSD), were performed to scrutinize the effect of the group on each FA. The Dunn-Sidak procedure was used to adjust the associated significance level of the family-wise type-I error (Quinn and Keough, 2002). A total of 7 comparisons were applied (7 taxonomic groups), resulting in a significance level of 0.007.

Unless stated otherwise, a significance level of 0.05 was considered in the analyses. The software Statistica 12.0 (Statsoft, Inc., Tulsa, OK 74104, USA) was used.

3. Results

3.1. Fatty acid composition of two open ocean pleustonic hydrozoans

Detailed FA profiles of *Velella velella* and *Physalia physalis* are shown in Table 1.

The major saturated fatty acid (SFA) in both species was palmitic acid (16:0; 22% of total FA in *P. physalis* against 16% in *V. vellela*; Table 1; *t*-test: $P < 0.001$, Fig. 1) followed by stearic acid (18:0; 10% *vs* 5%; Table 1; *t*-test: $P < 0.001$; Fig. 1). The SFAs found in lower concentrations were 15:0 (1.3% in *P. physalis vs* 0.3% in *V. velella*; Table 1; *t*-test: $P < 0.001$) and 17:0 (1.2% *vs* 0.4%; Table 1; *t*-test: $P < 0.001$). Overall, *P. physalis* presented a significantly higher percentage of SFAs than *V. velella* with a value of approximately 41% (of total FA) in the former against 28% in the latter (Table 1; *t*-test: $P < 0.001$).

Regarding monounsaturated fatty acids (MUFA), vaccenic acid (18:1*n*-9) dominated in *V. velella* (7.3%) which exhibited a significantly higher proportion than that found in *P. physalis* (4.9%; Table 1; *t*-test: $P < 0.05$; Fig. 2). Conversely, paulinic acid (20:1*n*-7) dominated in *P. physalis* (6%), being present in significantly higher levels when compared to *V. vellela* (0.1%; Table 1; *t*-test: $P < 0.001$). No significant differences were found between these two species regarding MUFA fraction (Table 1; *t*-test: $P > 0.05$). However, it should be noted that *V. velella* exhibited some MUFAs that were not present in *P. physalis*, namely cetoleic (22:1*n*-11) and erucic (22:1*n*-9) acids (Table 1).

The major polyunsaturated fatty acid (PUFA) in both species was docosahexaenoic acid (DHA, 22:6*n*-3), followed by eicosapentaenoic acid (EPA, 20:5*n*-3), both found in significantly greater concentration in *V. velella* (Table 1; *t*-tests: $P < 0.05$; Fig. 3). Unlike what was observed in the SFA fraction, PUFA fraction content was significantly greater in *V. velella* (Table 1; *t*-test: $P < 0.05$). It is worth noting that this species exhibited several PUFAs that were not observed in *P. physalis*, namely hexadecatetraenoic (16:4*n*-3), γ-Linolenic acid (GLA, 18:3*n*-6), octadecatrienoic (18:3*n*-4), α-linolenic (ALA, 18:3*n*-3) and arachidonic (ARA, 20:4*n*-6) acids (Table 1).

As to *n*-3/*n*-6 ratio, no significant differences between species were found, with values of 10.79% and 12%, for *V. velella* and *P. physalis*, respectively (Table 1).

3.2. Intra-phylum variations in fatty acid composition

In order to ascertain FA composition differences among species examined in the present study (*V. velella* and *P. physalis*) and other cnidarian species belonging to seven orders, a PCA based on 26 FAs was performed (Fig. 4).

A clear crosswise separation between temperate and tropical species was observed mainly along the first principal component (PC1; explaining 20% of the variance), with temperate species [*V. velella* (Anthoathecata *V.*), *P. physalis* (Siphonophorae) and *V. cynomorium* (Pennatulaceae)] being placed to the left whilst tropical species were placed to the right (dashed line in Fig. 4A). The FAs that contributed the most to the separation of *V. velella* and *P. physalis* from other cnidarians were 16:2*n*-4, 22:6*n*-3 (see also Fig. 3B; Table SIII), 20:1*n*-9 (Fig. 2E; Table SIII) and 22:1*n*-11. Furthermore, *V. velella* was isolated due to a higher percentage of 20:1*n*-9 (Figs. 2E and 4B; Table SIII) and 22:1*n*-11 (Fig 4B, Table SIII). On the other hand, a high percentage of vaccenic (18:1*n*-7) (Figs. 2D and 4B; Table SIII), pentadecanoic (15:0), margaric (17:0), hexadecadienoic (16:2*n*-4), eicosatrienoic (20:3*n*-3) and 20:1*n*-7 acids were responsible for the separation of *P. physalis* from other cnidarian species (Fig. 4B, Table SIII).

A clear distinction between species with and without photosynthetic symbionts (PS) was achieved with a transversal separation along the PC2 (explaining 19% of the variance), with symbiotic species being placed in a right-lowermost position and the asymbiotic ones in a left-uppermost position (dotted line in Fig. 4A). This separation occurred mainly due to the contribution of the FAs 17:0, 15:0, anteiso 16:0, and iso 17:0 (Fig. 1B), together with 18:1*n*-7 (Figs. 2D and 4B; Table SIII) and tetracosahexaenoic (THA, 24:6*n*-3) (Figs. 3D and 4B; Table SIII), generally present in high percentage in species without photosynthetic symbionts (although not always statistically confirmed by analyses of variance). Conversely, the FAs 18:4*n*-3

Table 1
Fatty acid composition (% of total FA) of *Velella velella* and *Physalia physalis*.* Asterisks represent significant differences between species.

Fatty acids	*Velella velella*	*Physalia physalis*
Satured (SFA)		
11:0	0.01 ± 0.03	0.03 ± 0.02
14:0*	3.83 ± 0.22	4.57 ± 0.92
Iso 15:0*	0.33 ± 0.03	0.23 ± 0.04
15:0*	0.31 ± 0.04	1.26 ± 0.24
Anteiso 16:0	–	0.15 ± 0.03
16:0*	15.99 ± 0.66	22.36 ± 3.53
Iso 17:0	0.33 ± 0.03	0.34 ± 0.04
17:0*	0.37 ± 0.03	1.22 ± 0.10
18:0*	5.02 ± 0.18	9.94 ± 0.30
19:0*	0.38 ± 0.02	0.29 ± 0.02
20:0*	1.63 ± 0.15	0.34 ± 0.05
22:0	0.40 ± 0.04	–
Σ Branched	0.66 ± 0.02	0.72 ± 0.11
Σ SFA*	28.58 ± 1.08	40.73 ± 5.05
Monounsaturated (MUFA)		
16:1*n*-9	1.20 ± 1.01	0.75 ± 0.28
16:1*n* -7*	0.21 ± 0.25	2.37 ± 0.38
18:1*n* -9*	7.29 ± 0.40	4.86 ± 0.23
18:1*n* -7*	0.48 ± 0.12	1.20 ± 0.06
20:1*n* -9*	4.23 ± 0.52	0.47 ± 0.10
20:1*n* -7*	0.09 ± 0.01	6.00 ± 4.74
22:1*n* -11	0.89 ± 0.04	–
22:1*n* -9	0.38 ± 0.03	–
Σ MUFA	14.77 ± 0.67	15.64 ± 5.46
Polyunsaturated (PUFA)		
16:2*n* -4*	0.26 ± 0.04	1.26 ± 0.15
16:3*n* -4*	0.27 ± 0.05	0.34 ± 0.04
16:3*n*-3*	2.62 ± 0.33	0.86 ± 0.04
16:4*n* -3	0.07 ± 0.07	–
18:2*n* -6*	1.20 ± 0.11	0.91 ± 0.05
18:3*n* -6	0.11 ± 0.02	–
18:3*n* -4	0.12 ± 0.01	–
18:3*n* -3	0.98 ± 0.09	–
18:4*n* -3*	3.81 ± 0.14	2.07 ± 0.05
20:2*n* -6*	0.42 ± 0.02	0.21 ± 0.02
20:4*n* -6	0.33 ± 0.02	–
20:3*n* -3*	0.11 ± 0.02	2.24 ± 2.05
20:4*n* -3	0.68 ± 0.05	0.67 ± 0.05
20:5*n* -3*	7.77 ± 0.61	6.46 ± 0.55
21:5*n* -3	0.24 ± 0.03	0.27 ± 0.06
22:4*n* -6*	0.47 ± 0.06	0.28 ± 0.06
22:5*n* -6*	0.49 ± 0.04	1.59 ± 0.27
22:5*n* -3*	1.37 ± 0.09	1.86 ± 0.39
22:6*n* -3*	27.60 ± 1.24	22.94 ± 4.43
Σ PUFA*	48.92 ± 1.84	41.95 ± 7.22
Σ *n* -3	32.74 ± 15.00	37.36 ± 7.12
Σ *n* -6	3.01 ± 0.13	2.99 ± 0.30
***n* -3/*n* -6**	10.79 ± 4.78	12.43 ± 1.25
DHA/EPA	3.57 ± 0.28	3.53 ± 0.38

(Fig. 4B), 18:2*n*-6 (Fig. 4B) and 22:6*n*-3 (Fig. 3B, Table SIII) were generally found in greater amount in symbiotic organisms, also contributing to species separation (Fig. 4B).

In regard to tetracosapolyenoic acids [tetracosapentaenoic (TPA, 24:5*n*-6) and tetracosahexaenoic (THA, 24:6*n*-3)], it is worth noting that 24:6*n*-3 was found in the subclass discomedusae representative (*C. tagi*) while absent in the subclass hydroidolina representatives (*P. physalia*, *V. velella* and *Millepora sp.*; Fig. 3C, D; Table SI).

4. Discussion

4.1. Fatty acid differences of two pleustonic hydrozoans

Fatty acids (FAs) are useful qualitative markers that can be used to trace or confirm predator-prey relationships as well as organisms' taxonomic position and presence/absence of symbionts (e.g. Dalsgaard et al., 2003; Imbs et al., 2007a, 2014).

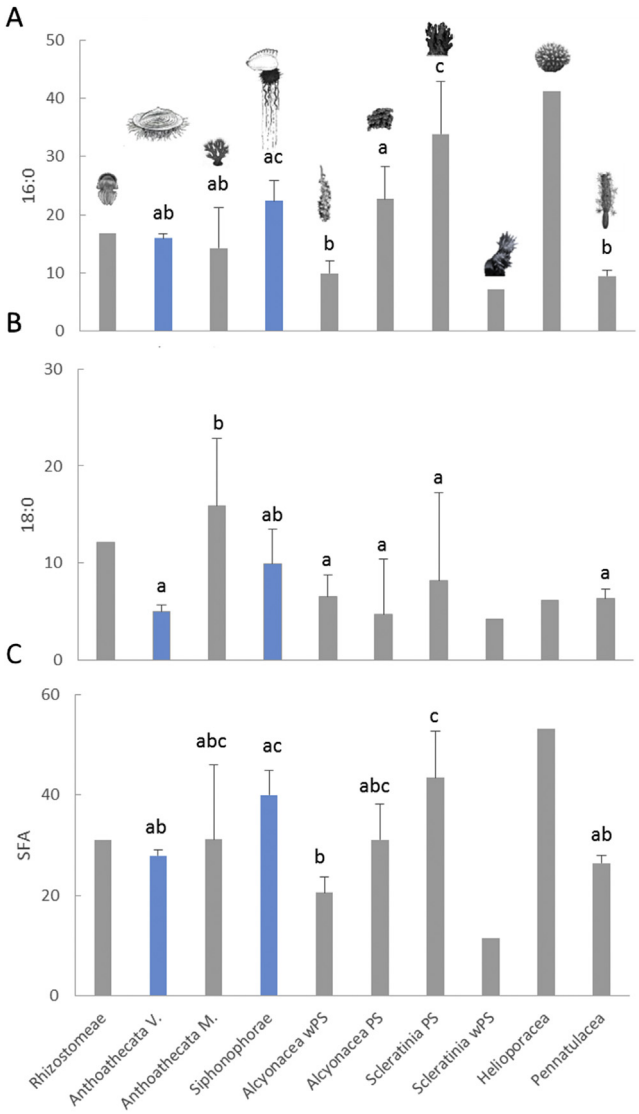

Fig. 1. Major saturated fatty acids and respective fraction (SFA) profile of 7 cnidarian orders. Values are means (±SD). "PS" and "wPS" stand for photosynthetic symbionts and without photosynthetic symbionts groups, respectively; "*Anthoathecata V.*" and "*Anthoathecata M.*" represent *Vellela vellela* and *Millepora* sp. (see Statistical Analysis section for more details). Letters denote significant differences between groups (Unequal N HSD post-hoc test).

Nonetheless, they should be used with caution since they may be metabolized and transformed after its consumption (Dalsgaard et al., 2003).

The present work describes the FA profiles of *V. velella* and *P. physalis* and establishes a chemotaxonomic discrimination in relation to other cnidarian taxonomic groups. When comparing *V. velella* and *P. physalis*, several differences in the FA composition become apparent, providing insights into distinct life traits between these species. *Velella velella* exhibited greater C18 PUFA proportion along with other PUFAs such as 20:5*n*-3 and 22:6*n*-3, which indicates the presence of photosynthetic symbionts. Indeed, while studying the distribution of FAs in reef building corals regarding their taxonomic position and presence of photosynthetic symbionts, Imbs et al. (2010) identified γ-linolenic acid (18:3*n*-6), stearidonic acid (18:4*n*-3), 20:5*n*-3 and 22:6*n*-3 as markers of zooxanthellae, especially the PUFAs 18:4*n*-3 and 22:6*n*-3 which are often dominant in dinoflagellates (Dalsgaard et al., 2003; Imbs et al., 2010). Although in the present study the presence/absence of symbiotic dinoflagellates was not assessed, other studies reported the presence of dinoflagellates in association with *V. velella* colonies from the Pacific ocean and Mediterranean sea (Banaszak et al., 1993; Trench, 1993; Gast and Caron, 1996) with any study reporting zooxanthellae associated with *P. physalis*.

In addition to photosynthetic symbionts, the presence of bacteria may also be detected through the specific FAs, particularly large quantities of odd-numbered and branched FAs as well as 16:1*n*-7 and 18:1*n*-7 (Dalsgaard et al., 2003). The present study shows that *P. physalis* has high proportion of 16:1n-7 and 18:1*n*-7 (considerably greater than those found in *V. velella*) as

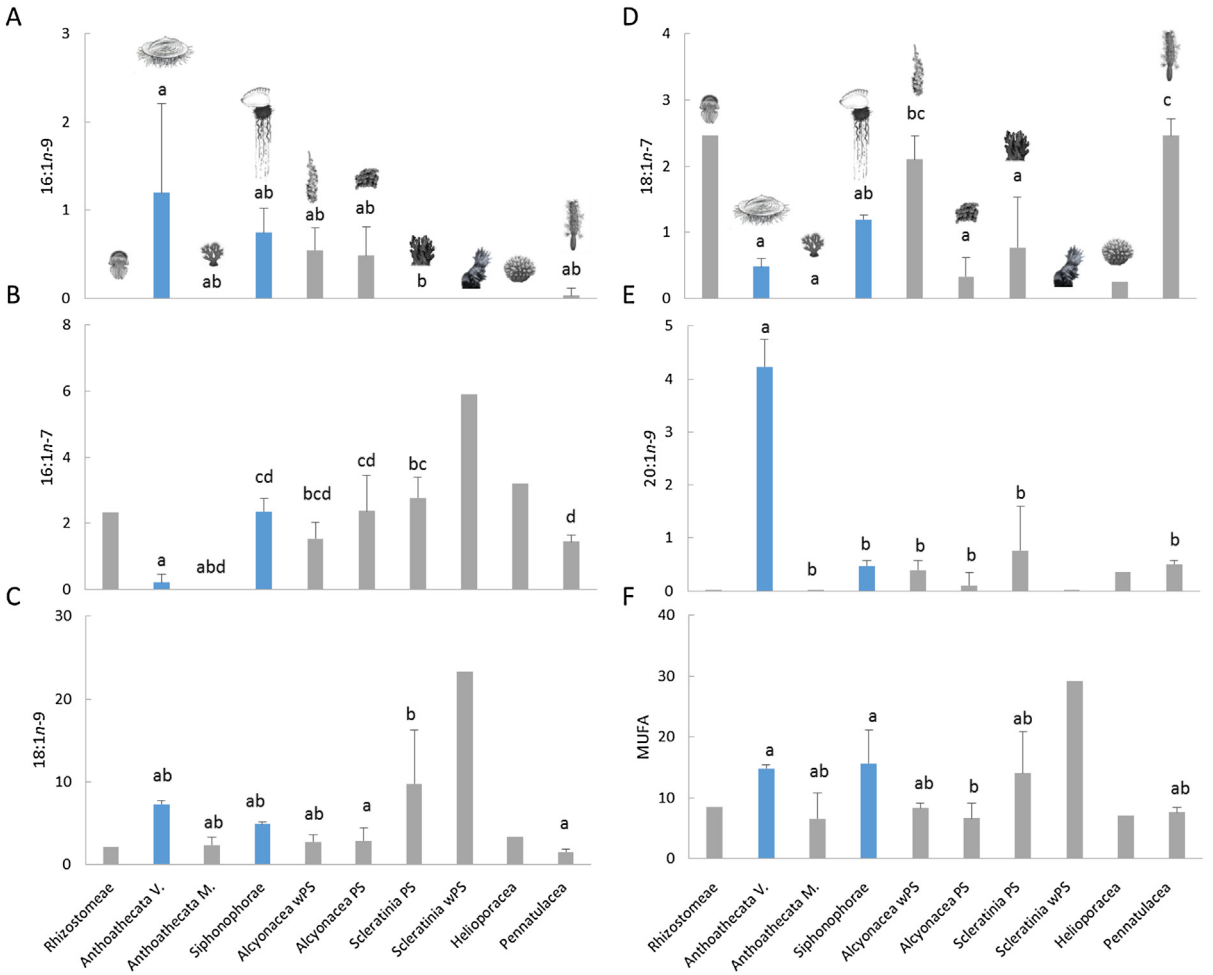

Fig. 2. Major monounsaturated fatty acids and respective fraction (MUFA) profile of 7 cnidarian orders. Values are means (±SD). "PS" and "wPS" stand for photosynthetic symbionts and without photosynthetic symbionts groups, respectively; "*Anthoathecata V.*" and "*Anthoathecata M.*" represent *Vellela vellela* and *Millepora* sp. (see Statistical Analysis section for more details). Letters denote significant differences between groups (Unequal N HSD post-hoc test).

well as of odd-numbered FAs (e.g. 15:0 and 17:0) thus indicating bacterial presence. As proposed by Imbs et al. (2007a), a possible explanation for the higher percentages observed for those FAs in *P. physalis* is that they occur as an adaptive response to the absence of symbiotic microalgae. A greater bacterial community living on and/or inside *P. physalis*, when comparing to *V. velella* should, therefore, be responsible for the increase in FA percentages.

Significantly higher percentage of 20:5*n*-3 and 22:6*n*-3 were found in *V. velella* when compared to *P. physalis*. This is probably explained by exposure of these species to different temperature regimes (Purcell, 1984; Purcell et al., 2012). In fact, membrane fluidity is largely determined by the balance between saturated and unsaturated fatty acids which in turn is affected by temperature (Holland, 1978; Beninger and Stephan, 1985; Ojea et al., 2004). According to previous studies, the general trend is an increase in unsaturated FAs at lower temperatures and an increase in saturated FAs at higher temperatures (especially in the phospholipid fraction; (Pazos et al., 1996; Hall et al., 2002)). This compositional adaptation of membrane lipids - homeoviscous adaptation, helps maintaining the correct membrane fluidity at the new conditions (Sinensky, 1974). This way, greater levels of the PUFAs 20:5*n*-3 and 22:6*n*-3 in *V. velella* could be linked to homeoviscous adaptation since water temperature was colder upon the stranding event of *V. velella* (Cascais, 15–17 °C) than that of *P. physalis* (Azores, 17–18 °C, max 22.8 °C). Still, one should keep in mind that the present FA analyses refers to total FA proportion and not to the phospholipid fraction alone. Another important factor that could help explain the differences in 20:5*n*-3 and 22:6*n*-3 levels in both species is food intake (Dalsgaard et al., 2003). The most common preys of *P. physalia* are leptocephalus and fish larvae (Purcell, 1984), both exhibiting a predominance of these FAs (Deibel et al., 2012). In accordance, the present study shows high levels of these FAs, similarly to what has been previously reported in other studies (e.g. Stillway, 1976). Contrarily, the diet of *V. velella* is mainly composed of harpacticoid copepods (Purcell et al., 2012), which exhibit high levels of 20:1*n*-9 and 22:1*n*-11 (Dalsgaard et al., 2003). Accordingly, *V. velella* exhibited a greater percentage of these FAs in comparison to *P. physalia*.

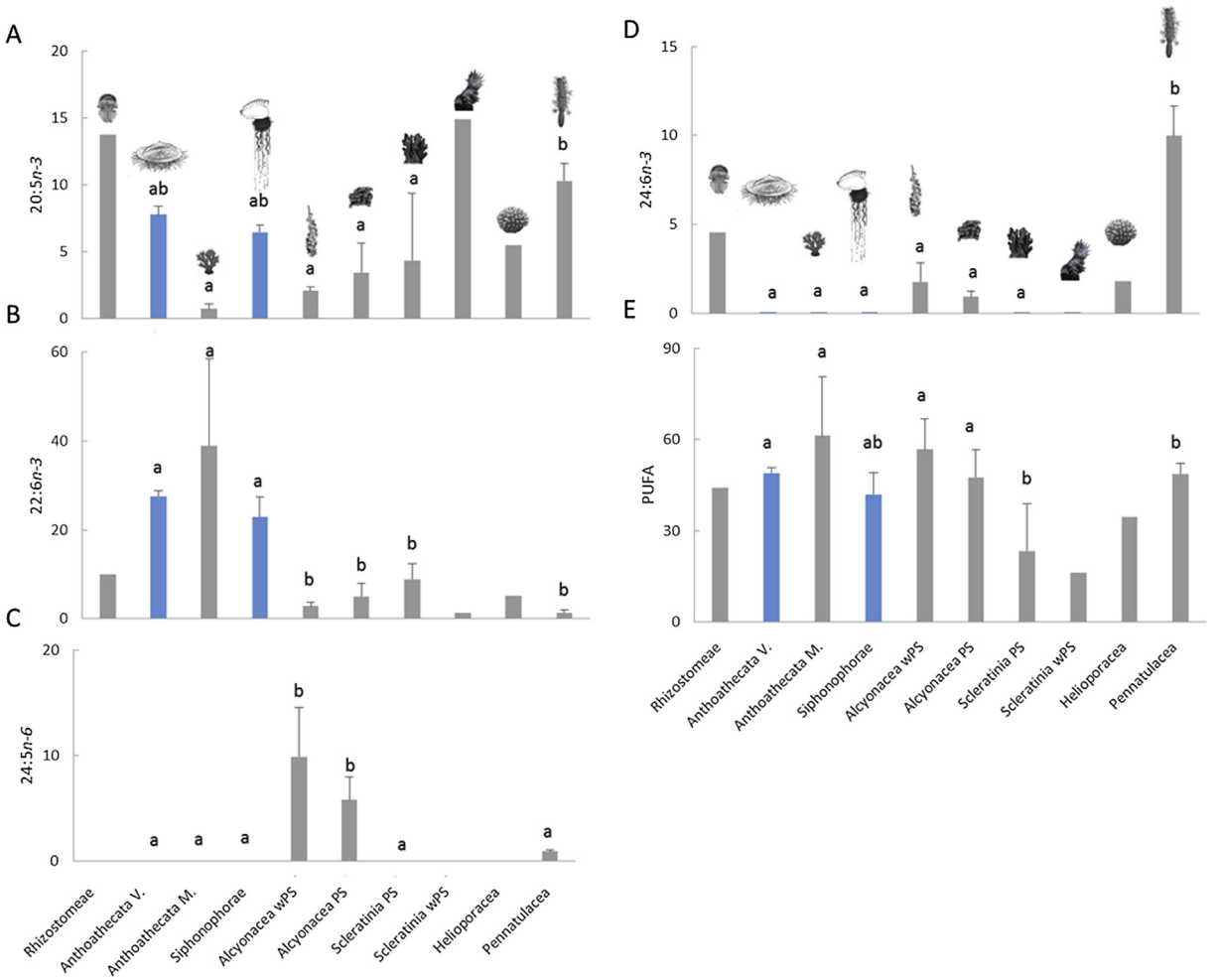

Fig. 3. Major polyunsaturated fatty acids, respective fraction (PUFA) and total fatty acid (total FA) profile of 7 cnidarian orders. Values are means (±SD). "PS" and "wPS" stand for photosynthetic symbionts and without photosynthetic symbionts groups, respectively; "*Anthoathecata V.*" and "*Anthoathecata M.*" represent *Vellela vellela* and *Millepora* sp. (see Statistical Analysis section for more details). Letters denote significant differences between groups (Unequal N HSD post-hoc test).

4.2. Intra-phylum differences in fatty acid composition

The PCA based on 26 FAs of 28 cnidarian species provided insights into the FA composition similarities/dissimilarities among the phylum. Three major factors contributing to species separation were identified: (i) presence/absence of symbionts, (ii) temperature profile of sampling region, and (iii) presence/absence of tetracosapolyenoic acids.

Differences between groups were mainly driven from the presence/quantity of dinoflagellate and bacterial FA markers. Species with photosynthetic symbionts (PS) were clearly separated from those not exhibiting PS, with the exception of Scleractinia. Hence, a separation was obtained between the species belonging to Anthoathecata, Helioporacea and Scleractinia orders and those belonging to Rhizostomae, Siphonophorae and Pennatulacea orders presenting higher percentage of C18 PUFAs (dinoflagellate markers) (Dalsgaard et al., 2003). Moreover, in general, higher percentage of bacterial markers (15:0, 17:0, Iso 17:0, Anteiso 16:0 and 18:1*n*-7) were found in species without PS (Dalsgaard et al., 2003) which confirms the theory proposed by Imbs et al. (2007a) which states that bacterial presence occur as an adaptive response to the absence of symbiotic microalgae.

Species inhabiting different latitudinal habitats such as temperate, sub-tropical and tropical, are exposed to distinct temperature regimes which are known to affect FA profiles (Holland, 1978; Beninger and Stephan, 1985; Ojea et al., 2004). In our study, species captured in temperate waters (belonging to Pennatulacea, Siphonophorae and Anthoathecata V. orders), were shown to possess distinct FA profile than those from species captured in tropical waters (Anthoathecata *M.*, Scleractinia, Alcyonacea, and Helioporacea orders). Interestingly, *C. tagi* (Rhizostomae) while collected in Portuguese waters (Morais et al., 2009), exhibited a similar FA profile with tropical species. This may be explained by the water temperature registered in Portuguese waters by the time of these organisms' collection (approximately 23 °C; Morais et al., 2009), which is similar to the

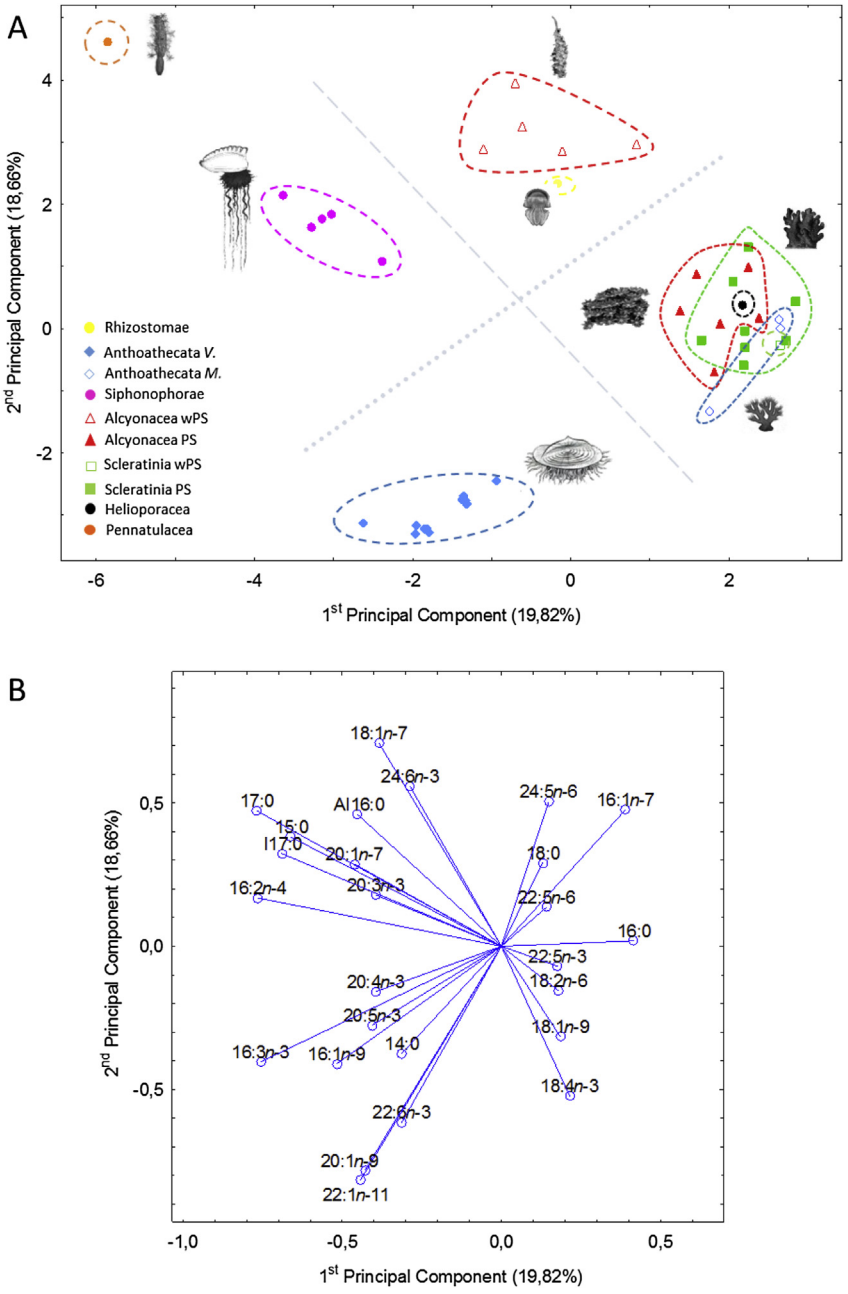

Fig. 4. Principal component analysis (PCA) based on total fatty acid (FA) composition (26 FAs of 28 cnidarian species). A) Principal component plot; Broken and dotted lines are only represented for visualization purposes and do not represent any data; "PS" and "wPS" stand for photosynthetic symbionts and without photosynthetic symbionts groups, respectively; "*Anthoathecata V.*" and "*Anthoathecata M.*" represent *Vellela vellela* and *Millepora* sp. (see Statistical Analysis section for more details); B) loading plot of FAs and their contribution to the spread along PC1 and PC2.

average temperature in tropical waters. On the other hand, the temperature upon species' collection in Portuguese waters of the other studies ranged between 15 °C and 18 °C (Baptista et al., 2012 and present study). The latitude-related separation of species was determined by the occurrence of high proportions of 20:5*n*-3, 22:6*n*-3, 16:3*n*-3, 16:1*n*-9, 20:1*n*-9 and 22:1*n*-11 in *V. cynomorium*, *V. velella* and *P. physalis*. Some of these FAs, namely 20:5*n*-3 and 22:6*n*-3, can be recognised as an adaptation to the low temperatures occurring in a temperate marine environment (Holland, 1978; Beninger and Stephan, 1985; Ojea et al., 2004). Still, temperature variation with latitude is one among a group of factors possibly dictating the FA profile differences observed between temperate and tropical species. The potential influence of dietary items, genetic inherent ability to

synthesize FAs and life cycle stage, among others, should not be disregarded when analysing the FA profiles of the aforementioned species (Arts et al., 2001; Dalsgaard et al., 2003; Sara, 2009).

Several FAs can act as unique chemical markers of some taxonomic groups. The PUFAs 24:5*n*-6 and 24:6*n*-3, for example, are considered chemotaxonomic markers of the subclass Octocorallia (e.g. Svetashev and Vysotskii, 1998; Imbs and Dautova, 2008; Baptista et al., 2012). In accordance, from all species analysed, only octocorals exhibit 24:5*n*-6 (at a concentration higher than that of trace levels). However, 24:6*n*-3 was also found in the scyphozoan *C. tagi* and in a generally higher concentration than in the octocorals reported herein, with the exception of the pennatulacean *V. cynomorium*. A similar result was found by Nichols et al. (2003) in the pelagic jellyfish *Aurelia* sp., where this unusual long-chain fatty acid constituted about 9.3% of total fatty acid. Therefore, we conclude that the presence of 24:6*n*-3 alone is not a suitable chemotaxonomic marker of the subclass Octocorallia.

This study gives an enormous contribution on the knowledge of the lipids biochemistry of hydrozoans. Moreover, it supports the use of FA profile as chemotaxonomic biomarkers, not only for the distinction between V. vellela an P. physalis but also between these species and other cnidarians.

Acknowledgments

Lopes A. R., Baptista, M. and Dionísio, G. were supported by PhD scholarships funded by the Fundação para a Ciência e Tecnologia (QREN-POPH-Type 4.1 – Advanced training, subsidized by the European Social Fund and national funds MEC). Gomes-Pereira, J. was supported by the doctoral grant from the Regional Directorate for Education, Science and Culture, of the Regional Government of the Azores (M3.1.2/F/062/2011). This work would not have been possible without the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation of this study mainly Luís Pires from DOP, Univ. of Azores.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2016.03.016.

References

Arts, M.T., Ackman, R.G., Holub, B.J., 2001. “Essential fatty acids” in aquatic ecosystems: a crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci. 58, 122–137.

Banaszak, A.T., Iglestas-Prieto, R., Trench, R.K., 1993. *Scrippsiella velellae sp*. nov. (peridiales) and *Gloeokinium viscum sp.* nov. (phytodiniales), dinoflagellate symbionts of two hydrozoans (cnidaria). J. Phycol. 29, 517–528.

Baptista, M., Lopes, V.M., Pimentel, M.S., Bandarra, N., Narciso, L., Marques, A., Rosa, R., 2012. Temporal fatty acid dynamics of the octocoral *Veretillum cynomorium*. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 161, 178–187.

Baptista, M., Repolho, T., Maulvault, A., Lopes, V., Narciso, L., Marques, A., Bandarra, N., Rosa, R., 2014. Temporal dynamics of amino and fatty acid composition in the razor clam Ensis siliqua (Mollusca: Bivalvia). Helgo.I Mar. Res. 1–18.

Beninger, P.G., Stephan, G., 1985. Seasonal variations in the fatty acids of the triacylglycerols and phospholipids of two populations of adult clam (*Tapes decussatus* and *T. philippinarum*) reared in a common habitat. Comp. Biochem. Physiol. B 81, 591–601.

Bergé, J.-P., Barnathan, G., 2005. Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. Mar. Biotechnol. 49–125.

Colaço, A., Prieto, C., Martins, A., Figueiredo, M., Lafon, V., Monteiro, M., Bandarra, N.M., 2009. Seasonal variations in lipid composition of the hydrothermal vent mussel *Bathymodiolus azoricus* from the Menez Gwen vent field. Mar. Environ. Res. 67, 146–152.

Dalsgaard, J., St John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. Adv. Mar. Biol. Academic Press., 225–340.

Deibel, D., Parrish, C., Grønkjær, P., Munk, P., Nielsen, T.G., 2012. Lipid class and fatty acid content of the leptocephalus larva of tropical eels. Lipids 47, 623–634.

Drazen, J.C., Phleger, C.F., Guest, M.A., Nichols, P.D., 2008. Lipid, sterols and fatty acid composition of abyssal holothurians and ophiuroids from the North-East Pacific Ocean: food web implications. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 151, 79–87.

Freites, L., Labarta, U., Fernández-Reiriz, M.J., 2002. Evolution of fatty acid profiles of subtidal and rocky shore mussel seed (Mytilus galloprovincialis, Lmk.). Influence of environmental parameters. J. Exp. Mar. Biol. Ecol. 268, 185–204.

Freites, L., García, N., Troccoli, L., Maeda-Martínez, A.N., Fernández-Reiriz, M.J., 2010. Influence of environmental variables and reproduction on the gonadal fatty acid profile of tropical scallop Nodipecten nodosus. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 157, 408–414.

Gast, R., Caron, D., 1996. Molecular phylogeny of symbiotic dinoflagellates from planktonic foraminifera and radiolaria. Mol. Biol. Evol. 13, 1192–1197.

Hall, J.M., Parrish, C.C., Thompson, R.J., 2002. Eicosapentaenoic acid regulates scallop (Placopecten magellanicus) membrane fluidity in response to cold. Biol. Bull. 202, 201–203.

Harland, A., Navarro, J., Davies, P.S., Fixter, L., 1993. Lipids of some Caribbean and Red Sea corals: total lipid, wax esters, triglycerides and fatty acids. Mar. Biol. 117, 113–117.

Holland, D., 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In: Malins, D.C., Sargent, J.R. (Eds.), Biochemical and Biophysical Perspectives in Marine Biology, 4. Academic press, London and New York, pp. 85–123.

Imbs, A., Dautova, T., 2008. Use of lipids for chemotaxonomy of octocorals (Cnidaria: Alcyonaria). Russ. J. Mar. Biol. 34, 174–178.

Imbs, A.B., Demidkova, D.A., Dautova, T.N., Latyshev, N.A., 2009. Fatty acid biomarkers of symbionts and unusual inhibition of tetracosapolyenoic acid biosynthesis in corals (Octocorallia). Lipids 44, 325–335.

Imbs, A.B., Demidkova, D.A., Latypov, Y.Y., Pham, L.Q., 2007b. Application of fatty acids for chemotaxonomy of reef-building corals. Lipids 42, 1035–1046.

Imbs, A.B., Latyshev, N.A., Dautova, T.N., Latypov, Y.Y., 2010. Distribution of lipids and fatty acids in corals by their taxonomic position and presence of zooxanthellae. Mar. Ecol. Prog. Ser. 409, 65–75.

Imbs, A.B., Latyshev, N.A., Zhukova, N.V., Dautova, T.N., 2007a. Comparison of fatty acid compositions of azooxanthellate *Dendronephthya* and zooxanthellate soft coral species. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 148, 314–321.

Imbs, A.B., Yakovleva, I.M., 2012. Dynamics of lipid and fatty acid composition of shallow-water corals under thermal stress: an experimental approach. Coral Reefs 31, 41–53.

Imbs, A.B., Yakovleva, I.M., Dautova, T.N., Bui, L.H., Jones, P., 2014. Diversity of fatty acid composition of symbiotic dinoflagellates in corals: evidence for the transfer of host PUFAs to the symbionts. Phytochemistry 101, 76–82.
Kaneda, T., 1991. Iso-and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol. Rev. 55, 288–302.
Morais, Z.B., Pintão, A.M., Costa, I.M., Calejo, M.T., Bandarra, N.M., Abreu, P., 2009. Composition and in vitro antioxidant effects of jellyfish *Catostylus tagi* from sado estuary (SW Portugal). J. Aquat. Food Prod. Technol. 18, 90–107.
Mortillaro, J.-M., Pitt, K.A., Lee, S.Y., Méziane, T., 2009. Light intensity influences the production and translocation of fatty acids by zooxanthellae in the jellyfish *Cassiopea sp*. J. Exp. Mar. Biol. Ecol. 378, 22–30.
Napolitano, G.E., Pollero, R.J., Gayoso, A.M., Macdonald, B.A., Thompson, R.J., 1997. Fatty acids as trophic markers of phytoplankton blooms in the bahia blanca estuary (Buenos Aires, Argentina) and in trinity bay (Newfoundland, Canada). Biochem. Syst. Ecol. 25, 739–755.
Nichols, P.D., Danaher, K.T., Koslow, J.A., 2003. Occurrence of high levels of tetracosahexaenoic acid in the jellyfish *Aurelia sp*. Lipids 38, 1207–1210.
Ojea, J., Pazos, A., Martınez, D., Novoa, S., Sanchez, J., Abad, M., 2004. Seasonal variation in weight and biochemical composition of the tissues of *Ruditapes decussatus* in relation to the gametogenic cycle. Aquaculture 238, 451–468.
Oku, H., Yamashiro, H., Onaga, K., Sakai, K., Iwasaki, H., 2003. Seasonal changes in the content and composition of lipids in the coral Goniastrea aspera. Coral Reefs 22, 83–85.
Parrish, C.C., 2013. Lipids in marine ecosystems. ISRN Oceanogr. 2013.
Patton, J.S., Battey, J.F., Rigler, M.W., Porter, J.W., Black, C.C., Burris, J.E., 1983. A comparison of the metabolism of bicarbonate 14C and acetate 1-14C and the variability of species lipid compositions in reef corals. Mar. Biol. 75, 121–130.
Pazos, A.J., Ruíz, C., García-Martin, O., Abad, M., Sánchez, J., 1996. Seasonal variations of the lipid content and fatty acid composition of crassostrea gigas cultured in E1 Grove, Galicia, N.W. Spain. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 114, 171–179.
Purcell, J.E., 1984. Predation on fish larvae by *Physalia physalis*, the Portuguese man of war. Mar. Ecol. Prog. Ser. 19, 189–191.
Purcell, J.E., Clarkin, E., Doyle, T.K., 2012. Foods of *Velella velella* (Cnidaria: Hydrozoa) in algal rafts and its distribution in Irish seas. Hydrobiologia 690, 47–55.
Quinn, G.P., Keough, M.J., 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press.
Rodrigues, L.J., Grottoli, A.G., Pease, T.K., 2008. Lipid class composition of bleached and recovering *Porites compressa* Dana, 1846 and *Montipora capitata* Dana, 1846 corals from Hawaii. J. Exp. Mar. Biol. Ecol. 358, 136–143.
Rosa, R., Calado, R., Narciso, L., Nunes, M., 2007. Embryogenesis of decapod crustaceans with different life history traits, feeding ecologies and habitats: a fatty acid approach. Mar. Biol. 151, 935–947.
Sara, J.E., 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds.), Lipids in Aquatic Ecosystems. Springer New York, New York, pp. 281–308.
Sargent, J., Whittle, K., 1981. Lipids and hydrocarbons in the marine food web. In: Longhurst, A. (Ed.), Analysis of Marine Ecosystems. Acad. Press., London, pp. 491–533.
Sinensky, M., 1974. Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in Escherichia coli. Proc. Natl. Acad. Sci. U. S. A. 71, 522–525.
Stillway, L.W., 1976. Fatty acids of the portuguese man-of-war *Physalia physalis*. Comp. Biochem. Physiol. B Comp. Biochem. 53, 535–537.
Svetashev, V.I., Vysotskii, M.V., 1998. Fatty acids of *Heliopora coerulea* and chemotaxonomic significance of tetracosapolyenoic acids in coelenterates. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 119, 73–75.
Trench, R.K., 1993. Microalgal-invertebrate symbioses: a review. Endocytobiosis Cell. Res. 9, 135–175.
Volkman, J.K., Barrett, S.M., Blackburn, S.I., Mansour, M.P., Sikes, E.L., Gelin, F., 1998. Microalgal biomarkers: a review of recent research developments. Org. Geochem 29, 1163–1179.
Ward, S., 1995. Two patterns of energy allocation for growth, reproduction and lipid storage in the scleractinian coral *Pocillopora damicornis*. Coral Reefs 14, 87–90.