



RESEARCH ARTICLE

# Biochemical profile of the native seaweed fly *Fucellia maritima* (Haliday, 1838) (Diptera: Anthomyiidae) reared on five different substrates

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## Abstract

The sustainability of aquaculture production is crucial for the industry's long-term viability. Diversification of feed ingredients is a key component in achieving this sustainability. Considered a promising alternative to traditional feed ingredients, terrestrial insect meals often lack sufficient n-3 polyunsaturated fatty acids (PUFA). This study explores the potential of marine insect species, specifically evaluating the influence of five different substrates on the growth and composition of the European native seaweed fly, *Fucellia maritima*. The substrates tested include a brown seaweed (*Fucus* sp.), a green seaweed (*Ulva* sp.), a red seaweed (the invasive *Agarophyton vermiculophyllum*), the invasive freshwater hyacinth (*Eichhornia crassipes*), and fish processing waste (codfish *Gadus morhua* frames). Results show no significant difference in the number of individuals per substrate, although *Fucus* sp. had a higher total number of individuals completing their life cycle. In contrast, feeding on codfish frames resulted in a lower number of completed life cycles, while *E. crassipes* led to no survival. Protein content in adult flies ranged from 55.2% to 56.7%, and in pupae, it ranged from 46.1% to 48.7% across different feeding substrates. Notably, pupae fed with *Ulva* sp., exhibited the highest protein content at 48.7%. Lipid content in adult flies ranged from 10.0% to 13.0%, while in pupae, it ranged from 8.8% to 11.4%. Codfish frames had the highest lipid content in both pupae (11.4%) and adults (13%). The most abundant fatty acid in *Fucus* sp. – fed pupae and adult flies was oleic acid (18:1 n-9), while palmitoleic acid (16:1 n-7) dominated in other treatments. It is worth highlighting that docosahexaenoic acid (22:6 n-3) was reported for the first time in a seaweed fly, namely when supplied with fish processing waste. These findings suggest that *Fucellia maritima* may be a promising complementary ingredient for formulating marine aquafeeds.

## Keywords

fatty acids – insect farming – novel ingredients – protein – PUFA

## 1 Introduction

As estimates point to a global population of 9.8 billion people by 2050, concerns about producing enough food

will also increase (UN, 2022). Current estimates suggest that we need to increase food production by more than 50% to sustainably meet the demand of this expanding population for wholesome food (van Dijk *et al.*,

2021). Aquaculture is an important food production system that can contribute to many countries' food security (FAO *et al.*, 2022). In 2022, aquaculture surpassed fisheries for the first time in the annual production of aquatic animals, accounting for 51% of the total production worldwide (FAO, 2024).

Marine carnivorous fish and shrimp production requires a diet high in animal protein and certain lipids (FAO, 2022; Glencross, 2009). To meet these nutritional requirements, two ingredients are commonly used in aquafeed formulation: fishmeal and fish oil; the latter is rich in long-chain n-3 polyunsaturated fatty acids (LC-PUFA) (FAO, 2020). The need for these two ingredients has caused great pressure on fisheries stocks, encouraging the search for more sustainable alternative ingredients, such as plants (not so environmentally sustainable and may contain anti-nutritional factors), single-cell proteins (expensive and not easily obtainable), livestock sources (not environmentally friendly and a potential vector for diseases) and insect meals (terrestrial insects are poor on n-3 polyunsaturated fatty acids, PUFA) (Ameixa *et al.*, 2020).

Despite insects being the most diverse group of animals on the planet, with nearly one million species described (Bánki *et al.*, 2024), only eight species are authorised to be used as animal feed in the European Union. In 2017, the European Commission (2017) authorised the use of seven insect species as aquafeed ingredients, namely, two species of Diptera, the black soldier fly (*Hermetia illucens*, BSF) and the common housefly (*Musca domestica*, CHF), two species of Coleoptera, the yellow mealworm (*Tenebrio molitor*, YM) and the lesser mealworm (*Alphitobius diaperinus*), and three species of Orthoptera, the house cricket (*Acheta domesticus*), the banded cricket (*Gryllobates sigillatus*), and the field cricket (*Gryllus assimilis*). In 2021, another species was added to this list, the Lepidoptera silkworm (*Bombyx mori*) (EU Regulation 2021/1925, 2021). This authorisation was extended to other farmed animal species in 2021, including poultry, swine, pets (e.g. dogs, cats, birds, or reptiles), and fur animals (e.g. *Mustela lutreola*) with the Regulation (EU) 2021/1372 of 17 August 2021 (EU, 2021). In 2024, the European Commission authorised the use of live insects in animal feed in the European Union. This includes feed for farmed fish, poultry, pigs, and pets, excluding ruminant animals.

Despite the knowledge gaps that remain when it comes to up-scaling the use of these eight species, the list is comparatively small compared to the estimated insect diversity. In fact, due to their particular life histories, several species may present characteristics that

enrich the diets of farmed animals. For instance, evidence shows that insects that live in coastal areas and feed on organic matter of marine origin present a nutritional composition richer in n-3 (Duarte *et al.*, 2021). For this reason, they can potentially be good candidates as aquafeed ingredients destined for marine fish. One example is the seaweed flies, which are known to feed on seaweed and dead fish in coastal areas (Biancarosa *et al.*, 2018b; Cheng, 1976).

The seaweed fly *Fucellia maritima* (Haliday, 1838) (Figure 1), native to western and northern Europe, is a member of the family Anthomyiidae and can be seen throughout the year overflying the beach wrack along the shores of Portugal (Lourenço *et al.*, 2020). It dwells on the beach wrack, predominantly composed of brown seaweed, mostly from the genera *Laminaria* and *Fucus*. It is also the only seaweed fly species known to date that can feed on decaying animal matter (Egglisshaw, 1960). This species has a relatively short life cycle of approximately 36 days at room temperature (15–18 °C), with three larval instar and pupae phases (Egglisshaw, 1960). In captivity, this species is described as reproducing on moderately decomposed brown seaweed *Laminaria* sp. and *Fucus* sp. with adult flies freely mating and laying their eggs on the seaweed (Egglisshaw, 1960). Like many others, this species is understudied, and the potential to be used as a feed ingredient is unknown. Hence, it is not authorised for feed production in the EU.

In the Centre region of mainland Portugal, namely in the coastal areas of the Aveiro region, beach wrack is predominantly composed of a mixture of *Fucus* sp. and *Ulva* sp., but at certain times of the year, large amounts of the invasive aquatic plant, the water hyacinth (*Eichhornia crassipes*), can also be observed (O. Ameixa, pers. obs.). To the authors' best knowledge, no study has ever investigated the biochemical profile of *F. maritima* reared in captivity. Hence, information on its fatty acid (FA) profile, namely in PUFA on different substrates, has yet to be determined in both pupae and adult flies.

In this work we assessed the potential of marine insect species, through the evaluation of the influence of five different substrates on the growth (biomass) and nutritional composition of the European native seaweed fly, *Fucellia maritima*. For this assessment we evaluated the nutritional composition of *F. maritima* through the use of feeding substrates commonly found in the composition of beach wrack on the coast of Aveiro region, namely: (a) the brown seaweed *Fucus* sp. which was used as the control group (CG), due to the fact that this is the preferential feeding substrate for this fly species; (b) the green seaweed *Ulva* sp.; (c) the inva-



FIGURE 1 *Fucellia maritima* adult fly on the sand at Barra beach, Aveiro, Portugal (40°38'31.1"N; 8°44'57.7"W).

sive red seaweed *Agarophyton vermiculophyllum*; (d) the invasive freshwater plant, the water hyacinth (*Eichhornia crassipes*), which can be very abundant in the local ecological niche of the fly; and (e) frames of Atlantic codfish (*Gadus morhua*, a common co-product of local codfish salting industries). Whole seaweeds are listed as authorised feed ingredients in the European catalogue of feed materials (Reg. EU 68/2013) (European Commission, 2013). However, the use of whole codfish frames is currently not authorised as a substrate for insects in the EU (European Parliament and Council, 2009).

## 2 Material and methods

### *Substrates sampling and preparation*

The brown seaweed *Fucus* sp. and the green seaweed *Ulva* sp. were sampled at Ria de Aveiro coastal lagoon in April 2021 (40°38'04.4"N; 8°39'40.2"W), while the invasive red seaweed *A. vermiculophyllum* was harvested in July 2020 in the same location. The invasive water hyacinth *E. crassipes* was sampled at the mouth of the Vouga River, also in Ria de Aveiro, in April 2021 (40°41'41.6"N; 8°36'06.8"W), with whole thalli being collected in plastic buckets along with some water to maintain the conditions during transport. In the laboratory at

ECOMARE (University of Aveiro, Portugal), on the same day of sampling, all seaweed and water hyacinth were rinsed in cold fresh water to remove salt, debris, and other algal or animal species present and were subsequently stored in small plastic bags at -20 °C for further use in the experiment. Frames of Atlantic codfish (*G. morhua*) were supplied by an industrial codfish-salting unit (Bacalhau do Barents, Produtos Alimentares Lda, Gafanha da Nazaré, Portugal) and stored at -20 °C until further use.

The feeding substrates being tested were later defrosted and minced using a 200 W Selecline Hand Blender to increase the surface-to-area ratio and facilitate their consumption by the larvae. The exception was codfish frames, which were cut into small pieces using scissors. Two hundred grammes of each substrate were placed into 1.1 L plastic food containers with a mesh net in the lid for air circulation.

### *Insect collection*

Adult specimens of *F. maritima* overflying the beach wrack were captured using a sweeping net at Barra beach, Aveiro, Portugal, in April 2021 (40°37'44.3"N; 8°44'42.0"W). Collected specimens were stored in 50-mL polypropylene flasks for transportation to the insectarium facility at ECOMARE (University of Aveiro, Por-

tugal) and kept under controlled conditions (a photoperiod of 16 h L:8 h D, at  $40 \pm 5\%$  Relative Humidity and  $25 \pm 3$  °C temperature).

### Experimental design

In the insectarium, adult flies were transferred to plastic boxes of 1.1 L containing the feeding substrates being tested (as described above). Fifty adult flies were placed in each box, with the number of males and females unknown since it is impossible to distinguish sexes without dissecting this species. Five independent replicates were tested for each substrate, with 50 flies  $\times$  5 feeding substrates  $\times$  5 replicates = 1,250 adult flies being used. The adult flies were allowed to freely mate and lay their eggs on the substrate for 48 hours, and the eggs deposited were not counted. The containers were supplied with tap water daily to maintain humidity, especially when supplying seaweed, as desiccation induced larval mortality. Within 4 days, larvae started to moult into pupae, and half of the pupae were sampled and frozen at  $-20$  °C. After one week, adult flies emerged from the remaining puparium and were frozen at  $-20$  °C until further analysis. Therefore, the experiment stopped when the adult flies emerged from the remaining puparium.

### Sample processing

All frozen material was transported to the Instituto de Investigaciones Marinas (IIM-CSIC, Vigo, Spain), where it was flash-frozen at  $-80$  °C before being freeze-dried. After freeze drying, all samples, except for codfish frames (see below), were ground, with a porcelain mortar and pestle being used to homogenise samples of pupae and adults of *F. maritima* and a blender (La Moulinette 700 W from Moulinex) being used for seaweed and freshwater hyacinth. Skin and meat from codfish frames were separated from the bone and minced using a blender (Moulinette Compact mini hachoir 270 W from Moulinex).

### Nutrient content analysis

Due to the low biomass of the initial five sample replicates per treatment, these were mixed and redistributed into two replicates per treatment to analyse nutrient content. Moreover, it was impossible to repeat the experiment to increase the biomass necessary for the analysis due to the start of the cleaning period at the beaches in Portugal, which happens yearly during spring to autumn, which removes the beach wrack from the beaches, affecting the local populations of seaweed flies. Therefore, we decided to follow the analysis nonethe-

less, due to the novelty of the work. Analytical determinations of water content (moisture), crude protein, total lipid, and crude ash were performed. The moisture content was determined by the gravimetric difference between the final weight and the weight of the sample after being dried for 24 hours at 105 °C. Crude ash was determined using the AOAC method (AOAC, 2003). Protein content was determined via the Kjeldahl method (AOAC, 2003), and total nitrogen of the substrates was multiplied by a 6.25 conversion factor. For both pupae and adult flies, a conversion factor of 4.76 was used. This value is more suitable to avoid an overestimation of insects' protein content (see Janssen *et al.*, 2017). Results are expressed as dry matter.

### Chemicals

Methanol (CH<sub>3</sub>OH), Chloroform (CHCl<sub>3</sub>), Dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>), Toluene (C<sub>7</sub>H<sub>8</sub>), Hexane HPLC (C<sub>6</sub>H<sub>14</sub>), Sulfuric Acid (H<sub>2</sub>O<sub>4</sub>S) and Petroleum Ether were purchased from Fischer Scientific (Loughborough, UK) MILLIQ Water was obtained by a purification system (Synergy, Millipore Corporation, Burlington, MA, USA). Amino acid standards mixture containing acidic, neutral, and basic amino acids were purchased from Waters (Milford, MA, USA). The AccQ-Tag eluent concentrates, and AccQ-Tag Fluor Reagent Kit derivation for hydrolysate Amino Acid Analysis were purchased from Waters Corporation (Milford, MA, USA). Tryptophane, OH-Proline, taurine, and ornithine were purchased from Merck (St. Louis, MO, USA). Acetonitrile and Water HPLC grade were purchased from Fisher Scientific.

### Lipid extraction and fatty acids analysis

The lipids from the pupae, the adult flies, and the codfish were extracted using a method based on the Bligh-Dyer protocol with a proportion of CH<sub>3</sub>OH: CH<sub>2</sub>Cl<sub>2</sub> 1:1 (Bligh and Dyer, 1959). Approximately 0.065 g was weighed for the adult flies, and 4 mL of CH<sub>3</sub>OH + 2 mL CH<sub>2</sub>Cl<sub>2</sub> were added and mixed with Turrax for 60 seconds. Then, more 2 mL CH<sub>2</sub>Cl<sub>2</sub> + 2.5 MILLIQ Water were added before mixing in Turrax again for 60 seconds. They were centrifuged at 3500 r.p.m. for 10 minutes at 4 °C (Allegra X-12R from Beckman Coulter, Brea, CA, USA). Lipids were recovered from the organic phase.

For the pupae and the codfish samples, 6 mL of CH<sub>3</sub>OH and 3 mL of CH<sub>2</sub>Cl<sub>2</sub> were added and mixed for 60 seconds with Turrax. This procedure in pupae was followed by adding more 3 mL CH<sub>2</sub>Cl<sub>2</sub> and 4 mL Milli-Q water. As for the codfish, 3 mL of Milli-Q water was added because the sample was not freeze-dried; hence, less water was needed to separate the phases. For the

seaweed and the water hyacinth, a modification of the method described by Lopes *et al.* (2019), using  $\text{CH}_2\text{Cl}_2$  instead of  $\text{CHCl}_3$ , was employed.

Fatty acids (FA) were analysed as Fatty Acid Methyl Esters (FAME). Briefly, lipids were methylated with 1% sulfuric acid methanolic solution by incubation at 50 °C for 18 h; after that, an aqueous 5% NaCl solution was added, and the resulting methyl esters were extracted into toluene. FAMES were determined in duplicates in a Gas chromatograph (Perkin Elmer Clarus 500 GC, Madrid, Spain) equipped with a flame ionisation detector and a fused silica capillary column (SP-2330, 0.25 mm × 30 m × 0.2 µm, Supelco, catalogue no.: 24019). Identification of FAME was achieved using commercially available certified standard material (Supelco 37 Component FAME Mix, CRM47885), and nonadecanoic fatty acid was used as an internal standard.

#### Separation and quantification of lipids by Thin Layer Chromatography (TLC)

TLC plates (Silica gel on TLC plates, 20 cm × 20 cm, Merck) were prewashed with  $\text{CH}_3\text{OH}$ . At the time of use, the plates were activated in an oven at 120 °C for one hour. Lipid samples at a concentration of 25 µg/µL in  $\text{CH}_2\text{Cl}_2$  were injected using Hamilton TLC syringes. TLC plates were eluted in a mobile phase of hexane, ether, and acetic acid (80:20:2 by volume). The spots were visualised with a 10% copper sulphate solution in 8% phosphoric acid and heated at 160 °C for 10 minutes. Lipids were identified and quantified using a standard mixture of triglycerides, free fatty acids, monoglycerides, diglycerides, cholesterol, phospholipids, and waxes.

#### Amino acids analysis of the substrates for fly rearing

Amino Acids (aa) analysis was performed using the Waters procedure (AccQ·Tag Kit, Milford USA). Briefly, aa were extracted from defatted samples by hydrolysis with HCL 6N, 1% Phenol at 110 °C for 24 hours, and Tryptophan was determined directly by HPLC-FL after basic hydrolysis with NaOH 4N at 110 °C for 24 hours. Amino Acids from acid hydrolysis derivatization with AccQ·Tag reagents were prepared according to the manufacturer's protocol. The derivatised aa and tryptophan were then analysed by HPLC coupled with a Fluorescence detector (Waters Alliance 2695 HPLC system, Multi λ Fluorescence detector 2475). The separation column was a Waters AccQ·Tag for Hydrolysate Amino Acid Analysis working at 37 °C. The Fluorescence detector was set at  $\lambda_{\text{ex}} = 250 \text{ nm}$ - $\lambda_{\text{em}} = 395 \text{ nm}$  and for Tryptophane was set at  $\lambda_{\text{ex}} = 280 \text{ nm}$ - $\lambda_{\text{em}} = 345 \text{ nm}$ .

#### Statistical analysis

Statistical tests were carried out to compare the influence of the type of substrate on the number of pupae and adults. Larval weight was not measured individually; rather, it was presented as a ratio of the total weight for each treatment by the number of individuals. A two-way ANOVA was performed using linear models (independent variables: substrate used with four levels, namely: *Fucus* sp., *A. vermiculophyllum*, *Ulva* sp., and cod fish frames). Dependent variables: life cycle stage with two levels: number of adult flies and number of pupae). Homogeneity in variances and normal distribution was verified by graphical evaluation using the plot function in R (residuals vs fitted, normal Q-Q plot) as well as numerically by the Shapiro-Wilks test, and a post-hoc test was performed using the Tukey Honest Significant Differences (Tukey HSD). All statistical analyses were performed using the software R (R Development Core Team, 2022).

### 3 Results

#### *Seaweed fly productivity under different feeding substrates*

There were no significant differences in the total number of individuals between pupae and adults reared on the different substrates (not accounting for *E. crassipes*). It is worth mentioning that replicates displayed high variability, namely those supplied with *Fucus* sp. as feeding substrate, as seen in Table 1. No specimens could complete their life cycle using *E. crassipes* as a feeding substrate; however, female flies laid eggs on this substrate, and larvae emerged, so no specimens survived beyond the first larval instar. For this reason, this substrate was excluded from further analysis related to the composition of the pupae and adult flies. The substrate on which more larvae moulted into pupae was *Fucus* sp. (see Figure 2), followed by *A. vermiculophyllum* and *Ulva* sp. The feeding substrate on which fewer pupae developed was codfish frames, even though individual mean weight was higher in larvae fed with this substrate (14.5 mg) when compared to *Fucus* sp. (5.2 mg).

#### *Nutritional composition of feeding substrates, pupae and adult flies*

The nutritional composition of the feeding substrates employed in the present study is shown in Table 2. Codfish frames presented the highest values of lipid and protein content. The lowest lipid content was recorded in *Ulva* sp., and the lowest protein content was recorded

TABLE 1 Productivity of pupae (P) and adult (A) flies of *Fucellia maritima* under different feeding substrates. Mean number of individuals (mean  $\pm$  standard deviation,  $n = 5$ ), and individual weight (obtained by dividing the total weight recorded by the total number of individuals,  $n = 5$ )

	Substrate	Mean number of individuals	Individual weight (mg)
Pupae	Codfish (P)	66.2 $\pm$ 23.8 <sup>a</sup>	14.5
	<i>Fucus</i> (CG) (P)	97.6 $\pm$ 71.6 <sup>a</sup>	5.2
	<i>A. vermiculophyllum</i> (P)	88.8 $\pm$ 15.9 <sup>a</sup>	8.0
	<i>E. crassipes</i> (P)	0	0
	<i>Ulva</i> (p)	73.0 $\pm$ 24.8 <sup>a</sup>	8.3
Adult flies	Codfish (A)	75.2 $\pm$ 29.6 <sup>a</sup>	8.3
	<i>Fucus</i> (CG) (A)	110.8 $\pm$ 80.7 <sup>a</sup>	5.4
	<i>A. vermiculophyllum</i> (A)	96.4 $\pm$ 24.1 <sup>a</sup>	7.4
	<i>E. crassipes</i> (A)	0	0
	<i>Ulva</i> (A)	82.6 $\pm$ 26.9 <sup>a</sup>	6.5

Different letters on the same column mean statistically significant differences ( $P < 0.05$ ).



FIGURE 2 *Fucellia maritima* pupa on on the brown seaweed *Fucus* sp.

in *Fucus* sp., which also presented the highest ash content.

The composition of pupae and adult flies reared under the feeding substrates detailed above is also shown in Table 2. Pupae fed with *Ulva* sp. and *A. vermiculophyllum* had the highest protein content compared to pupae reared with other feeding substrates. Pupae fed with codfish and *Ulva* sp. had the highest lipid content and the lowest ash content. Pupae fed with *A. vermiculophyllum* had the lowest lipid content and the highest ash content. Regarding adult flies, those fed *A. vermiculophyllum* and *Fucus* sp. showed the lowest lipid

content, while flies fed with codfish showed the lowest protein content.

#### *Amino acids composition of feeding substrates*

Pupae and adult flies fed with *Ulva* sp. showed higher protein content despite this substrate's lower amount of protein. Therefore, it was important to consider the digestibility of the protein and the bioavailability of the different amino acids. For this reason, the full amino acid profile of the substrates was assessed to confirm the protein quality of the substrate composition used to rear *F. maritima*. The amino acid composition of the dif-

TABLE 2 Average composition of feeding substrates (S) tested in the present study, pupae (P), and adult (A) specimens of seaweed fly *Fucellia maritima* cultured using those feeding substrates, in terms of lipid, protein (substrates (Kp) = 6.25; adults and pupae (Kp) = 4.76), and ash content (dry weight basis, n = 2)

Substrates	Lipid (%)	Ash content (%)	Protein (%)
<b>Substrate composition</b>			
Codfish (S)	7.8	2.4	82.4
<i>Fucus</i> sp. (CG) (S)	4.1	21.7	14.1
<i>A. vermiculophyllum</i> (S)	1.1	11.9	18.1
<i>Eichornia crassipes</i> (S)	1.9	18.8	16.6
<i>Ulva</i> sp. (S)	0.5	20.8	22.0
<b>Pupae</b>			
Codfish (P)	11.5	4.8	46.1
<i>Fucus</i> sp. (CG) (P)	10.4	5.4	46.3
<i>A. vermiculophyllum</i> (P)	8.8	6.1	48.2
<i>Ulva</i> sp. (P)	11.1	5.7	48.7
<b>Adults</b>			
Codfish (A)	13	5.2	55.2
<i>Fucus</i> sp. (CG) (A)	10	6.1	56.4
<i>A. vermiculophyllum</i> (A)	10.5	5.1	56.7
<i>Ulva</i> sp. (A)	11.1	5.0	56.3

ferent seaweeds used as feeding substrates (*A. vermiculophyllum*, *Fucus* sp., and *Ulva* sp.), the aquatic plant substrate (*E. crassipes*), and the marine animal substrate (codfish frames) are all displayed in Table 3. All feeding substrates presented Essential Amino Acids (EAA). Compared to codfish, seaweed substrates had a greater concentration of five of the nine essential amino acids (EAAs): threonine (Thr), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), and tryptophan (Trp). Despite its interesting composition of amino acids, *E. crassipes* was not used as a substrate by *F. maritima*.

#### Fatty acids composition of feeding substrates

The composition of fatty acids varied between feeding substrates, as shown in Table 4. The most abundant fatty acids present in the different feeding substrates tested were 22:6 n-3 DHA (18.5%) in codfish frames, 18:1 n-9 oleic acid (OA) (32.7%) in *Fucus*, and 16:0 palmitic acid (PA) in *A. vermiculophyllum*, *E. crassipes*, and *Ulva* (with 45.9%, 27.6%, and 41.6%, respectively). The brown seaweed *Fucus* sp. displayed the highest abundance of 20:5 n-3 EPA (4.5%) among the seaweed species tested, and the abundance of 18:3 n-3 alpha-linoleic acid (ALA) (3.9%) on this seaweed is also noteworthy. Codfish frames displayed the highest n-3/n-6 ratio, with *E. crassipes* featuring high percentages of PUFA due to its content in 18:2 n-6 linoleic acid (LA) and ALA (18:3 n-3);

the lowest levels of PUFA abundance were recorded in *Ulva* sp., with the lowest n-3/n-6 ratio being recorded in *A. vermiculophyllum*, which also presented the highest levels of saturated fatty acids and the lowest levels of monounsaturated fatty acids (Table 4).

#### Lipid classes and fatty acids composition of pupae and adult flies

The lipid classes identified in pupae and adult flies were triacycloglycerols (TG), free fatty acids (FFA), and cholesterol (CH) and their abundance is shown in Table 5. The highest value of TG was from pupae fed with codfish; for FFA, the highest value was from pupae fed with *A. vermiculophyllum*; and for CH, the highest value was from adult flies fed with *A. vermiculophyllum*.

The most abundant fatty acid was OA, both in pupae and adult flies fed with *Fucus* sp., while for all the other treatments, the most abundant FA was palmitoleic acid (16:1 n-7), as shown in Table 6. Levels of EPA higher than 5% of the total pool of fatty acids were only found on pupae and adult flies fed with codfish frames.

Noteworthy, the abundance of PUFAs (Table 6) was higher in pupae fed with *Fucus* sp., followed by codfish frames, and was lower in larvae fed with *A. vermiculophyllum*. Among these PUFAs, the amount of PUFA n-3 in pupae fed with *Fucus* sp. was notable. The results were similar for adult flies. The n-3/n-6 ratio was higher in pupae fed with codfish frames, followed by those fed

TABLE 3 Amino Acid profile of feeding substrates employed to culture the seaweed fly *Fucellia maritima*. Data are shown as the mean of two replicates (% of total amino acids)

% of total aa	Substrates				
	<i>A. vermiculophyllum</i> (S)	<i>Fucus</i> sp. (S)	<i>Eichhornia crassipes</i> (S)	<i>Ulva</i> sp. (S)	Codfish (S)
Non-essential amino acids					
Aspartic Acid	14.5	13.9	20.9	13.6	9.9
Serine	6.2	5.1	5.8	5.8	4.9
Glutamic Acid	11.8	20.2	7.4	13.7	16.9
Glycine	6.4	5.9	8.7	6.7	6.9
Ornithine	1.2	0.0	0.0	0.0	0.0
Taurine	1.8	0.0	0.0	0.0	0.9
Arginine	6.8	4.9	6.5	7.0	6.9
Alanine	3.6	3.7	2.7	4.7	6.5
Proline	6.0	4.5	4.8	5.3	4.0
Cysteine	0.0	0.0	0.0	0.0	0.0
Tyrosine	2.7	2.9	3.8	3.0	3.1
Hydroxyproline	0.0	0.0	0.0	1.3	0.1
∑NEAA	60.8	61.1	60.6	61.0	60.1
Essential amino acids					
Valine	6.6	5.9	6.2	6.7	5.0
Methionine	0.5	2.4	1.1	1.9	3.0
Histidine	1.3	1.7	2.3	1.3	1.8
Threonine	5.9	5.0	6.3	5.6	4.2
Lysine	5.0	5.4	3.3	4.8	9.6
Isoleucine	5.6	4.9	5.0	4.7	4.1
Leucine	8.4	7.9	7.8	7.7	8.2
Phenylalanine	5.3	4.9	6.2	5.5	3.1
Tryptophan	0.6	0.9	1.3	0.9	0.8
∑EAA	39.2	38.9	39.4	39.0	39.8

(S) = substrate; ∑NEAA = sum of non-essential amino acids; ∑EAA = sum of essential amino acids.

with *Ulva* sp. Lower values were obtained in individuals fed with *A. vermiculophyllum*. The same results were observed for adult flies. All samples had a higher percentage of MUFA when compared to PUFA. Still, pupae and adult flies fed with *A. vermiculophyllum* had the highest percentage of monounsaturated fatty acids.

#### 4 Discussion

The present study investigated the nutritional profile of an understudied European native insect species that was only recently recorded from the coastal area of mainland Portugal (Lourenço *et al.*, 2020). Its nutritional profile and ability to breed in captivity make the seaweed fly *F. maritima* a suitable candidate as a novel ingredient for marine aquafeed production, affording

new potential insect species for this purpose (Lourenço *et al.*, 2022).

Seaweed flies were able to complete their life cycle on most feeding substrates tested in this study. In our facilities they were maintained in captivity for several generations in insect cages (350 mm × 350 mm × 600 mm) under stable climatic conditions (unpubl. data). It is noteworthy to mention that there are no known life tables for this species or mass-rearing protocols described to date.

In this experimental setting, we doubled the population's initial size (50 adult flies per replicate) to more than 100 individuals per replicate (sum of pupae and adult flies in Table 1). However, there was a high deviation between the replicates, which can be caused by the unknown ratio of females in the initial population, where a high ratio of females is needed in a mass-rearing context (Fitz-Earle and Barclay, 1989). As men-

TABLE 4 Fatty acid relative abundance (%) of the substrates tested. Data are expressed as mean values of two replicates for *Fucus* sp. *A. vermiculophyllum*, *E. crassipes* and *Ulva* sp., while four replicates were used for the codfish frames

FA	Substrates				
	Codfish (S)	<i>Fucus</i> sp. (CG) (S)	<i>A. vermiculophyllum</i> (S)	<i>E. crassipes</i> (S)	<i>Ulva</i> sp. (S)
11:0	3.9	–	0.0	0.1	0.0
12:0	0.0	0.1	0.3	0.7	0.1
13:0	–	0.2	0.1	0.1	0.2
14:0 iso	–	0.1	0.2	0.2	0.7
14:0	3.1	12.0	7.9	1.8	0.9
15:0	0.4	0.4	0.5	1.4	1.7
16:0	18.3	15.4	45.9	27.6	41.6
17:0	1.3	0.4	0.1	1.3	0.4
18:0	4.4	1.5	1.4	2.4	1.7
20:0	0.2	0.6	0.1	0.5	0.2
22:0	0.0	0.2	0.1	0.7	2.1
24:0	0.	0.3	0.1	2.1	0.4
SFA	31.4	31.3	56.9	39.1	50.3
14:1 n-5	0.1	0.2	0.0	0.5	0.0
15:1 n-5	0.1	–	0.0	0.2	0.2
16:1 n-9	0.5	0.1	0.1	0.8	0.4
16:1 n-7	3.3	1.5	1.9	3.9	7.7
16:1 n-5	0.4	0.2	0.1	1.4	0.3
18:1 n-9	14.2	32.7	8.0	3.4	3.4
18:1 n-7	3.7	0.6	2.2	3.8	18.8
20:1 n-9	5.2	0.4	0.3	0.2	0.3
22:1 n-11	1.9	0.5	0.1	–	0.0
22:1 n-9	0.8	0.1	0.3	0.9	1.2
24:1 n-9	1.9	–	0.4	–	0.0
MUFA	31.9	36.3	13.6	15.3	32.5
16:2 n-6	0.0	0.1	0.0	0.5	0.1
16:2 n-4	0.4	0.1	0.5	0.2	0.1
16:3 n-3	0.1	0.0	0.2	0.2	0.3
18:2 n-6	1.1	7.8	1.2	22.1	2.7
18:3 n-6	0.1	0.6	0.6	0.3	0.4
18:3 n-3	0.3	3.9	0.2	17.9	5.0
18:4 n-3	0.4	1.7	0.0	0.2	2.6
20:3 n-6	0.1	0.8	2.7	0.3	0.5
20:4 n-6	2.4	11.1	23.4	0.7	0.8
20:4 n-3	0.4	0.4	–	–	0.7
20:5 n-3	10.1	4.5	0.2	0.9	1.3
22:4 n-6	0.2	0.8	0.1	0.0	0.5
22:5 n-6	0.3	0.1	0.1	1.1	0.4
22:5 n-3	1.4	0.1	0.0	0.7	1.3
22:6 n-3	18.5	0.1	0.0	0.3	0.2
PUFA	36.7	32.3	29.5	45.6	17.2
n-3	31.9	10.8	0.7	20.2	11.5
n-6	4.3	21.3	28.2	25.1	5.5
n-3/n-6	7.3	0.5	0.03	0.8	2.1

1 SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-3 = omega 3 fatty acids; n-6 = omega 6 fatty acids; n-3/n-6 = omega-3 to omega-6 ratio; (S) = substrate.

tioned earlier, it is impossible to distinguish sexes in *F. maritima* without dissecting. Since the experiment ended with the first generation, it was impossible to confirm the exponential population buildup. However, the observed duplication of the number of individuals in the first generation seems to indicate this possibility.

To clarify this, more studies on *F. maritima* population dynamics to test the likelihood of its mass-rearing are necessary.

The invasive plant *E. crassipes* was not a suitable substrate, and *F. maritima* larvae could not complete their life cycle using this substrate. It is known that *E. cras-*

TABLE 5 Lipid classes of *Fucellia maritima* pupae (P) and adult (A) flies fed on different feeding substrates. Data are shown as mean values (% of total lipid classes, n = 2)

	Feed	TG	FFA	CH
Pupae	Codfish (P)	76.7	22.0	1.3
	<i>Fucus</i> sp. (CG) (P)	72.0	27.0	1.0
	<i>A. vermiculophyllum</i> (P)	66.7	31.0	2.4
	<i>Ulva</i> sp. (P)	71.1	27.6	1.3
Adult flies	Codfish (A)	75.2	20.8	4.1
	<i>Fucus</i> sp. (CG) (A)	61.9	34.2	3.9
	<i>A. vermiculophyllum</i> (A)	60.5	33.9	5.7
	<i>Ulva</i> sp. (A)	69.0	26.5	4.5

TG = triacylglycerols; FFA = free fatty acids; CH = cholesterol.

*sipes* produces secondary metabolites that deter insect feeding, namely quercetin (a flavonoid) and tomatine (a glycoalkaloid), which have documented insecticidal and larvicidal activity against Diptera (Ben Bakrim *et al.*, 2022). Annually, in Ria de Aveiro, these plants are washed from the Vouga River to the beach and comprise about 90% of the beach wrack, overlapping with the naturally most abundant seaweeds, *Fucus* sp. and *Ulva* sp. (O. Ameixa, pers. obs.) (Figure 3). This finding reinforces the negative impact that this invasive freshwater plant species can have on the ecosystems it invades and adjacent ones, especially for seaweed flies.

Overall, the type of feeding substrate seems to influence the weight of the pupae of *F. maritima*, especially when larvae are fed with codfish frames, although this was not confirmed by statistical analysis. As with other insect orders, weight is known to change in Diptera when different feeding substrates are used; examples are known for CHF (Ganda *et al.*, 2022), BSF (Liland *et al.*, 2017), *Coelopa frigida*, and *Coelopa pilipes* (Biancarosa *et al.*, 2018b). However, we also observed that the weight of the larvae and adult flies was inversely proportional to the number of individuals, indicating a possible effect of density on the weight of these specimens. Although, as mentioned earlier, there is no statistical test on the weight of individuals, the density was not tested, and the number of individuals presents no significant difference. Nevertheless, the rearing density is known to have a negative effect on the weight of different insect species. For instance, a higher density of BSF can significantly impact the individual larval weight when compared to a treatment with a low density (Yakti *et al.*, 2022). Similar results are noticed in the pupal weight of CHF, with the lower weight seen on the highest density (Kökdener and Kiper, 2021). With YM, there is a similar effect, with the highest larval weight seen

in the lower-density treatment (Koutsogeorgiou *et al.*, 2024).

The lipid content recorded for *Fucus* sp. was above the range of other studies available in the literature (1.4-3.7%) (da Costa *et al.*, 2019; Lorenzo *et al.*, 2017; Neto *et al.*, 2018). Ash content, however, was in the range reported by the same authors (20.7-25.5%), the same being true for protein content (12.9-15.1%). In comparison to other studies, *A. vermiculophyllum* showed a lower lipid content (0.7-2.0%), while *Ulva* sp. had a lipid content within the range of other studies (0.9-2.53%) (Afonso *et al.*, 2021; Neto *et al.*, 2018). Both *A. vermiculophyllum* and *Ulva* sp. showed lower ash content (19.6-28.9% and 26.4-37.1%, respectively) and protein content inside the range for *A. vermiculophyllum* and higher for *Ulva* sp. (14.7-23.6% and 9.3-17.7%) (Afonso *et al.*, 2021; Lopes *et al.*, 2019; Neto *et al.*, 2018). Seasonal changes in protein and lipid values are common in seaweeds, with the highest values being found in winter and the beginning of spring and lower values being found in summer and autumn (Afonso *et al.*, 2021; da Costa *et al.*, 2019). *Eichhornia crassipes* lipid content (0.9-14.9%) and ash content (12.4-39.8%) were in the range of other studies, but protein content was higher (6.6-15.2%) (Arayana *et al.*, 1984; Hosain *et al.*, 2015; Okoye *et al.*, 2000). The high range found in other studies was probably due to the separation of plant parts by those authors, with roots having the lowest protein and lipid content and leaves having the highest; in our study, the whole plant tissue was ground and homogenised, likely promoting the contrasting results recorded. Codfish frames had a higher lipid (5.2%) and protein (58.9%) content and a lower ash content (30.4%) in comparison to what was previously found in another study that also used codfish frames from the same company but to culture black sol-

TABLE 6 Fatty acid composition (Relative abundance %) of pupae (P) and adult (A) flies of *Fucellia maritima* fed with different substrates (codfish frames, *Fucus* sp. *A. vermiculophyllum*, *E. crassipes*, *Ulva* sp.). Data are expressed as mean values of two replicates

FA	Pupae				Adults			
	Codfish (P)	<i>Fucus</i> sp. (CG) (P)	<i>A. vermiculophyllum</i> (P)	<i>Ulva</i> sp. (P)	Codfish (A)	<i>Fucus</i> sp. (CG) (A)	<i>A. vermiculophyllum</i> (A)	<i>Ulva</i> sp. (A)
11:0	0.8	0.8	0.9	0.7	2.1	1.9	1.9	2.1
12:0	0.5	0.4	0.5	0.5	0.5	0.2	0.3	0.3
13:0	0.0	0.2	0.1	0.2	0.0	0.1	0.1	0.1
14:0 iso	0.3	0.8	1.8	0.8	0.2	0.4	1.6	0.3
14:0	3.2	7.9	3.7	3.8	2.4	4.7	2.2	2.3
15:0	0.5	1.3	1.0	1.9	0.4	0.9	0.7	1.4
16:0	22.4	18.9	21.8	25.0	19.3	17.9	17.6	21.3
17:0	0.8	0.4	0.9	0.8	0.8	0.3	0.8	0.6
18:0	1.9	1.4	2.1	1.9	1.9	1.4	1.8	1.8
20:0	0.3	0.3	0.4	0.4	0.3	0.5	0.5	0.5
22:0	0.1	0.2	0.3	0.2	0.5	0.6	0.6	0.5
24:0	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.1
SFA	31.0	32.6	33.6	36.3	28.7	29.3	28.5	31.5
14:1 n-5	0.8	0.9	3.4	1.3	0.6	0.5	3.1	0.9
15:1 n-5	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.0
16:1 n-9	4.2	6.3	1.2	1.1	3.9	4.8	1.1	0.9
16:1 n-7	28.9	19.5	37.2	38.1	26.3	18.4	35.0	36.0
16:1 n-5	0.4	0.3	0.9	0.3	0.3	0.2	0.5	0.2
18:1 n-9	21.0	23.3	15.4	13.6	25.0	25.9	21.2	18.0
18:1 n-7	2.5	1.4	3.1	3.2	1.8	1.3	2.1	2.8
20:1 n-9	0.7	0.3	0.4	0.4	0.6	0.3	0.3	0.4
22:1 n-11	0.1	0.0	0.1	0.1	0.5	0.7	0.7	0.6
22:1 n-9	0.1	0.3	0.4	0.1	0.2	0.3	0.2	0.2
24:1 n-9	0.1	–	0.0	–	0.3	0.2	0.4	0.2
MUFA	58.8	52.7	62.1	58.4	59.7	52.8	64.9	60.4
16:2 n-6	0.1	0.4	0.0	0.2	0.1	0.3	0.0	0.1
16:2 n-4	0.4	0.4	1.2	0.6	0.3	0.4	1.5	0.8
16:3 n-3	0.2	0.0	–	0.0	0.1	0.0	–	0.0
18:2 n-6	0.7	5.5	0.5	1.3	1.0	5.8	0.7	2.0
18:3 n-6	0.1	0.5	0.2	0.2	0.1	0.4	0.4	0.4
18:3 n-3	0.1	2.4	0.2	0.8	0.3	3.5	0.4	1.5
18:4 n-3	1.0	0.6	0.0	0.2	1.1	0.6	0.1	0.4
20:3 n-6	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0
20:4 n-6	0.7	2.3	0.8	0.2	0.7	2.9	1.5	0.4
20:4 n-3	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.1
20:5 n-3	5.1	1.5	0.3	0.6	6.4	2.8	0.5	1.2
22:4 n-6	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.0
22:5 n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
22:5 n-3	0.4	0.2	0.4	0.5	0.6	0.5	0.8	0.7
22:6 n-3	0.9	0.1	–	–	0.5	0.0	0.0	0.1
PUFA	10.1	14.6	4.2	5.4	11.6	17.8	6.6	8.1
n-3	7.9	5.2	1.0	2.4	9.2	7.8	2.0	4.1
n-6	1.8	8.9	1.9	2.2	2.0	9.6	3.0	3.2
n-3/n-6	4.3	0.6	0.5	1.1	4.5	0.8	0.7	1.3

1 SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-3 = omega 3 fatty acids; n-6 = omega 6 fatty acids; n-3/n-6 = omega-3 to omega-6 ratio; (P) = pupae; (F) = adult flies.

dier fly (Rodrigues *et al.*, 2021). The differences in our results may arise from our analyses only including the flesh and skin of codfish frames, while in the study by Rodrigues *et al.* (2021), the whole ground frames, which

contained a considerable mass of bones, were also being used.

All the tested substrates presented EAAs. The vegetable samples had a higher value on five of the nine EAAs when compared to the amino acids present on



FIGURE 3 *Eichhornia crassipes* composing most of the biomass of beach wrack (40°38'31.1"N; 8°44'57.7"W).

codfish frames, making them a source of good-quality protein with high digestibility for the seaweed fly *F. maritima*. The EAA/total AA ratio suggests that almost 40% of the total AA was EAA on all samples; these values are close to the ones reported from other studies addressing *Fucus* sp. (40.9%) (Lorenzo *et al.*, 2017), and *Ulva* sp. (40.7%) (Machado *et al.*, 2020), but lower than those reported for *A. vermiculophyllum* (51%) (Afonso *et al.*, 2021), and *E. crassipes* (50.3%) (for leaf protein concentrate) (Hontiveros and Serrano, 2015).

The fat content was lower, but the protein content of *F. maritima* (as a total percentage of dry matter as shown in Table 2) was within the range observed in other terrestrial insect species produced for food and feed, namely BSF and YM (30.7-58.8% and 51.8-59.8%, respectively) (Nogales-Mérida *et al.*, 2019). Moreover, *F. maritima* has a higher protein content than other seaweed flies, like *C. frigida* and *C. pilipes* (Biancarosa *et al.*, 2018b). However, the higher protein content observed in adult flies (55.2-56.7%) could be overestimated, as adult insects have a higher content of chitin and non-digestible protein (Jonas-Levi and Martinez, 2017). Considering this, we recommend the study of the total amino acids profile of *F. maritima* to have a more accurate estimate of its protein content and to select the most adequate conversion factor, as suggested by Janssen *et al.* (2007).

Considering FAs, *F. maritima* showed a considerable amount of n-3 PUFAs in their composition, especially in pupae fed with codfish frames, which had a higher

n-3/n-6 ratio and a higher level of EPA and DHA than those found in other seaweed flies, *C. frigida* and *C. pilipes* (Biancarosa *et al.*, 2018b), but in a lower abundance than what was found in the coastal long-legged fly, *Machaerium maritima* (Duarte *et al.*, 2021). Despite these differences, the levels of PUFA were, in general, still higher than those found on terrestrial species, even when these were fed with substrates rich in these biomolecules (Nogales-Mérida *et al.*, 2019; Oonincx *et al.*, 2020). This was most evident in pupae whose larvae were fed codfish frames. Moreover, higher inclusions of n-3 PUFA-rich substrates in the diets presented a negative trade-off, such as reduced larval growth in terrestrial species, namely BSF (Ewald *et al.*, 2020; Liland *et al.*, 2017; Rodrigues *et al.*, 2022). In the study done by Liland *et al.* (2017) it is hypothesised that this trade-off occurs mostly because of the low protein content of the seaweed used and due to its larger particle size when compared to the control diets, suggesting that a further reduction of the particle size of seaweed could improve the growth of the larvae (Liland *et al.*, 2017).

The most abundant fatty acid found on both pupae and adult flies of *F. maritima* in all feeding treatments (except *Fucus* sp., for which the most abundant fatty acid was OA) was the monounsaturated palmitoleic acid (16:1 n-7), which, when supplemented in the diet of the Pacific white shrimp *Litopenaeus vannamei*, can help to protect the growth rate and enzymatic activity of this species against aflatoxins that are often present in aquafeeds (García-Pérez *et al.*, 2020).

The saturated PA (the most abundant saturated fatty acid found in animals) and the monounsaturated OA were the other abundant fatty acids recorded. These FAs are important in maintaining the cellular membrane's physical properties; however, an excess of PA in the diet may increase an inflammatory response in mammals' intestinal and hepatic tissues (Ghezal *et al.*, 2020). These damages to the gut tissue can be mitigated by including EPA in farmed fish's diets (Shi *et al.*, 2022). However, for zebrafish *Danio rerio*, a higher inclusion of PA increased their antiviral protection (Librán-Pérez *et al.*, 2019). Even though many animal and vegetable fats and oils naturally contain OA, fish raised in aquafeeds that include OA have higher immune responses to bacterial infections, which promotes fish growth and survival rates when infected with *Vibrio vulnificus*. (Natnan *et al.*, 2022).

Palmitoleic acid and OA are the most abundant fatty acids in other seaweed flies, such as *C. frigida* and *C. pilipes* (Biancarosa *et al.*, 2018b). However, for *M. maritima*, also a coastal insect, the abundance of PA was much lower when compared to these three seaweed fly species (Duarte *et al.*, 2021). The reason for this might be the difference in their diets, as *M. maritima* is an amphibiotic insect, and as with most Dolichopodidae species, both larvae and adult flies may be predaceous, while palmitoleic acid is mostly a product of PA metabolism in the body or can be acquired trophically through the consumption of seaweed (McAlpine *et al.*, 1981; Vannice and Rasmussen, 2014).

Regarding n-3 fatty acids, EPA was much higher in insects fed with codfish frames, suggesting that *F. maritima* larvae can assimilate the fatty acid content of their feeding substrate, which was much richer on this FA than all others tested in this study. The abundance of EPA in pupae fed with codfish frames was similar to the abundance found in another study using wild-caught *F. maritima*, with approximately 6% of relative abundance (Lourenço *et al.*, 2024). The abundance of DHA was lower than 1% of the total pool of FA, even in pupae and adult flies whose larvae were fed with codfish frames (this FA was not detected on pupae from specimens fed with *A. vermiculophyllum* or *Ulva* sp.). The presence of DHA is reported for the first time in a seaweed fly in the present study, although it has already been found in another marine fly species, namely *M. maritima* (Duarte *et al.*, 2021).

Despite the lower abundance of EPA and DHA in seaweed flies than in fish or shrimp, both adult flies and pupae of *F. maritima*, namely those fed with codfish frames, still present an interesting composition in terms

of these essential fatty acids, which can be used as insect meals for the formulation of marine aquafeeds.

## 5 Conclusions

Due to its short life cycle duration (from 20 to 28 days, egg to adult fly), *F. maritima* can be a potential novel ingredient for marine aquafeed formulations despite its small size compared with other insect species normally used in insect meals. Preliminary rearing trials by the authors revealed that exponential population growth can be achieved relatively quickly. Since the need for heating room temperature during production is also lower compared to other insect species currently reared, the costs of producing *F. maritima* as an insect meal may eventually be lower than for other 'mainstream' insect species. Furthermore, one of the advantages of using native insect species in comparison with exotic ones is that it minimises the potential risks of invasion when commercial-scale farming is put forward in Europe (Lourenço *et al.*, 2022). In addition, one must highlight that *F. maritima* can also be used to add value to the biomass of the invasive red seaweed *A. vermiculophyllum*, as it can be used as a feeding substrate for this insect. From this study, one can suggest that to maintain a successful culture of *F. maritima*, one should most likely use a mixture of *Fucus* sp. and cod fish frames to achieve higher survival and insect biomass production, which has a more suitable PUFA content for marine aquafeed formulation.

However, the authors know this study lacks strong statistical testing to confirm our findings. Still, overall, the data suggests that the seaweed fly *F. maritima* has the potential to be used as an aquafeed ingredient, considering their fatty acid profile and high protein content. However, there needs to be more research regarding their capacity to be mass-produced, the stability of their nutritional profile, and developing life tables to guarantee an optimum product for commercialisation, before this species can be considered a true candidate to be used as an aquafeed ingredient. Moreover, like other seaweed flies, *F. maritima* is dependent on marine organic matter as a substrate, and it is known that seaweeds can accumulate undesirable metals like arsenic, mercury, and cadmium (Biancarosa *et al.*, 2018a). However, Ferrari *et al.* (2002) have demonstrated that bioaccumulation of mineral and trace elements in reared insects can also be positive, opening new scenarios for feed and food production (Ferrari *et al.*, 2022). Therefore, studies on the bioaccumulation of metals in *F.*

*maritima* are needed to truly consider this species as a candidate for aquafeed ingredients.

Overall, *F. maritima* shows the potential to contribute to a higher diversification of insect production, considering that this sector is still in its infancy. It may join the list of potential insect species to be explored as novel ingredients for the formulation of marine aquafeeds.

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### Conflict of interest

The authors have no conflict of interest to declare.

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