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Harvesting amphipods applying the integrated multitrophic aquaculture (IMTA) concept in off-shore areas

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ABSTRACT

Current trends in aquaculture research are towards more sustainable aquaculture, implementing more environment-friendly models such as integrated multi-trophic aquaculture (IMTA) systems. One possible IMTA strategy involves taking advantage of the species that naturally grow on aquaculture facility structures. Since amphipods have been cultured on a small and medium scale in laboratory conditions with the aim of using them as natural food for farmed fish or live prey for cephalopods, the high density of them in farm fouling could be pointing to a potential accessory culture, bringing new possibilities for commercial production and diversification of cultivable species. Two IMTA experiments focusing on harvesting amphipods were carried out between May and September 2014, testing two collector types, two depths, at 5 m and 15 m, and two experimental times. Extraction method selected to detach and recover the amphipods recovered more than 80% of the fauna gathered by the collectors, obtaining a final product with more than 86% purity in amphipods. Monthly production was estimated in 10 g wet-weight per collector (5 l volume) and it did not vary with the depth, indicating it is possible to use the entire water column occupied by the cages (down to 20 m depth) for this culture. This is the first pilot trial of an amphipod culture within an offshore IMTA facility. Nutrient uptake from wastes of the main culture was established, promoting a more sustainable development of aquaculture in the marine environment. Moreover, nutritional composition of the amphipod-based product is of great utility as a suitable natural ingredient in aquafeed compositions, and also as a potential food supplement for human nutrition.

1. Introduction

Current trends in aquaculture research are towards more sustainable aquaculture, searching for alternative protein sources for feed supply, diversifying cultivable species and implementing more environment-friendly models such as integrated multi-trophic aquaculture (IMTA) systems (Bostock et al., 2010). The latter involve the culture of fed species (e.g. fin-fish) together with secondary extractive species such as marine invertebrates and/or algae that feed on detritus from the main species and convert it into valuable products (Chopin and Robinson, 2004). The IMTA system has received significant interest from researchers and regulators because of the win/win situation which both eliminates a large part of aquaculture waste and increases productivity (e.g. Soto, 2009; Troell et al., 2009; Chopin et al., 2012). However, some issues need to be solved in order to successfully move forward from pilot-scale IMTA experiments to commercial scale development, assessing the real financial benefits, increased complexity of the system or extra space requirements (Hughes and Black, 2016). One IMTA strategy involves taking advantage of the species that naturally grow on aquaculture facility structures, thus avoiding the introduction of new species into the system and also its derived initial costs. The natural occurrence and even increase in populations of commercially valuable species such as mussels *Mytilus* spp. (Cook et al., 2006; Fitridge et al., 2012), sea urchins (Gonzalez-Silvera et al., 2015) or lobsters *Homarus* spp. (Drouin et al., 2015) in farm areas may lead to exploring several possible accessory cultures. In fact, some of these species have already been tested in co-culture systems (Peharda et al., 2007; Cook and Kelly, 2007; Perez-Benavente et al., 2010). Thus, the high density of amphipods in farm fouling could be pointing to an obvious potential accessory culture (Fernandez-Gonzalez, 2017), bringing new possibilities for commercial production and diversification of cultivable species. Amphipods contribute to the daily diet of fish and play an important role in energy exchange between lower and upper levels of the trophic chain (Jiménez-Prada et al., 2015). Promising nutritional

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analyses of amphipods have been reported, regarding general chemical composition (Baeza-Rojano et al., 2014), fatty-acid profiles (e.g. Guerra-García et al., 2004; Kolanowski et al., 2007; Cook et al., 2010), macro and micromineral contents (Guerra-García et al., 2010a, 2010b), phospholipids (Baeza-Rojano et al., 2014; Guerra-García et al., 2016) and amino acids (Köprücü and Özdemir, 2005). For these reasons, amphipods have been cultured on a small and medium scale in laboratory conditions with the aim of using them as natural food for farmed fish (Parsons et al., 1985; Moren et al., 2006; Baeza-Rojano et al., 2013a) or live prey for cephalopods (Baeza-Rojano et al., 2010, 2013b; González et al., 2011).

In addition to their commercial value, organisms selected as accessory cultures should act as biofilters and if possible ensure aquaculture wastes are not spread over the surrounding environment, hopefully aiding in their eventual removal. Fatty acids (FA) have been used as a tool to detect how the fauna associated with coastal aquaculture processes trophic resources derived from the activity (Fernandez-Jover et al., 2011; Gonzalez-Silvera et al., 2015; Arechavala-Lopez et al., 2015; Squadrone et al., 2016). The increasing utilization of vegetable or alternative animal oils in the production of aquafeeds usually makes cultivated fish and associated fauna contain higher levels of terrestrial FA in their tissues (see Fernandez-Jover et al., 2011 for review). Modified FA profiles have been observed in amphipods feeding on aquaculture wastes (Gonzalez-Silvera et al., 2015; Guerra-García et al., 2016), which would reveal the potential uptake of wastes by this accessory culture. Other biomarkers like trace elements (TE) have been used to determine the impact of aquaculture on wild fauna (Arechavala-Lopez et al., 2015 and references therein), since aquafeeds are also enriched with copper, iron, manganese, zinc, selenium and cobalt among others (CIESM, 2007). This technique would additionally detect the copper or nickel content involved in antifouling treatments of net-pens and related equipment (Fitridge et al., 2012), which could lead to unwanted metal concentrations in amphipod culture.

Previous studies have suggested the potential of amphipod cultures associated with IMTA systems (Woods, 2009; Baeza-Rojano, 2013; Guerra-García et al., 2016). This study aims to test the applicability of amphipods as an accessory culture in an offshore IMTA system with finfish as main product. Specifically, our aims were to: a) Determine the efficiency and selectivity in terms of purity and species composition of the final product, and of an extraction method to detach and recover the amphipods. b) Assess the potential exploitable biomass of amphipods and their assemblage structure, given the characteristics of the collectors and the effects of deployment depth on the harvesting system; c) Verify the efficiency of amphipods as biofilters of aquaculture wastes, through the analysis of their fatty acids profile and of selected trace-element content. d) Provide a complete nutritional analysis of the harvested product, with special emphasis on their amino-acid composition and some essential trace minerals unreported for marine amphipods in the bibliography.

2. Material and methods

2.1. Study area and experimental design

Two IMTA experiments focusing on harvesting amphipods were carried out between May and September 2014. The first was performed in the coastal waters of Málaga (Spain, 30S 378,484 N; 4,062,640 W) with a duration of 10 weeks. The second was carried out in Almería (Spain, 30S 541,345 N; 4,074,262 W) and lasted 16 weeks, due to operational constraints of the company. The cultured species at both fish farms were sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). Two collector types were deployed in order to test their amphipod harvesting efficiency: the first was based on those used in Fernandez-Gonzalez (2017), being a net bag containing a lifeless artificial habitat (plastic raffia) but with a volume of approximately 5 l; the second also consisted of a 5 l net bag, but filled with dried mussel shells. Collectors were deployed at three different sites along the fish farm facility, at 5 m and 15 m depth and three replicates at each depth. Thus, a total of 72 collectors were used, 36 in each experiment. At the end of each experimental period, amphipods were extracted by introducing the collectors into containers with fresh water for 3 min. They were then sieved through a 500 μm mesh and preserved in ice until frozen. The collectors were finally immersed in alcohol at 70% to detach any remaining mobile fauna from the collector (with the aid of a soft brush if necessary) and also thus test if the fresh water extraction was efficient. Subsamples of at least 20% of the samples extracted by fresh water and by alcohol were sorted and amphipods identified at species level to determine the potential selectivity of the extraction methods for some species. Moreover, other macrofaunal groups were additionally quantified to assess the purity of the extracted sample. Subsamples of fresh amphipods were separately stored for the various nutritional and trace element analyses.

2.2. Chemical analysis

Nutritional analyses of amphipods were performed according to AOAC (1995). Dry matter was calculated by weight loss after drying for 24 h at 105 °C and ash by incinerating for 24 h at 550 °C in a muffle furnace. Crude protein was measured using the Kjeldahl technique after acid digestion (Kjeltec 2300 Auto Analyser, Tecator, Höganas, Sweden) and multiplying N by 6.25. Crude lipid was quantified by ether extraction with an Ankom XT10 Extraction System (NY, USA) (AOCS, 2005). Energy content was calculated according to Brouwer (1965), from the C (g) and 181 N (g) balance (GE = 51.8 × C − 19.4 × N).

Following the method previously described by Bosch et al. (2006), the amino acid contents of amphipods were determined using a Waters HPLC system (Waters 474, Waters, Milford, MA, USA) consisting of two pumps (Model 515, Waters), an auto sampler (Model 717, Waters), a fluorescence detector (Model 474, Waters) and a temperature control module. Aminobutyric acid was added as an internal standard before hydrolysis. The amino acids were derivatized with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). Methionine and cysteine were determined separately as methionine sulphone and cysteic acid after oxidation with performic acid. The amino acids were separated with a C-17 Waters Acc. Tag reverse-phase column (150 mm × 3.9 mm) and then transformed into methionine and cysteine. Lipids and fatty acids (FA) were extracted by homogenization in chloroform/methanol (2:1, v/v) according to Folch et al. (1957). The organic solvent was evaporated under a stream of nitrogen, the lipid content being gravimetrically determined (Christie, 1982) and stored in chloroform/methanol (2:1) containing 0.01% butylated hydroxytoluene (BHT) at −20 °C until further analysis. The lipid extract was subjected to acid-catalysed transmethylation with 1% sulphuric acid (v/v) in methanol, and the resultant fatty acid methyl esters (FAME) purified by thin layer chromatography (TLC). Individual FA concentrations were expressed as mg/100 g and as percentages of the total FA composition.

A total of 4 major elements: calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na). and 26 minor elements: lithium (Li), beryllium (Be), boron (B), aluminium (Al), vanadium (V), chrome (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), arsenic (As), selenium (Se), strontium (Sr), molybdenum (Mo), silver (Ag), cadmium (Cd), indium (In), antimony (Sb), barium (Ba), mercury (Hg), tantalum (Tl), lead (Pb) and bismuth (Bi) were analysed through inductively coupled plasma mass spectrometry (ICP-MS). Approximately 1 g of amphipods were subjected to wet mineralization following homogenization using a mixture of seven ml of nitric acid and 2 ml of hydrogen peroxide to extract TEs from a sample matrix through a microwave digestion system. Minority and majority elements were expressed in parts per million (p.p.m.). All analyses were performed in triplicate.

2.3. Statistical analyses

Due to bad weather conditions, 13 replicates of the first experiment and 16 replicates of the second were lost. Thus, in Málaga, the effects of collector type and depth were tested through an unbalanced experimental design considering two factors: “Collector” with three levels: ‘Shallow shells’, ‘Shallow artificial habitat’ and ‘Deep shells’; and “Site” random and nested in “Collector”. The Almería experiment was also analysed according to an unbalanced model: “Collector” with two levels: ‘Artificial habitat’ and ‘Shells’; and orthogonal and fixed ‘Depth’, with two levels ‘Shallow’ and ‘Deep’. Consequently, the variables ‘biomass’ and ‘total abundance’ were analysed using a univariate permutational analysis of variance (PERMANOVA), which is feasible even when there are unequal numbers of replicate samples within each factor level of the design (Anderson et al., 2008). The Resemblance matrix of univariate analyses was based on a Euclidean distances matrix and they were tested using 4999 random permutations of residuals under a reduced model (Anderson, 2001b), with appropriate units as required by the design (Anderson and ter Braak, 2003). When the number of possible permutable units was not enough for a reasonable test by permutation, a *p*-value was obtained using a Monte Carlo test (Anderson and Robinson, 2003). In the case of significant differences, data were subsequently investigated using a pair-wise test. A multivariate permutational ANOVA based on the Bray–Curtis dissimilarities of the untransformed data (PERMANOVA; Anderson, 2001a; McArdle and Anderson, 2001) was carried out to analyse the differences in the overall amphipod species composition in the same way as univariate tests.

The efficiency of the extraction method was assessed by the mean value of extracted amphipods with fresh water with respect to the total biomass obtained per collector, i.e. the sum of both extractions (fresh water and alcohol). The selectivity of the method was tested by comparing biomass, total abundance, abundances of main amphipod species, and species composition of the shallow water samples collected by each liquid. Univariate and multivariate PERMANOVA of repeated measures based on binomial deviance were used to test extraction method selectivity. For this, an additional factor “Efficiency” was included in the above-mentioned experimental designs. Thus, efficiency of extraction method in Málaga was tested under a three-factor model: “Collector”, ‘Site’ and “Efficiency”; and under a two-factor model in Almería: ‘Collector’ and ‘Efficiency’. Analyses were based on the same model and number of permutations than the previous PERMANOVAs.

Statistical analyses were performed using PRIMER-E software (PRIMER software; Clarke and Gorley, 2006) with the add-on package PERMANOVA+ (Anderson et al., 2008).

3. Results

3.1. Efficiency and selectivity of extraction method

The fresh water extraction method recovered 81.7 ± 1.4% and 84.4 ± 2.2% of the associated fauna from the collectors in the Málaga and Almería experiments respectively (Fig. 1).

The resulting product showed 89.5 ± 0.8% purity in amphipods in the shorter experiment in Málaga and 86.1 ± 4.0% in the longer one (Almería). Other taxonomic groups were found attached to the collectors, mainly mussel juveniles (*Mytilus* sp.) in Málaga and other bivalve species (e.g. *Musculus* spp.) and tanaidaceans in Almería.

The amphipod-based product obtained with the extraction method showed similar biomass, total abundance and species composition to the total fauna associated with the collectors in Almería. However, in Málaga, fresh water extraction rendered significantly lower amphipod abundances when compared to the total abundances in the collectors (*pseudo-F* = 67.14, *p* = 0.003). The extraction method showed positive selectivity (i.e. higher extraction rates) towards *Jassa* spp. (*pseudo-F* = 23.60, *p* = 0.03) and negative selectivity for *Caprella dilatata* (*pseudo-F* = 13.945; p = 0.03) and *Caprella equilibra* (*pseudo-F* = 137.65, *p* = 0.0008) (Fig. 1), which mostly remained attached to the collector after the three minutes in fresh water.

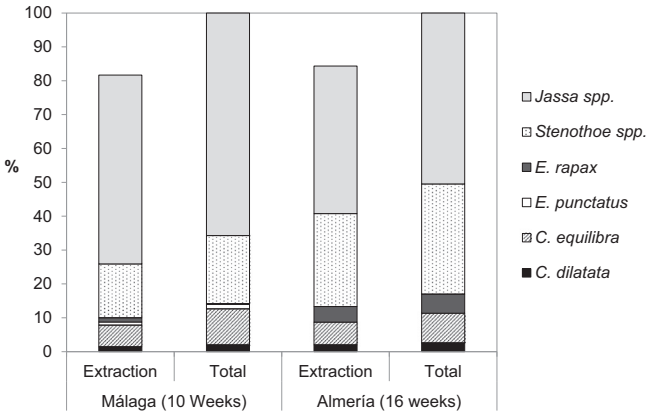

Fig. 1. Species composition (%) of the amphipod-based product extracted with fresh water and the total assemblages composition associated to the collectors extracted with alcohol.

3.2. Effects of depth and type of collector in the harvested amphipods

Mean total biomass per collector obtained in the Málaga experiment was 27.9 ± 2.3 g and 36.3 ± 5.4 g in Almería, resulting in a monthly production of 12.1 ± 0.7 and 9.1 ± 1.4 g per collector respectively. No significant differences were detected between the two collector types and the two depths in the Málaga experiment, however the loss of replicates in this locality may reduce the statistical power of the analysis (Fig. 2). On the other hand, significant differences for total amphipod abundance (*pseudo-F* = 7.07, *p* = 0.019) and biomass (*pseudo-F* = 5.09, *p* = 0.02) were found between both collectors in Almería experiment, in such a way that collectors formed by dried shells cumulated more amphipods than that formed by artificial habitat (Fig. 2).

Different species composition was detected between the collectors in both experiments. In Málaga, PERMANOVA results reflected a different amphipod assemblage in deeper collectors, due to a higher presence of *Jassa* spp. (*pseudo-F* = 6.93, *p* (MC) = 0.043) and *C. equilibra* (*pseudo-F* = 13.002, *p* (MC) = 0.014; Fig. 3). In Almería, collectors with dried shells accumulated more gammarids belonging to *Stenothoe* spp. (*pseudo-F* = 12.65, *p* = 0.002), while artificial habitat collectors tended to accumulate more *Jassa* spp. and caprellids but without detecting significant differences between them (Fig. 3).

3.3. Nutritional analysis and aquaculture influence of amphipods

The water content (humidity) of amphipod-based product of the Málaga and Almería experiments was 83.3 and 83.8%, respectively. Samples were characterized in both experiments by high levels of protein (32.0–35.0%) and ash (30.2–33.1%) and low levels of lipids (12.8–13.1%) and carbohydrates (7.2–9.1%) (Fig. 4). Gross energy was 12.88 kJ/g and 13.73 kJ/g in amphipods from Málaga and Almería respectively.

Similar results were obtained for amino acids contents in both experiments. Arginine, leucine and lysine were the most abundant essential amino acids in amphipods, with contents around 23.8, 19.03 and 18.8 mg/g respectively. Non-essential amino acids were mainly represented by glutamic (42.4 mg/g) and aspartic acids (29.8 mg/g). Valuable contents were also obtained for glycine, valine and proline while deficient values were found for methionine (3.29–3.74 mg/g; Table 1).

The polyunsaturated fatty acids (PUFA) in amphipods from Málaga constituted 41.6% of the total, while the saturated FA (SFA) were

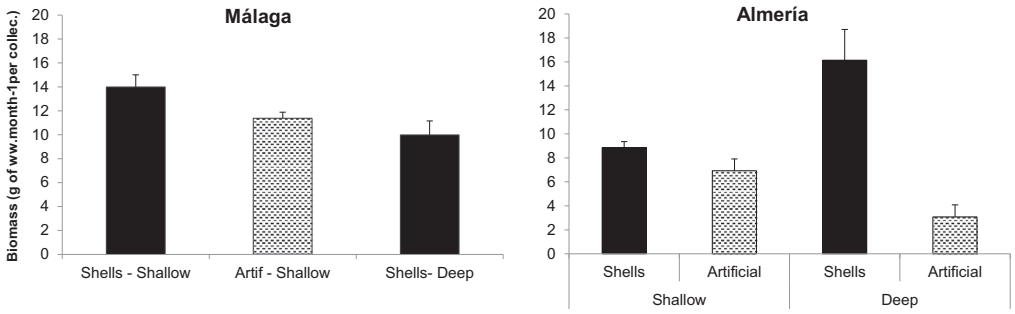

Fig. 2. Mean total biomass (wet weigh) cumulated by each kind of collector and depth in Málaga (10 weeks) and Almería experiment (16 weeks).

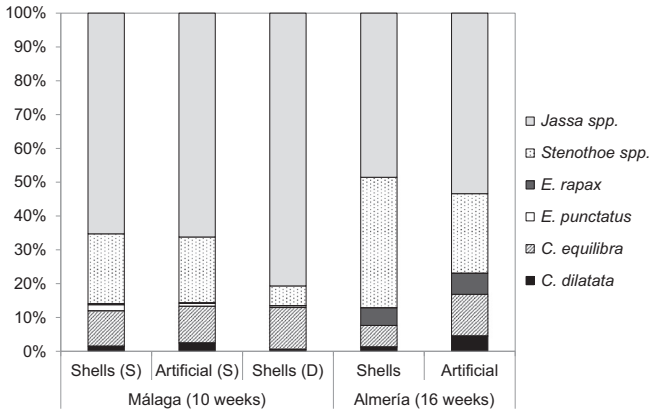

Fig. 3. Species composition (%) on collectors in Málaga (10 weeks) and Almería experiment (16 weeks). S: Shallow, D: Deep.

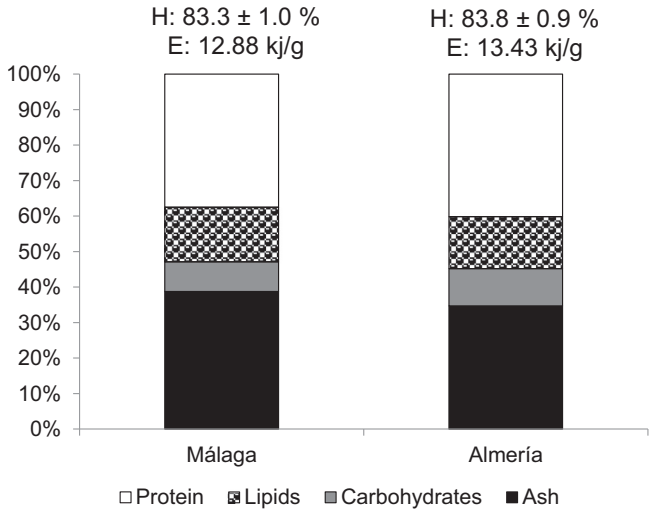

Fig. 4. General chemical composition (% wet weigh), humidity (H) and energy (E) of amphipods collected in Málaga and Almería experiments.

36.0% and monounsaturated fatty acids (MUFA), 22.8%. Amphipods in the Almería samples contained 35.9% PUFA, 39% SFA and 25.2% MUFA. FA predominant in amphipod samples from both experiments were: the SFAs palmitic (24.6–26.3%) and stearic acid (5.7–6.6%), the MUFA oleic acid (16.0–18.0%), and the PUFAs: eicosapentaenoic (EPA; 13.9–10.7%), docosahexaenoic (DHA; 16.9–13.2%) and linoleic acid (3.6–4.2%) (Table 2).

Values of all major and trace elements measured in amphipods from Málaga and Almería experiments are included in Table 3. Na was the most abundant of the four major elements, with 10.22 ± 0.57 and 8.82 ± 0.36 mg/g in amphipods from Málaga and Almería

Table 1
Amino acids contents in amphipod mixture from Málaga (10 weeks) and Almería (16 weeks) experiments.

	Aa (mg/g)	Málaga	Almería
Essential amino acids (EAA)	Histidine	6.14 ± 0.48	6.73 ± 0.30
	Arginine	24.44 ± 1.23	23.21 ± 0.95
	Threonine	12.95 ± 0.94	13.15 ± 0.38
	Valine	15.16 ± 1.06	15.08 ± 0.65
	Methionine	3.29 ± 0.07	3.74 ± 0.29
	Lysine	19.06 ± 1.82	19.0 ± 1.38
	Isoleucine	13.08 ± 0.36	12.22 ± 0.68
	Leucine	18.90 ± 1.31	18.78 ± 0.92
	Phenylalanine	12.88 ± 0.75	12.81 ± 0.20
Non-essential amino acids (NEAA)	Aspartic acid	28.98 ± 2.55	30.60 ± 1.90
	Serine	14.61 ± 1.01	15.24 ± 0.54
	Glutamic acid	41.88 ± 3.46	42.92 ± 2.58
	Glycine	17.72 ± 1.03	17.47 ± 0.75
	NH_3	16.89 ± 1.63	15.89 ± 0.40
	Alanine	14.98 ± 1.16	15.14 ± 0.74
	Proline	11.51 ± 0.81	11.17 ± 0.51
	Cysteine	3.35 ± 0.11	3.58 ± 0.13
	Tyrosine	10.36 ± 0.68	10.53 ± 0.23
	EAA/NEEA	0.79 ± 0.02	0.77 ± 0.01

respectively. All essential TE were represented in both experiments with similar values, except for the higher concentrations of Fe found in Málaga (150.19 vs 79 mg/kg) and the higher quantities of Cu in Almería (6.31 vs 1.67 mg/kg). The analysis of non-essential TE reflects high concentrations of Al, being more important in Málaga (244.94 ± 33.38 mg/kg) than Almería (96.37 ± 52.26), and Sr, with similar values in both experiments, around 220 mg/kg. Finally, heavy metals content was 0.06–0.05 ppm for Cd and 0.14–0.23 ppm for Pb while concentrations of Hg were not detectable.

4. Discussion

This is the first pilot trial of an amphipod culture within an offshore IMTA facility. The extraction method recovered more than 80% of the fauna gathered by the collectors, obtaining a monthly production of around 10 g wet-weight per collector (5 l volume). Nutrient uptake from wastes of the main culture was established, promoting a more sustainable development of aquaculture in the marine environment. Moreover, an amphipod-based product with more than 86% purity in amphipods is of great utility as a suitable natural ingredient in aquafeed compositions, and as a potential food supplement for human nutrition.

4.1. Amphipod culture in an offshore IMTA system

The artificial structures used as collectors for harvesting amphipods showed differences regarding accumulated biomass and species composition, as well as their resistance to local oceanographic conditions. Harvest collectors containing recycled dried shells accumulated more biomass, tending to recruit more gammarids and better resist the

Table 2
Mean values (± SE) and proportions of total fatty acids in amphipods of Málaga (10 weeks) and Almería experiments (16 weeks). nd: non detected.

	Fatty acids (FA)	Málaga		Almería	
		mg/100 mg	%	mg/100 mg	%
Saturated (SFA)	Myristic Acid (14:0)	0.08 ± 0.001	2.4	0.08 ± 0.002	2.7
	Pentadecanoic Acid (15:0)	0.02 ± 0.000	0.8	0.02 ± 0.001	0.7
	Palmitic Acid (16:0)	0.80 ± 0.004	24.6	0.80 ± 0.013	26.3
	Heptadecanoic Acid (17:0)	0.06 ± 0.000	1.8	0.06 ± 0.001	2.0
	Stearic Acid (18:0)	0.19 ± 0.003	5.7	0.20 ± 0.005	6.6
	Arachidic Acid (20:0)	0.02 ± 0.000	0.5	0.01 ± 0.000	0.4
	Behenic Acid (22:0)	0.01 ± 0.000	0.2	0.01 ± 0.000	0.3
	Lignoceric Acid (24:0)	n.d	–	n.d.	–
	Total Saturated	1.18 ± 0.090	36	1.18 ± 0.090	39
Monounsaturated (MUFA)	Myristoleic Acid (14:1)	0.03 ± 0.000	0.9	0.03 ± 0.001	1.0
	Palmitoleic Acid (16:1)	0.04 ± 0.000	1.4	0.05 ± 0.002	1.8
	cis-10-Heptadecenoic Acid (17:1)	0.01 ± 0.000	0.2	0.00 ± 0.000	0.2
	Vaccenic (18:1 n-7)	0.07 ± 0.001	2.2	0.06 ± 0.001	2.1
	Elaidic Acid (18:1 n-9 t)	0.00 ± 0.001	0.1	0.00 ± 0.000	0.1
	Oleic Acid (18:1 n-9c)	0.52 ± 0.006	16.0	0.55 ± 0.019	18.0
	cis-11-Eicosenoic Acid (20:1)	0.04 ± 0.001	1.3	0.04 ± 0.001	1.4
	Erucic Acid (22:1 n-9)	0.01 ± 0.000	0.2	0.01 ± 0.000	0.2
	Nervonic Acid (24:1)	0.01 ± 0.001	0.5	0.01 ± 0.000	0.4
	Total MUFA	0.73 ± 0.052	22.8	0.75 ± 0.055	25.2
Poliunsaturated (PUFA)	Linoleic Acid (18:2 n-6c)	0.12 ± 0.002	3.6	0.13 ± 0.006	4.2
	γ-Linolenic Acid (18:3 n-6)	n.d.	–	n.d.	–
	α-Linolenic Acid (18:3 n-3)	0.05 ± 0.001	1.5	0.03 ± 0.002	1.0
	Eicosadienoic Acid (20:2)	0.06 ± 0.001	1.9	0.06 ± 0.002	1.8
	Eicosatrienoic Acid (20:3 n-6)	n.d.	–	n.d.	–
	Eicosatrienoic Acid (20:3 n-3)	0.01 ± 0.000	0.5	0.01 ± 0.001	0.4
	Arachidonic Acid (20:4 n-6)	0.05 ± 0.001	1.7	0.09 ± 0.006	3.0
	Docosadienoic Acid (C22:2)	0.01 ± 0.000	0.4	0.01 ± 0.001	0.2
	Eicosapentaenoic Acid (C20:5 n-3)	0.45 ± 0.009	13.9	0.32 ± 0.023	10.7
	Docosatetraenoic Acid (22:4 n-6)	0.02 ± 0.000	0.5	0.03 ± 0.003	0.9
	Docosapentaenoic Acid (22:5 n-3)	0.02 ± 0.001	0.7	0.01 ± 0.001	0.5
	Docosahexaenoic Acid (C22:6 n-3)	0.55 ± 0.01	16.9	0.40 ± 0.035	13.2
	Total PUFA	1.34 ± 0.051	41.6	1.09 ± 0.037	35.9
	Total n-3 PUFA	1.09 ± 0.02	33.2	0.78 ± 0.06	25.8
	Total n-6 PUFA	0.18 ± 0.00	5.85	0.25 ± 0.01	8.09
	Ratio n-3/n-6	5.68 ± 0.13		3.08 ± 0.32	

experimental time than collectors with plastic raffia. Moreover, shell collectors were developed as a means of recycling part of the fouling, mainly mussels, that are removed during routine mechanical cleaning of the cages carried out by fish farm staff. Physical structure of the substrate is a factor greatly influencing the abundance of associated small crustaceans (Aikins and Kikuchi, 2001) in such a way that selection of the interior matrix for collectors may provide different amphipod composition. The natural preference of caprellids for branched substrates (Lacerda and Masunari, 2011; Baeza-Rojano et al., 2013a; Fernandez-Gonzalez, 2017) could make nets a more suitable substrate for them, which has already been tested in land-based cultures (Baeza-Rojano et al., 2013a). This would explain their lower presence in the dried shell collectors tested in this study.

The amphipod biomass production did not vary with depth, indicating it is possible to use the entire water column occupied by the cages (down to 20 m depth) for this culture. Moreover, the maximum biomass was collected in the deeper layer of the second experiment. This result is in agreement with the proposed depth for bivalve culture in non-eutrophic marine waters such as the Mediterranean Sea, where the higher chlorophyll *a* concentration in deeper strata of the water column may determine the optimum depth (Sanz-Lázaro et al., unpublished data).

The longer the collectors were deployed the more amphipod biomass was retrieved, but after 16 weeks the presence of other faunal groups increased 4-fold when compared to the 10 weeks experiment. More studies should be performed to establish an optimum time for harvesting, but it may be around 12 weeks, when amphipod biomass reached 30 g or more per collector and the presence of other fauna was around 2%. Potential improvements are further discussed in the Section 4.2.

4.2. Efficiency of the extraction method

The proposed extraction method was efficient to remove the amphipods, mainly gammarids, recovering more than the 80% of the fauna associated with the collector. In fact, no differences were detected when comparing fresh water extraction and the total fauna attached to the collector in the Almería experiment. However, this method was not enough to force caprellids like *C. equilibra* to release their hold; they remained attached to the collector even when submerged in alcohol to quantify the total fauna (personal observation). Caprellids are morphologically specialized for a clinging mode of life due to their degenerating abdomen and pleopods (Takeuchi and Hirano, 1992), the latter in turn probably lead to a weaker swimming escape response in adverse conditions. The fresh water extraction method has beneficial aspects such as avoiding their exposure to irritant solutions (Woods, 2009) that could otherwise affect the possible utility of the final product, but it also prevents the use of amphipods as live prey as proposed by other authors (Baeza-Rojano et al., 2010, 2013a, 2013b).

The biomass obtained in this study showed a high amphipod purity (86% of the total biomass). This could be improved by implementing further steps in the extraction process. For instance, an additional sieve with a wider mesh aperture (e.g. 1 cm), could be used to separate larger accompanying fauna such as decapods. Furthermore, given that the main non-targeted fauna were bivalves, the inclusion of an intermediate step of resuspension and sedimentation could eliminate most of the sessile organisms present in the samples, even if they are post-larval. This would greatly enhance the purity of the final product.

Table 3
Mean values (± SE) of majority and minority elements concentrations in amphipods of Málaga (10 weeks) and Almería (16 weeks) experiments. nd: non detected.

		TEs	Málaga	Almería
Majority E. (mg/g)		Ca	3.31 ± 0.29	3.79 ± 0.35
		K	0.59 ± 0.03	0.54 ± 0.03
		Na	10.22 ± 0.57	8.82 ± 0.36
		Mg	2.58 ± 0.17	2.53 ± 0.20
Trace elements (mg/kg)	Essentials	Fe	150.19 ± 18.77	79.00 ± 33.13
		Co	0.05 ± 0.01	0.03 ± 0.01
		Cu	1.67 ± 0.08	6.31 ± 0.54
		Cr	0.47 ± 0.13	0.64 ± 0.25
		Mn	2.57 ± 0.28	2.08 ± 0.39
		Mo	0.06 ± 0.00	0.04 ± 0.00
		Zn	11.12 ± 1.04	11.88 ± 0.60
		Se	0.17 ± 0.01	0.28 ± 0.03
	Nonessentials	Ni	2.31 ± 0.10	2.77 ± 0.43
		V	0.57 ± 0.07	0.29 ± 0.13
		Li	0.30 ± 0.07	0.24 ± 0.05
		Be	n.d.	n.d.
		B	12.53 ± 1.04	10.56 ± 0.63
		Al	244.94 ± 33.38	96.37 ± 52.26
		Ga	0.49 ± 0.05	0.42 ± 0.08
		As	0.55 ± 0.02	1.60 ± 0.19
		Sr	212.61 ± 16.72	227.25 ± 24.70
		Ag	n.d.	0.01 ± 0.00
		In	n.d.	n.d.
		Sb	n.d.	n.d.
		Ba	2.16 ± 0.22	1.94 ± 0.32
		Bi	5.09 ± 0.16	4.84 ± 0.65
		Tl	n.d.	n.d.
		Pb	0.14 ± 0.02	0.23 ± 0.09
		Cd	0.06 ± 0.00	0.05 ± 0.00
		Hg	n.d.	n.d.

Further studies are required to test the effectiveness of these new steps.

4.3. Use of subproducts by the accessory amphipod culture

Higher body-fat content was observed in our samples compared to the published bibliography (Köprücü and Özdemir, 2005; Opstad et al., 2006; Cook et al., 2010; Baeza-Rojano et al., 2014), which together with an altered FA profile reflected the influence of the main culture (Fernandez-Jover et al., 2007, 2011). There was a dominance of some FA (EPA, DHA, oleic and palmitic acids), in concordance with other studies where amphipods showed no influence from the aquaculture stock (e.g. Guerra-García et al., 2004, 2016; Cook et al., 2010; Baeza-Rojano et al., 2014). However, here the influence of finfish aquaculture was clear, owing to the higher levels of oleic and linoleic acids, and lower levels of EPA, DHA and arachidonic acid detected. These reflect the oleic- and linoleic-rich composition of the aquafeed and fish faeces (Gonzalez-Silvera et al., 2015). These changes in the fatty acid profile have been also detected in aquaculture-associated fauna such as aggregated wild fish (e.g. Skog et al., 2003; Fernandez-Jover et al., 2007), demersal shrimps (Olsen et al., 2012), mussels (Gao et al., 2006; Gonzalez-Silvera et al., 2015) and fouling communities including amphipods (Cook et al., 2010; Gonzalez-Silvera et al., 2015). Thus, the amphipod FA profile provides evidence of an uptake of organic waste generated by the main culture, also observed in land-based experiments (Guerra-García et al., 2016), confirming the role of amphipods as biofilters. This clearly justifies their inclusion as accessory culture in IMTA facilities.

Little is known about TE content in amphipods in comparable conditions, so it is difficult to determine the influence of aquaculture due to the enriched diets or antifouling treatments at fish farms. However, most of the results for minor elements (Fe, Cu and Zn etc.) obtained in this study were lower than those described for littoral and planktonic amphipods (Rainbow, 2002; Moren et al., 2006; Guerra-García et al., 2010b), not reaching undesirable concentrations for their subsequent use (see Section 4.4).

4.4. Nutritional value of amphipod-based products and potential applications

The obtained amphipod mixture is rich in proteins (32–35% of ww), with a content of approx. 13% lipids and low levels of carbohydrates (less than 10% ww). These results, together with the high levels of PUFAs, mainly n-3 PUFA (30% of the total FA), are in agreement with previous studies performed with amphipods from littoral areas (Guerra-García et al., 2004, 2016; Baeza-Rojano et al., 2014) and from fish-farms (Cook et al., 2010; Gonzalez-Silvera et al., 2015). Gross energy was also similar to that reported for gammarids used as fish-feed ingredient (Köprücü and Özdemir, 2005).

General chemical composition in relation to proteins, lipids and n-3 PUFA fulfill the estimated adult nutrient requirements of crustaceans such as *Homarus* spp., penaid shrimps and fish including the European sea bass and Gilthead sea bream, the most widely cultured fish in the Mediterranean Sea. However, higher levels of proteins and essential FA are needed for fish larvae and early juveniles to complete their growth requirements (NRC, 1993; Shiau, 1998; Halver and Hardy, 2002).

The amphipod-based product showed a high ash content in accordance with previous data on fresh and marine amphipods (Köprücü and Özdemir, 2005; Opstad et al., 2006; Baeza-Rojano et al., 2014). Ash is not known to be harmful to cultured organisms per se (De Silva and Anderson, 1995), but high ash content may affect the digestibility of the diets (Köprücü and Özdemir, 2005; Goda et al., 2007 and references therein) and decrease feed efficiency and mineral absorption. This can be alleviated by supplements such as copper or zinc (NRC, 1993). However, dietary inclusion levels for practical diets of fish and crustaceans heavily rely on ash content (Hertrampf and Piedad-Pascual, 2000; Halver and Hardy, 2002; Moutinho et al., 2017).

Amphipods showed adequate levels of most of the essential amino acids (e.g. arginine and glycine) for fish and crustacean nutrition, like those reported for fresh gammarids (Köprücü and Özdemir, 2005), but methionine supplementation would be needed to avoid pathologies related to its deficiency (NRC, 1993; De Silva and Anderson, 1995; Halver and Hardy, 2002). The amphipod-based product also contain notable levels of non-essential amino acids such as glutamic and aspartic acids, that act on herbivorous fish as feeding stimulants, and glycine and valine that stimulate the feeding response of carnivores (NRC, 1993).

As mentioned above (see Section 4.3.), the major and minor element contents in this study were lower than those recorded in the bibliography (Rainbow, 2002; Moren et al., 2006; Guerra-García et al., 2010a, 2010b). Even so, concentrations of Fe, Cu, Mn, Co and Se in amphipod mixture fulfill the criteria of trace minerals requirements for fish and crustacean nutrition, but Zn (for fish nutrition) and Cu (for crustaceans) showed lower levels than needed (Davis and Gatlin, 1996; Watanabe et al., 1997; Halver and Hardy, 2002). Regarding macrominerals, fish usually absorb most of them from the environment (NRC, 1993), but amphipod feed mixtures would also contribute to the concentration of dietary Ca, Na, K and Mg (Halver and Hardy, 2002). Unexpectedly high concentrations of Al and Sr were found in our samples. Similar concentrations of Al have been reported in zooplankton (Battuello et al., 2016) and mussels in the Mediterranean Sea (Squadrone et al., 2016), but it does not seem to result in toxic or beneficial effects for fish (Handy, 1996). Sr is a calcium analogue that accumulates in bones and other calcium-rich structures such as exoskeleton, its regulation being closely related to the concentrations found in seawater (Funge-Smith et al., 1995). Thus elevated concentrations of Sr have also been reported for prawns (Funge-Smith et al., 1995), amphipods (Campbell et al., 2005; Kalantzi et al., 2014) and molluscs (Kalantzi et al., 2014; Pavlov et al., 2015). The main source of Sr in marine fish is sea water, thus freshwater fish depend on dietary sources and therefore marine amphipods could be used as feed for them

(Walther and Thorrold, 2006 and references therein). Finally, maximum values set for heavy metals Hg, Cd and Pb were never exceeded in the examined samples (NRC, 1993; Commission Regulation EC No 1881/2006).

5. Conclusions

The development of commercial scale IMTA systems must overcome some issues regarding the feasibility of these systems for the farmers (Hughes and Black, 2016). The use of wild fauna already growing in fish farms emerges as a workable solution to reduce the required investment costs, mainly arising from obtaining seed for the different cocultures. Moreover, accessory cultures within an existing farming activity should limit the use and number of new infrastructures, with the aim of reducing the costs and complexity of farm systems. The same materials and infrastructures present at the fish farms (floating rings, depth rings, ropes, nets, mussel shells, etc.) were used in the present harvesting system, in order to avoid complex structures and tedious methods for farm staff and facilitate production of the new secondary culture.

The nutritional value of the amphipod-based product obtained in this study makes it an excellent natural ingredient to fish meal, comparable in various aspects (e.g. crude protein, ash content, mineral and amino acid composition) to those commonly used in fish feeds as alternative sources of proteins such as maize, seafood, meat and bone or poultry meals (NRC, 1993; Hertrampf and Piedad-Pascual, 2000), but rich in n-3 PUFA as fish oil. Some important compounds for fish nutrition, such as the minerals phosphorus and iodine or the amino-acid tryptophan, still need to be determined so as to properly address the suitability of amphipods as aquafeed ingredient.

The incorporation of amphipods into finfish aquafeeds has been shown to be possible (Suontama et al., 2007) but many varied uses could be explored, including aquafeeds for crustaceans (Shiau, 1998; Halver and Hardy, 2002), frozen food for high-value ornamental fish such as seahorses or pipefish (Woods, 2009; Olivotto et al., 2011) or even as a food supplement for human nutrition. Only the use as live prey is restricted due to the extraction method with fresh water. The latter could be avoided by placing the collectors directly inside tanks as proposed by Woods (2009) and Baeza-Rojano (2013), for cultured species like octopus which may need to consume live prey (Baeza-Rojano et al., 2013b).

In the light of the results, coupling drop-ropes with amphipod collectors to the existing floating cages could achieve an estimated annual production of one ton of amphipods within an aquaculture facility with approximately 24 cages dedicated to finfish production. This means that one year's potential aquaculture wastes, otherwise lost to and eutrophying the marine environment would be invested back into 335 kg of protein and 10 kg of marine lipids in the form of amphipods. These could then be reused as fish feed. This study shows that harvesting amphipods is viable, as well as an attractive alternative for developing IMTA systems that might diversify cultivable species. However, further studies are necessary to assess the annual variability and the economic efficiency of amphipod production, with the aim of offering real opportunities to increase productivity in a way that will prove economically sound to the farmer.

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