Omega-3 on the fly: long-legged fly *Machaerium maritimae* as a potential source of eicosapentaenoic acid for aquafeeds

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Abstract

The need for more sustainable aquafeeds has prompted the exploration of insects as potential ingredients. However, commonly reared insects are not satisfactory regarding their fatty acid composition, lacking omega-3 long chain polyunsaturated fatty acids (n-3 LC PUFA), important nutrients for marine organisms. In this study, we present in detail the fatty acid profile of the long-legged fly *Machaerium maritimae* Haliday (Diptera: Dolichopodidae), an amphibiotic insect native to European coastal habitats. Specimens of this fly were collected in Ria de Aveiro coastal lagoon (Portugal) and the fatty acid profile was analysed through gas chromatography coupled with mass spectrometry. Results revealed a remarkably high level of n-3 LC PUFA, (11 µg/mg dry weight, 20% of the fatty acid profile) namely eicosapentaenoic acid, and a low level of saturated fatty acids. This value is higher than what is found in *Hermetia illucens* (black soldier fly), while comparable to other aquatic insects. We also report the presence of 50 distinct fatty acids, including multiple branched-chain and PUFA, and discuss potential implications of their presence on the ecology of this species. Variation in the fatty acid profile along the landscape is also presented. Our results indicate that *M. maritimae* can potentially be a high-quality lipid source for marine aquafeeds. Current legislation in Europe allowing the use of insects in aquaculture is restricted to a few selected species, often non-native, however, further research should consider native insects as potential alternatives to current ingredients in aquafeed.

Keywords: coastal insects, Diptera, Dolichopodidae, fatty acids, novel ingredients

1. Introduction

With a global population expected to surpass 9.7 billion by 2050 (DESA UN, 2017), the demand for limited resources, such as energy and food, will increase accordingly. Regarding food security, it is estimated that the demand for animal origin products will double, not only due to the increased population, but also to the improvement of economic conditions (FAO/WHO, 2019).

Aquaculture is the food production system which is benefiting the most from this increasing demand, compensating for the apparent stagnation in fisheries (FAO, 2020). However, the high dependence on marine ingredients for feed production (i.e. fishmeal and fish

oil) raises doubts about the long-term sustainability of this activity and its capacity to sustain a growing population. These feed ingredients are the main source of important lipids for cultured fish, specifically omega-3 long chain polyunsaturated fatty acids (LC-PUFA), such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (Henry *et al.*, 2015). These lipids promote various health benefits (Shahidi and Ambigaipalan, 2018), and their inclusion in the diet is essential for farmed fish growth, especially for marine species, and for consumers' own health benefits (FAO, 2020). Other alternatives such as plant-based, animal-based ingredients or single cell protein, do not present all the nutrients required for marine organisms and their intensive production has high environmental impacts or production costs (Ameixa *et al.*, 2020).

Amongst the alternative feed sources investigated, insects have been suggested as a natural, low-carbon emitting and potentially cost-effective source of high quality proteins and other important nutrients (Ameixa *et al.*, 2020; Henry *et al.*, 2015; Van Huis *et al.*, 2013). It has been demonstrated that insects can be included in fish feeds with positive results, depending on the species used, inclusion rate, and overall composition of the diet (Barroso *et al.*, 2014; Belghit *et al.*, 2019; Dobermann *et al.*, 2017; Sánchez-Muros *et al.*, 2014). Although insect meals are frequently a valid alternative protein sources for fish, their fatty acid profile is often inadequate. Terrestrial insects, typically lack n-3 LC-PUFA, being richer in n-6 PUFA (Makkar *et al.*, 2014; Nogales-Mérida *et al.*, 2019; Rumpold and Schlüter, 2013). To overcome this constraint, the possible solutions are: (1) the manipulation of the fatty acid profile of the insects through their diets, which has been accomplished with some restrictions (Liland *et al.*, 2017; St-Hilaire *et al.*, 2007); or (2) proceed the screening of more viable insect species. The former may not always be economically viable, as n-3 rich substrates are expensive and can often be consumed directly by fish (Henry *et al.*, 2015; Stoneham *et al.*, 2018); the second option seems more promising, especially if these new species can efficiently recycle marine wastes that fish cannot (Sánchez-Muros *et al.*, 2014; Tran *et al.*, 2015).

From over 5.5 million insect species estimated to exist worldwide (Stork, 2018), aquatic and amphibiotic (with aquatic larvae and terrestrial, often winged, adults) species have great potential for aquaculture feeds. These have been implicated in nutrient transport from aquatic to terrestrial ecosystems, especially LC-PUFA (Popova *et al.*, 2017; Twining *et al.*, 2018), and their distinct fatty acid profiles might present a viable solution to meet nutritional requirements for fish feed. In addition, these insects are part of the natural diet of fish (Bell *et al.*, 1994; Nogales-Mérida *et al.*, 2019). The key difference in their fatty acid profile, is the relatively high values of EPA (around 15%), in comparison with terrestrial insects, which only have trace levels (Bell *et al.*, 1994; Fontaneto *et al.*, 2011; Sushchik *et al.*, 2003; Yang *et al.*, 2006). For this reason, it can be expected that coastal and marine insects may accumulate higher levels of n-3 fatty acids through their diet.

Dolichopodidae are small amphibiotic flies that inhabit a variety of humid environments (Miller *et al.*, 2008), with more than 7500 species described worldwide (Pollet *et al.*, 2019). Many are habitat specialists, well adapted to coastal and estuarine areas, such as mangroves and reed marshes. One of these species is *Machaerium maritimae* Haliday, a small metallic-green fly widely spread along the north-western European coast (Pollet *et al.*, 2019). The larvae and pupae of this insect have structural and physiological adaptations to thrive in brackish waters (d'Assis Fonseca,

1978), and the adults are commonly observed moving close or above water, in search of food, from May to September.

In the present study we characterised the fatty acid profile of *M. maritimae*, considering the species spatial distribution, by evaluating if it varied along collection sites in the Ria de Aveiro coastal lagoon (Portugal), a heterogenous mosaic landscape. Results are discussed in the context of the natural diet of this species and its potential as a novel aquafeed ingredient.

2. Materials and methods

Study area and experimental design

The study took place in Ria de Aveiro coastal lagoon (Portugal), characterised by wetlands habitats, e.g. mud and sand flats, seagrasses and salt marshes, classified under the Natura 2000 network and recognised as International Long-Term Ecosystem Research (ILTER) site (Lillebø *et al.*, 2015). The field work was carried out within one-week period in late July 2017. In order to account with the natural spatial variability, the specimens were collected on seven sites along the studied area, most of which are submerged during high tides (Figure 1). The vegetation in these sites is dominated by halophyte species, forming more or less continuous and homogenous patches, depending on the degree of tolerance to submersion and salinity (Asensi and Diez-Garretas, 2017).

Insect specimens were collected on the vegetation, in the selected locations, using sweeping nets (300 sweeps per plant species in each site (Figure 1), and then transferred with an insect aspirator to small flasks, which were preserved at -80 °C for later processing. Thus, a distinct number of specimens was collected in each sampling site, reflecting the heterogenous natural distribution of *M. maritimae* in Ria de Aveiro.

Lipid extraction and quantification

Five representative composed samples (n=5), consisting of 10 individuals each, originating from all sites, were selected exclusively to determine the lipid content (Figure 2). Total lipid extracts were obtained by a modified Folch method (Folch *et al.*, 1957). Briefly, 3 ml 2:1 (v/v) dichloromethane/methanol and 1 ml ultra-pure water were added to grinded freeze-dried insects, followed by vigorous vortexing and centrifugation (5 min at 2,000 rpm). The organic phase was recovered, and the process was repeated twice, by adding 1 ml of dichloromethane/methanol, vortex and centrifugation. The lipid content was measured gravimetrically.

Figure 1. Sampling sites in Ria de Aveiro: (1) Bico (40°43'33.0"N 8°38'46.3"W) dominated by *Halimione portucaloides* L. and *Juncus maritimus* L.; (2) Farinha (40°42'19.3"N 8°40'33.6"W), dominated by *J. maritimus* and *H. portucaloides*; (3) Salinas (40°40'35.0"N 8°40'20.0"W), dominated by *H. portucaloides*, *J. maritimus* and *Sarcocornia perennis* (Mill.) A.J. Scott, *Phragmites australis* (Cav.) Trin ex.Steud.; (4) Rio Novo (40°40'50.8"N 8°40'18.6"W), dominated by *Spartina maritima* (Curtis) Fernald; (5) Cacia (40°41'45.0"N 8°37'32.2"W), dominated by *J. maritimus*, *H. portucaloides* and *Atriplex patula* L.; (6) Reboxo (40°39'42.9"N 8°41'39.9"W) dominated by *H. portucaloides*, *S. perennis*, *S. maritima* and *Limonium vulgare* Mill.; and (7) Triângulo (40°38'38.6"N 8°39'54.3"W) dominated by *H. portucaloides*, *Salicornia ramosissima* J. Woods, and *Tamarix africana* Poir. Map was generated in ArcGIS version 10.6.1.

Fatty acid analysis by gas chromatography-mass spectrometry

The fatty acids were analysed directly through trans-methylation, with no prior extraction, by a procedure adapted from Aued-Pimentel *et al.* (2004). Samples (1 adult insect per each sample, for a total of 5-20 replicate samples per site; Figure 2 and Table 2) were freeze-dried, weighed (Table 2), grinded to dust and dissolved in 1 ml of n-hexane containing nonadecanoic acid methyl ester as internal standard (C19:0, 2.5 µg/ml), followed by 200 µl of methanolic solution of KOH (2 M). The mixture was then well homogenised. Finally, 2 ml of a saturated solution of

NaCl was added and the mixture was centrifuged at 2,000 rpm for 5 min. The phase with the fatty acid methyl esters was recovered and 2 µl were used as injection volume.

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted in an Agilent Technologies 6890 N Network (Santa Clara, CA, USA) equipped with a DB-FFAP column, 30 m of length, 0.32 mm of internal diameter and 0.25 µm film thickness (J&W Scientific, Folsom, CA, USA), connected to an Agilent 5973 Network Mass Selective Detector operating with the electron impact mode at 70 eV, scanning range m/z 50-550, 1 s cycle in full scan mode acquisition. Oven temperature was programmed with an

Figure 2. Overview of the workflow applied to the samples used for lipid extraction and to samples used for fatty acids analysis.

initial temperature of 80 °C, with a linear increase at 14.4 °C/min up to 220 °C, followed by a linear increase at 10 °C/min up to 240 °C, and finally 5 °C/min to 250 °C. The injector was set to 220 °C and the detector to 280 °C. Helium was used as carrier gas, at a flow rate of 1.4 ml/min. All solvents were of HPLC grade, purchased from Fisher Scientific (Loughborough, UK).

Data processing and statistical analysis

Individual fatty acid methyl esters peaks were analysed using PARADISE version 3.87 (Johnsen *et al.*, 2017) and identified by their retention time and using the NIST/EPA/NIH Mass Spectral Library with Search Program, version 2.2 (NIST 14, Gaithersburg, MD, USA). PARADISE is an implementation of the Parallel Factor analysis 2 (PARAFAC2) model, which allows for deconvolution, identification and quantification of chromatographic peaks in all samples simultaneously,

enabling measurements of co-eluting and low signal-to-noise ratio chromatographic peaks.

Quantification relative to the internal standard was carried out assuming a response factor of 1, and values were presented as relative abundances, in percentages. Data used in statistical analysis were those of fatty acids with overall abundance above 0.5%, plus the sum of all branched chain fatty acids (BCFA). Statistical analysis was conducted on PRIMER 6 with PERMANOVA+ add on (Anderson *et al.*, 2008; Clarke and Gorley, 2006).

Differences between sites were examined with a one-way analysis of similarity (ANOSIM) using a Bray-Curtis dissimilarity matrix on raw (untransformed) data ($\alpha=0.05$) (Happel *et al.*, 2017). This test calculates a global R statistic, with values close to one meaning high separation between groups, while values closer to zero imply a lower explicatory effect of the factor 'site' (Anderson *et al.*, 2008; Anderson

and Walsh, 2013; Ricardo *et al.*, 2015). Similarity percentages analysis (SIMPER) was conducted to determine the fatty acids that contributed the most for the dissimilarities between statistically different sites (cut-off 80%).

3. Results

Lipid extraction and quantification

The composed samples of ten individuals each ($n=5$, 50 adult *M. maritima* specimens in total) had 13.16 ± 0.27 mg (SE) in dry weight (DW). These samples yielded lipid

extracts containing 1.56 ± 0.09 mg (SE). Thus, these flies had a lipid content of $12.0 \pm 0.76\%$ (SE); moreover, each specimen weighed around 1.3 mg and contained around 0.15 mg of lipid.

Fatty acid analysis

Concerning the samples for GC-MS fatty acid analysis (individual flies collected in the study sites), these weighed (DW) on average 1.26 ± 0.04 mg (SE). The global fatty acid profile, obtained from these ($n=74$), was remarkably diverse, encompassing fifty-one different fatty acids (Table 1).

Table 1. Full list of the fatty acids identified in *M. maritima*, mean relative abundance (%) and absolute quantification (μg of fatty acid per mg of insect), $n=74$.^{1,2}

Fatty acid	Relative abundance % $\pm$ SE	$\mu\text{g}/\text{mg DW} \pm \text{SE}$	Fatty acid	Relative abundance % $\pm$ SE	$\mu\text{g}/\text{mg DW} \pm \text{SE}$
12:0	0.097 ± 0.007	0.067 ± 0.009	16:2 n-6	0.102 ± 0.023	0.085 ± 0.030
13:0	0.033 ± 0.003	0.024 ± 0.004	18:2 n-6	3.275 ± 0.203	1.824 ± 0.128
14:0	0.870 ± 0.123	0.690 ± 0.169	18:3 n-6	0.375 ± 0.027	0.251 ± 0.036
15:0	0.329 ± 0.046	0.250 ± 0.052	20:2 n-6	0.205 ± 0.015	0.121 ± 0.013
16:0	17.131 ± 0.555	10.448 ± 0.760	20:3 n-6	0.220 ± 0.011	0.127 ± 0.009
17:0	1.007 ± 0.086	0.591 ± 0.071	20:4 n-6	4.046 ± 0.214	2.301 ± 0.148
18:0	8.185 ± 0.341	4.516 ± 0.235	22:4 n-6	0.192 ± 0.015	0.112 ± 0.012
20:0	0.281 ± 0.011	0.152 ± 0.006	Σ n-6	8.414 ± 0.400	4.821 ± 0.309
Σ SFA	27.934 ± 0.416	16.738 ± 1.039			
12:0, 11-methyl	0.023 ± 0.003	0.017 ± 0.003	16:4 n-3	0.050 ± 0.011	0.034 ± 0.008
13:0, 3-methyl	0.015 ± 0.001	0.008 ± 0.001	18:3 n-3	0.919 ± 0.089	0.555 ± 0.071
13:0, 12-methyl	0.035 ± 0.004	0.025 ± 0.004	18:4 n-3	0.275 ± 0.030	0.194 ± 0.039
14:0, 13-methyl	0.145 ± 0.016	0.100 ± 0.015	18:5 n-3	0.232 ± 0.035	0.144 ± 0.032
14:0, 12-methyl	0.095 ± 0.010	0.063 ± 0.009	20:3 n-3	0.099 ± 0.006	0.057 ± 0.005
15:0, 14-methyl	0.149 ± 0.015	0.098 ± 0.013	20:4 n-3	0.171 ± 0.013	0.102 ± 0.011
15:0, 13-methyl	0.047 ± 0.003	0.027 ± 0.002	20:5 n-3	17.287 ± 0.772	9.423 ± 0.507
16:0, 15-methyl	0.373 ± 0.038	0.234 ± 0.031	21:5 n-3	0.144 ± 0.010	0.086 ± 0.010
16:0, 14-methyl	0.348 ± 0.037	0.210 ± 0.026	22:5 n-3	0.462 ± 0.037	0.275 ± 0.033
17:0, 16-methyl	0.103 ± 0.009	0.061 ± 0.007	22:6 n-3	0.810 ± 0.076	0.496 ± 0.067
17:0, 15-methyl	0.085 ± 0.006	0.046 ± 0.003	Σ n-3	20.449 ± 0.870	11.367 ± 0.654
16:1, 7-methyl	0.140 ± 0.013	0.086 ± 0.010			
Phytanic acid	0.068 ± 0.009	0.041 ± 0.007	16:2 n-4	0.122 ± 0.024	0.104 ± 0.033
Σ BCFA	1.628 ± 0.136	1.016 ± 0.117	16:3 n-4	0.066 ± 0.017	0.058 ± 0.022
14:1 n-5	0.150 ± 0.012	0.101 ± 0.015	17:2 n-5	0.125 ± 0.019	0.082 ± 0.018
16:1 n-9	7.958 ± 0.583	5.392 ± 0.821	20:2 n-7	0.150 ± 0.009	0.086 ± 0.008
17:1 n-7	1.293 ± 0.114	0.784 ± 0.108	22:2 $\Delta 5, 13$	0.183 ± 0.014	0.107 ± 0.012
18:1 n-9 + n-7	29.834 ± 0.824	16.523 ± 0.710	Σ other PUFA	0.645 ± 0.054	0.437 ± 0.070
19:1 n-9	0.425 ± 0.034	0.246 ± 0.030			
20:1 n-9 + n-7	1.272 ± 0.131	0.816 ± 0.124	Σ PUFA	29.508 ± 0.891	16.624 ± 0.824
Σ MUFA	40.931 ± 0.825	23.862 ± 1.315	n-6/n-3	0.535 ± 0.055	0.535 ± 0.055
			Total FA		58.240 ± 2.937

¹ n-6/n-3 ratio was calculated as the mean of ratios from each sample.

² BCFA = branched chain fatty acids; DW = dry weight; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SE = standard error; SFA = saturated fatty acids

Saturated fatty acids (SFA) comprised 28% of the profile, with 16:0 being the most abundant of the SFA and the third most abundant in the total fatty acid profile (17%). Monounsaturated fatty acids (MUFA) constituted 41% of the profile, dominated by 18 carbons long fatty acids (18:1 n-9 and n-7), representing 30% of the total. PUFA exhibited high content (30%) and diversity, with twenty-two different fatty acids being identified. Among these, the most abundant contained 20 carbon atoms: 20:5 n-3 (EPA, 17%) and 20:4 n-6 (arachidonic acid, 4%). Total n-6 and n-3 averaged around 8% and 20%, respectively, resulting in a low n-6/n-3 ratio (around 0.4 to 0.5; Table 1). Diversity was also higher in the n-3 family (10 fatty acids), with a length between 16 and 22 carbons. Other PUFA families identified include n-7, n-4 and n-5. Lastly, 13 BCFA were detected (1.6% of the profile), including iso- and anteiso-(mono) methyl BCFA, an unsaturated branched-chain (7-methyl-6-hexadecenoic acid) and the (poly)methyl phytanic acid (3,7,11,15-tetramethylhexadecanoic acid). While the FA profile mostly consists of even-carbon chains, odd-chain fatty acids represent a considerable portion of the total ($\approx 3.8\%$), especially 17 carbon fatty acids.

Concerning the fatty acid content, 100 mg of *M. maritima* meal would provide, on average, 5.8 mg of fatty acids. Of these, 1.7 mg are PUFA, of which 1.1 mg is n-3. The average fatty acid profile of *M. maritima*, recorded in each sampling site, is summarised in Table 2.

Statistical analysis

The dominant fatty acids present in the flies were roughly the same across the different sites, albeit with differences in their relative contributions. The ANOSIM test revealed these differences to be highly significant (Global $R=0.342$, $P<0.001$). The relatively low value of R denotes high natural variability within groups (samples from the same site), with some overlap between them. Thus, not all sites were found to be different in the subsequent pair-wise comparisons (Table 3).

Reboxo was statistically different from all the others, while Cacia differed from all locations except Triângulo. The remaining five sites were not statistically distinct, with the exception of Salinas and Triângulo ($P<0.001$). Between site dissimilarity is mostly explained by 18:1 and 20:5 n-3 (EPA), according to the SIMPER results (Supplementary Material Table S1). Other relevant fatty acids are 16:1, 16:0 and 18:0, that were not only the most abundant, but also had the highest standard deviations (Tables 1 and Supplementary Material Table S1).

The proportions of each fatty acid varied along the landscape. The differences observed between Cacia and the other sites were mainly due to the lower levels of 20:5 n-3 ($7.7\pm 3.3\%$) in this site, accompanied by an increase in 18:1 ($\approx 38\%$). In Reboxo, levels of 18:1 were also high

Table 2. *M. maritima* fatty acid characterisation per study site, expressed as relative abundances (%) $\pm$ SE. Sample mean weight (mg) $\pm$ SE is provided in the first table row. Only the top contributing fatty acids are shown in the table (average abundance $>0.5\%$).¹

	Bico (n=10)	Cacia (n=5)	Farinha (n=5)	Reboxo (n=20)	Rio Novo (n=5)	Salinas (n=15)	Triângulo (n=14)
Weight (mg)	0.8 $\pm$ 0.1	1.5 $\pm$ 0.1	1.1 $\pm$ 0.2	1.6 $\pm$ 0.1	1.3 $\pm$ 0.2	1.0 $\pm$ 0.1	1.4 $\pm$ 0.1
14:0	0.5 $\pm$ 0.1	0.6 $\pm$ 0.0	1.2 $\pm$ 0.2	0.7 $\pm$ 0.0	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	1.9 $\pm$ 0.4
16:0	13.8 $\pm$ 0.9	19.1 $\pm$ 0.8	17.2 $\pm$ 1.2	20.2 $\pm$ 0.9	16.1 $\pm$ 1.4	14.1 $\pm$ 0.7	18.1 $\pm$ 1.1
16:1	4.7 $\pm$ 0.3	12.4 $\pm$ 0.6	5.8 $\pm$ 0.3	8.8 $\pm$ 0.3	6.1 $\pm$ 1.1	4.9 $\pm$ 0.5	12.2 $\pm$ 1.7
17:0	1.6 $\pm$ 0.3	0.4 $\pm$ 0.1	1.3 $\pm$ 0.2	0.5 $\pm$ 0.0	1.3 $\pm$ 0.2	1.5 $\pm$ 0.1	0.8 $\pm$ 0.1
17:1	2.0 $\pm$ 0.5	0.5 $\pm$ 0.1	1.3 $\pm$ 0.1	0.9 $\pm$ 0.0	1.4 $\pm$ 0.1	1.5 $\pm$ 0.1	1.3 $\pm$ 0.1
18:0	10.4 $\pm$ 0.7	7.1 $\pm$ 0.3	9.0 $\pm$ 0.4	5.8 $\pm$ 0.2	9.7 $\pm$ 1.0	10.9 $\pm$ 0.5	6.7 $\pm$ 0.5
18:1	27.3 $\pm$ 0.9	38.3 $\pm$ 0.9	23.6 $\pm$ 2.4	35.1 $\pm$ 0.3	30.8 $\pm$ 2.4	27.3 $\pm$ 1.4	25.6 $\pm$ 2.0
18:2n-6	3.2 $\pm$ 0.3	4.4 $\pm$ 0.4	2.8 $\pm$ 0.4	2.7 $\pm$ 0.2	2.9 $\pm$ 0.5	3.1 $\pm$ 0.2	4.2 $\pm$ 0.6
18:3n-3	0.9 $\pm$ 0.1	0.4 $\pm$ 0.1	1.3 $\pm$ 0.2	0.6 $\pm$ 0.0	0.7 $\pm$ 0.1	1.1 $\pm$ 0.1	1.4 $\pm$ 0.3
20:1	1.0 $\pm$ 0.1	0.6 $\pm$ 0.1	2.5 $\pm$ 0.6	0.9 $\pm$ 0.1	1.5 $\pm$ 0.5	1.6 $\pm$ 0.2	1.4 $\pm$ 0.3
20:4n-6	4.0 $\pm$ 0.4	4.4 $\pm$ 0.4	4.1 $\pm$ 0.7	4.0 $\pm$ 0.3	3.4 $\pm$ 0.3	3.8 $\pm$ 0.2	4.5 $\pm$ 0.5
20:5n-3	22.4 $\pm$ 1.3	7.7 $\pm$ 1.5	19.3 $\pm$ 1.0	15.0 $\pm$ 0.8	18.4 $\pm$ 1.3	22.2 $\pm$ 1.0	14.0 $\pm$ 1.8
22:6n-3	1.0 $\pm$ 0.1	0.2 $\pm$ 0.1	1.7 $\pm$ 0.4	0.5 $\pm$ 0.0	0.8 $\pm$ 0.1	1.1 $\pm$ 0.1	0.7 $\pm$ 0.1
BCFA	1.4 $\pm$ 0.1	1.1 $\pm$ 0.1	2.7 $\pm$ 0.6	1.4 $\pm$ 0.1	2.3 $\pm$ 0.9	1.4 $\pm$ 0.2	1.9 $\pm$ 0.3
Others	5.8 $\pm$ 0.7	2.7 $\pm$ 0.2	6.2 $\pm$ 1.0	3.0 $\pm$ 0.1	3.9 $\pm$ 0.3	5.0 $\pm$ 0.3	5.4 $\pm$ 0.5

¹ BCFA = branched chain fatty acids; SE = standard error.

Table 3. Results from the ANOSIM pairwise comparisons between sites.

Paired sites	R statistic	P-value
Bico, Cacia	0.777	<0.001
Bico, Farinha	0.179	0.095
Bico, Reboxo	0.648	<0.001
Bico, Rio Novo	0.057	0.297
Bico, Salinas	-0.04	0.698
Bico, Triangulo	0.116	0.062
Cacia, Farinha	0.836	0.008
Cacia, Reboxo	0.404	0.004
Cacia, Rio Novo	0.476	0.016
Cacia, Salinas	0.734	<0.001
Cacia, Triangulo	-0.107	0.776
Farinha, Reboxo	0.706	<0.001
Farinha, Rio Novo	-0.008	0.452
Farinha, Salinas	0.111	0.123
Farinha, Triangulo	-0.117	0.826
Reboxo, Rio Novo	0.341	0.021
Reboxo, Salinas	0.565	<0.001
Reboxo, Triangulo	0.389	<0.001
Rio Novo, Salinas	0.054	0.282
Rio Novo, Triangulo	-0.145	0.903
Salinas, Triangulo	0.234	<0.001

(≈35%) but the content in EPA was twice as much as in Cacia (≈15%). By contrast, samples from Bico and Salinas had the highest content in EPA (≈22±4%). Some flies from Bico had maximum abundances of EPA, up to 30%. The n-6/n-3 ratio was, on average, 0.5. However, it should be noted the very high variability between samples, even within groups, with many shorter fatty acids being almost absent from most samples.

4. Discussion

This study is the first characterisation of the fatty acid profile of a dolichopodid fly. A wide diversity of fatty acids was identified in *M. maritima*, particularly of methyl-branched with less than 16 carbons and PUFA of varying length and unsaturation degree. From the latter, the diversity and high abundance of n-3 fatty acids is of particular interest to ensure the good condition of captive fish (Bou *et al.*, 2017; Henry *et al.*, 2015).

Concerning the dietary value of *M. maritima*, this insect can be considered as an interesting source of n-3 PUFA (11 µg/mg DW), namely EPA. A relative abundance of n-3 fatty acids up to 20% or more is well above that recorded for other insects currently used in aquafeeds, such as *Hermetia illucens* (black soldier fly). Even when *H. illucens* was supplemented with fish offal or fishmeal, n-3 fatty

acids comprised only 4 and 5.3% of the fatty acid profile, respectively (Barroso *et al.*, 2017; St-Hilaire *et al.*, 2007). Whenever higher values for these fatty acids were reported, a negative trade-off occurred in the form of reduced growth and nutritional imbalances (Liland *et al.*, 2017).

The fatty acid profile of *M. maritima* is richer in n-3 fatty acids than other insects currently investigated for aquafeeds (Nogales-Mérida *et al.*, 2019), but it is rather comparable to that of other semi-aquatic insects. For instance, freshwater insects can exhibit values of total n-3 ranging from 20 to 30% (Bell *et al.*, 1994; Sushchik *et al.*, 2013). However, in some species, much of the n-3 PUFA content corresponds to alpha-linolenic (18:3 n-3, or ALA) (Bell *et al.*, 1994; Ghioni *et al.*, 1996). In *M. maritima* however, the main n-3 PUFA was EPA, which is known to be required in the diet of marine organisms (Henry *et al.*, 2015), due to their low conversion efficiency from ALA. Another issue, regarding the conversion of fatty acids, is the enzymatic competition with the n-6 family (Lee *et al.*, 2016). Nevertheless, the lipids of *M. maritima* were low in n-6 fatty acids, and the main one from this group was the arachidonic acid (20:4 n-6), also a LC-PUFA. By contrast, the precursor linoleic acid (18:2 n-6) is usually the main PUFA in terrestrial food sources, including insects (Bayes *et al.*, 2014; Fontaneto *et al.*, 2011; Nogales-Mérida *et al.*, 2019; Yang *et al.*, 2006).

The branched fatty acids, both saturated and unsaturated, iso and anteiso, are typically associated with bacterial sources (Meziane *et al.*, 1997; Tsunoda *et al.*, 2017) but can sometimes be synthesised by insects (Blomquist *et al.*, 1994; Finck *et al.*, 2016). We also recorded the presence of C16 n-4 PUFA, a typical diatom marker, and 18:5 n-3, which is associated with dinoflagellates (Dalsgaard *et al.*, 2003; Dunstan *et al.*, 1993; Ricardo *et al.*, 2017; Zhukova, 1995). However, since these fatty acids were present in low quantities, the microalgal communities were most likely dominated by other microalgal taxa, such as cryptophytes, prymnesiophytes and green microalgae (Dalsgaard *et al.*, 2003; Rossi *et al.*, 2006; Sushchik *et al.*, 2013). *M. maritima* are evidently part of the estuarine trophic chain, as revealed by these fatty acid trophic markers, presumably as consumers of small invertebrates.

Traces of phytanic acid were also recorded, which is a by-product of chlorophyll digestion, highly associated with both herbivory and herbivore-consumption (van den Brink and Wanders, 2006). In contrast, long chain SFA (e.g. 22:0, 24:0), which are characteristic of terrestrial plants, including *Salicornia ramosissima*, *Halimione portucaloides* (Maciel *et al.*, 2018) and *Spartina maritima* (Bergamino *et al.*, 2014), were not found. The presence of these fatty acids could imply direct consumption of the plants where the species was found, but that was not the case. This information may help clarify the trophic ecology of *M. maritima*. Dolichopodids are classically predators of aquatic larvae

and other insects (Miller *et al.*, 2008). This hypothesis is reinforced by the lower levels of EPA in the individuals collected furthest away from sea water, in Cacia (Figure 1), where flies would be restricted to terrestrial insect prey. Consumption of macroalgae by adult flies seems unlikely, since adults were collected in the vegetation and the fatty acid profile is fairly distinct from seaweed flies (Biancarosa *et al.*, 2018). On the other end, our results suggest that the larval stages may feed on decomposing organic matter of aquatic (likely algal) origin, thus explaining the presence of bacterial and microbial trophic markers.

The MUFA portion of *M. maritima* seem to be less informative. Despite oleic acid being often used as a trophic marker of carnivory in marine animals, micro- and macroalgae sometimes contain substantial amounts of this fatty acid (Dalsgaard *et al.*, 2003). For instance, in seaweed flies, the dominant MUFA is 16:1 n-7 (Biancarosa *et al.*, 2018), while values around 30% of 18:1 (n-9) have been reported in the black soldier fly (Barroso *et al.*, 2017).

In *M. maritima*, the 18:1 fatty acids appears to be associated to the reduced levels of PUFA, especially EPA, as seen in Cacia and Reboxo. The higher abundance of 18:1 in these sites may be explained by a compensatory effect, since insects assimilate PUFAs when available, but replace them with MUFA when these are scarce (Stanley-Samuelson *et al.*, 1988). There seems to be a negative correlation, in insects, between MUFA (16:1 n-7 specifically) and PUFA (Bell *et al.*, 1994).

Several PUFA routinely found in marine oils were also present in *M. maritima* lipids, including n-7 FAs and 10 different n-3 PUFA, such as 16:4, 21:5 and DHA (Wasta and Mjøs, 2013).

Although it is possible that *M. maritima* may be able to convert alpha-linolenic into EPA and DHA, as some insects can (Stanley-Samuelson *et al.*, 1988), it is clear that this fatty acid profile results from the direct assimilation of dietary fatty acids. Additionally, a selective retaining effect may occur, resulting in elevated LC-PUFA content, especially when comparing with other insects.

Various fatty acids recorded in this study had never before been identified in insects, including 21:5 n-3 (heneicosapentaenoic acid) and 7-methyl-6-hexadecenoic acid. The discovery and quantification of these compounds was facilitated by the usage of the PARADISE tool. Because it analyses all samples simultaneously, provides a deconvoluted spectra for each chromatographic peak, enabling identification even on overlapping and/or low signal-to-noise ratio peaks (Johnsen *et al.*, 2017). Yet, the inconsistent abundance of unusual fatty acids contributed to a high variability between individuals and is likely related to opportunistic feeding, a common trait in many amphibiotic

insects (Williams, 1996). However, some variance can also be attributed to the natural heterogeneity among individuals collected in the wild, since these had different weights, age, sex and other uncontrolled factors, besides their trophic regime (Fontaneto *et al.*, 2011; Nogales-Mérida *et al.*, 2019).

The data provided in this study only concerns the fatty acid profile of adult flies, despite dipteran larvae being usually preferred as food or feed, both in the scientific and industrial applications. This preference arises from the higher dry mass of larvae (Liu *et al.*, 2017) and from the chitin-based exoskeleton, further developed in adult insects, that is believed to impair digestion (Tran *et al.*, 2015). However, studies with other insect species have frequently found a beneficial effect in defatting them, thus suggesting that the high saturated lipid content of larvae or prepupae may actually be detrimental (Henry *et al.*, 2015; Tran *et al.*, 2015). Chitin, in turn, can constitute an anti-nutrient, as it lowers protein digestibility (Dobermann *et al.*, 2017). However, this is not always what occurs in marine fish, since these can digest chitin naturally present in dietary items in the wild, such as crustaceans and insects (Henry *et al.*, 2015; Nogales-Mérida *et al.*, 2019). In this sense, it is perhaps unadvised to overlook the potential use of adult insects as a valid alternative for aquafeeds.

By displaying a fatty acid profile poor in SFA and generally rich in EPA, as well as other PUFA, *M. maritima* could potentially be a good alternative ingredient for marine aquafeeds. Moreover, the occupation of habitats in the vicinity of brackish and seawater seems to be determinant for the assimilation of dietary EPA by this fly. The high variability of its fatty acid profile indicates some dietary flexibility, hinting that with an appropriate substrate, *M. maritima* could probably reach even higher levels of n-3 (and more consistently) than those observed in the wild. Another advantage of this species, and others present in European marine or coastal areas, is the opportunity to use native insects instead of the exotics ones, thus minimising the risks of unwanted introductions and disease spill-over (Vilcinskis, 2019). Furthermore, tropical insects require warmer environments, which ultimately represents an increase in production costs in temperate regions (Makkar *et al.*, 2014).

5. Conclusions

This study presents for the first time the fatty acid profile of a dolichopodid. Furthermore, it shows that the coastal *M. maritima* has a fatty acid profile rich in n-3 PUFA, albeit with some variation, which is to be expected from wild insects. However, it casts a light on the hidden potential of native marine and coastal insect biodiversity, which deserves more attention.

On that regard, there are several crucial aspects that require further study: (1) the analysis of the fatty acid profile of larvae and pupae of this species; this would reveal if n-3 fatty acids are accumulated during the aquatic larval stages of the insect life cycle; (2) what is the larval primary food source (decomposing matter, benthonic algae, invertebrate prey, etc.); and (3) the difference between males and females.

However, the possibility that adult flies may continue to consume and accumulate fatty acids should not be discarded. For instance, in other insect species, PUFA play crucial roles during mating and oviposition periods (Stanley-Samuelson *et al.*, 1988).

Despite this contribution to the knowledge on the ecology of *M. maritima*, the next step would necessarily be to successfully rear the species in a laboratorial environment, which is evidently still far from an industrial scale operation. For now, several aspects of the ecology of *M. maritima* remain unknown, making this task more difficult. While the industrial sector is funnelling towards few well-studied species, thus limiting the options regarding food biodiversity, it may be pertinent to develop captive breeding of native insects as well.

The search for viable, sustainable aquafeed ingredients continues, but coastal insects, due to their high-quality lipids, may become an important part of the solution for the ongoing nutrient crisis currently impacting animal production worldwide, namely aquaculture.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/JIFF2020.0112>.

Table S1. Between sites fatty acid profile dissimilarity and main contributors to dissimilarities. Only statistically relevant comparisons are shown.

References

- Ameixa, O.M.C.C., Duarte, P.M. and Rodrigues, D.P., 2020. Insects, food security and sustainable aquaculture. In: Leal-Filho, W., Azul, A., Brandli, L., Özyay, P. and Wall, T. (eds.) Zero hunger. Encyclopedia of the UN Sustainable Development Goals. Springer Nature, Cham, Switzerland, pp. 1-11. https://doi.org/10.1007/978-3-319-69626-3_111-1
- Anderson, M.J. and Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecological Monographs 83: 557-574. <https://doi.org/10.1890/12-2010.1>
- Anderson, M.J., Gorley, R.N. and Clarke, K.R., 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth, UK, 214 pp.
- Asensi, A. and Diez-Garretas, B., 2017. Coastal vegetation. In: Loidi, J. (ed.) The vegetation of the Iberian Peninsula. Springer, Cham, Switzerland, pp. 397-432. https://doi.org/10.1007/978-3-319-54867-8_8
- Aued-Pimentel, S., Lago, J.H.G., Chaves, M.H. and Kumagai, E.E., 2004. Evaluation of a methylation procedure to determine cyclopropanoids fatty acids from *Sterculia striata* St. Hil. Et Nauds seed oil. Journal of Chromatography A 1054: 235-239. <https://doi.org/10.1016/j.chroma.2004.07.090>
- Barroso, F.G., De Haro, C., Sánchez-Muros, M.J., Venegas, E., Martínez-Sánchez, A. and Pérez-Bañón, C., 2014. The potential of various insect species for use as food for fish. Aquaculture 422: 193-201. <https://doi.org/10.1016/j.aquaculture.2013.12.024>
- Barroso, F.G., Sánchez-Muros, M.-J., Segura, M., Morote, E., Torres, A., Ramos, R. and Guil, J.-L., 2017. Insects as food: enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. Journal of Food Composition and Analysis 62: 8-13. <https://doi.org/10.1016/j.jfca.2017.04.008>
- Bayes, S.K., Hellerstein, M.K., Fitch, M., Mills, N.J. and Welter, S.C., 2014. You are what you eat: fatty acid profiles as a method to track the habitat movement of an insect. Oecologia 175: 1073-1080. <https://doi.org/10.1007/s00442-014-2976-9>

- Belghit, I., Liland, N.S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagbø, R., Krogdahl, Å. and Lock, E.-J., 2019. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). *Aquaculture* 503: 609-619. <https://doi.org/10.1016/j.aquaculture.2018.12.032>
- Bell, J.G., Ghioni, C. and Sargent, J.R., 1994. Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*): a comparison with commercial diets. *Aquaculture* 128: 301-313. [https://doi.org/10.1016/0044-8486\(94\)90319-0](https://doi.org/10.1016/0044-8486(94)90319-0)
- Bergamino, L., Dalu, T. and Richoux, N.B., 2014. Evidence of spatial and temporal changes in sources of organic matter in estuarine sediments: stable isotope and fatty acid analyses. *Hydrobiologia* 732: 133-145. <https://doi.org/10.1007/s10750-014-1853-1>
- Biancarosa, I., Liland, N.S., Day, N., Belghit, I., Amlund, H., Lock, E.-J. and Gilburn, A.S., 2018. The chemical composition of two seaweed flies (*Coelopa frigida* and *Coelopa pilipes*) reared in the laboratory. *Journal Insects as Food Feed* 4: 135-142. <https://doi.org/10.3920/JIFF2018.0008>
- Blomquist, G.J., Guo, L., Gu, P., Blomquist, C., Reitz, R.C. and Reed, J.R., 1994. Methyl-branched fatty acids and their biosynthesis in the housefly, *Musca domestica* L. (Diptera: Muscidae). *Insect Biochemistry and Molecular Biology* 24: 803-810. [https://doi.org/10.1016/0965-1748\(94\)90108-2](https://doi.org/10.1016/0965-1748(94)90108-2)
- Bou, M., Berge, G.M., Baevefjord, G., Sigholt, T., Østbye, T.-K. and Ruyter, B., 2017. Low levels of very-long-chain n-3 PUFA in Atlantic salmon (*Salmo salar*) diet reduce fish robustness under challenging conditions in sea cages. *Journal of Nutritional Science* 6: e32. <https://doi.org/10.1017/jns.2017.28>
- Clarke, K.R. and Gorley, R.N. (2006) PRIMER v6: user manual/tutorial (Plymouth routines in multivariate ecological research). PRIMER-E, Plymouth, UK, 192 pp.
- d'Assis Fonseca, E.C.M., 1978. Diptera: Orthorrhapha, Brachycera, Dolichopodidae, handbooks for the identification of British insects. Royal Entomological Society of London, London, UK, 90 pp. <https://doi.org/10.1111/j.1946-150X.1940.tb00204.x>
- Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D. and Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* 46: 225-340. [https://doi.org/10.1016/S0065-2881\(03\)46005-7](https://doi.org/10.1016/S0065-2881(03)46005-7)
- Dobermann, D., Swift, J.A. and Field, L.M., 2017. Opportunities and hurdles of edible insects for food and feed. *Nutrition Bulletin* 42: 293-308. <https://doi.org/10.1111/nbu.1229>
- Dunstan, G.A., Volkman, J.K., Barrett, S.M., Leroi, J.-M. and Jeffrey, S.W., 1993. Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). *Phytochemistry* 35: 155-161. [https://doi.org/10.1016/S0031-9422\(00\)90525-9](https://doi.org/10.1016/S0031-9422(00)90525-9)
- Finck, J., Berdan, E.L., Mayer, F., Ronacher, B. and Geiselhardt, S., 2016. Divergence of cuticular hydrocarbons in two sympatric grasshopper species and the evolution of fatty acid synthases and elongases across insects. *Scientific Reports* 6: 33695. <https://doi.org/10.1038/srep33695>
- Folch, J., Lees, M. and Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* 226: 497-509.
- Fontaneto, D., Tømmaseo-Ponzetta, M., Galli, C., Risé, P., Glew, R.H. and Paoletti, M.G., 2011. Differences in fatty acid composition between aquatic and terrestrial insects used as food in human nutrition. *Ecology of Food and Nutrition* 50: 351-367. <https://doi.org/10.1080/03670244.2011.586316>
- Food and Agriculture Organisation (FAO), 2020. The state of world fisheries and aquaculture 2020. Sustainability in action. FAO, Rome, Italy, 206 pp. <https://doi.org/10.4060/ca9229en>
- Food and Agriculture Organisation / World Health Organisation (FAO/WHO), 2019. Hazards associated with animal feed. Report of the Joint FAO/WHO expert meeting. May 12-15, 2015. FAO, Rome, Italy, 265 pp. <http://www.fao.org/3/ca6825en/ca6825en.pdf?eloutlink=imf2fao>
- Ghioni, C., Bell, J.G. and Sargent, J.R., 1996. Polyunsaturated fatty acids in neutral lipids and phospholipids of some freshwater insects. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 114: 161-170. [https://doi.org/10.1016/0305-0491\(96\)00019-3](https://doi.org/10.1016/0305-0491(96)00019-3)
- Happel, A., Czesny, S., Rinchar, J. and Hanson, S.D., 2017. Data pre-treatment and choice of resemblance metric affect how fatty acid profiles depict known dietary origins. *Ecological Research* 32: 757-767. <https://doi.org/10.1007/s11284-017-1485-9>
- Henry, M., Gasco, L., Piccolo, G. and Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: past and future. *Animal Feed Science and Technology* 203: 1-22. <https://doi.org/10.1016/j.anifeedsci.2015.03.001>
- Johnsen, L.G., Skou, P.B., Khakimov, B. and Bro, R., 2017. Gas chromatography – mass spectrometry data processing made easy. *Journal of Chromatography A* 1503: 57-64. <https://doi.org/10.1016/j.chroma.2017.04.052>
- Lee, J., Lee, H., Kang, S. and Park, W., 2016. Fatty acid desaturases, polyunsaturated fatty acid regulation, and biotechnological advances. *Nutrients* 8: 23. <https://doi.org/10.3390/nu8010023>
- Liland, N.S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C.G., Waagbø, R., Torstensen, B.E. and Lock, E.-J., 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. *PLoS ONE* 12: e0183188. <https://doi.org/10.1371/journal.pone.0183188>
- Lillebø, A., Ameixa, O., Sousa, L., Sousa, A., Soares, J., Dolbeth, M. and Alves, F., 2015. The physio-geographical background and the ecology of Ria de Aveiro. In: Lillebø, A., Stålnacke, P. and Gooch, G.D. (eds.) *Coastal lagoons in Europe: integrated water resource strategies*. International Water Association, London, UK, pp. 21-28.
- Liu, X., Chen, X., Wang, H., Yang, Q., ur Rehman, K., Li, W., Cai, M., Li, Q., Mazza, L., Zhang, J., Yu, Z. and Zheng, L., 2017. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLoS ONE* 12: e0182601. <https://doi.org/10.1371/journal.pone.0182601>
- Maciel, E., Lillebø, A., Domingues, P., Da Costa, E., Calado, R. and Domingues, M.R.M., 2018. Polar lipidome profiling of *Salicornia ramosissima* and *Halimione portulacoides* and the relevance of lipidomics for the valorization of halophytes. *Phytochemistry* 153: 94-101. <https://doi.org/10.1016/j.phytochem.2018.05.015>

- Makkar, H.P.S., Tran, G., Heuzé, V. and Ankers, P., 2014. State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology* 197: 1-33. <https://doi.org/10.1016/j.anifeedsci.2014.07.008>
- Meziane, T., Bodineau, L., Retiere, C. and Thoumelin, G., 1997. The use of lipid markers to define sources of organic matter in sediment and food web of the intertidal salt-marsh-flat ecosystem of Mont-Saint-Michel Bay, France. *Journal of Sea Research* 38: 47-58. [https://doi.org/10.1016/S1385-1101\(97\)00035-X](https://doi.org/10.1016/S1385-1101(97)00035-X)
- Miller, L.T., Stange, L., MacVean, C., Rey, J.R., Frank, J.H., Mizell, R.F., Heppner, J.B., Heppner, J.B., Capinera, J.L., Feir, D., Borgemeister, C., Hangay, G., Flowers, R.W., Heppner, J.B., Jaffe, K., Dietrich, C.H., Boucher, S., Heppner, J.B., Cranshaw, W., Cranshaw, W., Heppner, J.B., Behmer, S.T., Punzo, F., Boucias, D., Gayubo, S.F., Kima, P.E., Aldryhim, Y., Capinera, J.L., Calabrese, D.M., Heppner, J.B., Nation, J.L., Harman, D.M., Heppner, J.B., Wang, Q., Pollet, M.A.A., Brooks, S.E., Heppner, J.B., Leppla, N.C., Norris, D.E. and Heppner, J.B., 2008. Long-legged flies (Diptera: Dolichopodidae). In: Capinera, J.L. (ed.) *Encyclopedia of entomology*. Springer, Dordrecht, the Netherlands, pp. 2232-2241. https://doi.org/10.1007/978-1-4020-6359-6_2087
- Nogales-Mérida, S., Gobbi, P., Józefiak, D., Mazurkiewicz, J., Dudek, K., Rawski, M., Kierończyk, B. and Józefiak, A., 2019. Insect meals in fish nutrition. *Reviews in Aquaculture* 11: 1080-1103. <https://doi.org/10.1111/raq.12281>
- Pollet, M., Andrade, R., Gonçalves, A., Andrade, P., Jacinto, V., Almeida, J., De Braekeleer, A., Van Calster, H. and Brosens, D., 2019. Dipterological surveys in Portugal unveil 200 species of long-legged flies, with over 170 new to the country (Diptera: Dolichopodidae). *Zootaxa* 4649: 1-69. <https://doi.org/10.11646/zootaxa.4649.1.1>
- Popova, O.N., Haritonov, A.Y., Sushchik, N.N., Makhutova, O.N., Kalachova, G.S., Kolmakova, A.A. and Gladyshev, M.I., 2017. Export of aquatic productivity, including highly unsaturated fatty acids, to terrestrial ecosystems via Odonata. *Science of the Total Environment* 581: 40-48. <https://doi.org/10.1016/j.scitotenv.2017.01.017>
- Ricardo, F., Maciel, E., Domingues, M.R. and Calado, R., 2017. Spatio-temporal variability in the fatty acid profile of the adductor muscle of the common cockle *Cerastoderma edule* and its relevance for tracing geographic origin. *Food Control* 81: 173-180. <https://doi.org/10.1016/j.foodcont.2017.06.005>
- Ricardo, F., Pimentel, T., Moreira, A.S.P., Rey, F., Coimbra, M.A., Rosário Domingues, M., Domingues, P., Costa Leal, M. and Calado, R., 2015. Potential use of fatty acid profiles of the adductor muscle of cockles (*Cerastoderma edule*) for traceability of collection site. *Scientific Reports*. 5: 11125. <https://doi.org/10.1038/srep11125>
- Rossi, S., Sabatés, A., Latasa, M. and Reyes, E., 2006. Lipid biomarkers and trophic linkages between phytoplankton, zooplankton and anchovy (*Engraulis encrasicolus*) larvae in the NW Mediterranean. *Journal of Plankton Research* 28: 551-562. <https://doi.org/10.1093/plankt/fbi140>
- Rumpold, B.A. and Schlüter, O.K., 2013. Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies* 17: 1-11. <https://doi.org/10.1016/j.ifset.2012.11.005>
- Sánchez-Muros, M.-J., Barroso, F.G. and Manzano-Agugliaro, F., 2014. Insect meal as renewable source of food for animal feeding: a review. *Journal of Cleaner Production* 65: 16-27. <https://doi.org/10.1016/j.jclepro.2013.11.068>
- Shahidi, F. and Ambigaipalan, P., 2018. Omega-3 polyunsaturated fatty acids and their health benefits. *Annual Review of Food Science and Technology* 9: 345-381. <https://doi.org/10.1146/annurev-food-111317-095850>
- Stanley-Samuelson, D.W., Jurenka, R.A., Cripps, C., Blomquist, G.J. and De Renobales, M., 1988. Fatty acids in insects: composition, metabolism, and biological significance. *Archives of Insect Biochemistry and Physiology* 9: 1-33. <https://doi.org/10.1002/arch.940090102>
- St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C. and Irving, S., 2007. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *Journal of the World Aquaculture Society* 38: 309-313. <https://doi.org/10.1111/j.1749-7345.2007.00101.x>
- Stoneham, T.R., Kuhn, D.D., Taylor, D.P., Neilson, A.P., Smith, S.A., Gatlin, D.M., Chu, H.S.S. and O'Keefe, S.F., 2018. Production of omega-3 enriched tilapia through the dietary use of algae meal or fish oil: improved nutrient value of fillet and offal. *PLoS ONE* 13: e0194241. <https://doi.org/10.1371/journal.pone.0194241>
- Stork, N.E., 2018. How many species of insects and other terrestrial arthropods are there on Earth? *Annual Review of Entomology* 63: 31-45. <https://doi.org/10.1146/annurev-ento-020117-043348>
- Sushchik, N.N., Gladyshev, M.I., Moskvichova, A.V., Makhutova, O.N. and Kalachova, G.S., 2003. Comparison of fatty acid composition in major lipid classes of the dominant benthic invertebrates of the Yenisei river. *Comparative Biochemistry and Physiology – Part B: Biochemistry and Molecular Biology* 134: 111-122. [https://doi.org/10.1016/S1096-4959\(02\)00191-4](https://doi.org/10.1016/S1096-4959(02)00191-4)
- Sushchik, N.N., Yurchenko, Y.A., Gladyshev, M.I., Belevich, O.E., Kalachova, G.S. and Kolmakova, A.A., 2013. Comparison of fatty acid contents and composition in major lipid classes of larvae and adults of mosquitoes (Diptera: Culicidae) from a steppe region. *Insect Science* 20: 585-600. <https://doi.org/10.1111/j.1744-7917.2012.01582.x>
- Tran, G., Heuzé, V. and Makkar, H.P.S., 2015. Insects in fish diets. *Animal Frontiers* 5: 37-44.
- Tsunoda, T., Suzuki, J.-I. and Kaneko, N., 2017. Fatty acid analyses to detect the larval feeding preferences of an omnivorous soil-dwelling insect, *Anomala cuprea* (Coleoptera: Scarabaeidae). *Applied Soil Ecology* 109: 1-6. <https://doi.org/10.1016/j.apsoil.2016.09.020>
- Twining, C.W., Shipley, J.R. and Winkler, D.W., 2018. Aquatic insects rich in omega-3 fatty acids drive breeding success in a widespread bird. *Ecology Letters* 21: 1812-1820. <https://doi.org/10.1111/ele.13156>
- United Nations, Department of Economic and Social Affairs (UN DESA), 2017. World population prospects: the 2017 revision. Vol. I: comprehensive tables ST/ESA/SER.A/399. UN DESA, Population Division, New York, NY, USA. Available at: https://population.un.org/wpp/Publications/Files/WPP2017_Volume-I_Comprehensive-Tables.pdf

- Van den Brink, D.M. and Wanders, R.J.A., 2006. Phytanic acid: production from phytol, its breakdown and role in human disease. *Cellular and Molecular Life Sciences* 63: 1752-1765. <https://doi.org/10.1007/s00018-005-5463-y>
- Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G. and Vantomme, P., 2013. Edible insects: future prospects for food and feed security. FAO Forestry Paper no. 171. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, 187 pp. Available at: <http://www.fao.org/docrep/018/i3253e/i3253e.pdf>.
- Vilcinskas, A., 2019. Pathogens associated with invasive or introduced insects threaten the health and diversity of native species. *Current Opinion in Insect Science* 33: 43-48. <https://doi.org/10.1016/j.cois.2019.03.004>
- Wasta, Z. and Mjøs, S.A., 2013. A database of chromatographic properties and mass spectra of fatty acid methyl esters from omega-3 products. *Journal of Chromatography A* 1299: 94-102. <https://doi.org/10.1016/j.chroma.2013.05.056>
- Williams, D.D., 1996. Environmental constraints in temporary fresh waters and their consequences for the insect fauna. *Journal of the North American Benthological Society* 15: 634-650. <https://doi.org/10.2307/1467813>
- Yang, L.F., Siriamornpun, S. and Li, D., 2006. Polyunsaturated fatty acid content of edible insects in Thailand. *Journal of Food Lipids* 13: 277-285. <https://doi.org/10.1111/j.1745-4522.2006.00051.x>
- Zhukova, N., 1995. Fatty acid composition of 15 species of marine microalgae. *Phytochemistry* 39: 351-356. [https://doi.org/10.1016/0031-9422\(94\)00913-E](https://doi.org/10.1016/0031-9422(94)00913-E)