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Growth, protein retention and biochemical composition in *Octopus vulgaris* fed on different diets based on crustaceans and aquaculture by-products

J. Estefanell [a,*], J. Socorro [a], F. Tuya [b], M. Izquierdo [a], J. Roo [a]

[a] Grupo de Investigación en Acuicultura, Instituto Canario de Ciencias Marinas & Instituto Universitario de Sanidad Animal y Seguridad Alimentaria, PO Box 56, E-35200 Telde, Las Palmas, Canary Islands, Spain
[b] BIOGES, Dpto. Biología, Universidad de Las Palmas de Gran Canaria, 35017, Las Palmas de Gran Canaria, Canary Islands, Spain

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ABSTRACT

The octopus, *Octopus vulgaris*, is one of the main targets for aquaculture diversification in Mediterranean countries. However, the development of octopus farming is limited by the lack of information regarding nutritional requirements of this species during its life cycle. In this study, five diets were tested on the biological performance (growth, protein retention and biochemical composition) of individually reared octopuses (n = 8 per diet), including three single diets constituted by: an endemic crab (the white crab, *Plagusia depressa*), a commercial crab imported frozen (the blue crab, *Portunus pelagicus*), and bogue (*Boops boops*) discarded from fish farms (aquaculture by-product), as well as two mixed diets, containing a 60–40% of blue crab-bogue and white crab-bogue, respectively. The rearing period lasted 8 weeks. Octopuses that fed on a mixed diet constituted by blue crab-bogue showed a higher growth than those feeding on bogue as a single food item. No significant differences in growth were observed among individuals feeding on single food items. Highest protein retention was observed in octopuses fed on diets containing discarded bogue, associated with a high lipid and monoenes content in this food item, underlying the use of lipid as energy source in *O. vulgaris*. However, discarded bogue was deficient in ARA in comparison with octopus tissues, which did not seem to affect growth during the experimental period. These findings underline the potential of aquaculture by-products, particularly bogue, as an adequate diet for culturing *O. vulgaris*.

1. Introduction

Aquaculture production rose to ~73 million tons in 2009, representing nearly 45% of total aquatic products. However, aquaculture growth worldwide has slowed down over the last decade, and in some areas, like the European Union, a negative growth has been observed (FAO, 2011). The low number of species cultured at industrial scale is partially responsible, among other factors, for the relative market saturation in the EU. Accordingly, diversification is one of the main targets of European aquaculture for the next decades.

Cephalopods are potential candidates for the aquaculture industry for a number of reasons: they have short life cycles, fast growth and efficient conversion rates, high prices and increasing market demand in several countries (Semmens et al., 2004; Sykes et al., 2006; Vaz-Pires et al., 2004). Particularly, the octopus, *O. vulgaris* (Cuvier, 1797), is one of the main targets for aquaculture diversification in Mediterranean countries (Iglesias et al., 2000). Currently, the development of octopus farming is limited by the lack of specific enrichments and compound diets, which would maximize growth and survival along the life cycle of this species (García García and Cerezo Valverde, 2006; Iglesias et al., 2007). Indeed, high mortality in paralarval rearing is related to nutritional deficiencies of enriched *Artemia*, commonly used in fish larval rearing, especially in n−3 HUFA (Navarro and Villanueva, 2000, 2003). The ongrowing of wild sub-adults in floating cages using low price trash species as food (discarded from fisheries) has shown promising results (García García et al., 2009; Rodríguez et al., 2006), and a few companies in Spain have been pioneers in octopus farming. This activity, however, has showed a low profitability in comparison with other farmed species, due to the high cost of octopus juveniles and food (García García et al., 2004). The first experimental moist diets have been well accepted by octopods, producing a positive growth, although an octopus specific compound feed is not available yet (Cerezo Valverde et al., 2008; Estefanell et al., in press; Quintana et al., 2008; Rosas et al., 2007). A temporary solution to decrease production costs during octopus farming lays on the identification of low-price food items, easily available and with no interest for human markets, which may promote high growth rates in this species. In this sense, little attention has been given to aquaculture by-products, which represent a potential source of fish origin raw materials for the aquaculture industry. This is especially interesting nowadays, as a result of the increasing demand of such raw materials in the production of aqua-feeds (Sargent and Tacon, 1999). Large aggregations of small pelagic fishes are

* Corresponding author. Tel.: +34 928 13 29 00/04; fax: +34 928 13 20 08.
E-mail address: juan.estefanell@giaqua.org (J. Estefanell).

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typically found around off-shore cages (Dempster et al., 2006), and some species can get into the cages through the net mesh or occasional net holes, feeding on commercial compound diets together with target species until harvesting. Along the Mediterranean and eastern Atlantic, the bogue, *Boops boops* (L. 1758), is the most abundant discard, representing at least a 2–5% of total production in sea bream cages (Estefanell et al., 2010b, in press; Socorro et al., 2005).

Biochemical analyses of octopus tissues and diets could provide valuable information regarding nutritional requirements for *O. vulgaris* and nutritional quality changes for human consumption purposes (Fluckiger et al., 2008). Both octopus muscle (90–95% of total body weight) and those preys that have promoted a high growth (e.g. crab, squid) show high protein and low lipid content (Rosa et al., 2004; Cerezo Valverde et al., 2008; García Garrido et al., 2011). These findings are in agreement with the mainly protein-based metabolism and the low capacity to oxidize lipids, which have been observed in cephalopods (Lee, 1994; O'Dor et al., 1984). However, the use of dietary lipids as an energy source has been observed in *O. vulgaris*, through a decrease in lipid content in digestive gland in unfed octopuses (García Garrido et al., 2010), and by a high protein retention in octopus fed on a high lipid diet (Estefanell et al., in press). Also, several works have suggested that lipid digestibility in *O. vulgaris* largely depends on the quantity and quality of dietary lipids (Mazón et al., 2007; Sánchez et al., 2009; Seiça Neves et al., 2010).

The aim of this work was to evaluate the biological performance (growth, feeding rate and mortality rate) of octopuses feeding on three single diets constituted by: an endemic crab (the white crab, *Plagusia depressa* Fabricius 1775), a commercial crab (the blue crab, *Portunus pelagicus*, L. 1758) and discarded bogue (*B. boops*), the most abundant aquaculture by-product along the Mediterranean and eastern Atlantic. To test the effect of mixed diets, both crab species were also supplemented with discarded bogue. Diets, muscle and digestive gland from wild and reared animals were also analyzed, so changes on proximate composition and fatty acids profile between initial (wild) and final (reared) individual were assessed.

2. Material and methods

2.1. Capture and acclimatization of individuals

Wild octopuses were caught by local fishermen using cylindrical trawls (1.5 m diameter, 0.4 m high), placed at 20–30 m depth in the coast of Mogán (Canary Islands, Spain). Octopuses were transported to lab facilities in three 0.5 m^3 square tanks provided with pure oxygen. Individuals were acclimatizated for 1 week in rectangular 1.5 m^3 tanks, where PVC tubes were provided as shelters. Sea water circulation was open flow-through (1500 L/h) and each tank was covered with a shadowing net. During this period, octopuses were fed ad libitum once a day on a mixed diet containing blue crab (*P. pelagicus*), white crab (*P. depressa*) and discarded bogue (*B. boops*), supplied on alternate days.

2.2. Rearing conditions

Rearing trials were performed in 5 rectangular tanks (1.5 m^3). In order to minimize interactions between animals, each tank was internally individualized using a PVC net (2 cm mesh) into 8 compartments (each ca. $0.4\times1\times0.5$ m, 0.2 m^3), so each octopus was kept individually. Each compartment was provided with a T-shaped PVC tube (160 mm diameter) as a shelter, and all tanks were covered with shadowing nets, since benthic octopods spend most of their daily cycle out of light in dens in the natural environment (Hanlon and Messenger, 1996). The assays lasted 8 weeks (March 4th–April 29th) under natural photoperiod (approx. 12.5 h of light and 11.5 h of dark) using an open flow-through sea water system (1500 L/h). Mean water temperature and oxygen levels, measured once a day with a portable oxymeter (Oxiguard Handy, Point tour Systems Inc., Canada), were 20.3 ± 0.3 °C and 6.2 ± 0.3 ppm (85 ± 5%), respectively. Salinity was 35‰.

2.3. Experimental design and diets

A total of 40 octopuses (1176 ± 230 g, $n=8$ per diet) were PIT-tagged (Estefanell et al., 2011) and acclimatized to the individual rearing system for another week prior to the beginning of the experiment. In order to avoid reproductive processes during the experiment, only males were selected (Estefanell et al., 2010a). The following five diets were tested:

1. White crab, *P. depressa* (WC)
2. Blue crab, *P. pelagicus* (BC)
3. Bogue, *B. boops*, discarded from fish farms (DB)
4. White crab, *P. depressa* + discarded bogue, *B. boops* (60–40% of total food supplied) (WC + DB)
5. Blue crab, *P. pelagicus* + discarded bogue, *B. boops* (60–40% of total food supplied) (BC + DB)

Octopuses were fed ad libitum 6 days per week (aprox. at 10:00 am), removing uneaten food every 2 days (aprox. at 8:00 am). In mixed diets, crab and fish were provided on alternate days. White crab (55 ± 18 g), an endemic crab in the Canarian archipelago, was provided fresh by professional fishermen. Blue crab (517 ± 111 g), an indo-west Pacific crab, was provided frozen by a local fish trade company. Bogue, *B. boops* (166 ± 32 g), was provided by a local fish farm as a discarded species. Walking legs and main carapace were removed from crabs, while bogues were provided as a whole piece.

2.4. Biological parameters

All individuals were weighted at the beginning, at two intermediate points (3rd and 6th weeks) and at the end of the experimental period. The following parameters were calculated individually:

- Specific Growth Rate: $SGR = (\mathrm{Ln}W_f - \mathrm{Ln}W_i)*100/t$
- Daily Biomass Increment: $DBI = (B_f - B_i)/(B_i*t)$
- Specific Feed Intake: $SFI = (FI/t)*100/W_a$
- Specific Protein Intake: $SPI = (IP/t)*100/W_a$
- Specific Lipid Intake: $SLI = (IL/t)*100/W_a$
- Specific Energy Intake: $SEI = ((FI/t)*GE/1000)/W_a$
- Protein Efficiency Ratio: PER = (W_f in dry weight − W_i in dry weight)/IP
- Protein Productive Value in muscle: $PPVm = 100*((W_f*P_f - W_i*P_w)/IP)$
- Feed Efficiency: $FE = (W_f - W_i)*100/FI$
- Digestive Gland Index: $DGI = W_{DG}/W_f$

Where W_f = Final weight (in g); W_i = initial weight (in g); B_f = final biomass (in g); B_i = initial biomass (in g); FI = feed intake per octopus (in g); W_a = average weight between sampling (in g); t = total time in days; IP = ingested protein (in g); IL = ingested lipid (in g); GE = gross energy (kJ/g of feed); P_f = final% protein in muscle (wet weight) for each octopus; P_w = average% protein in muscle (wet weight) in wild octopuses; W_{DG} = digestive gland total weight (in g).

To estimate ingested food (FI), uneaten food was removed 3 times per week and dried in an oven at 105 °C to constant weight. In mixed diets, crab and fish were weighted separately. The following formula was applied: $F_i = F_p - (100*F_r/(100-M))$; where F_i is ingested food (in g); F_p is food provided (in g); F_r is dried removed food (in g); M is the diet moisture. Crumbs were also removed every week by water-vacuum the tanks, and were mainly small carapace bits, fish spines or fish scales. In mixed diets, those crumbs that were unequivocal from crab (carapace bits) or from fish (spines, scales) were also separated and quantified. Crumbs initial weight was estimated after drying in an oven at 105 °C to constant weight and subtracted

proportionally from each octopus total ingested food. Mortality was evaluated every day.

2.5. Sampling procedure

The edible fraction from each type of food was sampled three times during the experimental period. Each sample was obtained from a pool of 6 individuals, randomly selected every 2–3 weeks of feeding. After transportation from the sea, eight wild octopuses (males, weight: 1187 ± 277 g) and all reared octopuses from each dietary treatment at the end of the experimental period, were sacrificed by immersion in ice-cold sea water. A sample from muscle and digestive gland was taken from each octopus. Muscle samples were taken from whole left arms II. From each tissue, three pools of 3-3-2 specimens were homogenized from each dietary treatment and stored at −80 °C until biochemical analysis. In those treatments were mortality occurred during the experimental period, at least 2 samples were included on each pool.

2.6. Biochemical analysis

Biochemical analysis followed standard procedures of AOAC (1997). Moisture was determined after drying the sample in an oven at 105 °C to constant weight; ash by combustion in a muffle furnace at 600 °C for 12 h; protein content (N × 6.25) was determined by Kjeldahl method and crude lipid was extracted following the method described by Folch et al. (1957). Fatty acids from total lipids were prepared by transmethylation, as described by Christie (1982), and separated by gas chromatography under the conditions described by Izquierdo et al. (1992). Gross energy was estimated using the Miglavs and Jobling (1989) energy coefficients. All analyses were conducted in triplicate of pools. Proximate composition and gross energy in mixed diets were estimated from the profile of each single food item.

2.7. Estimation of proximate composition and fatty acid profile in mixed diets

The proximate compositions of mixed diets were estimated as a weighted average of the biochemical composition of each single food item and their contribution to total ingested food in the corresponding mixed diet. The fatty acid profiles of mixed diets were estimated as a weighted average of the fatty acid profile of each single food item and the contribution of each food item to total lipid content of each pool of mixed diet. Accordingly, the following formulas were used:

- $PC_{WC+DBi} = PC_{WCi} * (IF_{WC}/(IF_{WC}+IF_{DB1}) + PC_{DBi} * (IF_{DB1}/(IF_{WC}+IF_{DB1})$, i = pool 1, 2 or 3.
- $PC_{BC+DBi} = PC_{BCi} * (IF_{BC}/(IF_{BC}+IF_{DB2}) + PC_{DBi} * (IF_{DB2}/(IF_{BC}+IF_{DB2})$, i = pool 1, 2 or 3.
- $FA_{WC+DBi} = FA_{WCi} * ((L_{WCi} * (IF_{WC}/(IF_{WC}+IF_{DB1}))/L_{WC+DBi}) + FA_{DBi} * ((L_{DBi} * (IF_{DB1}/(IF_{WC}+IF_{DB1}))/L_{WC+DBi})$, i = pool 1, 2 or 3.
- $FA_{BC+DBi} = FA_{BCi} * ((L_{BCi} * (IF_{BC}/(IF_{BC}+IF_{DB2}))/L_{BC+DBi}) + FA_{DBi} * ((L_{DBi} * (IF_{DB2}/(IF_{WC}+IF_{DB2}))/L_{BC+DBi})$, i = pool 1, 2 or 3.

Where "PC_{WC+DBi}" is the proximate composition of the white crab–discarded bogue" mixed diet (% dw), "PC_{WCi}" is the proximate composition of the white crab (% dw), "IF_{WC}" is average total ingested white crab (g ww), "IF_{DB1}" is average total ingested discarded bogue in white crab–discarded bogue mixed diet (g ww), "PC_{DBi}" is the proximate composition of the discarded bogue (% dw), "PC_{BC+DBi}" is the proximate composition of the "blue crab–discarded bogue" mixed diet (% dw), "PC_{BCi}" is the proximate composition of the blue crab (% dw), "IF_{BC}" is the average total ingested blue crab (g ww), "IF_{DB2}" is the average total ingested discarded bogue in the blue crab–discarded bogue mixed diet, "FA_{WC+DBi}" is the fatty acid profile of the white crab–discarded bogue mixed diet (% of total fatty acids), "FA_{WCi}" is the fatty acid profile of the white crab (% of total fatty acids), "L_{WCi}" is the lipid content of the white crab (% dw), "L_{WC+DBi}" is the total lipid content of the white crab–discarded bogue mixed diet (% dw), "FA_{DBi}" is the fatty acid profile of the discarded bogue (% of total fatty acids), "L_{DBi}" is the lipid content of the discarded bogue (% dw), "FA_{BC+DBi}" is the fatty acid profile of the blue crab–discarded bogue mixed diet (% of total fatty acids), "FA_{BCi}" is the fatty acid profile of the blue crab (% of total fatty acids), "L_{BCi}" is the lipid content in the blue crab (% dw) and "L_{BC+DBi}" is the lipid content of the blue crab–discarded bogue mixed diet (% dw).

2.8. Statistical analysis

All data, presented as mean ± standard deviation, were tested for normality with the one-sample Kolmogorov–Smirnoff test as well as for homogeneity of variances (Levene's test). When necessary, arcsin square root transformation of the data was carried out, particularly when data was presented as% (Fowler et al., 1998). When normality or homogeneity of variances was not achieved, non parametric tests were used. Significant differences were considered when $p<0.05$.

Data were analyzed with the statistical computer package SPSS v15 (SPSS, Chicago, IL, USA), using a General Linear Model, where "diet" was established as a fixed factor. Significant differences were considered when $p<0.05$. Some data (initial weight, intermediate weights, final weight, SGR, SFI) were submitted to repeated measures ANOVA, since all these parameters were measured 3 times (3, 6 and 8 weeks) on the same experimental groups along the trial. Other data (SPI, SLI, SEI, PER, PPVm, FE, DGI, proximate composition and fatty acids profiles from diets, muscle and digestive gland from each treatment) were calculated at the end of the rearing period, and compared via a one way ANOVA. The weight of each individual at the start of the experimental period was considered as a covariate, to remove the potential effect of differences in the weight of individuals at the start of the experimental period on response variables. When differences were found, a post-hoc Bonferroni test was used to determine the homogeneous subsets ($p<0.05$). Survival was compared on transformed data (1 = survivors; 0 = deaths) by a Kruskal–Wallis non parametric test, and significant differences were considered when $p<0.05$.

To visualizate affinities in the fatty acid profiles (% of total fatty acids) between the diet and the digestive gland corresponding to each diet at the end of the experimental period, a nm-MDS ordination was carried out on untransformed data calculated from Bray–Curtis dissimilarities. The nm-MDS is robust to deviations from linear responses, because it uses ranks to visualizate similarities among treatments. To determine whether the fatty acid profile from the digestive gland overlapped the fatty acid profile from the diet, the ρ correlation coefficient was estimated between both dissimilarity matrices.

3. Results

3.1. Biochemical composition of the diets

3.1.1. Proximate composition of the diets

The highest lipid and energy content was observed in the discarded bogue diet, followed by mixed diets and single crab diets. In contrast, the highest protein and moisture content were observed in the single crab diets, followed by mixed diets and discarded bogue (Table 1).

3.1.2. Fatty acid profile of the diets

In this study, the most abundant fatty acids in wild octopus tissues were considered, accounting for a 91.2–93.2% of total fatty acids in the samples analyzed. Different lipid content among diets led to differences in the fatty acid profile expressed in relative or absolute terms. In relative terms, higher 20:4n−6 (ARA) and n−3 HUFA

Table 1
Proximate composition of each diet (% dry substance) and gross energy (GE, kJ/100 g food wet weight) (mean ± SD, n = 3).

Diets	WC	BC	DB	WC + DB	BC + DB
Lipids (%)	9.1 ± 1.8^{a}	7.0 ± 0.4^{a}	46.5 ± 1.6^{c}	26.3 ± 1.1^{b}	24.8 ± 1.0^{b}
Proteins (%)	79.1 ± 2.0^{c}	80.8 ± 2.3^{c}	46.5 ± 1.9^{a}	64.1 ± 1.0^{b}	65.4 ± 1.8^{b}
Moisture (%)	76.4 ± 0.6^{c}	79.4 ± 1.5^{c}	63.0 ± 2.8^{a}	70.2 ± 1.0^{b}	72.0 ± 1.6^{b}
Ash (%)	2.4 ± 0.0	2.4 ± 0.2	2.2 ± 0.5	2.3 ± 0.2	2.3 ± 0.3
GE (KJ/100 g)	530 ± 20^{a}	451 ± 42^{a}	1084 ± 83^{c}	784 ± 28^{b}	736 ± 47^{b}

WC = white crab; BC = blue crab; DB: bogue, discarded from fish farms; WC + DB = 60% white crab–40% discarded bogue; BC + DB = 60% blue crab–40% discarded bogue. Different superscript letters within a row denotes significant differences ($p < 0.05$).

content were observed in both single crab diets than in diets containing discarded bogue (Table 2). The high contribution of DB to total lipid content in mixed diets reflected in a similar fatty acid profile in comparison to DB, with the exception of ARA which showed higher values in mixed diets than in DB (Table 2). In absolute terms, the highest n−3 HUFA content was observed in DB, followed by mixed diets and finally both crab species. ARA showed the lowest value in DB, followed by mixed diets and BC, and WC showed the highest value (Table 3). In comparison with crab species, particularly high values of monoenes and n−9 (16:1n−7, palmitoleic acid; 18:1n−9, oleic acid), 18:2n−6 (linoleic acid) and 18:3n−3 (alpha-linolenic acid) were observed in DB (Table 3). Also, higher absolute values in ARA and EPA were observed in WC than in BC.

3.2. Biological parameters

Similar SGR and W_f were observed in octopuses that fed on single crab diets and DB (Table 4, Fig. 1). In general, a higher SGR was observed in octopuses that fed on mixed diets (crab-fish) in comparison to those fed on single diets, although significant differences were only found between octopuses that fed on BC + DB and those fed on DB.

Table 2
Fatty acids profiles from each diet (% of total fatty acids) (n = 3) (mean ± SD).

	WC	BC	DB	WC + DB	BC + DB
14:0	1.9 ± 0.1^{b}	0.8 ± 0.1^{a}	4.6 ± 0.2^{d}	4.1 ± 0.2^{c}	4.0 ± 0.2^{c}
16:0	18.5 ± 1.4^{b}	14.8 ± 0.9^{a}	17.3 ± 0.4^{b}	17.5 ± 0.3^{b}	16.9 ± 0.4^{b}
16:1 n−7	3.5 ± 0.6^{a}	5.5 ± 0.4^{b}	6.6 ± 0.3^{c}	6.0 ± 0.2^{bc}	6.5 ± 0.3^{bc}
18:0	6.5 ± 0.3^{b}	8.6 ± 0.8^{c}	4.7 ± 0.2^{a}	5.0 ± 0.1^{a}	5.3 ± 0.0^{a}
18:1 n−9	9.8 ± 0.6^{a}	13.2 ± 1.0^{b}	17.7 ± 0.1^{c}	16.2 ± 0.3^{c}	17.0 ± 0.3^{c}
18:1 n−7	2.5 ± 0.2^{a}	3.0 ± 0.4^{ab}	3.2 ± 0.0^{b}	3.1 ± 0.9^{b}	3.2 ± 0.1^{b}
18:1n−5	1.2 ± 0.2^{c}	2.5 ± 0.1^{d}	0.8 ± 0.0^{a}	0.9 ± 0.1^{ab}	1.1 ± 0.1^{bc}
18:2 n−6	2.6 ± 0.2^{b}	1.8 ± 0.1^{a}	15.7 ± 0.7^{d}	13.1 ± 0.9^{c}	13.5 ± 0.8^{c}
18:3 n−3	1.3 ± 0.3^{b}	0.4 ± 0.1^{a}	1.8 ± 0.1^{c}	1.7 ± 0.1^{c}	$1.6 \pm 0.1b^{c}$
20:1 n−9	0.9 ± 0.2	0.5 ± 0.3	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
20:2n−6	1.3 ± 0.4^{b}	0.9 ± 0.1^{b}	0.3 ± 0.0^{a}	0.5 ± 0.0^{a}	0.4 ± 0.0^{a}
20:4 n−6	13.5 ± 1.6^{c}	10.9 ± 1.3^{c}	0.7 ± 0.0^{a}	3.2 ± 0.4^{b}	2.3 ± 0.2^{b}
20:5 n−3	14.9 ± 1.2^{b}	9.9 ± 1.2^{a}	7.8 ± 0.7^{a}	9.2 ± 0.6^{a}	8.1 ± 0.5^{a}
22:4 n−6	0.5 ± 0.2^{b}	1.4 ± 0.3^{c}	0.2 ± 0.0^{a}	0.2 ± 0.0^{ab}	0.4 ± 0.1^{ab}
22:5 n−6	0.2 ± 0.1^{a}	1.1 ± 0.2^{b}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.4 ± 0.0^{a}
22:5 n−3	0.6 ± 0.1^{a}	1.8 ± 0.3^{b}	2.1 ± 0.1^{b}	1.8 ± 0.1^{b}	2.1 ± 0.1^{b}
22:6 n−3	12.0 ± 1.3^{b}	14.0 ± 0.6^{c}	8.4 ± 0.1^{a}	9.1 ± 0.2^{a}	9.3 ± 0.1^{a}
∑ Saturated	30.0 ± 1.7^{b}	27.0 ± 0.9^{a}	27.9 ± 0.1^{ab}	28.3 ± 0.5^{ab}	27.7 ± 0.2^{ab}
∑ Monoenes	19.1 ± 1.4^{a}	26.9 ± 0.5^{b}	30.1 ± 0.3^{d}	28.1 ± 0.2^{bc}	29.6 ± 0.2^{cd}
∑ n−3	31.0 ± 2.4^{c}	27.4 ± 1.3^{b}	22.4 ± 0.9^{a}	24.1 ± 0.4^{ab}	23.2 ± 0.6^{a}
∑ n−6	18.9 ± 2.2	16.6 ± 0.8	18.1 ± 0.7	18.2 ± 0.5	17.8 ± 0.8
∑ n−9	10.7 ± 0.6^{a}	14.2 ± 0.4^{b}	19.0 ± 0.1^{d}	17.5 ± 0.3^{c}	18.2 ± 0.2^{cd}
∑ n−3 HUFA	28.0 ± 2.0^{b}	26.0 ± 1.5^{b}	19.0 ± 0.9^{a}	20.8 ± 0.5^{a}	20.1 ± 0.6^{a}
DHA/EPA	0.8 ± 0.1^{a}	1.4 ± 0.1^{c}	1.1 ± 0.1^{b}	1.0 ± 0.1^{ab}	1.1 ± 0.1^{b}
DHA/ARA	0.9 ± 0.2^{a}	1.3 ± 0.1^{a}	12.2 ± 0.2^{d}	2.9 ± 0.3^{b}	4.1 ± 0.3^{c}
EPA/ARA	1.1 ± 0.1^{b}	0.9 ± 0.0^{a}	11.2 ± 0.7^{e}	2.9 ± 0.2^{c}	3.6 ± 0.3^{d}

WC = white crab; BC = blue crab; DB: bogue, discarded from fish farms; WC + DB = 60% white crab–40% discarded bogue; BC + DB = 60% blue crab–40% discarded bogue. Different superscript letters within a row denote significant differences ($p < 0.05$).

Table 3
Main fatty acids from each diet and estimation of mixed diets (mg fatty acids/g substance dw) (mean ± SD) (n = 3).

	WC	BC	DB	WC + DB	BC + DB
14:0	0.17 ± 0.05^{a}	0.06 ± 0.01^{a}	2.13 ± 0.10^{c}	1.03 ± 0.05^{b}	0.99 ± 0.04^{b}
16:0	1.70 ± 0.49^{a}	1.03 ± 0.10^{a}	7.93 ± 0.25^{c}	4.44 ± 0.38^{b}	4.14 ± 0.16^{b}
16:1 n−7	0.32 ± 0.14^{a}	0.39 ± 0.01^{a}	3.05 ± 0.17^{c}	1.52 ± 0.11^{b}	1.58 ± 0.07^{b}
18:0	0.58 ± 0.11^{a}	0.60 ± 0.09^{a}	2.15 ± 0.13^{c}	1.27 ± 0.11^{b}	1.30 ± 0.02^{b}
18:1 n−9	0.88 ± 0.19^{a}	0.92 ± 0.08^{a}	8.11 ± 0.20^{b}	4.10 ± 0.22	4.16 ± 0.04
18:1 n−7	0.23 ± 0.05^{a}	0.21 ± 0.04^{a}	1.48 ± 0.04^{c}	0.78 ± 0.05^{b}	0.78 ± 0.03^{ab}
18:1n−5	0.11 ± 0.04^{a}	0.18 ± 0.02^{ab}	0.36 ± 0.02^{d}	0.22 ± 0.30^{bc}	0.26 ± 0.02^{c}
18:2 n−6	0.23 ± 0.05^{a}	0.13 ± 0.00^{a}	7.22 ± 0.44^{c}	3.31 ± 0.19^{b}	3.32 ± 0.20^{b}
18:3 n−3	0.12 ± 0.04^{a}	0.03 ± 0.01^{a}	0.84 ± 0.04^{c}	0.43 ± 0.04^{b}	0.39 ± 0.02^{b}
20:1 n−9	0.08 ± 0.04^{a}	0.04 ± 0.02^{a}	0.46 ± 0.02^{c}	0.25 ± 0.03^{b}	0.23 ± 0.02^{b}
20:2n−6	0.12 ± 0.02^{bc}	0.06 ± 0.01^{a}	0.15 ± 0.01^{c}	0.13 ± 0.01^{bc}	0.10 ± 0.01^{b}
20:4 n−6	1.21 ± 0.24^{c}	0.76 ± 0.07^{b}	0.32 ± 0.01^{a}	0.82 ± 0.14^{b}	0.56 ± 0.03^{b}
20:5 n−3	1.34 ± 0.23^{b}	0.69 ± 0.1^{a}	3.57 ± 0.28^{d}	2.32 ± 0.23^{c}	1.98 ± 0.11^{c}
22:4 n−6	0.04 ± 0.03^{a}	0.10 ± 0.03^{b}	0.08 ± 0.01^{ab}	0.06 ± 0.02^{ab}	0.09 ± 0.02^{b}
22:5 n−6	0.02 ± 0.01^{a}	0.07 ± 0.01^{bc}	0.11 ± 0.00^{d}	0.06 ± 0.00^{b}	0.09 ± 0.01^{cd}
22:5 n−3	0.05 ± 0.01^{a}	0.13 ± 0.03^{a}	0.97 ± 0.07^{c}	0.45 ± 0.04^{b}	0.51 ± 0.05^{b}
22:6 n−3	1.09 ± 0.22^{a}	0.98 ± 0.05^{a}	3.87 ± 0.14^{c}	2.31 ± 0.18^{b}	2.28 ± 0.05^{b}
∑ Saturated	2.75 ± 0.82^{a}	1.89 ± 0.20^{a}	12.81 ± 0.40^{c}	7.18 ± 0.62^{b}	6.80 ± 0.20^{b}
∑ Monoenes	1.75 ± 0.55^{a}	1.88 ± 0.17^{a}	13.82 ± 0.38^{c}	7.10 ± 0.46^{b}	7.26 ± 0.14^{b}
∑ n−3	2.80 ± 0.59^{a}	1.91 ± 0.09^{a}	10.30 ± 0.45^{c}	6.10 ± 0.49^{b}	5.69 ± 0.14^{b}
∑ n−6	1.70 ± 0.35^{a}	1.16 ± 0.05^{a}	8.30 ± 0.47^{c}	4.60 ± 0.25^{b}	4.37 ± 0.19^{b}
∑ n−9	0.97 ± 0.23^{a}	0.99 ± 0.09^{a}	8.73 ± 0.20^{c}	4.42 ± 0.25^{b}	4.47 ± 0.06^{b}
∑ n−3 HUFA	2.52 ± 0.45^{a}	1.81 ± 0.07^{a}	8.73 ± 0.44^{c}	5.25 ± 0.42^{b}	4.93 ± 0.20^{b}

WC = white crab; BC = blue crab; DB: bogue, discarded from fish farms; WC + DB = 60% white crab–40% discarded bogue; BC + DB = 60% blue crab–40% discarded bogue. Different superscript letters within a row denote significant differences ($p < 0.05$).

Survival was high and similar among treatments, with mortality only registered in individuals that fed on diets containing white crab (Table 4). From total ingested food, the percentage of crab-bogue ingested was 44–56 ± 4% for those that fed on WC + DB and 45–55 ± 4% for octopuses that fed on BC + DB. A higher SFI was observed in octopuses that fed on diets containing blue crab than for those that fed on diets containing white crab and discarded bogue. Regarding SPI, highest values were observed in octopus fed on BC, followed by those fed on WC and finally those fed on DB, while specimens fed on mixed diets showed intermediate values. Also, a higher SLI and SEI was observed in octopuses that fed on DB than for those that fed on single crab diets, while mixed diets showed intermediate values (Table 4). Feeding rates were reduced towards the end of the experimental period for all treatments (Fig. 2). Higher PER and PPVm were observed in octopuses fed on diets containing discarded bogue in comparison to those fed in single crab species, although results were only significant between mixed diets and BC for PER and diets containing discarded bogue and BC for PPVm. A lower FE was observed in octopuses that fed on BC, in comparison with those that fed on diets containing discarded bogue. DGI in (initial) wild specimens was 2.5 ± 0.7%, a similar value to those of reared octopuses (Table 4).

3.3. Biochemical composition of muscle and digestive gland

3.3.1. Proximate composition of muscle and digestive gland

A higher lipid and lower protein content was detected in the digestive gland of octopuses that were fed on diets containing discarded bogue, either as a main item or mixed with crabs, than in initial wild octopuses (Table 5). Regarding the muscle, a higher protein content was observed in individuals that fed on single discarded bogue and blue crab–bogue mixed diet, confronted with initial wild octopuses and those that fed on blue crab as a single food item (Table 5).

3.3.2. Fatty acids profile in muscle and digestive gland

A lower level of DHA in the digestive gland was detected in reared octopuses at the end of the rearing period, in comparison with initial

Table 4
Initial weight, final weight and biological parameters in *O. vulgaris* after eight weeks of feeding (mean values ± S.D., n = 8).

	WC	BC	DB	WC + DB	BC + DB
Initial weight (g.)	1107 ± 241	1232 ± 303	1150 ± 182	1185 ± 281	1145 ± 167
Final weight (g.)	1898 ± 255^{a}	2189 ± 316^{ab}	1831 ± 360^{a}	2152 ± 451^{ab}	2490 ± 565^{b}
SGR (%/day)	1.0 ± 0.3^{ab}	1.1 ± 0.3^{ab}	0.9 ± 0.3^{a}	1.1 ± 0.3^{ab}	1.4 ± 0.4^{b}
Survival (%)	87.5	100	100	87.5	100
DBI (%/day)	1.3	1.4	1.1	1.5	2.1
SFI (%/day)	2.45 ± 0.34^{a}	3.36 ± 0.69^{b}	2.06 ± 0.46^{a}	2.28 ± 0.31^{a}	3.04 ± 0.20^{b}
SPI (%/day)	0.46 ± 0.06^{b}	0.56 ± 0.11^{c}	0.35 ± 0.08^{a}	0.41 ± 0.06^{ab}	0.51 ± 0.03^{bc}
SLI (%/day)	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.36 ± 0.08^{d}	0.20 ± 0.03^{b}	0.27 ± 0.02^{c}
SEI (J/g day)	130 ± 18^{a}	152 ± 31^{a}	223 ± 49^{c}	172 ± 23^{ab}	214 ± 14^{bc}
PER	0.31 ± 0.07^{ab}	0.26 ± 0.04^{a}	0.36 ± 0.12^{ab}	0.40 ± 0.09^{b}	0.41 ± 0.12^{b}
PPV (%)	23.1 ± 5.6^{ab}	16.0 ± 3.3^{a}	30.2 ± 10.2^{b}	34.3 ± 7.2^{b}	35.5 ± 9.9^{b}
FE (%)	39.7 ± 6.9^{ab}	30.3 ± 4.6^{a}	46.6 ± 9.6^{b}	45.9 ± 9.2^{b}	44.9 ± 9.4^{b}
DGI (%)	2.4 ± 1.1	2.5 ± 1.0	2.2 ± 0.9	1.9 ± 0.5	1.7 ± 0.8

WC = white crab; BC = blue crab; DB: bogue, discarded from fish farms; WC + DB = 60% white crab–40% discarded bogue; BC + DB = 60% blue crab–40% discarded bogue. Different superscript letters within a row denote significant differences ($p<0.05$).
SGR = standard growth rate; SFI = standard feed intake; SPI = standard protein intake; SLI = standard lipid intake; SEI = standard energy intake; PER = protein efficiency ratio; PPV = protein productive value; FE = feed efficiency; DGI = digestive gland index.

wild octopus. In general, the fatty acids profile of the diet was reflected in relative terms in the digestive gland (Fig. 3); in turn, a significant correlation ($\rho = 0.86$, $p = 0.05$) between the fatty acid profile on the diet and the digestive gland was detected. An increase in 18:2n−6, EPA and DHA, and a decrease in ARA, was observed in octopuses that fed on diets containing discarded bogue, in comparison with octopuses that fed on single crab diets (Table 6). Particularly, high values of saturated (16:0, palmitic acid; 18:0, stearic acid) and monoenes (18:1n−9, 20:1n−9, linolenic acid) were detected in octopuses that fed on blue crab.

Regarding muscle, a decrease in saturated (16:0, 17:0) was observed in octopuses that fed on crab diets in comparison to the initial wild octopus fatty acid profile. Also, a decrease in ARA and an increase in EPA content was detected in animals that fed on discarded bogue, either alone or supplemented with blue crab. DHA was not affected by the diet (Table 7).

4. Discussion

In this study, octopuses that fed on a blue crab-bogue mixed diet showed a higher growth than those that fed exclusively on bogue. No significant differences in growth, however, were observed among diets constituted by a single food item. This result contrasts with previous works, where diets exclusively constituted by crabs had promoted the highest growth for *O. vulgaris* (Aguado Giménez and García García, 2002; García García and Cerezo Valverde, 2006; Prato et al., 2010). Our results underline, therefore, the potential of rich-lipid bogue discarded from fish farms, as an adequate diet for the ongrowing of *O. vulgaris* (Socorro et al., 2005), especially in comparison with other rich-lipid fish (García García and Aguado Giménez, 2002; Petza et al., 2006). However, growth, feeding rates and FE induced by a diet constituted by discarded bogue were lower than previous experiments using the same food item under comparable rearing conditions (Estefanell et al., in press). Such a difference could be explained by the different proximate composition in discarded bogue, with more than a threefold lipid and nearly half protein content in the bogue used in this study. Recent data on octopus ongrowing performed in off-shore sea cages, also providing rich-lipid discarded bogue (45% dry weight) as single diet, showed high growth rates (1.8–1.9%/day) (Estefanell et al., 2010b). This is suggesting that individual rearing in a reduced space (200 l cages) could have a negative effect on growth, perhaps related to the lack of caloric burn or stress due to strict confinement.

Despite similar proximate composition, the blue crab induced a higher feed intake (SFI, SPI) than the white crab, either as a single or mixed diet. This outcome suggests its value as a potential diet for octopus culture. A high crab intake in *O. vulgaris* was previously observed, related to adequate palatability or to an effort to compensate lipid and amino-acid deficiencies relative to fish (Cerezo Valverde et al., 2008, 2009; García García and Cerezo Valverde, 2006). Also, mortality was observed in tanks where octopuses were fed on diets containing white crab. The presence of the coccidian parasite, *Aggregata octopiana*, has been recently described for both wild and reared *O. vulgaris* in the Canary Islands (Betancor et al., 2010). Since this parasite requires crustaceans as intermediary hosts, mortality in octopus that fed on local fresh white crab may be related to this parasite, which causes the so-called "malabsorption syndrome" in octopuses (Gestal et al., 2002). The combination of blue crab and fish (discarded bogue) maximized growth rates in *O. vulgaris*, suggesting that mixed diets may better cover the nutritional requirements of octopuses (Cagnetta and Sublimi, 1999; Smale and Bouchan, 1981). Indeed, octopus natural diet is based on a wide variety of prey, including crustaceans, fish and other molluscs (Quetglas et al., 1998; Rosa et al., 2004).

With respect to nutrient utilization, octopuses that fed on diets containing discarded bogue (highest lipid and energy intake) also showed the highest values of PER, PPVm and FE, which demonstrates the use of lipids as energy source in this species, allowing a more efficient utilization of dietary protein (Estefanell et al., in press). Indeed, discarded bogue is very abundant in monoenes, reported as energy substrates in marine organisms (Sargent et al., 1995). Other works

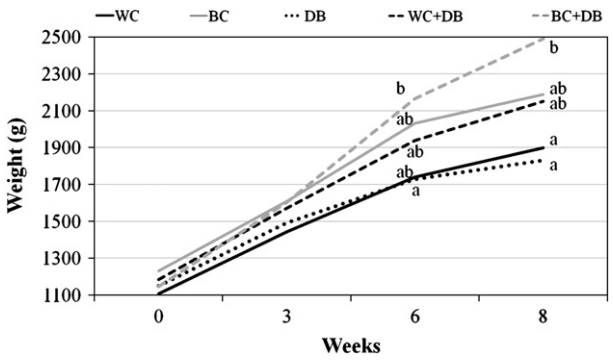

Fig. 1. Change in mean weight of individuals fed on each diet (n = 8) during the experimental period (8 weeks) (WC = white crab; BC = blue crab; DB: bogue, discarded from fish farms; WC + DB = 60% white crab–40% discarded bogue; BC + DB = 60% blue crab–40% discarded bogue). Different letters denote significant differences at 6 and 8 weeks ($p<0.05$).

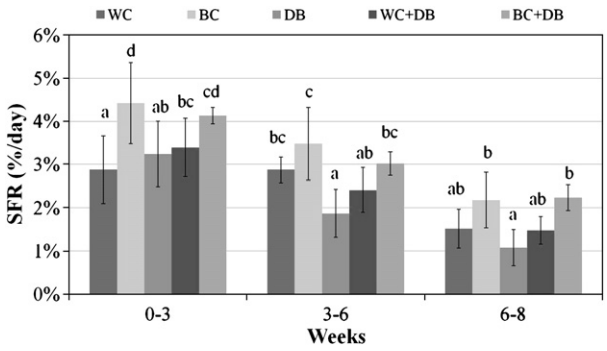

Fig. 2. Standard feeding rate (%/day) of individuals fed on each diet (n = 8) during the experimental period (8 weeks) (WC = white crab; BC = blue crab; DB: bogue, discarded from fish farms; WC + DB = 60% white crab–40% discarded bogue; BC + DB = 60% blue crab–40% discarded bogue). Different letters denote significant differences ($p<0.05$).

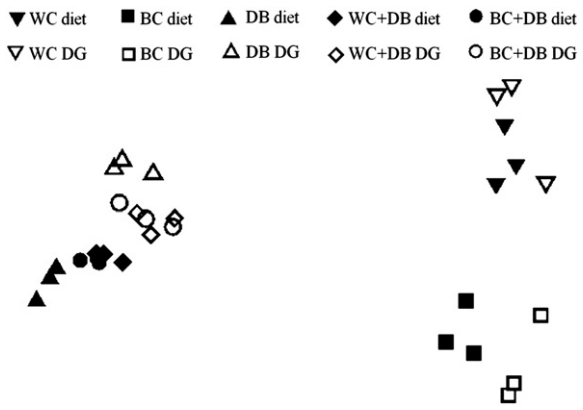

Fig. 3. Ordination (multidimensional scaling, MDS) of fatty acid profiles found in the diet and in the digestive gland of *O. vulgaris* after 8 weeks of feeding (stress = 0.04) (WC = white crab; BC = blue crab; DB: bogue, discarded from fish farms; WC + DB = 60% white crab–40% discarded bogue; BC + DB = 60% blue crab–40% discarded bogue; DG = digestive gland).

have also showed the use of lipids in *O. vulgaris* as an energy source during starvation (García Garrido et al., 2010). In this sense, recent findings support that lipid digestibility in *O. vulgaris* depends on the quantity and quality of dietary lipids (Mazón et al., 2007; Sánchez et al., 2009; Seiça Neves et al., 2010).

The edible fraction of crabs and octopus muscle showed a similar lipid and fatty acid profile, with high levels of ARA, EPA and DHA (Miliou et al., 2007; Prato et al., 2010; Rosa et al., 2004). In contrast, the fatty acid profile in bogue discarded from fish farms was very abundant in oleic and linoleic acid, since fish reflect the fatty acid profile of diets, and commercial aqua-feeds have increased levels of these fatty acids (Izquierdo et al., 2005). Also, bogue showed very low levels of ARA, in agreement with previous findings in wild bogue (Prato et al., 2010).

Lipid and fatty acid profiles in the digestive gland clearly reflected diets, denoting the use of this organ as a lipid and energy storage (Estefanell et al., in press; García Garrido et al., 2010). Octopuses that fed on mixed diets showed a similar lipid and fatty acid profile in the digestive gland in comparison with those that fed exclusively on discarded bogue, which suggests that this organ has a limited capacity to accumulate dietary lipids, and also the major contribution of bogue to the total lipid content in mixed diets. In other cephalopod species, the digestive gland also reflected the fatty acid profile of the diet (Fluckiger et al., 2008; Stowasser et al., 2006). The low n−3 HUFA absolute content in blue crab was reflected in the digestive gland after 8 weeks of feeding. However, n−3 HUFA reserves in this organ maintained n−3 HUFA levels in muscle at the end of the rearing period. This finding suggests that n−3 HUFA optimal content in octopus diets must be above 1.8 mg/g of lipid (dw). Proximate composition in octopus muscle was more constant than in the digestive gland, which is in agreement with previous reports in cephalopods (Almansa et al., 2006; Ferreira et al., 2009; García Garrido et al., 2011). However, the low content of ARA in discarded bogue reflected in octopus muscle, which apparently did not have a negative effect on growth, perhaps was associated to its substitution by EPA during phospholipids' esterification, as occurs in fishes (Bell et al., 1995). ARA is the main precursor of eicosanoids, involved in several physiological functions, such as immune response, neural function and reproduction (Tocher, 2003). Regarding previous rearing trials, specific retention of ARA in *O. vulgaris* after feeding with deficient diets (Navarro and Villanueva, 2000, 2003), or after long term starvation (García Garrido et al., 2010), underlined its importance for this species. Finally, low lipid content in crabs, despite its adequate fatty acid profile, was reflected in low saturates content in reared octopus muscle. This suggests the importance of saturated in muscle, perhaps for energy production.

In summary, these results have confirmed the potential of aquaculture by-products, particularly bogue, as an adequate diet to feed *O. vulgaris*, either as a single diet or supplemented with the blue crab *Blue crab*. The nutritional characteristics of a mixed diet constituted by blue crab-bogue should be taking into account in the development of octopus specific compound feeds.

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Table 5
Proximate composition (% dry weight) in digestive gland and muscle of wild (initial) and reared *O. vulgaris* after eight weeks of feeding (mean ± S.D., n = 3).

		Wild (initial)	WC	BC	DB	WC + DB	BC + DB
Digestive gland	Lipids (%)	19.9 ± 4.2^{a}	27.6 ± 4.3^{ab}	19.5 ± 0.6^{a}	45.5 ± 6.1^{c}	45.5 ± 8.8^{c}	42.6 ± 9.1^{bc}
	Proteins (%)	68.7 ± 4.2^{b}	60.5 ± 5.8^{b}	67.8 ± 2.0^{b}	42.3 ± 5.5^{a}	40.9 ± 7.7^{a}	44.7 ± 6.3^{a}
	Moisture (%)	71.5 ± 5.5	67.9 ± 2.4	69.2 ± 3.9	64.7 ± 3.7	60.8 ± 7.4	60.3 ± 7.7
	Ash (%)	1.8 ± 0.1^{ab}	1.7 ± 0.2^{ab}	2.0 ± 0.1^{b}	1.6 ± 0.2^{ab}	1.5 ± 0.1^{a}	1.5 ± 0.2^{a}
Muscle	Lipids (%)	5.5 ± 0.2	5.8 ± 0.3	5.7 ± 0.2	5.2 ± 0.5	5.4 ± 0.2	5.4 ± 0.3
	Proteins (%)	77.8 ± 3.2^{a}	81.9 ± 1.5^{ab}	78.3 ± 0.9^{a}	84.0 ± 0.6^{b}	82.7 ± 2.1^{ab}	83.9 ± 0.4^{b}
	Moisture (%)	83.6 ± 2.2	85.2 ± 0.6	85.9 ± 0.4	84.7 ± 1.0	84.1 ± 0.6	84.0 ± 0.9
	Ash (%)	1.8 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	1.7 ± 0.0	1.7 ± 0.1	1.7 ± 0.0

WC = white crab; BC = blue crab; DB: bogue, discarded from fish farms; WC + DB = 60% white crab–40% discarded bogue; BC + DB = 60% blue crab–40% discarded bogue. Different superscript letters within a row denote significant differences ($p<0.05$).

Table 6
Fatty acids profile (% of total fatty acids) in digestive gland of wild (initial) and reared *O. vulgaris* after eight weeks of feeding (mean ± SD, n = 3).

	Wild (initial)	WC	BC	DB	WC + DB	BC + DB
14:0	1.3 ± 0.4^{a}	1.9 ± 0.0^{a}	1.7 ± 0.3^{a}	3.7 ± 0.2^{b}	4.2 ± 0.1^{b}	3.9 ± 0.2^{b}
16:0	14.3 ± 0.1^{a}	16.2 ± 0.8^{bc}	17.7 ± 0.5^{c}	14.7 ± 1.1^{ab}	15.2 ± 0.5^{ab}	14.3 ± 0.5^{a}
16:1 n−7	2.8 ± 0.6^{a}	4.9 ± 0.6^{b}	6.0 ± 0.8^{b}	5.3 ± 0.2^{b}	6.2 ± 0.6^{b}	6.0 ± 0.4^{b}
18:0	7.8 ± 0.9^{bc}	6.4 ± 0.4^{ab}	9.4 ± 1.7^{c}	4.9 ± 0.7^{a}	4.6 ± 0.6^{a}	4.9 ± 0.5^{a}
18:1 n−9	10.7 ± 0.4^{b}	7.9 ± 0.3^{a}	12.1 ± 0.5^{bc}	11.8 ± 0.4^{bc}	12.5 ± 0.8^{c}	12.7 ± 0.7^{c}
18:1 n−7	3.1 ± 0.1^{bc}	3.3 ± 0.1^{c}	4.5 ± 0.4^{d}	2.6 ± 0.1^{a}	2.8 ± 0.0^{a}	2.8 ± 0.1^{ab}
18:1n−5	2.2 ± 0.2^{b}	2.1 ± 0.6^{b}	3.4 ± 0.2^{c}	0.7 ± 0.3^{a}	0.7 ± 0.2^{a}	0.8 ± 0.2^{a}
18:2 n−6	1.3 ± 0.3^{a}	2.9 ± 0.1^{b}	1.4 ± 0.2^{a}	13.5 ± 1.1^{c}	12.4 ± 0.3^{c}	12.8 ± 0.6^{c}
18:3 n−3	0.6 ± 0.1^{a}	1.6 ± 0.3^{b}	0.2 ± 0.1^{a}	1.2 ± 0.1^{b}	1.4 ± 0.1^{b}	1.3 ± 0.1^{b}
20:1 n−9	2.5 ± 0.1^{d}	1.5 ± 0.1^{b}	2.2 ± 0.1^{c}	1.2 ± 0.1^{a}	1.2 ± 0.1^{a}	1.2 ± 0.0^{a}
20:2n−6	0.9 ± 0.1^{a}	2.3 ± 0.3^{bc}	1.8 ± 0.3^{b}	3.3 ± 0.5^{c}	2.1 ± 0.7^{ab}	2.3 ± 0.4^{bc}
20:4 n−6	9.9 ± 2.5^{b}	18.9 ± 1.2^{d}	13.8 ± 1.6^{c}	2.2 ± 0.3^{a}	4.9 ± 0.7^{a}	3.9 ± 1.0^{a}
20:5 n−3	6.5 ± 0.9^{a}	11.7 ± 1.5^{b}	5.6 ± 0.3^{a}	11.8 ± 0.5^{b}	10.7 ± 0.5^{b}	10.6 ± 0.9^{b}
22:4 n−6	1.4 ± 0.3^{c}	1.0 ± 0.1^{b}	0.0 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}
22:5 n−6	1.8 ± 0.1^{c}	0.3 ± 0.1^{a}	1.0 ± 0.1^{b}	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}	0.5 ± 0.0^{a}
22:5 n−3	1.8 ± 0.2^{b}	1.1 ± 0.1^{a}	1.2 ± 0.1^{a}	2.3 ± 0.1^{c}	2.1 ± 0.1^{c}	2.3 ± 0.2^{c}
22:6 n−3	23.2 ± 0.1^{d}	9.0 ± 1.1^{a}	10.4 ± 0.7^{a}	14.9 ± 0.3^{c}	12.1 ± 0.2^{b}	13.1 ± 0.2^{b}
∑ Saturated	25.9 ± 1.7^{a}	26.3 ± 0.9^{a}	31.1 ± 1.5^{b}	24.1 ± 1.8^{a}	25.0 ± 1.0^{a}	24.1 ± 1.1^{a}
∑ Monoenes	23.3 ± 0.2^{bc}	21.1 ± 0.5^{a}	30.1 ± 0.3^{d}	22.4 ± 0.4^{ab}	24.4 ± 1.2^{c}	24.5 ± 0.9^{c}
∑ n−3	33.2 ± 1.0^{e}	25.0 ± 1.2^{b}	18.3 ± 0.7^{a}	31. ± 1.1^{de}	28.2 ± 1.0^{c}	29.1 ± 1.3^{cd}
∑ n−6	16.1 ± 2.5^{a}	26.5 ± 1.1^{c}	18.2 ± 1.5^{ab}	20.2 ± 0.7^{b}	20.9 ± 1.4^{b}	20.7 ± 1.1^{b}
∑ n−9	13.6 ± 0.6^{bc}	9.5 ± 0.3^{a}	14.8 ± 0.5^{c}	13.3 ± 0.4^{b}	14.0 ± 0.7^{bc}	14.2 ± 0.7^{bc}
∑ n−3 HUFA	32.0 ± 0.7^{e}	22.7 ± 0.7^{b}	17.5 ± 0.7^{a}	29.7 ± 1.0^{d}	25.7 ± 0.8^{c}	26.7 ± 1.0^{c}
DHA/EPA	3.6 ± 0.5^{c}	0.8 ± 0.2^{a}	1.9 ± 0.2^{b}	1.3 ± 0.0^{ab}	1.1 ± 0.0^{a}	1.2 ± 0.1^{ab}
DHA/ARA	2.4 ± 0.6^{b}	0.5 ± 0.0^{a}	0.8 ± 0.1^{a}	6.7 ± 1.0^{c}	2.5 ± 0.4^{b}	3.5 ± 0.9^{b}
EPA/ARA	0.7 ± 0.3^{a}	0.6 ± 0.1^{a}	0.4 ± 0.1^{a}	5.4 ± 0.9^{c}	2.3 ± 0.4^{b}	2.9 ± 1.0^{b}

WC = white crab; BC = blue crab; DB: bogue, discarded from fish farms; WC + DB = 60% white crab–40% discarded bogue; BC + DB = 60% blue crab–40% discarded bogue. Different superscript letters within a row denote significant differences ($p<0.05$).

Table 7
Fatty acids profile (% of total fatty acids) in muscle of wild (initial) and reared *O. vulgaris* after eight weeks of feeding (mean ± SD, n = 3).

	Wild (initial)	WC	BC	DB	WC + DB	BC + DB
14:0	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.1
16:0	17.8 ± 0.9^{b}	15.3 ± 0.7^{a}	15.4 ± 0.9^{a}	18.2 ± 0.5^{b}	17.0 ± 0.8^{ab}	17.0 ± 0.7^{ab}
16:1 n−7	0.7 ± 0.1^{c}	0.4 ± 0.1^{a}	0.5 ± 0.1^{bc}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{ab}
18:0	6.0 ± 0.5^{ab}	7.0 ± 0.1^{b}	6.3 ± 0.5^{ab}	5.9 ± 0.4^{a}	6.6 ± 0.3^{ab}	6.6 ± 0.3^{ab}
18:1 n−9	9.3 ± 0.7^{a}	11.6 ± 0.3^{b}	11.0 ± 0.4^{ab}	10.6 ± 0.8^{ab}	12.2 ± 0.7^{b}	11.8 ± 0.7^{b}
18:1 n−7	2.5 ± 0.4	2.6 ± 0.4	2.7 ± 0.5	2.4 ± 0.1	2.4 ± 0.2	2.5 ± 0.3
18:1n−5	1.6 ± 0.0	1.4 ± 0.2	1.4 ± 0.3	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.2
18:2 n−6	0.6 ± 0.2	0.8 ± 0.1	0.8 ± 0.3	0.8 ± 0.1	0.8 ± 0.0	0.9 ± 0.1
18:3 n−3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:1 n−9	3.0 ± 0.4	3.3 ± 0.1	3.2 ± 0.3	3.4 ± 0.3	3.3 ± 0.4	3.3 ± 0.3
20:2n−6	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.0	1.0 ± 0.1
20:4 n−6	13.0 ± 0.9^{c}	14.7 ± 0.9^{c}	13.6 ± 0.1^{c}	9.3 ± 1.6^{a}	12.3 ± 1.5^{bc}	10.1 ± 0.5^{ab}
20:5 n−3	10.1 ± 1.4^{a}	10.6 ± 0.8^{ab}	11.0 ± 0.5^{abc}	13.4 ± 1.2^{c}	12.0 ± 0.8^{abc}	13.3 ± 1.1^{bc}
22:4 n−6	1.8 ± 0.2	1.8 ± 0.2	1.3 ± 1.0	1.6 ± 0.5	1.5 ± 0.3	1.3 ± 0.1
22:5 n−6	1.4 ± 0.1^{b}	0.9 ± 0.1^{a}	1.2 ± 0.1^{ab}	1.1 ± 0.2^{ab}	0.9 ± 0.2^{a}	0.9 ± 0.0^{a}
22:5 n−3	1.5 ± 0.2	1.8 ± 0.0	1.8 ± 0.2	1.8 ± 0.1	1.7 ± 0.1	1.7 ± 0.2
22:6 n−3	23.3 ± 1.9	22.1 ± 1.0	23.8 ± 0.9	25.0 ± 1.2	22.6 ± 0.8	23.1 ± 1.3
∑ Saturated	26.7 ± 1.2^{b}	23.6 ± 0.5^{a}	23.1 ± 0.3^{a}	25.1 ± 0.6^{ab}	24.6 ± 1.0^{ab}	24.8 ± 0.6^{ab}
∑ Monoenes	18.9 ± 1.7	21.0 ± 1.1	20.8 ± 0.1	19.5 ± 0.8	21.2 ± 1.6	21.1 ± 1.7
∑ n−3	35.9 ± 3.6^{ab}	35.2 ± 1.8^{a}	37.4 ± 1.5^{ab}	41.0 ± 2.5^{b}	37.2 ± 0.3^{ab}	39.1 ± 1.7^{b}
∑ n−6	17.6 ± 1.4	19.3 ± 1.1	17.9 ± 1.2	13.9 ± 2.1	16.5 ± 1.9	14.3 ± 0.5
∑ n−9	12.5 ± 1.1^{a}	14.9 ± 0.3^{b}	14.3 ± 0.4^{ab}	14.1 ± 0.7^{ab}	15.6 ± 1.1^{b}	15.2 ± 1.1^{b}
∑ n−3 HUFA	35.4 ± 3.6	34.8 ± 1.8	36.9 ± 1.5	40.5 ± 2.4	36.6 ± 0.3	38.5 ± 1.8
DHA/EPA	2.3 ± 0.1^{c}	2.1 ± 0.1^{abc}	2.2 ± 0.1^{bc}	1.9 ± 0.1^{ab}	1.9 ± 0.2^{ab}	1.7 ± 0.2^{a}
DHA/ARA	1.8 ± 0.3^{a}	1.5 ± 0.2^{a}	1.7 ± 0.1^{a}	2.8 ± 0.6^{b}	1.9 ± 0.2^{a}	2.3 ± 0.1^{ab}
EPA/ARA	0.8 ± 0.2^{a}	0.7 ± 0.1^{a}	0.8 ± 0.0^{a}	1.5 ± 0.1^{b}	1.0 ± 0.2^{ab}	1.3 ± 0.2^{b}

WC = white crab; BC = blue crab; DB: bogue, discarded from fish farms; WC + DB = 60% white crab–40% discarded bogue; BC + DB = 60% blue crab–40% discarded bogue. Different superscript letters within a row denote significant differences ($p<0.05$).

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