Application of polychaetes in (de)coupled integrated aquaculture: production of a high-quality marine resource

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ABSTRACT: *Capitella* sp. and *Ophryotrocha craigsmithi* received a diet of salmon feces to evaluate their potential to convert fish waste into valuable marine products, e.g. ingredients for fish feed formulation. Production rate and body composition (focusing on fatty acid [FA] profiles) were determined for polychaetes fed fresh, acid-preserved or oven-dried salmon feces to evaluate their application in (de)coupled integrated multi-trophic aquaculture (IMTA) systems. Coupled production refers to direct integration of fish and polychaetes within the same (eco)system, while in decoupled production, units can be spatially or functionally separated. For decoupled production, preservation of fish waste is recommended. Although diets contained relatively low polyunsaturated FA (PUFA) levels (5–9% of total FAs), both species were rich in PUFAs (>30% of total FAs) and contained the essential FAs for fish. Feeding *Capitella* sp. the acid-preserved diet enriched its FA profile. Accumulation of PUFAs, de novo synthesis and/or transfer via bacterial biomass could have played a role in the upregulation of PUFA content. Amino acid profiles indicated that these polychaetes contained the amino acids essential for fish. Highest growth for both species was observed when fed fresh feces, whereas preserved diets resulted in negative growth rates for *O. craigsmithi*, suggesting an important role of microbes in polychaete diets. Our results indicate that both species are potential valuable marine products. Given growth rates with different diets, *O. craigsmithi* seems more suitable for integration in coupled systems, while *Capitella* sp. is interesting for both coupled and decoupled integrated systems.

KEY WORDS: Integrated multi-trophic aquaculture · IMTA · *Ophryotrocha craigsmithi* · *Capitella* sp. · Fatty acids · Amino acids · Alternative fish feed ingredients · Specific growth rate

1. INTRODUCTION

Facing the challenge of feeding the growing world population, an important role for aquaculture is foreseen (Duarte et al. 2009, Kobayashi et al. 2015). However, the predicted expansion of the aquaculture sector generates several environmental concerns (Ottinger et al. 2016). For example, discharges

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of metabolic wastes, uneaten feed and feces in cage aquaculture raise concerns about increasing nutrient loads to coastal ecosystems (Mente et al. 2006, Bostock et al. 2010, Holmer 2010). Expansion of the aquaculture sector also contributes to the growing need for high-quality feed (Tacon & Metian 2015). Ingredients traditionally used in fish feed, like fish meal and oil, have become costly and limited, especially fish oil (Gatlin et al. 2007, Froehlich et al. 2018). These traditional fish feed ingredients are rich in the essential n-3 long-chain polyunsaturated fatty acids (PUFAs), which need to be provided in fish diets (Izquierdo 2005, Tocher 2015). Although terrestrial-based plant materials are often suggested as alternative feed ingredients, they lack essential n-3 PUFAs (Miller et al. 2008). Furthermore, amino acid profiles are of importance for alternative resources for fish diets; in particular, the essential amino acids should be well balanced. Terrestrial plant-based alternatives are often deficient in the essential amino acids methionine and lysine, which must be supplemented in aquatic diets (Van Der Meer & Verdegem 1996, Gatlin et al. 2007). This highlights the need for sustainable and high-quality alternatives for fish meal and oil for aquatic diets.

Integrated multi-trophic aquaculture (IMTA) systems are a promising approach to tackle bottlenecks of high nutrient loading and the need for high-quality ingredients. IMTA combines fed culture with extractive culture in such a way that waste produced by the fed culture becomes input for the extractive cultures (Troell et al. 2003, Neori et al. 2004, Chopin 2013). By smart selection of extractive species, IMTA provides opportunities to re-use nutrients in waste products and to simultaneously produce ingredients for fish feed formulation, increasing productivity of the system (Chopin 2013, Hughes & Black 2016). It should be noted that, in Europe, the produced ingredients (i.e. extractive species) cannot be re-used in diets for the same species as the fed species in the IMTA system, due to EU regulations (European Commission 2001).

To date, most research has focused on seaweeds and bivalves as extractive species (Fang et al. 2016, Hughes & Black 2016, Buck et al. 2018). Seaweeds extract inorganic waste nutrients, and filter feeding bivalves are able to extract fine solid waste particles from the water column (Troell et al. 2003, Handå et al. 2012, Wang et al. 2014). However, major ecological concerns are directed to the benthic ecosystem below fish farms, due to the deposition of relatively high quantities of organic waste such as feed and feces (Sarà et al. 2006, Bannister et al. 2016). Extrac-

tive species that convert the solid waste fraction into valuable biomass are less studied.

Polychaetes have been suggested as extractive species in IMTA systems (Tsutsumi et al. 2005, Kinoshita et al. 2008, Brown et al. 2011, Fang et al. 2017), but their potential has not been fully explored. The high level of PUFAs measured in several polychaete species (Marsh et al. 1989, Bischoff et al. 2009, Brown et al. 2011, Salvo et al. 2015) make it interesting to explore the feasibility of using polychaetes to convert waste from fish farms into alternative resources for fish diets. Various opportunistic polychaete species are found in large densities underneath cage cultures in Norwegian fjords and coastal zones, showing their preference for organic-rich conditions (Kutti et al. 2007, Eikje 2013, Bannister et al. 2014). *Capitella* sp. and *Ophryotrocha craigsmithi* were the most abundant polychaetes found under the fish farms visited in the current study, and were therefore selected for the laboratory trials. Both species occur at high densities beneath fish farms (Kutti et al. 2007, Eikje 2013).

Species belonging to the genus *Capitella* are opportunistic, soft-sediment dwellers, classified as non-specialized subsurface deposit feeders, feeding on detritus and the associated microbes on this detritus (Fauchald & Jumars 1979, Tenore 1981, Findlay & Tenore 1982). The pioneering work of Tsutsumi et al. (2005) and Kinoshita et al. (2008) showed that cultivation of *Capitella* sp. underneath net pens in Kusuura Bay (Japan) resulted in rapid growth of the polychaete population and enhanced decomposition of organic matter in the enriched sediments, highlighting the potential of *Capitella* spp. as extractive species for IMTA systems.

Species belonging to the genus *Ophryotrocha* can be found in organically enriched soft sediments (Dahlgren et al. 2001), but the highest densities have been found in the deep sea on whale-falls (Dahlgren et al. 2006, Wiklund et al. 2009a,b, 2012, Salvo et al. 2014) and hard substrates underneath fish farms in Norway and Canada (Murray et al. 2012, Eikje 2013, Salvo et al. 2014). *Ophryotrocha* spp. are often found in communities that form a complex matrix of polychaetes, their mucus, organic waste and chemoautotrophic bacteria (Salvo et al. 2015). Fatty acid (FA) profiles revealed that both organic wastes and bacteria contributed to the diet of *O. cyclops* collected underneath finfish aquaculture sites in Canada (Salvo et al. 2015).

System- and site-specific characteristics determine the production success of extractive species in IMTA. In this study, polychaete species were selected with

potential for cultivation in connection with Atlantic salmon (*Salmo salar*) aquaculture in Norway. Salmon aquaculture is dominated by open-water cage systems where wastes are discharged into the water column and eventually settle on the bottom (Bergheim 2012, Lekang et al. 2016, Clarke & Bostock 2017). Recently, efforts have been made to develop (semi-)enclosed sea farm systems where waste can be collected (Lekang et al. 2016, Klebert et al. 2018). (Semi-)enclosed systems provide opportunities to develop so-called decoupled integrated systems. This term was introduced for aquaponics (Goddek et al. 2016) and refers to systems consisting of different compartments (i.e. fed species and extractive species) which are integrated as separate functional units. For these systems, spatial connection between the fish and extractive cultivation unit is not required, which provides the opportunity to collect organic wastes at the sea farm and cultivate extractive species on land or in separate sea tanks. To keep the diet for the extractive species of sufficient quality in decoupled systems, preserving the organic wastes is recommended, since fish waste degrades quickly (Beristain 2005). Drying and acidification are relatively fast and easy to apply, which is important from a commercial perspective. Both methods are known to inactivate microbial activity (Luckstadt 2008, Betoret et al. 2016) and may therefore interfere with feeding activity of the polychaetes. Acidification is also known to change the content of the diet (Hardy et al. 1984, Özyurt et al. 2016), which may affect feeding activity, but also the body content of the polychaetes. For example, FA profiles of *Capitella* sp. eggs differed depending on the food source of the adults (Marsh et al. 1990). During digestion, FAs are generally released from lipid molecules, but they are not always completely broken down like other nutrients (e.g. proteins). Therefore, FAs can be incorporated and stored in the body tissue in their basic form. This can subsequently result in bioaccumulation of specific FAs and can reflect an animal's diet (Iverson 2009).

Compared to FA profiles, amino acid profiles of an organism are fairly constant. Amino acids are the building blocks of proteins, and their sequence is determined in the genome. Deficiencies of essential amino acids in an animal's diet reduce the rate of protein synthesis but do not lower the level of the amino acids in proteins, resulting in amino acid profiles that are relatively constant (Mente et al. 2002, Sissener 2018). Polychaete amino acid profiles are therefore assumed to remain similar irrespective of diet, but differences between species are to be expected (Limin et al. 2006).

In the current study, we evaluated the production rate and body composition of *Capitella* sp. and *O. craigsmithi* fed with fresh and preserved salmon waste in order to evaluate their application in coupled and decoupled IMTA systems. Growth and survival were determined to evaluate performance. Body composition, with the focus on FA profiles, was measured to evaluate the feasibility of including polychaetes in fish feed. Amino acid profiles of the 2 polychaete species were also measured and compared. Given the impact of drying and acidification on microbial activity and diet content, we hypothesized that feeding fresh salmon waste to *Capitella* sp. and *O. craigsmithi* would result in higher production and higher FA content compared to feeding preserved salmon waste. Due to the lack of data on both polychaete species with respect to their diet preference, possible differences in the response of both species to the dietary treatments were also tested.

2. MATERIALS AND METHODS

2.1. Study site and species

Capitella sp. and *Ophryotrocha craigsmithi* were collected underneath a soft-bottom (125 m depth) and a hard-bottom (140 m depth) commercial Atlantic salmon (*Salmo salar*), respectively, both located in the coastal area of western Norway. *Capitella* sp. was collected using a van Veen grab. To collect *O. craigsmithi*, 3 iron trays (1.2 × 1.2 × 0.1 m with a perforated base to allow water to pass through) covered with different plastic substrate were deployed underneath the salmon farm. Depth of tray deployment varied between 50 and 150 m. After being submerged for 3 wk, these trays where brought to the surface and all polychaetes present in the substrates were collected. Van Veen and benthic tray samples were gently rinsed and polychaetes were collected. Immediately after collection, polychaetes were placed in an aerated tank containing water from 200 m depth, ensuring similar salinity and water quality parameters as their natural habitat. The polychaetes were then transported to the experimental facilities at Austevoll Research Station (Institute of Marine Research, Norway). Two different *Ophryotrocha* species were collected, but in this experiment only the most abundant, *O. craigsmithi,* was included (species determination was confirmed using cytochrome *c* oxidase subunit I barcode data). The genus *Capitella* is represented by a

group of morphologically similar (cryptic), but so far undescribed, species in Norwegian waters. The name 'Capitella capitata' that is often used for capitellids under fish farms around the world (e.g. Hanson & Tenore 1981, Heip 1995) refers to a species described from Greenland (Blake 2009), and since genetic barcode data from the type locality of that species are not available, it is currently not known to what extent this species occurs in Norway. A taxonomic revision of *Capitella* species and other annelids in nutrient-rich habitats such as underneath fish farms, funded by the Norwegian species initiative, is ongoing (T. G. Dahlgren unpubl. data). We have therefore used the name '*Capitella* sp.' for the species investigated in this study.

Polychaetes were acclimatized for 2 d in flow-through, circular tanks receiving filtered (1 µm) seawater (34.8 ± 0.1‰, 8.7 ± 0.2°C) pumped from 200 m depth. During this acclimation period, they were fed with salmon feces which were stored in a freezer (−20°C) and thawed before being fed.

At the end of the experiment, not enough polychaete material could be sampled to perform both FA and amino acid analyses. As amino acid profiles are not expected to be highly affected by the diets given, a new batch of individual *O. craigsmithi* and *Capitella* sp. were collected from the fish farming sites. These animals were not exposed to the experimental treatments and were solely analyzed for amino acid profiles.

2.2. Treatments

A 3 × 2 factorial design was used to evaluate the effect of diet (i.e. fresh, acid-preserved and oven-dried salmon feces) and polychaete species (i.e. *Capitella* sp. and *O. craigsmithi*) on growth, survival and final polychaete body composition (C, N and FAs).

For diet preparation, feces were collected twice a week from salmon (individual weight ca. 2–4 kg) kept at the sea cage facility of Austevoll Research Station. Feces were collected by stripping the fish. Using this method ensured that only feces were collected and not uneaten feed or other particulate matter. After collection, feces were directly centrifuged (6300 × g for 3 min; Eppendorf 5810R), and liquid was carefully removed. This process was repeated twice, after which the solid fraction was homogenized and used to formulate the experimental diets.

The fresh centrifuged feces diet was directly fed to the polychaetes. The acid diet was preserved by adding formic acid (80%) to the centrifuged feces to create a pH <4 (pH 3.4 ± 0.1). The acidified feces were left for 24 h at room temperature, after which excessive liquid was carefully removed. Before feeding, the feces were washed twice with seawater to reduce acidity. Washing was done by adding seawater to the acidified feces, centrifuging for 3 min (6300 × g) and removing leftover liquid. The dried diet was preserved by oven-drying the centrifuged feces for 48 h at 100°C. Due to the difference in time span needed for the preservation of the diets (direct use, 24 h and 48 h), treatments started on 3 successive days, i.e. Day 1: fresh treatment, Day 2: acid treatment and Day 3: dried treatment.

2.3. Experimental design

At the start of the experiment, for each species, individuals of comparable lengths were selected (visually) from the acclimation tanks and divided over the experimental chambers. Simultaneously, polychaetes were collected as start sample to determine initial body weight and body composition (n = 3 × 30 individuals of each species). Polychaetes collected for the start samples were then placed in a tank with clean seawater for 2 d in order to clear their guts. Thereafter, samples were rinsed with deionized water and stored in the freezer (−20°C). Initial body composition and average individual weight were determined for the start samples and used in growth calculations (see Section 2.4).

Capitella sp. (initial weight 1.7 ± 0.1 mg ash-free dry weight [AFDW], mean ± SD) were kept in 1000 ml, cylindrical chambers (n = 66 ind. chamber^{-1}, n = 4 chambers treatment^{-1}). Inflow was at the top of the chamber, and outflow was positioned opposite to the inflow, approximately 1 cm above the bottom of the chamber. Glass marbles (5 mm, VWR) were added to each chamber (approximately 1 cm of the bottom was covered), to mimic natural substrates while providing the opportunity to observe the polychaetes during daily check-ups. Similar experimental chambers were used for *O. craigsmithi* (initial weight 1.9 ± 0.1 mg AFDW), but because of different behaviors (i.e. mucus production, community formation, active swimming in the water column) compared to *Capitella* sp., the design was adapted. Water inflow was positioned at the bottom of the chamber, and the outflow consisted of 2 larger openings (3 cm diameter) placed opposite each other at the top of the chamber and covered with mesh (250 µm) to prevent polychaetes escaping. To mimic natural substrate conditions, a

pre-combusted stone was added to the chamber. To standardize between species, total biomass was kept similar using 50 individuals of *O. craigsmithi* per chamber (n = 4 chambers treatment^{-1}). Flow rate was set to 28 ± 2 ml min^{-1}, and the experimental chambers were kept in the dark during the experiment.

Polychaetes were fed 1 of the 3 experimental diets for a period of 4 wk. This time span was assumed to be long enough to distinguish potentially significant changes in FA profiles (Kirsch et al. 1998, Turner & Rooker 2005). Treatments were randomly assigned to the chambers using the random numbers function ('RAND()') in Microsoft Excel (2016). Animals were fed twice a week. Feed was provided in excess (~1.5 g chamber^{-1} feeding^{-1}), for which the amount was determined based on a pilot study. During the experiment, leftover feed was always observed, confirming that feed was provided in excess. During each feeding, leftover feed was first removed and the chambers were cleaned, after which new feed was added. Flow was terminated for a minimum of 30 min to allow the feed to settle. During each feeding, diet samples were collected from each diet treatment (i.e. fresh, acid or dried feces) (n = 6 samples diet^{-1} feeding^{-1}) and stored in the freezer (–20°C up to 4 wk, following protocols described by Budge et al. 2006) before further analyses.

At the end of the experiment, polychaetes were counted to determine survival. Chambers were cleaned and polychaetes were left for 2 d in clean seawater, enabling them to empty their guts, before the final sample was collected. Sampled polychaetes were rinsed with deionized water and stored in the freezer (similar protocol as above) before further analyses.

2.4. Analytical analyses and calculations

Diet and polychaete samples were freeze-dried and then ground using a bullet mill. For each diet, samples collected over the time span of the experiment were pooled in such a way that each sample contained a replicate from each feeding, resulting in a total of 6 samples per diet for analyses.

2.4.1. Subsampling

Diets and polychaetes were analyzed for dry matter (freeze-dried), ash (550°C for 6 h), gross energy (adiabatic bomb calorimetry; IKA-calorimeter C7000,

ISO 9831), C and N content and FA profiles. Gross energy was expressed in kJ g^{-1} AFDW, and C, N and total FAs were expressed in g kg^{-1} AFDW.

C and N samples were combusted with an element analyzer (Flash 2000, Thermo Fisher) at 1020°C, in the presence of oxygen, to convert C and N to CO_2 and NO_x, respectively. Thereafter, NO_x was reduced to N_2 in a reduction column.

FA profiles were analyzed according to the method described by Meier et al. (2006). Samples were methylated, and the respective FA methyl esters (FAMEs) were analyzed on an HP-7890A gas chromatograph (Agilent) with a flame ionization detector (GC-FID). The FA 19:0 was added as an internal standard. Dry HCl in methanol (2.5 M) was used as a methylation reagent. FAMEs were extracted using 2 × 2 ml of hexane. The extracted hexane was diluted or concentrated to obtain a suitable chromatographic response. One µl was injected splitless (i.e. the split was open after 2 min), and the injection temperature was set to 280°C. The column was a 25 m × 0.25 mm fused silica capillary, coated with polyethylene-glycol of 0.25 µm film thickness, CP-Wax 52 CB (Varian-Chrompack). Helium (99.9999%) was used as mobile phase at 1 ml min^{-1} for 45 min and then increased to 3 ml min^{-1} for 30 min. The temperature of the FID was set at 300°C. The oven temperature was programmed to hold at 90°C for 2 min, increase from 90 to 165°C at 30°C min^{-1} and then to 240°C at 2.5°C min^{-1}, and was then held there for 35 min. Total analysis time was 75 min. Fifty-eight well-defined peaks in the chromatogram were selected and identified by comparing retention times with a FAME standard (GLC-463 from Nu-Chek Prep.) and retention index maps and mass spectral libraries (GC-MS) (www.chrombox.org/index.html) performed under the same chromatographic conditions as the GC-FID (Wasta & Mjøs 2013). Chromatographic peak areas were corrected by empirical response factors calculated from the areas of the GLC-463 mixture. The chromatograms were integrated using the EZChrom Elite software (Agilent Technologies). FAs are given as % of total FAs. Complete FA compositions (g kg^{-1} AFDW) of the 2 polychaete species fed the 3 different diets are provided in Table S1 in the Supplement at www.int-res.com/articles/suppl/q011p221_supp.pdf.

Additionally collected polychaete samples (i.e. not exposed to the experimental treatments, n = 4 × 30 individuals for *Capitella* sp. and n = 3 × 75 individuals for *O. craigsmithi*) were freeze-dried, and amino acid composition was determined after hydrolysis, using an ultra-performance liquid chromatochraphy system as described by Espe et al. (2014).

2.4.2. Calculations

Based on the number of individuals at the start and end of the experiment, survival (%) per chamber was calculated. AFDW of the polychaete samples was used to calculate total biomass gain per chamber. Average individual body weight (mg AFDW) at the start and end of the experiment was determined by dividing the total AFDW of a polychaete sample (either start or end) by the number of individuals in that sample. Specific growth rate (SGR, % d^{-1}) was calculated using the following formula:

$$SGR = \{[\ln(W_f) - \ln(W_i)] / T\} \times 100 \qquad (1)$$

where W_f is the average individual final weight (mg AFDW), W_i is the average individual initial weight (mg AFDW), and T is the number of experimental days.

2.5. Statistical analyses

Statistical analyses were performed in R studio 3.4.0 and PRIMER v6. Prior to statistical analysis, residuals of the data were checked for homogeneity of variance and normality using Q–Q plots and Shapiro-Wilk and Levene tests. One-way ANOVA was used to test differences in composition between diets (C, N, energy, total FAs and FA profiles) and to test differences in growth (biomass gain and SGR) and survival within polychaete species when fed the 3 diets. Two-way ANOVAs were used to test the effect of diet, species and their interaction on polychaete final body composition (N, C, energy, total FAs and FA profiles). Due to the low sample size (n = 2), *O. craigsmithi* fed the dried diet was excluded from the 2-way ANOVA. Additionally, FA classes were compared, using all data, between the 2 polychaete species using a nonparametric Mann-Whitney *U*-test.

When assumptions of homogeneity of variance and normality where violated, data were transformed. If after transformation assumptions of homogeneity of variance and normality were still violated, we used a nonparametric Kruskal-Wallis test. If only the assumption of homogeneity of variance was violated, Welch's ANOVA was used. When the ANOVA tests were significant (p < 0.05), treatments were compared using Tukey's HSD post hoc multiple comparison tests. When results of Welch's ANOVA were significant, treatments were compared by the Games-Howell post hoc test. Significant results found with the non-parametric test were followed by Mann-Whitney *U*-test with Bonferroni correction.

To compare FA profiles of diets and polychaetes fed with these diets, a principal component analyses (PCA) was run on all data using PRIMER v6. Data were not transformed.

3. RESULTS

3.1. Diets

Preservation affected diet composition (Table 1). Compared to the other 2 diets, preserving salmon feces by acidification resulted in a significantly lower AFDW (Mann-Whitney U; p < 0.01), N (Tukey HSD; p < 0.001) and energy (Tukey HSD; p < 0.001) content, while the FA content was significantly higher (Tukey HSD; p < 0.001). Drying the salmon feces at 100°C resulted in a significantly higher energy content (Tukey HSD; p < 0.001), but a lower FA content (Tukey HSD; p < 0.001), compared to the fresh and acid diets. C content did not differ among the 3 diets (Kruskal-Wallis; p = 0.097).

3.2. Growth and survival

The number of individuals in all experimental chambers with *Ophryotrocha craigsmithi* reduced over the experimental period. This was partly the result of mortality, but we cannot rule out that some animals might have escaped. Survival of *O. craigsmithi* was (mean ± SD) 80 ± 6, 75 ± 13 and 48 ± 39% for the fresh, acid-preserved and oven-dried diet, respectively, with no statistical differences between the dietary treatments (Welch's ANOVA; p = 0.36). Despite the reduced number of individuals at the end

Table 1. Composition of the experimental diets, i.e. fresh salmon feces and salmon feces preserved by acidification (formic acid, pH <4) or by oven-drying at 100°C. Values are mean ± SD (n = 6 samples treatment^{-1}). AFDW: ash-free dry weight (g kg^{-1} dry matter); FA: fatty acid. C, N and total FAs are given in g kg^{-1} AFDW, energy is given in kJ g^{-1} AFDW. ***p < 0.001, **p < 0.01, *p < 0.05, ns: not significant. Means within a row lacking a common subscript differ significantly (p < 0.05)

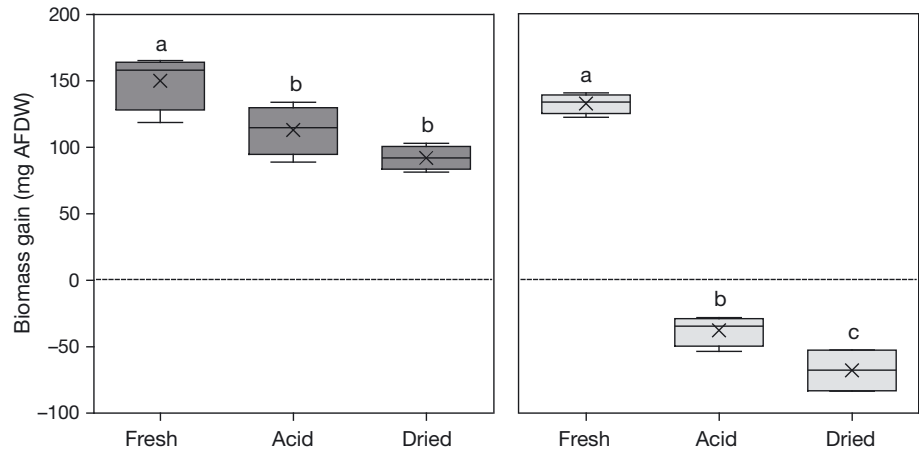|	Fresh	Acid	Dried	Significance
AFDW	777 ± 5[a]	675 ± 47[b]	774 ± 4[a]	**
C	425 ± 12	424 ± 15	441 ± 15	ns
N	24 ± 1[a]	15 ± 2[b]	24 ± 1[a]	***
Energy	18.0 ± 0.3[b]	16.9 ± 0.4[c]	18.5 ± 0.2[a]	***
Total FAs	62 ± 0.4[b]	72 ± 0.6[a]	59 ± 0.5[c]	***

Fig. 1. Biomass gain of (A) *Capitella* sp. and (B) *Ophryotrocha craigsmithi* fed 3 different diets (fresh, acid-preserved and oven-dried). Biomass gain was calculated based on total biomass at the start and end in each experimental chamber. Boxes represent the interval between the lower and the upper quartiles of the distributions. Crosses are the mean values (n = 4 tanks treatment^{-1}). Vertical bars show minimal and maximal values. Boxes lacking a common subscript letter differ significantly (p < 0.05)

of the experimental period, total biomass of *O. craigsmithi* fed the fresh diet increased during the experimental period, resulting in a biomass gain of 133 ± 8 mg AFDW (Fig. 1). Feeding *O. craigsmithi* the preserved diets resulted in a negative biomass gain (mg AFDW), which were both significantly lower compared to *O. craigsmithi* fed the fresh diet (Fig. 1; Tukey HSD; p < 0.001). The same pattern was observed for the SGR; *O. craigsmithi* fed the fresh salmon feces had a significantly (Games-Howell; p < 0.001) higher SGR (mean ± SD; 3.56 ± 0.30% d^{-1}) than *O. craigsmithi* fed the preserved diets (mean ± SD; −0.70 ± 0.32 and −1.02 ± 1.06% d^{-1} for the acid and dried treatments, respectively). The greatest reduction in biomass was observed for *O. craigsmithi* fed the dried diet, which had a significantly lower biomass gain than both other diets (Fig. 1; Tukey HSD; p = 0.001).

In most experimental chambers with *Capitella* sp. (except for 2 chambers), the number of individuals increased, probably due to reproduction, resulting in >100% of individuals present at the end of the experiment compared to the start (respectively 104 ± 6%,

121 ± 8% and 111 ± 9% for the fresh, acid and dried diets). At the end of the experiment, significantly more individuals were present in the chambers fed the acid diet compared to chambers assigned to the fresh treatment (Tukey HSD; p < 0.05). In all *Capitella* sp. treatments, biomass (mg AFDW) increased during the experimental period (Fig. 1). Total biomass gain (mg AFDW) was significantly higher in the fresh treatment compared to both other treatments (Fig. 1; Tukey HSD; p < 0.001). This was also reflected in the significantly higher SGR (Tukey HSD; p < 0.01) for *Capitella* sp. fed the fresh diet (2.56 ± 0.34% d^{-1}), compared to *Capitella* sp. fed the preserved diets (1.59 ± 0.36 and 1.58 ± 0.38% d^{-1} for the acid and dried treatments, respectively). Yet, in contrast to *O. craigsmithi*, there was a net gain of *Capitella* sp. biomass with all diets tested.

3.3. Body composition

The initial and final body composition of *O. craigsmithi* and *Capitella* sp. are shown in Table 2. Except

Table 2. Body composition of the 2 polychaete species (*Ophryotrocha craigsmithi* and *Capitella* sp.) fed 3 different diets (defined in Table 1). Values are mean ± SD. For initial and final body composition, n = 3 and 4 tanks treatment^{-1}, respectively, except for C and N content of *O. craigsmithi* (acid), where n = 3 tanks, and total FAs of *O. craigsmithi* (dried), where n = 2. For some samples, not enough material was available to analyze all body composition parameters. This is indicated by ND (not determined). D: diet; S: species; other abbreviations, units and significance as in Table 1

| | *O. craigsmithi* | | | | *Capitella* sp. | | | | Significance | | |
| | Initial | Final | | | Initial | Final | | | D | S | D*S |
		Fresh	Acid	Dried		Fresh	Acid	Dried			
AFDW	508 ± 84	730 ± 41	705 ± 49	794 ± 69	922 ± 19	902 ± 17	841 ± 26	860 ± 24	*	***	ns
C	396 ± 18	441 ± 14	428 ± 8	ND	469 ± 18	485 ± 9	488 ± 4	484 ± 4	ns	***	ns
N	95 ± 5	112 ± 1[a]	109 ± 3[a]	ND	96 ± 3	99 ± 1[b]	101 ± 3[b]	99 ± 0.3[b]	ns	***	*
C:N ratio	4.2 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	ND	4.9 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	4.9 ± 0.0	ns	***	ns
Energy	ND	23 ± 2	21 ± 2	ND	15 ± 5	22 ± 1	22 ± 0.5	22 ± 0.2	ns	ns	ns
Total FAs	ND	104 ± 11[b]	86 ± 8[b,c]	73	ND	97 ± 11[b,c]	125 ± 4[a]	80 ± 4[c]	***	ns	***

for the energy and total FA content, a species effect was observed for all other parameters (2-way ANOVA; p < 0.001). At the end of the experiment, *Capitella* sp. had a significantly higher AFDW, C and C:N ratio compared to *O. craigsmithi*, while the latter had a significantly higher N content (2-way ANOVA; p < 0.001).

Diets affected AFDW and FA content of the polychaetes (2-way ANOVA; p < 0.05). A significantly lower AFDW content was found when polychaetes were fed the acid diet, compared to polychaetes fed the dried diet (Tukey HSD; p < 0.05). Total FA content of polychaetes fed the dried diet (80 g kg^{-1} AFDW) was significantly lower compared to polychaetes fed the other 2 diets (Tukey HSD; p < 0.05).

An interaction effect between diet and species was found for N and total FA body content (2-way ANOVA; p < 0.05). At the end of the experiment, *O. craigsmithi* fed the fresh or acid diet had a significantly higher N content compared to *Capitella* sp. fed the fresh, acid or dried diet (Tukey HSD; p < 0.05). Total FA content was highest in *Capitella* sp. fed the acid diet (125 g kg^{-1} AFDW), which was significantly higher compared to the other treatments (Tukey HSD; p < 0.05). Interestingly, *O. craigsmithi* fed the acid diet had one of the lowest FA contents (86 g kg^{-1} AFDW), which was significantly lower than the FA content of *Capitella* sp. fed the acid diet (Tukey HSD; p < 0.001). The lowest FA content was observed for *O. craigsmithi* fed the dried diet (73 g kg^{-1} AFDW).

Amino acid analyses showed that both species contained all essential amino acids (Fig. 2). Higher levels of each essential amino acid were observed for *O. craigsmithi* compared to *Capitella* sp. In particular, lysine and isoleucine were high in *O. craigsmithi* (respectively 32.7 ± 0.6 and 23.6 ± 1.2 mg g^{-1} dry matter) (Fig. 2).

3.4. FAs

FA composition of the diets was dominated by saturated FAs (SFAs, >65%), followed by monounsaturated FAs (MUFAs, >25%) (Table 3). PUFAs only had a minor contribution to the total FA composition of the diets (<10%), resulting in a low (<0.15) PUFA: SFA ratio for the diets (Table 3). The main SFAs in the diets were 18:0, 16:0 and 22:0, while MUFAs were dominated by 18:1 (n-9) (Table 3). Preservation of the salmon feces affected the FA profile (Table 3). Fresh salmon feces had a significantly lower SFA content compared to the preserved salmon feces (Table 3; Mann-Whitney *U*; p < 0.01). MUFA and PUFA content, on the other hand, were significantly higher in the fresh feces compared to the preserved feces (Table 3; respectively Mann-Whitney *U* and Games-Howell; p < 0.01). The lowest PUFA content was measured in the feces preserved by acidification, of which both total PUFAs and the n-6 PUFAs were significantly lower than in the fresh and dried feces (Table 3; Games-Howell; p < 0.001).

All FA classes except MUFAs were significantly different between the 2 polychaete species (Fig. 3; Mann-Whitney *U*; p < 0.05). FA composition of *Capitella* sp. was dominated by MUFAs (40 ± 5% of total FAs), followed by PUFAs (30 ± 10% of total FAs) and SFAs (29 ± 5% of total FAs; Fig. 3). *O. craigsmithi* was rich in PUFAs (44 ± 1% of total FAs) and MUFAs (36 ± 2% of total FAs; Fig. 3). Both n-3 and n-6 PUFAs
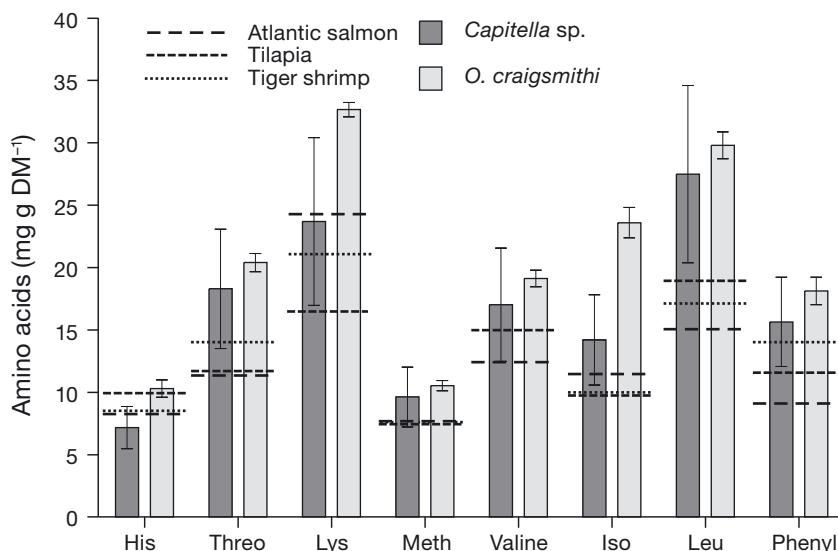

Fig. 2. Essential amino acid body composition of *Capitella* sp. and *Ophryotrocha craigsmithi* measured in this study and essential amino acid requirements for Atlantic salmon, tilapia and tiger shrimp as reported in NRC (2013). Bars are mean values (n = 4 samples for *Capitella* sp. and 3 samples for *O. craigsmithi*); error bars are SD. His: histidine; Threo: threonine; Lys: lysine; Meth: methionine; Iso: isoleucine; Leu: leucine; Phenyl: phenylalanine

Table 3. FA composition (% of total FAs) of the diets (defined in Table 1). Only FAs contributing >1% of the total FAs are considered here, as well as [1]linoleic acid, [2]arachidonic acid, [3]α-linolenic acid, [4]eicosapentaenoic acid and [5]docosahexaenoic acid. All FAs (>0.1% of total FAs) were used to calculate the sum of saturated, monounsaturated and polyunsaturated FAs (SFAs, MUFAs and PUFAs), n-6 and n-3 PUFAs or non-methylene interrupted (NMI) FAs. Values are given as mean ± SD (n = 6 samples diet^{-1}, except for the acid diet where n = 4). Significance as in Table 1

	Fresh	Acid	Dried	Significance
SFAs	67 ± 2[a]	71 ± 1[b]	71 ± 1[b]	**
14:0	2.4 ± 0.2	2.6 ± 0.0	2.5 ± 0.2	ns
16:0	18.6 ± 0.6[b]	20.8 ± 0.2[a]	19.2 ± 0.9[b]	***
18:0	20.9 ± 0.9	21.8 ± 0.3	22.5 ± 0.9	ns
20:0	4.9 ± 0.2	5.2 ± 0.1	5.3 ± 0.2	ns
22:0	18 ± 1	18.4 ± 0.3	20 ± 1	ns
24:0	0.95 ± 0.03[b]	1.04 ± 0.01[a]	1.04 ± 0.02[a]	**
MUFAs	33 ± 2[a]	29 ± 1[b]	29 ± 1[b]	**
18:1 (n-7)	1.33 ± 0.07	1.28 ± 0.03	1.20 ± 0.07	ns
18:1 (n-9)	14 ± 1	13 ± 1	12 ± 0.4	ns
20:1 (n-9)	2.1 ± 0.09[a,b]	2.2 ± 0.04[a]	1.97 ± 0.06[b]	**
22:1 (n-11)	2.89 ± 0.06[a]	2.91 ± 0.05[a]	2.73 ± 0.03[b]	***
24:1 (n-9)	1.17 ± 0.01[c]	1.29 ± 0.02[a]	1.20 ± 0.02[b]	***
PUFAs	9 ± 0.4[a]	5 ± 0.2[c]	6 ± 0.2[b]	***
PUFAs (n-6)	4.7 ± 0.3[a]	2.3 ± 0.1[c]	3.6 ± 0.1[b]	***
18:2 (n-6)[1]	4.3 ± 0.2[a]	2.0 ± 0.2[c]	3.3 ± 0.1[b]	***
20:4 (n-6)[2]	0.07 ± 0.004[a]	0.03 ± 0.005[c]	0.04 ± 0.003[b]	***
PUFAs (n-3)	3.6 ± 0.1[a]	2.3 ± 0.1[b]	2.4 ± 0.1[b]	***
18:3 (n-3)[3]	0.8 ± 0.08[a]	0.3 ± 0.02[c]	0.4 ± 0.02[b]	***
20:5 (n-3)[4]	0.6 ± 0.03[a]	0.4 ± 0.01[b]	0.4 ± 0.02[b]	***
22:6 (n-3)[5]	0.8 ± 0.01[a]	0.4 ± 0.02[b]	0.4 ± 0.02[b]	***
NMI FAs	0.4 ± 0.02[a]	0.4 ± 0.01[a]	0.4 ± 0.03[b]	**
PUFA:SFA	0.13 ± 0.009[a]	0.07 ± 0.004[c]	0.09 ± 0.004[b]	***
DHA:EPA	1.32 ± 0.09[a]	1.19 ± 0.02[b]	1.18 ± 0.04[b]	*

were significantly higher in *O. craigsmithi* than in *Capitella* sp. (Mann-Whitney *U*; p < 0.01). The difference in FA classes between the polychaete species is also reflected in their PUFA:SFA ratio, which was significantly higher for *O. craigsmithi* (Mann-Whitney *U*; p < 0.001).

An interaction effect between species and diet was found for all FA classes (Table 4; 2-way ANOVA; p < 0.05). This interaction effect revealed that except for non-methylene interrupted (NMI) FAs, *Capitella* sp. fed the fresh or dried diet significantly differed in FA classes from the other treatments (Table 4; Tukey HSD; p < 0.05); MUFA content was significantly higher (Tukey HSD; p < 0.001), while PUFA content was significantly lower (Tukey HSD; p < 0.001). This was also reflected in the n-3 and n-6 PUFAs, which were both lowest for *Capitella* sp. fed the fresh and dried diets (Tukey HSD; p < 0.001). An interaction effect between diet and species was also observed for the PUFA:SFA ratio (2-way ANOVA; p < 0.05). Feeding *Capitella* sp. with the acid diet significantly increased their
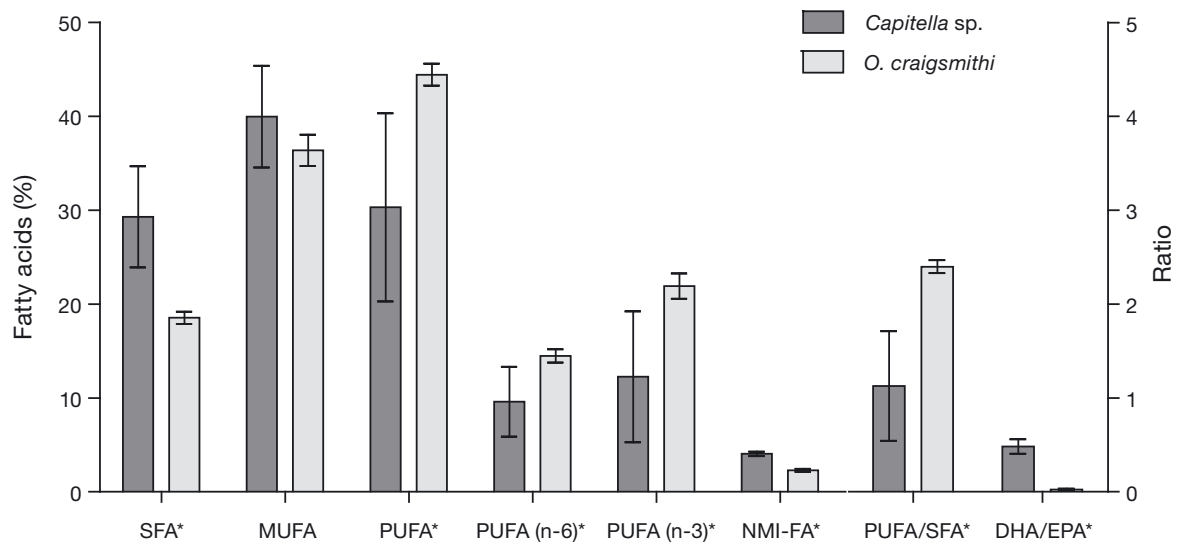

Fig. 3. FA classes (% of total FAs) and ratios (polyunsaturated [PUFA]:saturated FA [SFA] and docosahexaenoic [DHA]:eicosapentaenoic acid [EPA]) per polychaete species (*Capitella* sp. and *Ophryotrocha craigsmithi*). Bars are mean values (n = 12 samples for *Capitella* sp. and 10 samples for *O. craigsmithi*); error bars are SD. FA classes or ratios with an asterisk are significantly different between the 2 polychaete species (p < 0.05). All FAs (>0.1% of total FAs) were used to calculate the sum of SFAs, monounsaturated FAs (MUFAs), PUFAs, n-6 and n-3 PUFAs or non-methylene interrupted (NMI) FAs

Table 4. FA composition (% of total FAs, all defined in Table 3) of the 2 polychaete species (*Capitella* sp. and *Ophryotrocha craigsmithi*) fed with the 3 different diets (defined in Table 1). Values are given as means ± SD (n = 4 tanks treatment^{-1}, except for *O. craigsmithi* [dried], where n = 2). Significance as in Table 1. D: diet; S: species

	Capitella sp.			*O. craigsmithi*			Significance		
	Fresh	Acid	Dried	Fresh	Acid	Dried	D	S	D × S
SFAs	30 ± 3^b	23 ± 1^c	35 ± 2^a	18 ± 0.4^d	19 ± 0.5^d	19	***	***	***
14:0	6.6 ± 0.5^a	4.8 ± 0.4^b	7.3 ± 0.8^a	1.2 ± 0.1^c	1.0 ± 0.1^c	0.96	***	***	**
16:0	13 ± 1^{a,b}	10 ± 1^c	14 ± 1^a	12.6 ± 0.4^b	12.9 ± 0.3^{a,b}	12.9	***	ns	***
18:0	5.0 ± 0.6^b	4.0 ± 0.3^c	6.3 ± 0.3^a	3.2 ± 0.1^c	3.5 ± 0.2^c	4.0	***	***	**
20:0	0.9 ± 0.1^b	0.7 ± 0.1^c	1.1 ± 0.1^a	0.1 ± 0.01^d	0.1 ± 0.02^d	0.2	***	***	*
22:0	1.2 ± 0.6	1.3 ± 0.2	2.4 ± 0.5	0.08 ± 0.03	0.2 ± 0.1	0.4	**	***	ns
MUFAs	45 ± 1^a	33 ± 1^c	42 ± 2^a	38 ± 2^b	36 ± 1^{b,c}	35	***	***	***
16:1 (n-7)	3.2 ± 0.1	2.3 ± 0.2	2.9 ± 0.1	4.1 ± 0.6	4.0 ± 0.7	4.1	*	***	ns
16:1 (n-9)	0.8 ± 0.04^c	0.6 ± 0.04^c	0.7 ± 0.07^c	3.1 ± 0.2^a	3.2 ± 0.1^a	2.8	ns	***	*
18:1 (n-7)	4.2 ± 0.1^b	3.2 ± 0.1^b	4.0 ± 0.2^b	9.7 ± 0.6^a	9.8 ± 0.7^a	10.6	ns	***	*
18:1 (n-9)	20 ± 1^a	14 ± 0.4^c	17 ± 1^b	11 ± 1^d	9 ± 1^d	8	***	***	**
20:1 (n-7)	0.9 ± 0.04^b	0.6 ± 0.02^c	0.8 ± 0.02^b	0.9 ± 0.03^{a,b}	1.0 ± 0.08^a	1.3	***	***	***
20:1 (n-9)	3.7 ± 0.2^a	2.6 ± 0.1^c	3.2 ± 0.1^b	1.0 ± 0.2^d	0.6 ± 0.1^d	0.4	***	***	***
20:1 (n-11)	4.9 ± 0.5^a	3.7 ± 0.1^b	5.4 ± 0.3^a	4.0 ± 0.1^b	4.1 ± 0.2^b	4.6	***	***	***
22:1 (n-9)	0.8 ± 0.02^b	0.5 ± 0.04^c	0.8 ± 0.05^b	1.4 ± 0.1^a	1.6 ± 0.2^a	1.8	ns	***	***
22:1 (n-11)	2.5 ± 0.1^a	1.7 ± 0.1^c	2.2 ± 0.1^b	0.7 ± 0.2^d	0.4 ± 0.04^e	0.3	***	***	***
PUFAs	25 ± 2^b	43 ± 2^a	23 ± 4^b	44 ± 2^a	45 ± 1^a	45	***	***	***
16:4 (n-1)	3.5 ± 0.4^{b,c}	3.1 ± 0.1^c	4.3 ± 0.4^a	3.5 ± 0.4^{b,c}	4.1 ± 0.3^{a,b}	4.2	***	ns	*
18:5 (n-1)	0.7 ± 0.1^c	0.7 ± 0.01^c	0.8 ± 0.1^c	1.9 ± 0.2^b	2.2 ± 0.2^a	2.5	*	***	*
PUFAs (n-6)	8 ± 1^b	14 ± 0.4^a	6 ± 2^b	15 ± 0.5^a	14 ± 0.2^a	16	***	***	***
18:2 (n-6)[1]	4.6 ± 0.8^b	7.8 ± 0.2^a	3.4 ± 1.0^{b,c}	3.2 ± 0.4^c	2.3 ± 0.2^c	2.6	***	***	***
20:2 (n-6)	2.4 ± 0.2^b	3.8 ± 0.1^a	2.0 ± 0.5^{b,c}	2.1 ± 0.4^b	1.4 ± 0.1^c	1.2	***	***	***
20:4 (n-6)[2]	0.5 ± 0.1	1.4 ± 0.1	0.4 ± 0.2	7.7 ± 0.4	8.5 ± 0.2	10.0	***	***	ns
PUFAs (n-3)	8 ± 1^b	21 ± 1^a	7 ± 2^b	22 ± 1^a	23 ± 0.3^a	20	***	***	***
18:3 (n-3)[3]	1.1 ± 0.2^c	2.6 ± 0.2^a	1.0 ± 0.4^b	1.0 ± 0.2^b	0.6 ± 0.1^b	0.4	***	***	***
20:5 (n-3)[4]	3.1 ± 0.2^c	9.4 ± 0.6^b	2.9 ± 1.1^c	18 ± 2^a	20 ± 0.5^a	18	***	***	***
21:5 (n-3)	0.5 ± 0.04^b	1.1 ± 0.1^a	0.5 ± 0.2^b	0.3 ± 0.02^c	0.3 ± 0.02^c	0.3	***	***	***
22:5 (n-3)	0.6 ± 0.1^c	1.6 ± 0.1^a	0.5 ± 0.2^c	0.9 ± 0.1^b	0.9 ± 0.02^b	0.7	***	ns	***
22:6 (n-3)[5]	1.6 ± 0.3^b	5.1 ± 0.4^a	1.2 ± 0.4^{b,c}	0.6 ± 0.1^c	0.5 ± 0.1^c	0.2	***	***	***
NMI FAs	4.1 ± 0.3^{a,b}	3.8 ± 0.1^b	4.2 ± 0.1^a	2.2 ± 0.1^c	2.22 ± 0.02^c	2.6	*	***	*
22:2 NMI	1.8 ± 0.2^a	1.6 ± 0.1^b	1.9 ± 0.1^a	1.0 ± 0.1^c	1.0 ± 0.03^c	1.2	**	***	**
PUFA:SFA	0.8 ± 0.1^c	1.9 ± 0.2^a	0.7 ± 0.2^c	2.4 ± 0.1^b	2.4 ± 0.1^b	2.3	***	***	***
DHA:EPA	0.51 ± 0.06	0.54 ± 0.04	0.39 ± 0.01	0.03 ± 0.007	0.02 ± 0.005	0.01	***	***	ns

PUFA:SFA ratio compared to *Capitella* sp. fed with the fresh or dried diets (Tukey HSD; p < 0.001). The lowest docosahexaenoic (DHA):eicosapentaenoic acid (EPA) ratio was observed in *O. craigsmithi* fed the dried diet (Table 4).

The PCA run on all samples (Fig. 4) separated the polychaete samples from the diet samples. In addition, samples from the 2 polychaete species were also separated from each other (Fig. 4). The first principal component (PC1) explained 81.2% of the sample distribution and was mainly driven by, in order of relative importance, 22:0, 18:0, 20:5 (n-3), 16:0 and 18:1 (n-7). PC2 explained 15.7% of the sample distribution and was driven by 20:5 (n-3), 18:1 (n-9), 20:4 (n-6), 14:0, 22:0, 18:0 and 18:1 (n-7). Furthermore, the PCA showed that FA profiles of *O. craigsmithi* were independent of the diets given, which was not the case for *Capitella* sp. *Capitella* sp. fed the acid diet differed in FA profile from *Capitella* sp. fed the fresh or dried diets. For 8 of the 9 FAs mainly driving the differences between the polychaete species in the PCA, a significant species effect was found (Table 4; 2-way ANOVA; p < 0.05); *Capitella* sp. had a significantly higher content of 14:0, 18:1 (n-9), 18:2 (n-6), 20:2 (n-6) and 22:6 (n-3), while *O. craigsmithi* was richer in 18:1 (n-7), 20:4 (n-6) and 20:5 (n-3). The 2 species did not differ in 16:0 (Table 4; 2-way ANOVA; p = 0.067). Interestingly, for 7 of the 9 FAs mainly driving the difference between species in the PCA, *Capitella* sp. fed the acid diet was significantly different from the other treatments (Table 4, Tukey HSD; p < 0.05).

4. DISCUSSION

This study shows that, although diets fed to the polychaetes contained relatively low levels of PUFAs (5–9%), high levels were observed in the poly-
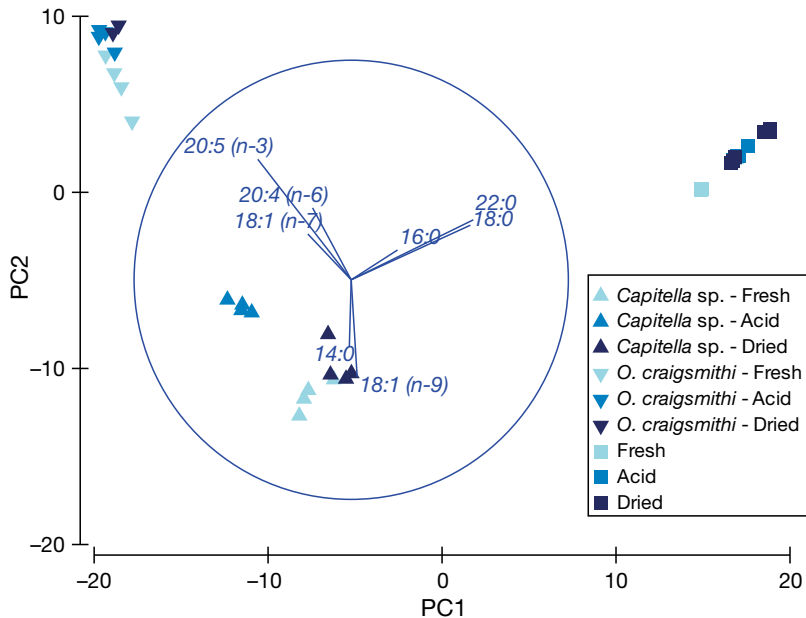
Fig. 4. Principal component analysis (PCA) run with FA profiles of all sample types (see Tables 3 & 4 for FA profiles) including the polychaetes (*Capitella* sp. and *Ophryotrocha craigsmithi*) and the diets (fresh, acid-preserved and oven-dried). PCA was run on untransformed data. Vectors show correlations of >0.2

chaetes (>30%), including the FAs essential for fish. These results indicate the potential for producing high-quality marine resources using *Capitella* sp. and *Ophryotrocha craigsmithi* as extractive species in integrated aquaculture systems. Especially for *O. craigsmithi*, we observed a stable FA profile with high levels of EPA and arachidonic acid (AA). Feeding *Capitella* sp. with acid-preserved salmon feces enhanced their FA profile in terms of essential FAs. Both species showed highest growth when fed fresh salmon feces. These differences in growth and composition between diets and species have implications for application in coupled and decoupled IMTA systems.

4.1. Polychaetes as high-quality marine resources?

O. craigsmithi and *Capitella* sp. used in the current study were rich in PUFAs (>30% of total FAs) and contained the essential FAs for fish, i.e. α-linolenic acid (ALA, 18:3 n-3), linoleic acid (LA, 18:2 n-6), AA (20:4 n-6), EPA (20:5 n-3) and DHA (22:6 n-3), making these polychaetes interesting candidates as alternative ingredients for fish diets. Freshwater fish and salmonids can produce AA, EPA and DHA when the precursors LA and ALA are provided via the diets. For freshwater fish and salmonids, part of their FA requirement can therefore be fulfilled when C_{18} PUFAs are present in the diet, but the C_{20} and C_{22}

PUFAs are often nutritionally more effective. Atlantic salmon and other marine fish have a restricted activity of Δ5 and Δ6 desaturase and elongase and therefore AA, EPA and DHA are required in their diets (Ruyter et al. 2000, Izquierdo 2005, Tocher 2010, 2015). Various alternatives for fish oil have been explored (reviewed by Gatlin et al. 2007). Most alternatives proposed from terrestrial origin do contain ALA and LA, but lack AA, EPA and DHA (Table 5), making them less suitable for diets of marine finfish, except for recent advances in the genetically modified crop *Camelina sativa*, which now has the potential to include EPA and DHA (Betancor et al. 2017) (Table 5). Alternatives proposed from marine origin, including marine polychaetes, often contain EPA and DHA (Table 5), and are therefore interesting for marine finfish diet formulation.

Although both polychaete species contained the FAs essential for fish, a distinctly different FA signature was observed based on the PCA and statistical comparison between the 2 species. It should be noted that statistical comparisons of 2 species are not always desirable, in particular when studying adaptation (Garland & Adolph 1994). In the current study, species were statistically compared with the aim of evaluating their potential as ingredients for aquatic diets. *O. craigsmithi* was richer in both n-3 and n-6 PUFAs. In particular, high levels of EPA (19%) and AA (8.5%) were observed for *O. craigsmithi*. Salvo et al. (2015) also reported high levels of EPA and AA in *O. cyclops* collected underneath aquaculture farms in Newfoundland (Canada), but reported lower values (EPA: <15% and AA: 4%) than observed in the current study. Similar to Salvo et al. (2015), we also found relatively low levels of DHA (0.2–0.6%) for *O. craigsmithi*, resulting in a low DHA:EPA ratio (0.02). The optimal DHA:EPA ratio reported for several marine finfish (e.g. sea bream, sea bass, halibut and turbot) ranges between 1.3 and 2.08 (Rodríguez et al. 1998, Sargent et al. 1999, Xu et al. 2016), but for Atlantic salmon it has been shown that when diets lack DHA, this can be converted from EPA (Bou et al. 2017). In the current study, *Capitella* sp. was 6 times richer in DHA compared to *O. craigsmithi*. The DHA content of *Capitella* sp. was higher in our study compared to that of Marsh et al. (1990), who reported a DHA content of

Table 5. FA composition in % of total FAs (g kg⁻¹ dry matter in parentheses) of fish oil and potential alternatives for fish oil reported in the literature. LA: linoleic acid; ALA: α-linolenic acid; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosa-hexaenoic acid; NI: no information; ND: not detectable

	SFAs	MUFAs	PUFAs (n-6)	PUFAs (n-3)	LA	ALA	AA	EPA	DHA	Ref.
Fish oil[a]	19–31 (170–277)	25–62 (223–554)	1.3–3 (12–27)	12–31 (107–277)	1.1–1.7 (10–15)	0.3–0.8 (3–7)	0.1–1.6 (0.9–14)	5–17 (45–152)	3–13 (27–116)	NRC (2013)
Vegetable oils[a]										
Crude palm	49 (463)	37 (350)	9 (85)	0.2 (1.9)	9 (85)	0.2(1.9)	ND	ND	ND	Turchini et al. (2009)
Soybean	14 (132)	23 (217)	51 (482)	7 (66)	51 (482)	7 (66)	ND	ND	ND	Turchini et al. (2009)
Rapeseed	5 (48)	63 (602)	20 (191)	12 (115)	20 (191)	12(115)	ND	ND	ND	Turchini et al. (2009)
Sunflower	10 (95)	20 (189)	66 (624)	0	66(624)	ND	ND	ND	ND	Turchini et al. (2009)
Genetically modified camelina seed	16	20	31	33	18–21	13–15	2	6–11	5–8	Ruiz-Lopez et al. (2014), Betancor et al. (2017)
Rendered animal products[a]										
Poultry	29 (260)	43 (385)	20 (179)	1 (9)	20(179)	1 (9)	ND	ND	ND	Turchini et al. (2009)
Pork lard	39 (349)	44 (393)	10 (89)	1 (9)	10 (89)	1 (9)	ND	ND	ND	Turchini et al. (2009)
Beef tallow	48 (429)	41 (366)	3 (27)	0.6 (5.4)	3 (27)	0.6(5.4)	ND	ND	ND	Turchini et al. (2009)
Marine microalgae										
Schizochytrium sp.	9	2	18	46	ND	ND	1	0.8	43	Sarker et al. (2016)
Marine macroalgae										
Ascophyllum nodosum	NI	NI	22 (10)	8 (4)	11 (5)	2 (0.7)	10 (5)	4 (2)	ND	Van Ginneken et al. (2011)
Undaria pinnatifida	NI	NI	21 (4)	35 (6)	4 (0.8)	7 (1)	16 (3)	16 (3)	ND	Van Ginneken et al. (2011)
Ulva lactuca	NI	NI	32 (7)	32 (7)	25 (6)	20 (4)	2 (0.4)	1 (0.2)	ND	Van Ginneken et al. (2011)
Polychaetes										
Hediste diversicolor	NI	NI	NI	NI	3 (0.8)	2 (0.5)	5 (1)	39 (11)	4	Bischoff et al. (2009)
Capitella sp.	23–35 (24–26)	33–45 (29–40)	6–14 (4–15)	7–21 (5–22)	3–8 (2–8)	1–3 (0.7–3)	0.5–1 (0.3–1.5)	3–9 (2–10)	1–5 (0.8–5)	This study
Ophryotrocha craigsmithi	18–19 (10–14)	35–38 (19–29)	14–16 (8–11)	20–23 (11–17)	2–3 (1–2)	0.4–1 (0.2–1)	8–10 (5–6)	18–20 (10–14)	0.2–0.5 (0.1–0.5)	This study

[a]For conversion from % of total FAs to g kg⁻¹ dry matter, we used FA concentrations given in CVB (2018)

<1%. Nevertheless, they reported EPA contents of 22%, which is more than 4 times higher than the EPA content of *Capitella* sp. measured in this study (5%). One possible explanation for these differences is that the *Capitella* sp. used by Marsh et al. (1990) and the *Capitella* sp. in our study might be different species.

Diets fed to the polychaetes contained low levels (<1%) of EPA, DHA and AA, while high levels were observed in the polychaetes themselves. Salvo et al. (2015) also observed high proportions of AA, EPA and 20:2 n-6 in the body of *O. cyclops*, while low levels were found in its food sources. To explain this result, Salvo et al. (2015) proposed 3 hypotheses: (1) even though low levels of certain FAs are present in food sources, accumulation in body tissue may result in increased levels measured in the polychaetes; (2) polychaetes contain enzymes which enable de novo synthesis of particular FAs; and (3) these FAs could have been transferred from bacterial biomass. The diets used in the current study did contain EPA, DHA and AA, albeit at low levels, and therefore all 3 hypotheses suggested by Salvo et al. (2015) could have played a role. Nevertheless, the high levels of AA and EPA in *O. craigsmithi* compared with *Capitella* sp. suggests an active enzyme system in *O. craigsmithi* that is able to strongly modify FAs from its diet through elongation and desaturation (Monroig et al. 2013), resulting in an enrichment of the salmon waste by *O. craigsmithi* into essential FAs. De novo synthesis of particular long-chain FAs, like EPA, have been demonstrated for some marine polychaetes, including *Arenicola marina* and *Nereis virens* (García-Alonso et al. 2008, Olive et al. 2009, Pairohakul 2013). In our study, FA profiles of polychaetes at the start of the experiment were lacking, and it is therefore unknown if the poly-

chaetes collected underneath salmon farms may already have had enhanced levels of certain FAs, if de novo synthesis did take place and/or if FAs were taken up via bacterial biomass.

FA profiles of *O. craigsmithi* were less influenced by the diets compared to *Capitella* sp. The multivariate analyses showed that the FA profile of *Capitella* sp. changed when fed with the acid diet; in particular, the amount of long-chain PUFAs increased. From an aquaculture point of view, feeding *Capitella* sp. with acidified salmon feces enhanced its FA profile, since a significant increase in ALA, LA, AA, EPA and DHA was observed. The influence of diet on *Capitella* sp. FA profiles was also shown by Marsh et al. (1990). They reported that FA profiles of *Capitella* sp. eggs could be distinguished based on the FA profile of diets fed to the individuals producing the eggs. Although in the current study a significantly higher total FA content was observed for the acid diet compared to the fresh and dried diets, the essential FAs were similar to the dried diet and even lower than the fresh diet (in terms of % of total FAs). Using formic acid to produce fish silage has also been shown to result in a decrease in PUFAs, including DHA, compared to fresh fish (Özyurt et al. 2016). It remains unclear why essential FAs (in terms of % of total FAs) increased in the *Capitella* sp. fed with the acid diet. Gene expression of the desaturation and elongation genes may give more insights, but was beyond the scope of this study.

4.2. Growth performance of polychaetes

Based on the FA profiles, it can be concluded that both *O. craigsmithi* and *Capitella* sp. are high-quality marine resources. Besides a suitable FA profile, growth performance on fish waste also determines the potential use of these polychaete species in integrated systems. This study shows that both polychaete species can grow on salmon waste. Other polychaete species studied in integrated aquaculture settings that can grow on fish feces are *Nereis virens* (halibut feces) (Brown et al. 2011), *Hediste diversicolor* (eel sludge) (García-Alonso et al. 2008) and *Perinereis nuntia vallata* (flounder feces) (Honda & Kikuchi 2002). Growth rates (SGRs) reported in those studies ranged between 0.45 and 2.09% d^{-1}, which were lower than the highest SGR of *O. craigsmithi* fed fresh salmon feces in our study (3.6% d^{-1}). Given the negative growth rates for *O. craigsmithi* fed the preserved diets, it is questionable if *O. craigsmithi* can be cultivated on salmon feces preserved by drying or by acid-

ification. This has implications for the potential use of *O. craigsmithi* in decoupled integrated aquaculture systems, since preservation of fish waste in these systems is recommended. In contrast, *Capitella* sp. seems to thrive on both preserved and fresh fish waste, as biomass increased in all treatments, although the highest growth rate for this species was also achieved with fresh salmon feces. Successful biomass production of *Capitella* sp. on fish wastes was also shown in a study by Tsutsumi et al. (2005), where *Capitella* sp. was cultivated underneath a fish farm in Kuusura Bay, Japan. The highest biomass production was observed in December and January, during which biomass gain was approximately 40–50 g wet weight m^{-2} mo^{-1}. Converting results of the current study gives a biomass gain of 58 g wet weight m^{-2} mo^{-1} for *Capitella* sp. fed with the fresh diet, indicating that the results of the current study are comparable to biomass production obtained in the field. Interestingly, in our study, reproduction of *Capitella* sp. was observed in all treatments. Members of the genus *Capitella* are opportunistic, and depending on sediment quality they can start reproduction at the age of 4 wk, after which they are known for continuous reproduction (Grassle & Grassle 1976, Levin et al. 1996, Linton & Taghon 2000). They are described as r-strategists, being able to reproduce fast under unstable conditions (Tsutsumi et al. 2005). In our study, highest reproduction was observed in the acid treatment. The acid diet had the lowest quality in terms of N and energy, and this contradicts with studies reporting fast reproduction for *Capitella* sp. in organically enriched sediments (Bridges et al. 1994, Levin et al. 1996, Linton & Taghon 2000). It remains unclear why we observed the highest reproduction of this species in the acid diet.

We had hypothesized that preserving salmon feces would inactivate microbial activity. Although their exact role is not fully understood, there are indications that microbes play a role in the diets of the 2 polychaete species used in the current study (Findlay & Tenore 1982, Wiklund et al. 2009a), and this might be an explanation for the lower performance on preserved waste. The difference in performance between *O. craigsmithi* and *Capitella* sp. may indicate that the role of microbes in the diet of *O. craigsmithi* is more profound. This is supported by Wiklund et al. (2009a) who suggested, based on the P-type jaw structure of *O. craigsmithi*, that microbes, in particular mat-forming large filamentous bacteria, make up an important part of the diet of *O. craigsmithi*. Also, it was shown that underneath fish farms in Canada, part of the diet of *O. cyclops* consists of bacterial mats, but the interaction between polychaete com-

munities, chemoautotrophic bacteria and organic waste underneath fish farms is not fully understood (Salvo et al. 2015). Furthermore, acidification of waste changed the quality of the diet. Although deterioration can be avoided, acidification is also known to break down proteins in short-chain peptides, and free amino acids (Hardy et al. 1984), which could have disappeared from the preserved waste, decreasing the N and energy content of the acid diet. The growth of *Capitella* sp. is related to both organic N and caloric content of diets (Tenore 1983), which may explain the low growth performance of the poly-chaetes. On the other hand, the dried diet had an even higher energy content than the fresh salmon feces, which seems to contradict the lower growth performance. However, the hard, condensed structure of this diet may have made it more difficult to consume by the polychaetes (visual observations).

4.3. Implications for integrated aquaculture

Results of this study demonstrate that *Capitella* sp. and *O. craigsmithi* are promising species for converting fish waste into a valuable product with potential for aquatic diets. To assess the full potential of *Capitella* sp. and *O. craigsmithi* as extractive species in integrated aquaculture, additional aspects should be considered. The current study focused on FA profiles of the polychaete species, and contributes to the exploration for alternatives for fish oil in aquatic diets. Not only fish oil alternatives, but also fish meal replacers are needed (Tacon & Metian 2015), and it would be interesting to explore if, based on protein levels and amino acid profiles, *Capitella* sp. and *O. craigsmithi* cultivated in integrated systems could be regarded as fish meal alternatives. Amino acid profiles of *Capitella* sp. and *O. craigsmithi* collected under the fish farms indicated that based on the essential amino acid levels, both polychaete species would fulfil the amino acid requirement reported by the National Research Council (NRC 2013) for Atlantic salmon, tilapia *Oreochromis* spp. and tiger shrimp *Penaeus monodon* (Fig. 2). Especially the methionine and lysine content of the polychaete species is of great interest, since these are often the first limiting amino acids reported for plant-based alternatives to replace fish meal in aquatic diets (Van Der Meer & Verdegem 1996, Furuya et al. 2004). The species-to-species ban of the EU (European Commission 2001) prohibits the re-use of polychaetes cultivated on salmon waste for salmon diets. Nevertheless, based on their amino acid profiles and FA profiles,

Capitella sp. and *O. craigsmithi* show potential as a high-quality marine resource for other products as well. Next steps in the realization of a polychaete-based aquatic diet should focus on digestibility, attractability and palatability of *Capitella* sp. and *O. craigsmithi* for fish and shrimp.

Technical issues for integrated cultures should also be considered (Fang et al. 2017). To our knowledge, cultivation and harvesting techniques for marine cultivation of polychaetes have not been developed, although some steps have been taken. Jansen et al. (2018) described a pilot study in which benthic cultivation trays were used to enhance and collect *Ophryotrocha* spp. underneath salmon farms.

Besides enhancing the productivity of an aquaculture production system, the other major goal of the IMTA approach is to mitigate wastes produced by the fed cultures via extractive species (Chopin 2013, Hughes & Black 2016). The biomitigation potential of both *Capitella* sp. and *O. craigsmithi* should thus be studied to evaluate the overall efficiency and success as extractive species for IMTA systems.

In conclusion, the presence of essential FAs makes both *Capitella* sp. and *O. craigsmithi* highly valuable as alternative resources for fish feed formulation. In particular, *O. craigsmithi* is an excellent candidate due to its stable FA profile and high levels of EPA and AA. Feeding *Capitella* sp. with acid-preserved fish feces enhanced its FA profile. Potential for incorporation in integrated aquaculture systems is not only based on FA content but also the production (growth) potential of polychaetes fed with fish wastes. Fresh feces is the best diet for both species, suggesting that microbes play an important role in the diet of poly-chaetes. Interestingly, *O. craigsmithi* showed poor growth performance on preserved fish waste, while *Capitella* sp. showed some growth, albeit less compared to the fresh diet. Despite the increased FA profiles for *Capitella* sp. fed on acid diets, growth, and thus production potential, was lower. Overall, *O. craigsmithi* seems suitable for use in integrated open-water systems, where fresh fish feces are continuously supplied to the benthic system, but less for decoupled systems. *Capitella* sp. could be an interesting species for both open-water and decoupled integrated systems, given that growth was observed on all diets.

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