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The chemical composition of two seaweed flies (*Coelopa frigida* and *Coelopa pilipes*) reared in the laboratory

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RESEARCH ARTICLE

Abstract

Two species of seaweed flies, *Coelopa frigida* and *Coelopa pilipes*, were reared in the laboratory and their larvae were sampled for composition of amino acids, fatty acids and elements. The larvae were grown on two different species of seaweed, *Laminaria digitata* and *Fucus serratus*. The aim was to gain knowledge on the influence of feeding media on the growth and composition of the larvae. *F. serratus* was more nutrient-dense than *L. digitata*, being richer in both protein and lipids, and thus led to ~70% higher larvae growth. The larvae grown on *F. serratus* also had higher lipid and protein content than the larvae grown on *L. digitata*; *F. serratus*-grown larvae had ~8-9% protein and ~18% lipid (total fatty acids) (both values of dry matter), while the larvae grown on *L. digitata* had only ~7.5% protein and ~13% lipids. All seaweed flies had a similar and balanced amino acid composition, suitable for animal and human nutrition. The fatty acid composition was not highly affected by either insect species or feeding media, with all groups containing high concentrations of the monounsaturated fatty acid, palmitoleic acid (16:1n-7). The larvae also contained some fatty acids characteristic of marine environments, like eicosapentaenoic acid (20:5n-3), likely originating from the seaweed. Both species of seaweed fly larvae accumulated As, Cd, and Pb, but not Hg. The elevated levels of As and Cd in the larvae (highest measured concentrations 18.4 and 11.6 mg/kg, respectively, based on 12% moisture content) could potentially limit the use of seaweed fly larvae as a feed ingredient.

Keywords: insects, fatty acid, amino acid, brown algae, heavy metals

1. Introduction

Insects are becoming increasingly important for use in human and animal food chains and may develop as important as sources of nutrients (Henry *et al.*, 2015). Insect larvae can be naturally rich in protein and micronutrients and have favourable compositions of essential amino acids (Barroso *et al.*, 2014; Makkar *et al.*, 2014). Most studies on insects have been performed on species that feed on matter of terrestrial origin, such as (*Tenebrio molitor*), superworm (*Zophobas morio*), silkworm (*Bombyx mori*) and black soldier fly (*Hermetia illucens*) (Henry *et al.*, 2015). Insects feeding on matter of marine origin have been found to be richer in omega-3 polyunsaturated fatty acids (PUFA) than insects growing on terrestrial media (Fontaneto *et al.*, 2011; Liland *et al.*, 2017; St-Hilaire *et al.*, 2007). There are small numbers of terrestrial insects that naturally feed upon decomposing matter of marine origin. Amongst these insects are the seaweed flies (Diptera: Coelopidae). This is a small family of coastal flies that are entirely dependent upon beached wrack for the development of their larvae. Two species, *Coelopa frigida* and *Coelopa pilipes*, are common in Northern Europe (Edward *et al.*, 2007). Both species inhabit wrack beds primarily composed of brown algae (Phaeophyta), especially the genera *Laminaria* and *Fucus* (Dobson, 1974). The deposition of fresh seaweeds upon beaches, following storm events and spring high tides, attracts adult flies and stimulates mating and oviposition (Dunn and Crean, 2002). The larvae of both species feed on the decaying seaweeds and the bacteria found on the seaweed surface (Cullen *et al.*, 1987; Dobson, 1974). The larvae are often washed out of the wrack by storm events

and spring high tides where they become natural prey to fish such as bass (Dobson, 1974). Both *C. frigida* and *C. pilipes* have been successfully cultured in the laboratory (Edward *et al.*, 2007). No data are available on the nutritional or contaminant composition of these fly species. We aim to address this knowledge gap.

In the current study, *C. frigida* and *C. pilipes* larvae were reared in laboratory-scale facilities, using *L. digitata* or *F. serratus* as sole feeding substrates. The main aim of the study was to investigate the chemical composition of the insect larvae, and to gain knowledge on the composition of insects that naturally feed on matter of marine origin.

2. Materials and methods

Laboratory culture

Two species of brown algae: *Laminaria digitata* (Huds.) and *Fucus serratus* (L.) were collected from wrack bed deposits in Fife, Scotland. Seaweed was collected at two different days at two locations. The reason for several collection days was the need to repeat some of the treatments, while different locations were used as individual locations are inconsistent in their availability of wrack due to the direction of prevailing winds. The collection locations were Kingsbarns (N 56° 18.200 W 002° 38.670) which faces north east and Cellardyke Harbour (N 56° 13.500 W 002° 41.000) which faces south west. The wrack was sorted on the beach and then frozen at -20 °C. The seaweed was then defrosted and washed prior to being minced using a buffalo meat mincer to increase the surface area and rate of decomposition of the seaweed. Two species of seaweed flies (*C. frigida* and *C. pilipes*) were reared in laboratory-scale facilities at the University of Stirling, Scotland. All culturing was carried out in 10 l food storage boxes (Addis, Bridgend, UK) kept in the same room at constant temperature of 25 °C, 60% humidity and a 12:12 light-dark cycle. A hole was cut in the lid of each box and covered with paper towels to allow aeration of the cultures while preventing escapes of insects. Two kg of minced seaweed were added to each box. Fifty adult flies with a 50:50 sex ratio were added to each box. These flies came from laboratory populations established from wild collections of both species of coelopids from Kingsbarns. Six replicates boxes were established for each of the four treatments: (1) *C. frigida* grown on *L. digitate*; (2) *C. frigida* grown on *F. serratus*; (3) *C. pilipes* grown on *L. digitata*; and (4) *C. pilipes* grown on *F. serratus*.

Sampling

On day four after establishing the cultures, one sample of approximately 150 g biomass (larvae + seaweed) was removed from each box using a metal spoon. This sampling point was selected as it was expected that the larvae were at the largest size with the highest lipid and protein content (N.S. Liland, unpublished data) before pupation. The sample was weighed and the larvae were subsequently collected. The larvae were collected with a spoon, rinsed in water, dried on tissue paper and finally frozen on dry ice. The weight of the collected larvae collected from each sample was registered.

Sample processing

Frozen samples of the seaweed feeding media and larvae were ground to a powder by using a blender (Knife Mill Frindomix GM 200; Retsch, Haan, Germany). Dry ice was added in the blending process to prevent thawing of samples. Due to poor growth in some crates resulting in small sample size, samples from two crates were pooled (crate 1+2, crate 3+4, crate 5+6 for each group). One sample per seaweed species (a total of two samples) and three samples per insect/seaweed group (a total of twelve samples) were thus analysed. Aliquots of the samples were lyophilized for the content of dry matter by first freezing 24 h at -20 °C in vacuum (0.2-0.01 mBar), then leaving in vacuum at 25 °C until constant weight.

Chemical analyses

Analysis of total amino acids (except cysteine and tryptophan) of feeding media and larvae was carried out in technical duplicates by ultra-performance liquid chromatography (UPLC, Acquity UPLsC system; Waters, Milford, MA, USA) coupled with a UV detector (Biancarosa *et al.*, 2017a). Wet, powdered samples (containing ~40 mg of protein) were hydrolysed in 6 M HCl at 110 °C for 22 h. Prior to hydrolysis, 3.125 mM Norvaline (Sigma-Aldrich, St. Louis, MO, USA) was added as internal standard, and 0.1 M dithiothreitol (Sigma-Aldrich) as an antioxidant agent to protect methionine from degradation during acid hydrolysis. For a further protective aid, sample tubes were topped up with nitrogen gas. During acid hydrolysis, cysteine and tryptophan are destroyed and are therefore not reported in the results. After hydrolysis, samples were cooled to room temperature and centrifuged in a vacuum centrifuge until complete dryness was reached. After centrifugation, the residue was diluted in deionized water (MilliQ-Plus, Billerica, MA, USA) and filtered through a syringe-driven filter. Prior to the instrumental analysis, a derivatisation agent (AccQ.Tag™; Waters) was added to each sample. Finally, amino acids were separated by UPLC (column: Aquity UPLC BEH C18 1.7 µM, flowrate 0.7 ml/min; Waters) and results integrated by Empower 3 (Waters). Protein content of media and larvae is here presented as sum of anhydrous amino acids (also called true protein), a more accurate estimation of protein than N-content × protein factors (Biancarosa *et al.*, 2017a).

Fatty acid composition was determined for feeding media and larvae and analysed in technical duplicates as

described by Torstensen *et al.* (2004). Briefly, lipids were extracted from wet, powdered samples by homogenisation in chloroform:methanol (2:1, v:v) and analysed using gas chromatography coupled with a flame ionisation detector. The following instrumentation was used: Autosystem XL (Perkin Elmer, Waltham, MA, USA) with pre-column Silica 0.53 mm i.d. (Imperial Eastman Tubing, Baltimore, MD, USA) and a CP-sil-88™ column, 50 m × 0.32 mm i.d. Helium was used as carrier gas at 1.5 ml/min and hydrogen as a detector gas at 45 ml/min. The peaks were identified with the software Chromeleon® version 6.8 (Dionex, Sunnyvale, CA, USA) and individual methyl esters were identified by comparison to known standards and on the basis of published values (Ackman, 1980). Quantification of fatty acids was done by using 19:0 methyl ester as an internal standard.

Element concentrations in freeze-dried, powdered material of feeding media and larvae were analysed by inductively coupled plasma-mass spectrometry (ICP-MS) after wet digestion in a microwave oven, based on Julshamn *et al.* (2001). Briefly, the samples (approximately 0.2 g of dry sample) were digested in 69% nitric acid (2 ml; Sigma-Aldrich) and 30% hydrogen peroxide (0.5 ml; Merck Millipore, Billerica, MA, USA) using a microwave digestion system (UltraWAVE; Milestone, Sorisole, Italy). The solutions were diluted to 25 ml with deionized water (MilliQ plus; Merck Millipore). Element concentrations in the samples were quantified by ICP-MS (iCapQ ICPMS; Thermo Fisher Scientific, Waltham, MA, USA) equipped with an autosampler (FAST SC-4Q DX; Elemental Scientific, Omaha, NE, USA). Data were collected and processed using the Qtegra ICPMS Software (Thermo Fisher Scientific).

Statistical analysis

All statistical analyses were performed using the statistical environment R (R Development Core Team, 2017). Two-way ANOVA was performed using linear models (variables: insect species and seaweed feeding media). Homogeneity in variance and normal distribution were verified by graphical evaluation using the plot function in R (residuals vs fitted, normal Q-Q plot, scale-location plot, residuals vs leverage plot) as well as numerically by Levene's test and a Shapiro-Wilks test. Data not suitable for ANOVA analysis was analysed using Kruskal-Wallis.

3. Results

Growth of the larvae

The larvae that had grown on *F. serratus* had a larger biomass at the end of the four-day growth period compared to the larvae grown on *L. digitata* (~70% higher biomass per crate, Figure 1). Due to loss of data, only four replicate values for growth are reported in the *C. frigida* grown on *L. digitata*, while there are six for the other groups.

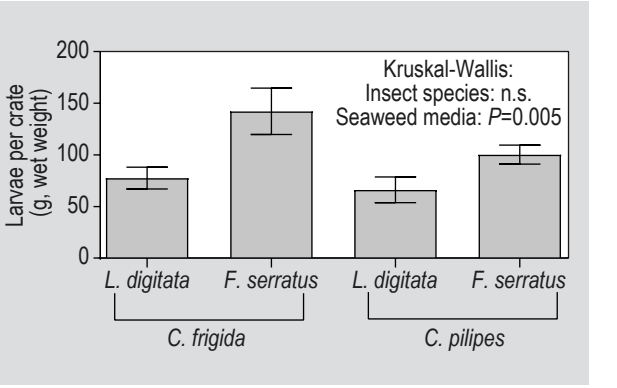

Figure 1. Total mass of seaweed larvae (*Coelopa frigida* and *Coelopa pilipes*) after a four-day growth period on either *Laminaria digitata* or *Fucus serratus* (g per crate). Data are expressed as mean values of six replicate crates per treatment ± SEM (n=4 for *C. frigida* on *L. digitata*).|

Composition of the seaweed feeding media

F. serratus had lower content of dry matter than *L. digitata*, but *F. serratus* had higher protein content (on dry weight basis) than *L. digitata* (Table 1). Both seaweed species were, however, low in several essential amino acids, namely phenylalanine that was below the quantification limit in *L. digitata*, while histidine and methionine were below the quantification limit in both algae species (Table 2). The lipid concentrations were also higher in *F. serratus* (~9% lipids on dry weight basis) than in *L. digitata*, that had less than 1% lipid (Table 1). Both seaweed species had similar concentrations of saturated, monounsaturated and polyunsaturated fats, but *L. digitata* had a higher n-3/n-6 ratio than *F. serratus* (Table 3).

The seaweed feeding media contained similar levels of macrominerals, although the concentration of Na was lower in *L. digitata* than in *F. serratus* (Table 4). There were differences in the concentrations of microminerals, *L. digitata* contained higher concentrations of Fe, Cu, and Zn, and lower concentrations of Mn, than *F. serratus*, while the level of Se was similar in the two species (Table 4). The levels of the heavy metals Cd, Hg, and Pb were low, while the levels of As were high in both species (Table 4). The levels of As were twice as high in *L. digitata* compared to *F. serratus.*

Composition of the larvae

The insect larvae grown on *F. serratus* had higher content of dry matter, protein, and total fatty acids than the larvae grown on *L. digitata* (Table 1). There was a lower concentration of the essential amino acid histidine in *C. pilipes* than in *C. frigida* (Table 2). *C. pilipes* also contained less phenylalanine than *C. frigida*, while *C. frigida* contained more lysine, methionine, and valine

Table 1. Proximate composition of the seaweed media (the brown algae *Laminaria digitata* and *Fucus serratus*) and the two species of seaweed fly larvae (*Coelopa frigida* or *Coelopa pilipes*) grown on either *L. digitata* or *F. serratus*. Data are expressed as mean values of three replicates ± standard deviation for insects and the mean of two technical replicates for the seaweed media.[1,2]

	Seaweed media		*C. frigida*		*C. pilipes*		I	S	I×S
	L. digitata	*F. serratus*	*L. digitata*	*F. serratus*	*L. digitata*	*F. serratus*			
Dry weight (%)	23.8	10.4	19.1±2.0	21.5±1.4	16.4±0.8	23.1±1.0	NS	*	NS
True protein (g/100 g dm)	1.0	2.3	7.3±0.6	7.8±0.4	7.4±0.5	8.9±0.6	NS	*	NS
Total FA (g/100 g dm)	0.8	8.8	12.1±3.4	19.7±3.7	14.2±5.0	16.8±0.2	NS	*	NS

[1] True protein = sum of anhydrous amino acids; I = effects of insect species; S = effects of seaweed growth media; I×S = interaction effects between the two variables; dm = dry matter.

[2] *P<0.05; NS = no significant effect (two-way ANOVA).

Table 2. Amino acid composition (% of total amino acids) of the seaweed media (the brown algae *Laminaria digitata* and *Fucus serratus*) and the two species of seaweed fly larvae (*Coelopa frigida* or *Coelopa pilipes*) grown on either *L. digitata* or *F. serratus*. Data are expressed as mean values of three replicates ± standard deviation for insects and the mean of two technical replicates for the seaweed media.[1,2]

	Saweed media		*C. frigida*		*C. pilipes*		I	S	I×S
	L. digitata	*F. serratus*	*L. digitata*	*F. serratus*	*L. digitata*	*F. serratus*			
Essential amino acids									
Histidine[3]	<LOQ	<LOQ	3.5±0.1	3.3±0.0	3.0±0.1	3.2±0.1	*	NS	**
Isoleucine	4.9	4.1	4.2±0.1	4.2±0.1	4.3±0.1	4.2±0.1	NS	NS	NS
Leucine	8.2	6.7	7.0±0.1	7.0±0.1	7.1±0.1	6.9±0.0	NS	NS	NS
Lysine	9.0	6.4	7.8±0.2	8.2±0.4	8.4±0.4	8.4±0.1	*	NS	NS
Methionine[3]	<LOQ	<LOQ	2.6±0.0	2.6±0.1	2.5±0.1	2.5±0.1	*	NS	NS
Phenylalanine[4]	<LOQ	4.1	5.9±0.2	6.0±0.2	5.2±0.2	5.6±0.3	**	NS	NS
Threonine	7.0	4.9	5.0±0.1	4.8±0.1	5.0±0.1	4.7±0.1	NS	**	NS
Valine	7.2	5.2	5.8±0.1	5.7±0.1	5.9±0.1	5.9±0.1	**	NS	NS
Non-essential amino acids									
Alanine	7.5	6.0	6.3±0.2	6.2±0.1	6.6±0.1	6.6±0.3	*	NS	NS
Arginine[4]	<LOQ	4.1	6.1±0.1	5.9±0.2	6.0±0.3	5.6±0.1	NS	*	NS
Aspartic acid	18.8	18.3	10.9±0.2	11.3±0.4	11.2±0.4	11.4±0.2	NS	NS	NS
Glutamic acid	17.9	23.2	15.3±0.2	15.3±0.1	16.0±0.2	15.8±0.2	***	NS	NS
Glycine	6.5	4.9	5.0±0.2	4.8±0.2	4.9±0.2	4.6±0.0	NS	*	NS
Proline	5.8	3.6	4.5±0.0	4.6±0.1	4.7±0.2	4.6±0.0	NS	NS	NS
Serine	7.3	4.9	4.9±0.2	4.8±0.2	4.0±0.3	4.7±0.1	NS	NS	NS
Tyrosine[4]	<LOQ	3.7	5.2±0.2	5.3±0.3	4.5±0.1	5.2±0.3	**	*	NS

[1] LOQ = limit of quantification; I = effects of insect species; S = effects of seaweed growth media; I×S = interaction effects between the two variables.

[2] *P<0.05; **P<0.01; ***P<0.001; NS = no significant effect(two-way ANOVA).

[3] LOQ = 0.7 mg/g.

[4] LOQ = 0.8 mg/g.

than *C. pilipes*. The larval concentrations of saturated and monounsaturated fats were similar between both species and feeding media (Table 3). The same was true for the polyunsaturated fatty acids, with the exception of *C. frigida* grown on *F. serratus*, which had almost 20% PUFA (of total fatty acids), compared to the other groups with values ~13% of total fatty acids. All the larvae samples had high concentrations of the monounsaturated fatty acid palmitoleic acid (16:1n-7, 25-30% of total fatty acids) and stable concentrations of eicosapentaenoic acid (EPA; ~3.5% of total fatty acids).

Table 3. Fatty acid composition (area %) of two species of seaweed fly larvae (*Coelopa frigida* or *Coelopa pilipes*) grown on two species of brown algae (*Laminaria digitata* or *Fucus serratus*). Data are expressed as mean values of three replicates ± standard deviation for insects and the mean of two technical replicates for the seaweed media.[1,2]

	Seaweed media		*C. frigida*		*C. pilipes*		I	S	I×S
	L. digitata	*F. serratus*	*L. digitata*	*F. serratus*	*L. digitata*	*F. serratus*			
14:0	4.5	8.7	6.6±0.8	6.2±0.8	4.8±0.9	4.6±0.5	*	NS	NS
16:0	19.0	14	16.8±1.5	17.7±0.8	19.2±1.0	19.1±0.3	*	NS	NS
18:0	0.9	0.5	1.5±0.4	1.6±0.2	2.1±1.1	2.3±0.2	NS	NS	NS
Total SFA	25.6	24.5	27.0±2.0	26.0±0.6	27.1±0.2	26.5±0.3	NS	NS	NS
16:1n-7	7.4	1.4	30.6±3.8	25.1±1.9	28.6±3.4	30.3±1.0	NS	NS	NS
18:1n-9	11.3	22	13.9±1.7	17.7±1.5	17.3±4.1	17.1±0.5	NS	NS	NS
18:1n-7	0.6	0.3	4.6±1.8	3.3±1.1	4.3±0.3	4.4±0.6	NS	NS	NS
Total MUFA	20.5	24.5	51.4±6.7	48.4±1.1	52.4±5.3	53.3±0.5	NS	NS	NS
18:2n-6 LA	2.9	9.5	2.4±0.7	5.3±0.9	3.1±1.7	3.3±0.8	NS	*	NS
18:3n-3 ALA	5.7	5.3	1.5±1.0	2.5±0.2	1.6±0.8	1.8±0.2	NS	NS	NS
18:4n-3 SA	10.7	5.9	1.6±1.2	1.7±0.1	1.3±0.5	1.4±0.1	NS	NS	NS
20:4n-6 ARA	9.3	12.7	3.8±1.1	4.4±0.4	3.1±0.9	2.7±0.2	*	NS	NS
20:5n-3 EPA	13.6	9.1	3.9±1.9	4.3±0.4	3.1±1.0	2.9±0.3	NS	NS	NS
22:6n-3 DHA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	–	–	–
Total n-3	33	22	6.1±4.0	8.6±0.7	6.0±2.2	6.1±0.5	NS	NS	NS
Total n-6	13	24	6.5±2.1	10.7±1.4	6.7±2.6	6.6±0.9	NS	NS	NS
n-3/n-6	2.5	0.9	0.9±0.3	0.8±0.1	0.9±0.1	0.9±0.1	NS	NS	NS
Total PUFA	46	46	12.6±6.1	19.5±2.2	13.5±4.5	12.8±1.3	NS	NS	NS

[1] SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; LA = linoleic acid; ALA = α-linolenic acid; SA = stearidonic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; PUFA = polyunsaturated fatty acids; LOQ = limit of quantification (1 mg FA / g sample); I = effects of insect species; S = effects of seaweed growth media; I×S = interaction effects between the two variables.

[2] *P<0.05; NS = no significant effect (two-way ANOVA).

In the fly larvae, the concentrations of the macrominerals Ca, Mg and P were generally higher in *C. pilipes* than in *C. frigida*, while the concentrations of microminerals where more affected by which species of seaweed they had been grown on (Table 4). Larvae grown on *F. serratus* had higher concentrations of Mn than larvae fed *L. digitata*, reflecting the higher concentration of Mn in *F. serratus*. Similarly, larvae grown on *L. digitata* had higher concentrations of Fe than larvae fed *F. serratus*, reflecting the higher concentration of Fe in *L. digitata*. The larval concentrations of Cu, Zn, and Se were only lightly affected by the feeding media. The levels of Hg and Pb in larvae were low in all groups, while elevated levels of As and Cd were found. The level of As was higher in larvae grown on *L. digitata* compared to larvae fed *F. serratus*, reflecting the higher level of As in *L. digitata* than in *F. serratus*. Cd accumulated in all groups of fly larvae. Interestingly, the level of Cd was lower in *C. frigida* fed *L. digitata* than in the three other groups of seaweed fly larvae.

4. Discussion

Seaweed fly larvae were successfully grown in the lab, although no large quantities of larvae were harvested in the process, and as such, the production of seaweed fly larvae for use in feed or food purposes is still not practically viable. The lower growth of both seaweed fly species on *L. digitata* is a consequence of a poorer nutrient content of this seaweed species, being lower in both protein and lipids than *F. serratus*. This was also reflected in the lower nutrient content of the larvae that had eaten *L. digitata*.

Lipid and especially protein content of the larvae were low compared to larvae of insect species commonly used in commercial production, such as black soldier fly pre-pupae (~40% protein and 40% fat) or pre-pupae of the common housefly (60% protein and 20% fat) (Veldkamp *et al.*, 2012). The concentrations of histidine in the larvae were lower than in other animal sources, like fishmeal, which typically has histidine concentrations at around 8% of amino acids (NRC, 2011). The protein from seaweed flies are thus more similar to proteins of plant origin in this aspect.

Table 4. Element composition of the seaweed media (the brown algae *Laminaria digitata* and *Fucus serratus*) and the two species of seaweed fly larvae (*Coelopa frigida* or *Coelopa pilipes*) grown on either *L. digitata* or *F. serratus* Data are expressed as mean values of three replicates ± standard deviation for insects and the mean of two technical replicates for the seaweed media.[1,2]

	Seaweed media		*C. frigida*		*C. pilipes*		I	S	I×S
	L. digitata	*F. serratus*	*L. digitata*	*F. serratus*	*L. digitata*	*F. serratus*			
(g/kg dw)									
Ca	3.2	4.3	28±4.1	24±1.3	46±5.3	30±4.0	***	**	*
Na	1.7	6.0	11±0.8	8.8±1.1	16±6.1	7.7±0.6	NS	*	NS
K	5.1	7.7	18±2.0	18±2.4	31±12	15±1.4	NS	NS	NS
Mg	0.8	1.7	2.9±0.4	4.3±1.0	7.4±2.5	7.0±1.3	**	NS	NS
P	0.3	0.5	13±0.7	15±3.8	27±6.7	20±2.7	**	NS	NS
(mg/kg dw)									
Mn	14	42	16±3.1	116±51	77±49	118±9.6	NS	**	NS
Fe	276	89	154±48	86±9.2	111±28	70±19	NS	*	NS
Cu	3.8	0.8	11±1.7	7.4±0.4	8.1±0.5	7.9±0.3	NS	**	*
Zn	18	8.2	84±1.1	95±7.5	80±3.6	83±3.1	*	*	NS
Se	0.08	0.03	0.10±0.01	0.08±0.00	0.09±0.00	0.07±0.01	NS	*	NS
As	24	12	19±1.8	11.3±0.6	15.0±6.3	11.3±4.5	NS	*	NS
Cd	0.11	0.31	1.5±0.8	9.4±3.4	6.8±5.3	11.4±1.7	NS	*	NS
Hg	0.02	0.00	0.03±0.00	0.01±0.00	0.01±0.01	0.01±0.00	**	**	NS
Pb	0.40	0.14	0.37±0.27	0.27±0.11	0.29±0.06	0.21±0.03	NS	NS	NS

[1] I = effects of insect species; S = effects of seaweed growth media; I×S = interaction effects between the two variables.

[2] *P<0.05; **P<0.01; ***P<0.001; NS = no significant effect (two-way ANOVA).

The remaining amino acid composition of the seaweed fly larvae was balanced and suitable for optimal nutrition of both animals and humans (NRC, 2011; WHO/FAO/UNU, 2007). Although there were some significant differences in amino acid composition due to insect species and seaweed feeding media in the current trial, the differences were only minor and not likely to give any difference in nutritional value of the protein fraction of the larvae.

Palmitoleic acid (16:1n-7), a minor fatty acid in plant sources, but present in fats of animal origin (Harwood *et al.*, 1994; Orsavova *et al.*, 2015), was high in all the seaweed fly larvae, irrespective of their growth media. Palmitoleic acid is a marker of endogenous lipid production (Paillard *et al.*, 2008), as it is a product of the conversion of acetyl Co-A to fatty acids, here likely high in the larvae due to their accelerated growth and high rate of lipid deposition in the current developmental stage. The scientific community is not sure if the health effects of this fatty acid are purely positive or negative (De Fabiani, 2011), as it has been connected to both positive and negative health effects in dietary trials with rodents and human (Matthan *et al.*, 2009; Nestel *et al.*, 1994; Yang *et al.*, 2011). Some 20:5n-3 (EPA) was also found in the larvae (~3.5% of total fatty acids), most likely just having been taken up from the algae and accumulated in the fat depositions. The concentration of EPA was much lower in the larvae than in the algae, so large parts of the EPA eaten by the larvae is probably oxidised and used as an energy source. No fatty acids longer than the 20-carbon EPA and none of the fatty acids being typical intermediates in the synthesis of PUFA were found, suggesting the larvae cannot perform the elongation steps necessary for these conversions. The sparse effects of the feeding media fatty acid composition on the fatty acid composition of the larvae also indicates that this insect species sent little dietary lipids directly to storage. Large parts of the fatty acids are therefore likely oxidised for energy and excess energy stored as their endogenously produced lipids, such as palmitoleic acid. This is in contrast with other insect species, such as black soldier fly, known to have a highly changed fatty acid composition when reared on different feeding media (Liland *et al.*, 2017; St-Hilaire *et al.*, 2007; Tschirner and Simon, 2015).

The concentrations of the macrominerals Ca, Na, and P were higher in *C. pilipes* than in *C. frigida*, suggesting that the uptake and use of these elements differ between the two insect species. The accumulated levels of Mn, Fe and Cd reflected the levels found in the seaweed feeding media: the higher the concentrations in the feeding media, the higher the concentrations in the larvae. This observation is in disagreement with findings for black soldier fly larvae;

increased concentrations of Fe in feed media for black soldier fly larvae did not give any increase in larvae Fe concentrations (Liland *et al.*, 2017). Both species of seaweed fly larvae accumulated As, Cd, and Pb, but not Hg (the concentrations of Hg in the growth media were very low). This is partly in agreement with findings for black soldier fly larvae, which accumulated As and Cd, but not Hg and Pb (Biancarosa *et al.*, 2017b; Diener *et al.*, 2015; Purschke *et al.*, 2017; Van der Fels-Klerx *et al.*, 2016). Other minerals measured showed much less tendency to vary with the concentrations in the seaweed, suggesting the uptake of these might be regulated.

There is a great interest in the use of insect larvae as a feed ingredient in animal feed, including fish. In Europe, the level of undesirable substances are controlled through the feed legislation, which set maximum levels for a range of compounds, including arsenic and the heavy metals Cd, Hg, and Pb, in feed ingredients and feed (Directive 2002/32/EC and amendments; EC, 2002). The levels of Hg and Pb in the seaweed fly larvae were low, and well below the current maximum levels set for feed ingredients (0.1 and 10 mg/kg feed ingredient, respectively, based on 12% moisture content; Figure 2). The levels of As and Cd in the seaweeds used in the growth media were below the current maximum levels, however the As and Cd levels in the fly larvae were above the current limits (Figure 2). The current maximum level for As in feed material are 2 mg As/kg, with an exception for seaweed meal and feed material derived from seaweed for which the maximum level is 40 mg/kg (both based on 12% moisture content). The current maximum levels for Cd are 1 mg/kg in feed materials of vegetable origin and 2 mg/kg in feed materials of animal origin (based on 12% moisture content). The elevated levels of As and Cd in seaweed larvae (highest measured concentrations 18.4 and 11.6 mg/kg, respectively) could potentially limit the use of seaweed fly larvae as a feed ingredient. The European Food Safety Authority has assessed the risks related to the production of insects for feed and food (EFSA, 2015). Several factors, such as growth substrate, insect species, growth stage of the insect and length of life cycle, will affect the accumulation and levels of undesirable substances in the insects. Of these, the growth substrate may have the largest affect, and occurrence of e.g. heavy metals and arsenic in insects can be controlled by managing the substrate, i.e. choosing a substrate with low levels of these elements.

In conclusion, seaweed flies could be a good source of nutrients for both human and animal nutrition, having a balanced composition of amino acids and being rich in unsaturated fatty acids. The high concentrations of As and Cd are, however, of concern and should be taken into account if production of these species should be upscaled for food or feed purposes.

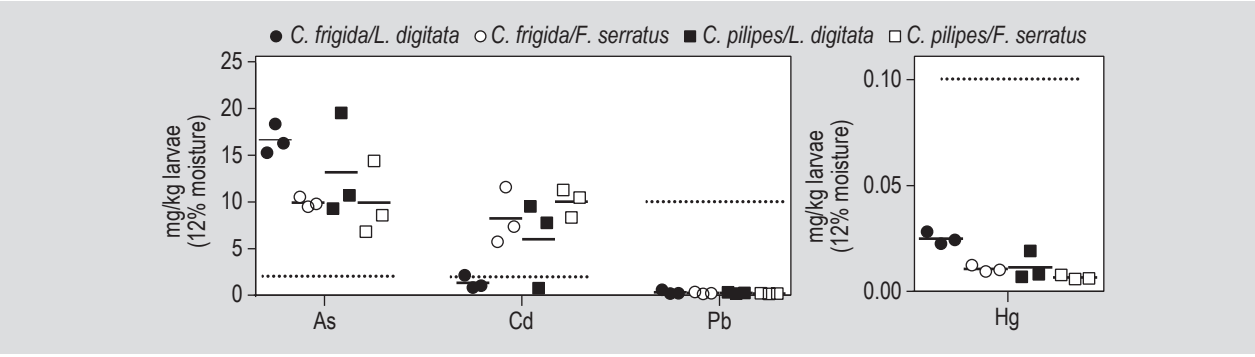

Figure 2. Composition of selected elements in two species of seaweed fly larvae (*Coelopa frigida* or *Coelopa pilipes*) grown on either *Laminaria digitata* or *Fucus serratus*. Data are expressed as the mean concentration of each mineral at 12% moisture level to be compared to EU's official maximum levels for each element in animal feed (Directive 2002/32/EC and amendments; EC, 2002). All three data points per group are plotted together with the mean value of each group plotted as a solid line. The stapled lines indicate the maximum permitted level in feed material for each element set by the EU.

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