Aquaculture 502 (2019) 232–241

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Growth and nutritional composition of the polychaete *Hediste diversicolor* (OF Müller, 1776) cultivated on waste from land-based salmon smolt aquaculture

Haiqing Wang[a,b,*], Inka Seekamp[a], Arne Malzahn[b], Andreas Hagemann[b], Ana Karina Carvajal[b], Rasa Slizyte[b], Inger Beate Standal[b], Aleksander Handå[b], Kjell Inge Reitan[a]

[a] Norwegian University of Science and Technology, Department of Biology, 7491 Trondheim, Norway
[b] SINTEF Ocean, 7465 Trondheim, Norway

ARTICLE INFO

Keywords:
Hediste diversicolor
Salmon smolt waste
Nutrient recycling
Growth
Amino acids
Fatty acids
Feed ingredients

ABSTRACT

Increased aquaculture production will result in increased amounts of waste produced, and these wastes are highly underexploited. Polychaetes might effectively consume such waste and transfer them into compounds as marine proteins and lipids. In our study, growth and nutritional composition of the polychaete worm *Hediste diversicolor* (O.F. Müller, 1776) were analyzed to evaluate i) the capability of *H. diversicolor* to utilize waste from land-based salmon smolt farms for growth and ii) the nutritional composition of *H. diversicolor* cultivated on salmon smolt waste. The worms were fed iso-carbonic diets comprised of fish feed, smolt waste, microalgae paste, and a 1:5 mixture (based on carbon content) of microalgae paste and smolt waste for a period of 30 days. *H. diversicolor* reared on fish feed grew significantly faster (wet weight basis) than worms grown on the other diets (SGR = 0.025 d^{-1}). Worms fed with the mixture of smolt waste and microalgae showed the lowest growth (SGR = 0.003 d^{-1}), while no significant differences were found between worms cultivated on smolt waste and microalgae paste (0.012 d^{-1}*vs.* 0.014 d^{-1}, respectively). The lipid content in *H. diversicolor* ranged between 12 and 16% of DW for all treatments, whereof approximately 45% of the total fatty acids were comprised of polyunsaturated fatty acids (PUFA). Palmitic acid (C16:0) and eicosapentaenoic acid (C20:5 *n*-3; EPA) were found to be the most abundant fatty acids in the worms. Docosahexaenoic acid (C20:6 *n*-3; DHA) content increased significantly from 1.5% to 4.6–7.8% of the total fatty acids during the experiment for all treatments. The protein content ranged between 54 and 58% of DW, and the most abundant essential amino acids (EAA) were found to be lysine and leucine. We calculated that potential polychaete biomass produced *via* recycling smolt waste nutrients will account for 8% of smolt production, indicating that *H. diversicolor* can not only be successfully reared on waste sludge from land-based salmon smolt aquaculture, but they also contain high valuable compounds, and therefore can help to increase the protein and lipid availability meanwhile decreasing the environmental impact from aquaculture activities.

1. Introduction

Aquaculture is the world's fastest growing food production sector and further growth is needed to meet the dietary requirements of the human population (FAO, 2017). In 2016, 47% of fish consumed globally came from aquaculture, and this share is expected to exceed 57% by 2025 (FAO, 2017). Along with a production increase, the demand for aquafeed also increases. Marine fish depend on a rich supply of omega-3 fatty acids, which are usually of marine origin and harvested in the form of forage fish being processed to fish meal and fish oil (Tacon and Metian, 2015; Cashion et al., 2017). As most wild fish stocks are already overexploited (Pauly and Zeller, 2016), relying on natural sources of marine feed ingredients will always be linked to fluctuating availability. To overcome such fluctuations, searching for more sustainable feed ingredients have received a lot attention in recent decades. Land-based materials such as soybean, maize and oilseed cakes are widely used as cheaper substitutes for fishmeal and fish oil (Fournier et al., 2004; Montero et al., 2005; Alam et al., 2018). However, these plant-derived ingredients lack essential amino acids and fatty acids, and contain antinutritional factors such as protease inhibitors, phytates, tannins, alkaloids and antivitamins, which can have adverse effects on fish growth and health (Francis et al., 2001).

* Corresponding author at: Norwegian University of Science and Technology and SINTEF Ocean, 7010, Trondheim, Norway.
E-mail address: haiqing.wang@ntnu.no (H. Wang).

https://doi.org/10.1016/j.aquaculture.2018.12.047
Received 9 October 2018; Received in revised form 15 December 2018; Accepted 15 December 2018
Available online 17 December 2018

Polychaete worms of the family Nereididae contain high levels of proteins and *n*-3 long-chain polyunsaturated fatty acids, which are essential ingredients in aquafeeds (Narciso and da Fonseca, 2000; Bischoff et al., 2009; Brown et al., 2011; Santos et al., 2016). Polychaetes are considered to have a well-balanced nutritional profile for use as fish broodstock diets (Palmer et al., 2014) and is an indispensable maturation supplement for fish and shrimp broodstocks (Norambuena et al., 2012; Leelatanawit et al., 2014). In addition to an adequate nutritional quality, using polychaetes as feed can have supplementary advantages as demonstrated in a study by Barata et al. (2007), that showed that amino acids and other odorants released by the ragworm *H. diversicolor* can attract feeding of the Senegalese sole (*Solea senegalensis*). Leelatanawit et al. (2014) demonstrated that male shrimps (*Panaeus monodon*) exclusively fed with the polychaete *Perinereis nuntia* had higher survival, growth and sperm performance than those fed with commercially available brood stock diets.

There is a high potential for intensive production of *H. diversicolor* reared on waste streams from land based aquaculture due to its natural, coprophagous feeding behavior. It has been shown that *H. diversicolor* can feed on fecal pellets from crustacean *Euphausiids* and copepods (Uttal and Buck, 1996), carpet shell clam (Batista et al., 2003), mysid crustaceans (Bradshaw et al., 1990), and fish faeces (Brown et al., 2011), but also on detritus and plant materials (Olivier et al., 1996),. Several studies have shown that polychaetes maintained in polychaete assisted sand filters (PASF) can efficiently handle sludge from land based aquaculture and simultaneously gain biomass (Palmer, 2008; Palmer et al., 2014; Fang et al., 2016; Marques et al., 2017). Palmer et al. (2014) showed that the nutritional composition of *Perinereis helleri* grown on wastes from prawn ponds was well-balanced in terms of amino acid, fatty acid, vitamin and mineral profiles. Pajand et al. (2017) demonstrated that *H. diversicolor* had great growth potential, and a favorable fatty acid profile, when integrated in culture with European sturgeon (*Huso huso*). *H. diversicolor* have a predominant metabolic rate, energy demand and uptake rate as their small body mass (Norkko et al., 2013), and moreover, they can reach the commercial size (10–12 cm) and maturation around four months in high density under control environment (Nesto et al., 2012). Accordingly, integrating polychaete production with finfish aquaculture, to exploit waste streams and convert it to valuable biomass, has a large potential.

This study aimed to evaluate the potential of utilizing waste from land-based salmon smolt aquaculture as feed for *H. diversicolor,* and assess worm production. We used three different types of feed for our experiment: 1) salmon smolt sludge waste, as the recycling potential of the worms was the main focus of the study, 2) fish feed, as this is the high quality component of salmon sludge waste, before fish digestion, and 3) microalgae paste, as this is a lipid and protein rich diet which mimics a more natural feeding situation, as microalgae form a good part of the detritus at times in nature. In order to test for deficiencies of salmon smolt sludge as the sole source of nutrition for *H. diversicolor*, we mixed 20% of microalgae paste to the sludge. The worms were analyzed for growth, lipid content and fatty acid composition, protein content and amino acid composition. The hypothesis was that *H. diversicolor* gain biomass by consuming waste from land-based aquaculture and convert to high value biomass suitable for use in aquafeeds.

2. Material and methods

2.1. Collection and maintenance of H. diversicolor

The polychaetes (*H. diversicolor*) used in the experiment were collected from intertidal flats of Leangenbukta, Trondheim, Norway (63°26′24.5″N, 10°28′27.7″E) in October 2016 and transferred to the laboratories at SINTEF SeaLab the same day. The worms were acclimated in flow-through tanks ($n = 12$, 52 × 36 × 18 cm W × D × H) with a 10 cm natural sediment layer (collected on-site) for 3 weeks during which time they were fed live microalgae (*Rhodomonas baltica*). The water temperature was increased by 1–2 °C per day until it reached a temperature of 19 °C.

2.2. Feeding experiments

The experiment was conducted in a multi-rack system operated as a flow-through system (XHAB XR3 stand alone, AQUATIC HABITATS®, Pentair Aquatic Eco-Systems Inc., USA) consisting of 20 polycarbonate tanks (16-L, 47 × 26 × 21 cm W × D × H) with density of 275 ind m^{-2}. At the end of the acclimation period, alive and intact polychaetes were selected for the feeding experiment and randomly stocked to the experimental units. We did not select for sex or maturation. Even though the season in which we conducted the experiment falls within the time of previtellogenesis of this species in mid-Norway, we are confident that our light treatment (simulation a summer situation) suppressed further maturation, as it has been shown by Olive (1999). The worms were weighed individually on a balance (Science Education, SE 622, VWR, Italy) after a starvation period of ≥2 h to assure the gut was empty upon weighing. The tanks were filled with a layer of crushed chamotte (8 cm depth, ceramic clay with grain size 0.5–2 mm, Alt for Keramikk AS, Norway) and seawater before the polychaetes ($n = 30$, 0.20 ± 0.06 g wet weight ind.$^{-1}$) were placed in each tank. Salinity, pH and dissolved oxygen levels (% DO) were recorded daily throughout the experiment. Water temperature and salinity was kept constant (19 ± 1 °C, 33 ± 1 ppt salinity), flow rate was set to 6 L $tank^{-1}$ h^{-1} over the course of 8 h day^{-1} (to allow stagnation during feeding) to ensure oxygen level above 70%, and the photoperiod was set to 16 h:8 h light:dark. The tanks were continuously aerated with atmospheric air supplied through an air stone placed at the sediment surface. Dead worms that were visible at the sediment surface were immediately removed from the tanks. The worms were fed four different diets: (1) Ground fish feed (GEMMA Diamond 1.0, Skretting); (2) Smolt waste (from SalMar Settefisk AS, Follafoss) which was centrifuged at 3000 rpm for 10 mins to discard the supernatant. The remaining paste-like sediment was frozen at −20 °C in individual feeding doses and thawed before use; (3) Microalgae paste (Shellfish Diet 1800®, Instant Algae ®, Reed Mariculture, USA) and (4) a mix of microalgae paste and smolt waste at a 1:5 ratio, respectively (Table 1). There were five replicate tanks for each treatment. The fish feed group was fed 3% of the worm biomass (WW) day^{-1}. The other treatments were given the carbon equivalent of the fish feed of their respective diets, which amounted to 37.8% and 21.4% of the worm biomass (WW) for the microalgae paste and smolt waste, respectively. The polychaetes were fed daily for a period of 30 days.

At the end of the experiment, the worms were collected and placed in fresh seawater for 2–4 h for gut clearance (based on observation and weight measure). Thereafter, the worms were weighed, freeze-dried and stored in nitrogen atmosphere at −80 °C for further analysis. Samples of fish feed, smolt waste and microalgae paste were freeze dried and homogenized with a mortar and pestle for lipid and amino acid analysis and sieved through a 1 mm sieve for CHN analysis. In addition, field-collected polychaetes (hereafter referred to as "Wild") and polychaetes sampled during the acclimatization period (hereafter

Table 1
Proximate composition of diets (mean ± SD; g g^{-1} dry weight, n = 2 replicates). Note: the composition of microalgae paste was taken from the technical data of the products except for the carbon.

Diets	Fish feed	Smolt waste	Microalgae paste	Mix (calculated)
Carbon	0.47 ± 0.01	0.29 ± 0.00	0.28 ± 0.0	0.29
Ash	0.11 ± 0.0	0.43 ± 0.02	0.22	0.40
Protein	0.61 ± 0.0	0.25 ± 0.00	0.45	0.28
Lipid	0.15 ± 0.0	0.09 ± 0.02	0.14	0.10
Carbohydrate	0.13 ± 0.0	0.24 ± 0.01	0.17	0.23

Table 2
Specific growth rate (d^{-1}) and proximate nutritional composition (mean ± SD g g^{-1} dry weight, n = 5 replicates, n = 3 for Initial and Wild) of *H. diversicolor* for the different feeding regimes; fish feed, smolt waste, microalgae, mixed diet; and Wild (field-collected), Initial (at the end of the acclimatization period, fed with *Rhodomonas baltica*.). Different superscripts from a to d within rows denote significant ($p < 0.05$) differences in descending order.

	Treatments				Initial	Wild
	Fish feed	Smolt waste	Microalgae	Mix		
SGR (d^{-1})	0.025 ± 0.0^{a}	0.012 ± 0.0^{b}	0.014 ± 0.0^{b}	0.0025 ± 0.0^{c}	–	–
Ash	0.11 ± 0.02	0.13 ± 0.02	0.14 ± 0.02	0.12 ± 0.02	0.11 ± 0.06	–
Carbon	0.46 ± 0.00^{a}	0.44 ± 0.01	0.44 ± 0.01	0.45 ± 0.01	0.43 ± 0.00	0.43 ± 0.01
Nitrogen	0.09 ± 0.00^{b}	0.09 ± 0.00^{ab}	0.09 ± 0.00 ^{ab}	0.09 ± 0.00 ^{ab}	0.10 ± 0.00 ^{ab}	0.10 ± 0.00^{a}
Protein	0.54 ± 0.01^{c}	0.55 ± 0.01^{bc}	0.54 ± 0.01^{c}	0.58 ± 0.01^{ab}	0.60 ± 0.02^{a}	0.60 ± 0.01^{a}
Lipid	0.16 ± 0.01^{a}	0.12 ± 0.01^{bc}	0.14 ± 0.01^{ab}	0.13 ± 0.01^{bc}	0.11 ± 0.08^{c}	0.13 ± 0.01^{bc}
Carbohydrate	0.20 ± 0.01	0.20 ± 0.01	0.18 ± 0.02	0.17 ± 0.02	0.18 ± 0.02	–

referred to as “Initial”) were analyzed for elemental and biochemical composition.

2.3. Elemental and biochemical composition

The elemental and biochemical composition of the polychaetes were assessed by analyzing the content of ash, total carbon (C) and nitrogen (N), total lipid, fatty acid composition, and amino acid profiles. Ash was determined by combusting dry samples at 450 °C in a muffle furnace for 5 h. Total C and N was measured using an elemental analyser (Costech Instruments ECS 4010) using acetanilide (C_8H_9NO) as a known standard. Protein content was calculated by multiplying total N with a factor of 6.25 (Jones, 1941). The carbohydrate content was determined by subtracting ash, protein and fat content from the total dry weight of the samples.

Total lipids were extracted using the method of Bligh and Dyer (1959), while the lipids of the microalgae samples were extracted by the method of Jakobsen et al. (2008). The total lipid was determined gravimetrically by weighing an aliquot of extract in a pre-weighed vial. Fatty acids were esterified to fatty acid methyl esters using BF_3-methanol (Metcalfe et al., 1966; Li et al., 2015), and thereafter analyzed using a gas chromatograph (Agilent Technologies 7890B, USA) with helium carrier gas and WCOT fused-silica capillary column coating CP-wax 52CB (Holger CP7713).

Amino acids composition were determined according to method of Šližytė et al. (2017) using HPLC (Agilent Technologies Infinity 1260) coupled with an online post-column derivatization module (Pinnacle PCX, Pickering laboratories, Mountain View, CA, USA). Freeze-dried samples of 0.05–0.1 g were weighed in test tubes, hydrolyzed in 6 M HCl containing 0.4% mercaptoethanol at 110 °C for 24 h. After hydrolysis, samples were filtered (Whatman glass microfiber filters, grade GF/C, 47 mm) and the pH was adjusted to 2.2 before HPLC analysis.

2.4. Growth of polychaetes

The specific growth rate (SGR, d^{-1}) of the polychaetes was calculated by the formula:

$$\mathrm{SGR} = \frac{[\ln W_t - ln\, W_0]}{t}$$

where W_t is the mean final biomass (g WW), W_0 is the mean initial biomass (g WW) and t is duration of the experiment (d).

2.5. Statistical analysis

IBM SPSS Statistics V25.0 was used for statistical analysis. Data for growth, elemental composition, amino acids and fatty acids profiles were tested for normal distribution using the Kolmogorov–Smirnov test. As percent data has a tendency towards a binomial distribution, all percentage data were transformed using equation: $\mathrm{transf}_{data} = 180/\pi \times \sin^{-1}(\sqrt{data/100})$ before analyses. The nutritional components of the diets and polychaetes were compared using one-way ANOVA. Homogeneity of variance was tested with a Levene's test. If homogeneity of variance was nonsignificant, the Bonferroni method was used for multiple comparisons, otherwise the nonparametric Dunnett's T3 test was used. The statistical significance level was set to $p < 0.05$. Principal component analysis (PCA) was performed to obtain a more integrated interpretation of amino acid and fatty acid profiles among different treatments by using Minitab® v.18 statistical software (Minitab Inc., PA, USA), and correlation matrix was used to standardize the data. A cluster analysis was performed to identify the similarity or dissimilarity between treatments using package “cluster” with K-means method in RStudio 1.1.456.

3. Results

3.1. Growth and proximate composition of the polychaetes

Worms fed with fish feed showed a significantly higher growth rate compared to the other groups. There were no significant differences in SGR between the smolt waste and microalgae treatments, whereas the mixed diet treatment showed the lowest SGR ($p < 0.05$, Table 2). The worms reared on either fish feed, smolt waste or microalgae had lower protein content compared to the Initial and Wild worms ($p < 0.05$). The worms reared on fish feed showed a significantly higher fat content than all other treatments except for the microalgae treatment ($p < 0.05$). There were no significant differences in ash and carbohydrate content between treatments.

3.2. Amino acids

3.2.1. Feeds

The concentration of total amino acids was much higher in the fish feed (358 ± 7.9 mg g DW^{-1}) compared to the smolt waste (117 ± 4.5 mg g DW^{-1}) and microalgae paste (101 ± 2.4 mg g DW^{-1}) (Fig. 1). However, the content of essential amino acids (EAA) in fish feed, smolt waste and microalgae paste was similar at 46.2, 47.5 and 45.4% of the total AA, respectively. Glutamic acid + glutamine, alanine, aspartic acid + asparagine, leucine and proline were the most prevalent amino acids in the three diets. The fish feed contained more lysine and arginine than smolt waste and microalgae. Conversely, phenylalanine was lower in the fish feed than in the other diets.

3.2.2. Polychaetes

The total amino acid content of the worms decreased during the course of the experiment compared to the Initial worms ($p < 0.05$), except for the mixed treatment. The EAA concentration in the polychaetes ranged from 42.1–45.0% of total amino acids between treatments, and all treatments showed a higher EAA content than the Wild (41.8%) and Initial (41.7%) polychaetes (Fig. 1). The most abundant EAA's (% of total AA) were lysine (8.4–10.1%) followed by leucine

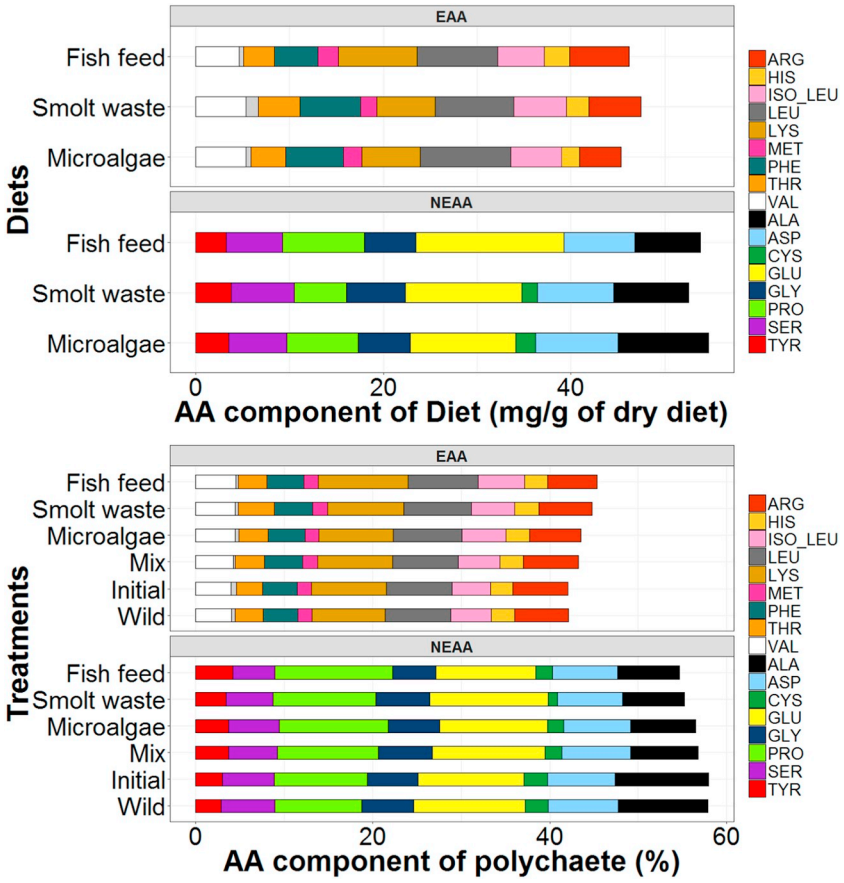

Fig. 1. Amino acid composition (% of total AA) of experimental diets: fish feed (n = 2 replicates), smolt waste (n = 2 replicates) and microalge (n = 3 replicates); and amino acid composition (% of total AA) of *H.diversicolor* under different food regimes: fish feed, smolt waste, microalgae and a mix (n = 5 replicates); and Wild (n = 1 replicates, natural field), Initial (n = 2 replicates, experiment start, fed with *Rhodomonas baltica*). ARG = arginine, HIS = histidine, ISO-LEU = isoleucine, LEU = leucine, LYS = lysine, MET = methionine, PHE = phenylalanine, THR = threonine, TRP = tryptophan, VAL = valine, ALA = alanine, ASP = aspartic acid + asparagine, CYS = cystine (Cys-Cys), GLU = glutamic acid + glutamine, GLY = glycine, PRO = proline, SER = serine, TYR = tyrosine. EAA = essential amoni acids, NEAA = non-essential amino acids.

(7.4–7.9%). The most abundant non-essential amino acids NEAA's (% of total AA) were glutamic acid + glutamine (11.7–13.3%) followed by proline (10.5–13.3) and aspartic acid + asparagine (7.4–7.8).

Initial and Wild worms had similar total AA concentrations as the worms fed with fish feed, but were higher than for the other treatments. The total AA concentrations in worms fed smolt waste, microalgae and mxed diet were 310.8 ± 6.6, 314.5 ± 11.2, and 331.7 ± 9.2 mg g DW^{-1}, respectively, which except for the fish feed treatment was higher than the AA concentrations in the diets alone. Polychaetes fed with fish feed showed the lowest total AA concentration (285.3 ± 11.5 mg g DW^{-1}), which was lower than in the diet. Worms fed with fish feed and smolt waste showed an increased EAA content compared to the Initial worms ($p < 0.05$). Furthermore, worms fed fish feed had a higher content of EAA compared to worms fed microalgae paste and mixed diet ($p < 0.05$). The proportions (% of total AA) of lysine and proline were both higher in the worms than in the diets ($p < 0.05$). The proportions of the amino acids tryptophan, alanine and serine in the worms were generally lower compared to those in their respective diets.

Principal component 1 of the amino acid profiles (PC-1, Fig. 2) explained 51% of the variation, and principal component 2 (PC-2) further explained 27% of the variation, hence, 78% of the variation was explained by the first two components. Worms from the fish feed treatment showed negative PC-1 scores and revealed clear differences in amino acid composition compared to Initial and Wild worms which showed higher, positive PC-1 scores. Polychaetes fed fish feed showed high proline and lysine content, which was distinctively different from Initial and Wild worms that had a high content of serine and alanine (Fig. 2). Worms fed with smolt waste, microalgae and the mixed diet were separated from worms fed fish feed by their higher PC-2 scores, indicating that they had higher percentages of glutamic acid + glutamine and glycine.

3.3. Fatty acids

3.3.1. Feeds

The total fatty acid content was highest in the fish feed and lowest in the smolt waste (Table 3). The diets differed significantly from each other with respect to saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). SFA and MUFA were highest in smolt waste and lowest in the microalgae paste. The highest PUFA content was found for the microalgae paste, followed by fish feed and smolt waste. The fish feed had higher levels of C20:4 *n*-6, C22:5 *n*-3 and C22:6 *n*-3, while the microalgae had higher levels of C14:0, C16:1 *n*-7, C18:3 *n*-3, C18:4 *n*-3 and C20:5 *n*-3. Conversely, the content of most saturated and monounsaturated fatty acids was higher in smolt waste than in the other diets.

3.3.2. Polychaetes

The total lipid content of the worms fed fish feed was similar to the worms fed microalgae, and was significantly higher than for worms fed smolt waste and the mixed diet, as well as the Initial and Wild worms ($p < 0.05$, Table 4).

The total FA content in worms from all treatments were significantly higher than for the Initial and Wild worms, whereof worms fed fish feed showed the highest total FA content. Worms fed microalgae showed significantly higher levels of SFA compared to the other treatments ($p < 0.05$), while for MUFAs the results showed the exact opposite. The PUFA levels in all polychaete samples accounted for 30–50% of the total FA. C16:0 and C20:5 *n*-3 were the most abundant fatty acids, and the levels were nearly the same for all treatments.

The worms fed microalgae paste showed a significantly lower content of linoleic acid (C18:2 *n*-6) than the other treatments, whereas worms fed fish feed and smolt waste showed the highest C18:2 *n*-6 content (6.92% and 6.47%, respectively). The C18:3 *n*-3 level in the worms decreased

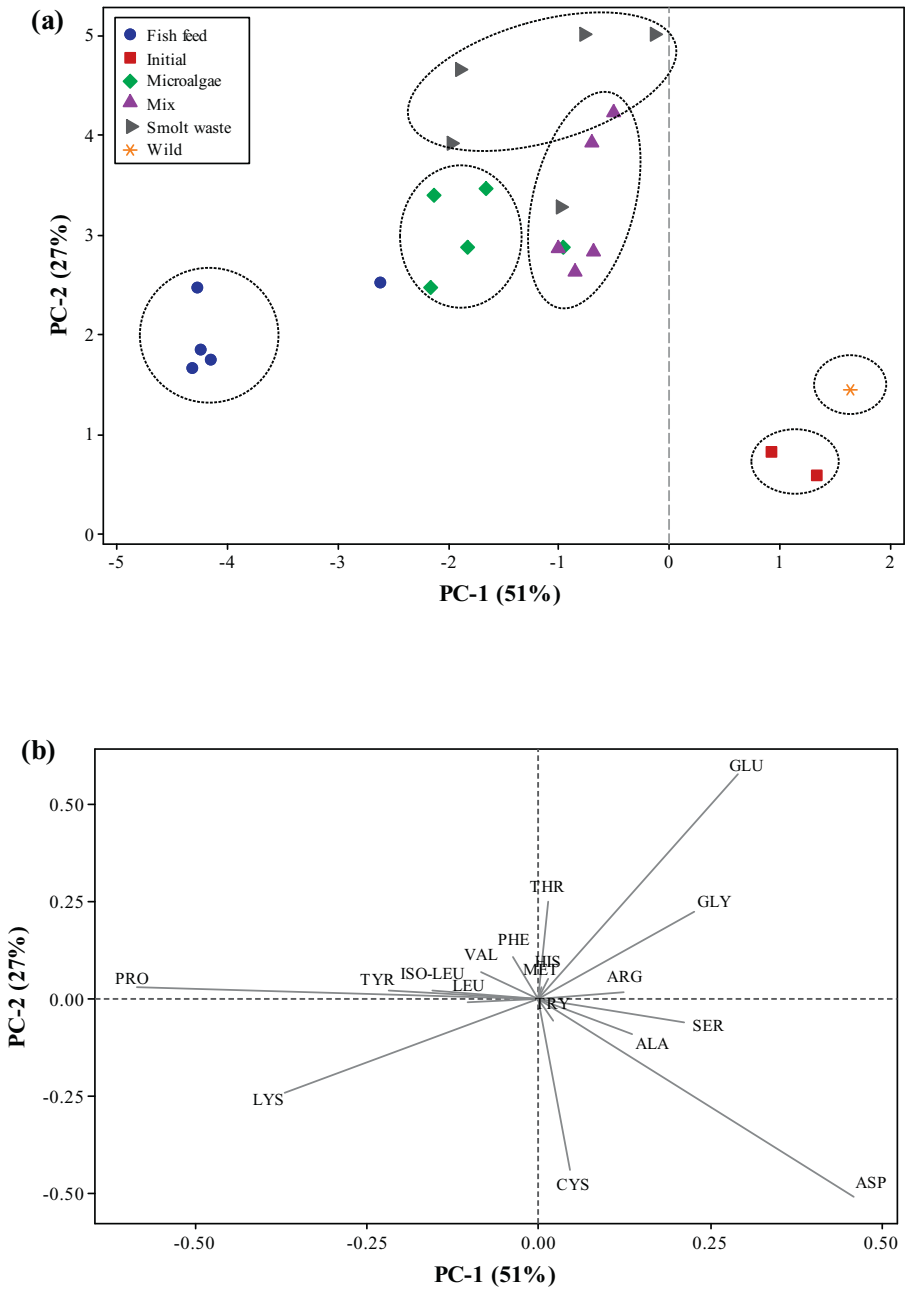

Fig. 2. PCA of amino acid profiles in polychaetes fed different diets: fish feed, smolt waste, microalgae and a mixed diet; Wild (field-collected), Initial (experiment start, fed with *Rhodomonas baltica.*). The upper panels (a) show sample scores plot (a), while the lower panels (b) show factor coefficients plots for the corresponding amino acids' contribution to the scores plot. Numbers at the x (PC-1)- and y-axis (PC-2) are the percentages of variance in amino acid profiles explained by principal components 1 and 2. The treatments were grouped into different clusters with circles.

significantly for all treatments compared to the Initial and Wild worms ($p < 0.05$). The content of C20:4 *n*-6 (ARA) for all treatments were significantly lower than for the Initial and Wild worms ($p < 0.05$), except for the worm fed with smolt waste. EPA (C20:5 *n*-3) content decreased significantly for all treatments compared to Wild worms ($p < 0.05$), apart from the worms fed microalgae. Moreover, the worms that were fed fish feed and smolt waste had lower EPA content than those fed with microalgae and the mixed diet. Worms from all treatments showed an elevated DHA (C22:6 *n*-3) content compared to the Initial and Wild worms (4.64–7.80% *vs.* 1.4–1.5%, respectively; $p < 0.05$), where the highest DHA content was found for worms fed fish feed ($p < 0.05$). The ratio of *n*-3 to *n*-6 varied from 2.3 ± 0.04 to 3.1 ± 0.14 across all treatments, where the highest and lowest ratios were found for worms fed microalgae paste and smolt waste, respectively.

The PCA of the proportion of fatty acids represented a compositional difference in polychaetes with the first two principal components explaining 97% of the variability of the data (Fig. 3). Moreover, worms fed with fish feed and smolt waste were strongly linked, while the characteristics of the mixed diet was closer to the smolt waste group than the microalgae group. Polychaetes fed fish feed was relatively enriched in C18:1 *n*-9, C18:2 *n*-6 and C22:6 *n*-3, while C16:0 and C20:5 *n*-3 were the dominant fatty acids in the Microalgae group (Fig. 3b). The level of C16:0 and EPA was higher in the microalgae group than in the other feeding groups, while conversely lower level of C18:1 *n*-9 and C18:2 *n*-6 were found in the microalgae fed group, separating them from smolt waste and mix groups.

Table 3
Fatty acid content (mg FA g DW^{-1}, means ± SD) and fatty acid composition (% of total FA) of experimental diets, fish feed (n = 2 replicates), smolt waste (n = 2 replicates) and microalgae (n = 3 replicates). SFA = saturated fatty acids, MUFA = monounsaturated FA and PUFA = polyunsaturated FA.

	Fish feed	Smolt waste	Microalgae
Total FA (mg g DW^{-1})	104.72 ± 2.02	47.24 ± 0.63	65.38 ± 3.46
% of total FA			
C14:0	6.34 ± 0.03	5.89 ± 0.04	9.34 ± 0.06
C15:0	0.46 ± 0.00	0.49 ± 0.00	0.47 ± 0.03
C16:0	22.7 ± 0.11	26.99 ± 0.13	15.92 ± 0.08
C18:0	3.94 ± 0.01	5.83 ± 0.03	0.46 ± 0.02
C20:0	0.31 ± 0.00	0.79 ± 0.01	1.01 ± 0.03
ΣSFA	34.68 ± 0.15	40.87 ± 0.15	27.20 ± 0.21
C16:*1 n*-7	6.88 ± 0.04	4.83 ± 0.03	13.47 ± 0.02
C18:1 *n*-9	14.4 ± 0.01	21.1 ± 0.04	6.02 ± 0.05
C18:1 *n*-7	3.25 ± 0.00	3.71 ± 0.01	1.05 ± 0.03
C20:1 *n*-9	2.18 ± 0.01	4.94 ± 0.03	–
C22:1 *n*-9	0.28 ± 0.00	0.94 ± 0.00	–
C24:1	0.55 ± 0.00	1.33 ± 0.03	–
ΣMUFA	27.55 ± 0.04	36.85 ± 0.02	20.54 ± 0.07
C18:2 *n*-6	9.32 ± 0.01	7.85 ± 0.05	4.62 ± 0.02
C18:3 *n*-3	1.90 ± 0.00	2.05 ± 0.00	5.07 ± 0.07
C18:4 *n*-3	1.63 ± 0.00	1.16 ± 0.01	11.91 ± 0.05
C20:4 *n*-6	1.16 ± 0.01	0.48 ± 0.01	0.64 ± 0.01
C20:5 *n*-3	11.15 ± 0.04	3.80 ± 0.03	20.94 ± 0.14
C22:5 *n*-3	1.20 ± 0.00	0.58 ± 0.01	0.17 ± 0.04
C22:6 *n*-3	11.04 ± 0.06	6.23 ± 0.00	8.91 ± 0.14
ΣPUFA	37.77 ± 0.19	22.29 ± 0.13	52.26 ± 0.28
n-3	27.01 ± 0.21	13.82 ± 0.03	47.00 ± 0.29
n-6	10.76 ± 0.02	8.47 ± 0.16	5.26 ± 0.03
n-3/*n*-6	2.51 ± 0.03	1.63 ± 0.03	8.93 ± 0.09
DHA/EPA	0.99 ± 0.00	1.64 ± 0.01	0.43 ± 0.00

4. Discussion

Our study showed that *H. diversicolor* can be reared successfully on salmon smolt waste, and that the worms can convert waste products to high value compounds. *H. diversicolor* showed positive growth in all treatments. The growth rates found in our study ranged from 0.012–0.025 d^{-1} except for the mix group. In comparison, Pajand et al. (2017) reported growth rates of *H. diversicolor* reared on *Huso huso* waste at 23 °C for 8 weeks being around 0.03 d^{-1}. Bischoff (2007) reared *H. diversicolor* on *Dicentrachus labrax* and *Sparus aurata* wastes and reported growth rates of up to 0.02 d^{-1}.

The low growth rates found in this study could have been caused by various reasons, where the quality of the diets is the most obvious one, but environmental and internal, development-related reasons might explain variability in growth rates. The specimens used in this experiment were collected in autumn, which commonly is when the onset maturation processes takes place (Kristensen, 1984). Maturation coincides with decreased growth rates due to the seasonal changes in day-length, food supply, temperature and resource partitioning. *H. diversicolor* has been shown to switch between somatic growth and gametogenic development (Last and Olive, 1999) as a reaction of artificial manipulation of day length. We followed this approach and simulated summer conditions to promote somatic growth and suppress maturation, however, there might still have been a substantial expenditure of energy like nucleolus synthesis during gametogenic development (Olive, 1999; Eckelbarger, 2005). Furthermore, growth rates generally decrease with animal size (Heip and Herman, 1979) and SGR around 0.06–0.07 d^{-1} have only been reported for small, juvenile worms (Nesto et al., 2012).

Food quality, quantity (Nielsen et al., 1995), availability and feeding behaviour (Scaps, 2002) play a major roles in determining growth rates. Video and visual observations during our study showed that *H. diversicolor* tended to stick their head out of their burrows, crawled near their burrows and ingested the feeds immediatley when added, suggesting they were hungry at feeding times. Surface deposit feeding is the preferred behaviour of the two feeding patterns typically observed in this species (Aberson et al., 2016), which is in turn defining territory size. *H. diversicolor* is known to be territorial (Miron et al., 1992; Scaps, 2002), and Reise (1979) reported that the size of an individual worms' territory is around 4 cm^2. Territory size is determined by worm size, as this species usually does not completely leave the burrow to search for food but tend to keep a fair proportion of their tail in the burrow to be prepared for immediate retraction when disturbed. We fed the polychates following an iso-carbonic approach, supplying fish feed at 3% of wet biomass d^{-1} as the baseline. This resulted in different amounts of feed being supplied to the polychaetes. The advantage of this approach is that the same amount of energy (carbon) was available in all treatments, but it also means that the total amount of food, hence the density of food particles at any given location on the sediment offered, differed between the treatments. This might have influenced the accessibility of food in the tanks. A more suited feeding strategy for future industrial applications is feeding *ad libitum*, especially if the aim is high density cultures, where growth rates tend to decrease (Scaps et al., 1993).

Protein is generally the most expensive basal component of fish feed, and finding other protein sources than fish meal stemming from capture fisheries has been in the limelight of research for some years (Francis et al., 2001; Halver and Hardy, 2002; Devic et al., 2018). Producing polychaetes on waste products from fin fish and crustacean aquaculture can be a future prospect to become less dependent of capture fisheries, and at the same time address the waste handling challenges of the aquaculture industry. For protein, *H. diversicolor* in our study showed high protein levels 54–58% in all treatments. In comparison, Pajand et al. (2017) reported a protein content of about 49%, for *H. diversicolor* fed with *H. huso* waste.

Moreover, protein content alone does not determine the quality. The protein quality is defined by the amino acid composition relative to the consumers's requirement. Amino acids serve a whole range of functions in living organisms. Limiting essential amino acids (LEAA) are the most important EAA as they can improve the utilization of other amino acids and reduce oxidation rates (Nunes et al., 2014). In aquaculture of many fishes, methionine, lysine, arginine and threonine are the most limiting dietary ingredients, especially if fishmeal is replaced by high levels of plant protein (Li et al., 2009). The nutritional requirements (% of the diet) of key limiting essential amino acids for Atlantic salmon (*Salmo salar*) have been estimated to be 2.0% for lysine, 0.7% for methionine, 1.1% for threonine and 2–2.2% for arginine (Anderson et al., 1993; Berge et al., 1998; Espe et al., 2008; Nunes et al., 2014). These LEAAs were found to be above or close to these limits in polychaetes fed smolt waste in our study. Compared with EAA content in *H. diversicolor* with dietary EAA requirements of fish and shrimp based on their varying dietary protein requirements (Tacon, 1987), most EAA can meet requirement of EAA content at high dietary protein level (55% of dry diet) for fish and shrimps.

Although the 10 EAA are indispensable for aquafeeds (Kaushik and Seiliez, 2010), some non-essential amino acids like glycine, proline, alanine and glutamic acid function as feed attractants and effectively affect behavior of shrimp and fish (Carr et al., 1996; Nunes et al., 2006). It is showed that *H. diversicolor* can release amino acids which can improve olfactory sensitivity of *S. senegalensis* (Velez et al., 2007). Li et al. (2009) showed that glycine can enhance feed intake and activate osmoregulatory responses; proline can also promote fish feed intake; alanine is essential to purine nucleotide synthesis and a preferred inner-organ carrier of nitrogen to metabolize AA; Glutamine is an important energy substrate in fish. These four amino acids, which are frequently used as feed attractants, accounted for 38% of the total amino acids on average in the polychaetes.

Lipids are used as energy for fish to oxidize amino acids. For fish and shrimp, dietary protein requirement is 24–57% (Tacon, 1987), which needs a general 10–20% of lipid (% of of dry diet) to oxidize these

Table 4
Fatty acid content (mg FA g DW^{-1}, means ± SD) and fatty acid composition (% of total FA) of *H. diversicolor* under different food regimes: fish feed, smolt waste, microalgae, mixed diet (n = 5 replicates); and Wild (n = 2 replicates), Initial (n = 3 replicates). Superscripts from a to d within the rows indicate significant descending concentration ($p < 0.05$). SFA = saturated fatty acids, MUFA = monounsaturated FA and PUFA = polyunsaturated FA Different superscripts from a to d within rows in table indicate significant ($p < 0.05$) differences in descending order.

	Feeding treatments				Initial	Wild
	Fish feed	Smolt waste	Microalgae	Mix		
Total lipid (mg g DW^{-1})	157.4 ± 10.4^{a}	123.6 ± 12.8^{bc}	140.0 ± 7.6^{ab}	131.1 ± 11.2^{bc}	113.7 ± 0.8^{c}	125.5 ± 8.3^{bc}
Total FA (mg g DW^{-1})	73.7 ± 6.2^{a}	56.9 ± 7.8^{b}	56.8 ± 3.2^{b}	53.5 ± 6.0^{bc}	41.16 ± 0.4^{c}	41.57 ± 0.4^{bc}
% of total FA						
C14:0	2.41 ± 0.48^{b}	1.67 ± 0.56^{bc}	3.68 ± 0.27^{a}	1.74 ± 0.21^{bc}	1.21 ± 0.04^{c}	1.86 ± 0.71^{bc}
C15:0	0.49 ± 0.07^{c}	0.60 ± 0.08^{bc}	0.90 ± 0.11^{a}	0.66 ± 0.06^{b}	0.62 ± 0.03^{bc}	0.63 ± 0.04^{bc}
C16:0	20.06 ± 0.99^{bc}	20.8 ± 1.01^{b}	22.96 ± 0.82^{a}	21.08 ± 0.22^{b}	18.57 ± 0.34^{c}	20.01 ± 1.04^{bc}
C17:0	0.70 ± 0.14^{b}	0.88 ± 0.14^{ab}	1.03 ± 0.08^{a}	0.99 ± 0.01^{a}	1.03 ± 0.02^{a}	0.96 ± 0.01^{ab}
C18:0	4.12 ± 0.61^{c}	5.00 ± 0.72^{abc}	4.59 ± 0.12^{b}	5.35 ± 0.12^{ab}	5.72 ± 0.09^{ab}	5.96 ± 0.04^{a}
ΣSFA	27.78 ± 1.29^{bc}	29.47 ± 1.00^{b}	33.16 ± 1.04^{a}	29.82 ± 0.30^{b}	26.97 ± 0.34^{c}	29.43 ± 1.82^{bc}
C16:1 *n*-7	5.29 ± 0.73^{ab}	4.06 ± 1.02^{b}	5.71 ± 0.18^{a}	4.15 ± 0.61^{b}	4.40 ± 0.29^{ab}	4.71 ± 0.10^{ab}
C18:1 *n*-9	11.51 ± 0.34^{a}	10.46 ± 0.81^{ab}	7.05 ± 0.42^{d}	9.54 ± 0.40^{bc}	8.83 ± 0.44^{c}	9.33 ± 0.36^{bc}
C18:1 *n*-7	5.34 ± 0.42^{ab}	5.64 ± 0.17^{a}	4.90 ± 0.16^{b}	5.43 ± 0.35^{ab}	5.43 ± 0.14^{ab}	5.63 ± 0.19^{ab}
C20:1 *n*-9	2.63 ± 0.58^{b}	4.11 ± 0.94^{a}	3.49 ± 0.27^{ab}	4.53 ± 0.19^{a}	3.45 ± 0.18^{ab}	3.66 ± 0.02^{ab}
C22:1 *n*-9	0.26 ± 0.10	0.78 ± 1.02	0.36 ± 0.34	0.95 ± 0.83	0.47 ± 0.81	1.03 ± 0.27
C24:1	0.30 ± 0.12	0.38 ± 0.10	–	0.23 ± 0.22	–	–
ΣMUFA	25.33 ± 0.76^{a}	25.43 ± 0.73^{a}	21.51 ± 0.30^{b}	24.83 ± 0.61^{a}	22.56 ± 0.14^{b}	24.36 ± 0.39^{a}
C18:2 *n*-6	6.92 ± 0.25^{a}	6.47 ± 0.29^{ab}	3.64 ± 0.13^{d}	5.33 ± 0.40^{c}	5.79 ± 0.68^{bc}	5.31 ± 0.31^{c}
C18:3 *n*-3	1.96 ± 0.09^{c}	2.06 ± 0.22^{c}	2.59 ± 0.10^{b}	2.27 ± 0.18^{bc}	3.35 ± 0.11^{a}	3.39 ± 0.10^{a}
C18:4 *n*-3	0.75 ± 0.18^{b}	0.39 ± 0.30^{b}	1.30 ± 0.13^{a}	0.59 ± 0.17^{b}	0.53 ± 0.04^{b}	0.59 ± 0.02^{b}
C20:2 *n*-6	4.86 ± 0.44	4.86 ± 0.44	4.31 ± 0.18	4.99 ± 0.22	4.73 ± 0.24	4.56 ± 0.22
C20:4 *n*-6	2.19 ± 0.53^{c}	3.26 ± 0.63^{abc}	2.29 ± 0.05^{c}	3.07 ± 0.09^{bc}	3.74 ± 0.21^{ab}	4.05 ± 0.08^{a}
C20:3 *n*-3	0.50 ± 0.06^{c}	0.57 ± 0.10^{bc}	0.36 ± 0.33^{c}	0.63 ± 0.06^{b}	0.81 ± 0.03^{a}	0.73 ± 0.02^{ab}
C20:5*n*-3 (EPA)	19.04 ± 0.87^{d}	19.13 ± 0.58^{d}	22.63 ± 0.51^{b}	20.59 ± 0.87^{c}	26.09 ± 0.41^{a}	22.82 ± 0.55^{ab}
C22:5 *n*-3	2.89 ± 0.10^{c}	2.94 ± 0.22^{bc}	3.20 ± 0.15^{bc}	3.23 ± 0.20^{bc}	3.77 ± 0.13^{a}	3.40 ± 0.07^{ab}
C22:6*n*-3 (DHA)	7.80 ± 1.09^{a}	5.43 ± 1.21^{b}	5.02 ± 0.21^{b}	4.64 ± 0.24^{b}	1.47 ± 0.06^{c}	1.36 ± 0.10^{c}
ΣPUFA	46.89 ± 1.64^{b}	45.11 ± 1.18^{b}	45.34 ± 0.9^{b}	45.34 ± 0.82^{b}	50.47 ± 0.47^{a}	46.21 ± 1.43^{b}
Σ *n*-3	32.93 ± 2.07^{ab}	30.52 ± 1.85^{b}	35.1 ± 0.92^{a}	31.95 ± 0.77^{b}	36.12 ± 0.09^{a}	32.28 ± 0.82^{ab}
Σ *n*-6	13.96 ± 0.57^{a}	14.59 ± 0.79^{a}	11.22 ± 0.64^{b}	13.39 ± 0.26^{a}	14.35 ± 0.56^{a}	13.93 ± 0.61^{a}
n-3/*n*-6	2.37 ± 0.23^{b}	2.10 ± 0.25^{b}	3.14 ± 0.14^{a}	2.39 ± 0.07^{b}	2.52 ± 0.11^{b}	2.32 ± 0.04^{b}
DHA/EPA	0.41 ± 0.04^{a}	0.28 ± 0.05^{b}	0.22 ± 0.01^{b}	0.23 ± 0.02^{b}	0.06 ± 0.0^{c}	0.06 ± 0.0^{c}

protein without excessive lipids deposited in their tissues (Halver and Hardy, 2002).

H. diversicolor differed way less in lipid and protein concentrations that the feeds did. This indicates that *H. diversicolor* regulates its' internal energy storage and aims at about 55–60% protein and about 10–15% lipids. *H. diversicolor* is known to be very efficient in removing minute amounts of lipids form their diets (Bradshaw et al., 1990). A relatively low degree in flexibility in protein and lipid concentrations is typically found in animals as compared to plants (Sterner and Elser, 2002). This indicates that *H. diversicolor* can efficiently transfer low-value smolt sludge into high-value products such as feed for fish and shrimp and provide enough energy to oxidize protein without the need of excessive lipid storage.

Essential fatty acids (EFA) as docosahexaenoic acid (DHA; 22:6 *n*-3), eicosapentaenoic acid (EPA; 20:5 *n*-3), arachidonic acid (ARA; 20:4 *n*-6), linolenic- (LNA; 18:3 *n*-3) and linoleic acids (LOA; 18:2 *n*-6), are known to affect animal growth, immune system, gonadal development and the fatty acids metabolism (Glencross, 2009). In particular, the *n*-3 highly unsaturated fatty acids (*n*-3 HUFA's) EPA and DHA, are considered the most valuable EFAs for feed producers globally. The EPA + DHA requirement of most juvenile and pre-adult fishes range between 0.3 and 1.4% of dry diet (Tocher, 2010). In this study, polychaetes fed with smolt waste had EPA + DHA levels around 1.4% of dry diet, hence meeting the requirement of most fish.

H. diversicolor can be extremely efficient in assimilating fatty acids deposited in sediments, which is considered important for the flux of lipids through the food web (Bradshaw et al., 1990). Oxtoby et al. (2016) reported that the surface deposit feeder *Leitoscoloplos pugettensis* consumed EPA from microbial FA pools which is degraded from phytodetritus and incorporated this fatty acid signature into its tissues. In our study, DHA accumulated evidently for all feeding groups, but conversely EPA decreased compared with the Wild worms for all treatments except for the worms fed microalgae paste. This means that *H. diversicolor* can efficiently retain the DHA from the diet. The ARA content of the worms in the smolt waste treatment were similar to the Wild worms, whereas for the other treatments the ARA levels decreased. It has been assumed that ARA and EPA are used as energy source for production of protein and phospholipid compound for egg yolk, thereby affect the larval survival and development (Glencross, 2009). Therefore, the decreasing content of ARA and EPA may indicate that the reproduction process was suppressed as we hypothesized.

Though long chain polyunsaturated fatty acids (LC-PUFA) is the most important in fish oil that can't be replaced by vegetable oil, but it showed that replacement of fish oil with MUFA-rich vegetable oil will not negatively affect the growth performance. MUFA can be used as preferential source of energy for mobilization of tissue than PUFA during starvation (Turchini et al., 2010). MUFA in this study account for 9–12% of the total lipids. Moreover, C20:1n-9 that in fish oil origin from zooplankton consuming fish at high latitude, which can be effectively absorbed by Atlantic salmon, and MUFA with C20:1n-9 and C22:1 *n*-9 can provide more energy than that deficient in C20:1 and C22:1 (Turchini et al., 2010). The C20:1n-9, C22:1 *n*-9 was also found in *H. diversicolor* in this study, which were 2.6–4.5%, and 0.3–1.0% of the total FA in our study.

In this study, feed conversion *ratio (FCR) (g dry faeces used*/g polychaete biomass produced) of worm reared on smolt waste was 3.55. Assuming an FCR for salmon smolt of 1.0 (Aas and Åsgård, 2017), for 1 kg of smolt feed (94% dry matter) used, 1 kg of smolt biomass was

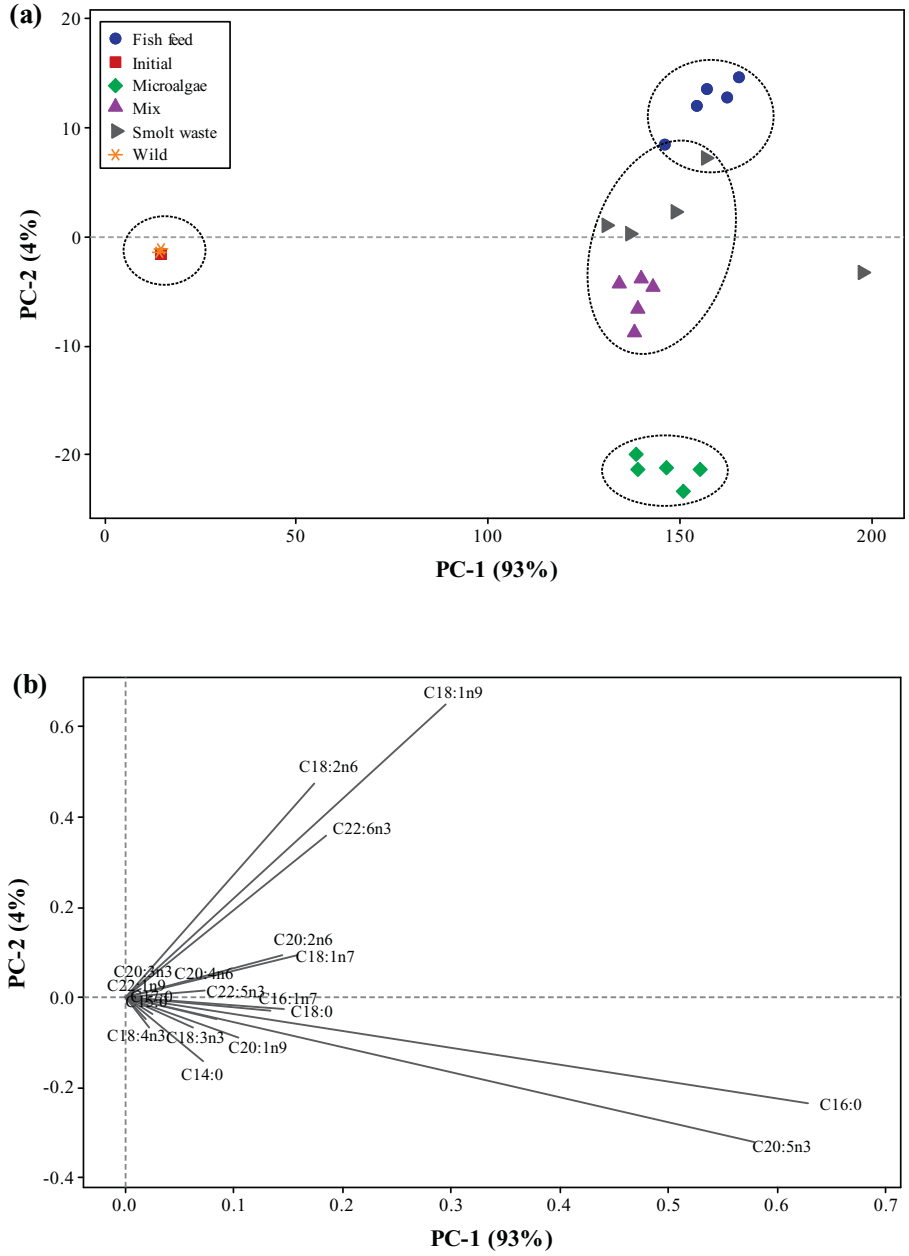

Fig. 3. PCA of fatty acid profiles in polychaete with different food regimes: fish feed, smolt waste, microalgae and a mix; and Wild (natural field), Initial (experiment start, fed with *Rhodomonas sp.*). The upper panel (a) shows factor coefficients plot (a), while the lower panel (b) shows the loading plot for the corresponding fatty acids' contribution to the scores plot. Numbers at the x (PC-1)- and y-axis (PC-2) are the percentages of variance in fatty acid profiles explained by principal components 1 and 2. The treatments were grouped into different clusters with circles.

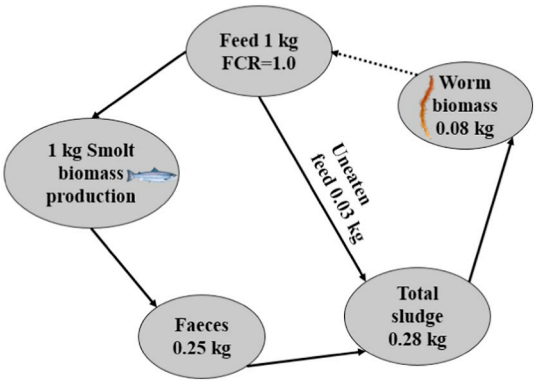

Fig. 4. Estimate biomass production of polychaete with sludge through one smolt of 1 kg production.

produced. According to carbon budget of salmon, 19% of feed carbon (C) will be released as faeces. The C content of faeces was 70% of that of feed (Wang et al., 2012). Therefore, the fecal dry mass production was 0.25 kg. The sludge will be 0.28 kg including 3% feed loss. This sludge can be utilized to produce 0.08 kg of polychaete biomass (WW) (Fig. 4) meaning that the biomass yield of H. diversicolor will account for 8% of smolt production.

Polychaetes are known to efficiently bioaccumulate minerals like zinc (Zn), copper (Cu), cadmium (Cd), and silver (Ag), and is considered as a suitable bio-monitor for contamination (Ghirardini et al., 1999; Gomes et al., 2013; Aas and Åsgård, 2017). Through comparing the minerals found in *Perinereis helleri* (Palmer et al., 2014) with the nutritional requirement of fish and shrimp (Tacon, 1987), such as phosphorus (P), calcium (Ca), manganese (Mn) and Cu that were near lower boundary of minerals typically found in fishmeal. Therefore, the

ability of polychaetes to recycle phosphorus, calcium, manganese, copper, and other mineral elements from fish sludge that can transfer these minerals to fish feed, and if the bioaccumulation of heavy metals such as Cd, lead (Pb) up to toxic concentration may need to be further studied.

5. Conclusion

The results of this study demonstrated that *H. diversicolor* can be reared on waste sludge from land-based salmon smolt aquaculture, and that polychaetes can utilize excess nutrients contained in sludge that are normally lost to the external environment. Protein and lipid contents meeting the dietary requirement of fishes and shrimps, and balanced amino acid profiles, except for a low content of methionine, suggests that polychaetes should be considered as an alternative feed source that could partly replace fishmeal and fish oil. The results indicate that cultivation of polychaetes on wastes from land-based salmon smolt aquaculture has the potential to increase sustainability in fish aquaculture through a more efficient use of globally limited feed resources.

Acknowledgement

The work was part of the Strategic Institute Project LOWTROPHIC: *Production and processing* of n*ew marine ingredients from low trophic species* at SINTEF OCEAN and the Research Council of Norway project POLYCHAETE (280836). The experiment was carried out within the framework of the research infrastructure Norwegian Center for Plankton Technology (245937/F50). The authors are grateful to Jan Ove Evjemo, Åsmund Johansen and Kjersti Rennan Dahl for their kind help. Haiqing Wang acknowledges financial support from the China Scholarship Council.

References

Aas, T.S., Åsgård, T.E., 2017. Estimated content of nutrients and energy in feed spill and faeces in Norwegian salmon culture. In: Nofima rapportserie.

Aberson, M.J.R., Bolam, S.G., Hughes, R.G., 2016. The effect of sewage pollution on the feeding behaviour and diet of *Hediste* (*Nereis diversicolor* (OF Muller, 1776)) in three estuaries in south-east England, with implications for saltmarsh erosion. Mar. Pollut. Bull. 105, 150–160.

Alam, M., Watanabe, W., Carroll, P., Gabel, J., Corum, M., Seaton, P., Wedegaertner, T., Rathore, K., Dowd, M., 2018. Evaluation of genetically-improved (glandless) and genetically-modified low-gossypol cottonseed meal as alternative protein sources in the diet of juvenile southern flounder *Paralichthys lethostigma* reared in a recirculating aquaculture system. Aquaculture 489, 36–45.

Anderson, J.S., Lall, S.P., Anderson, D.M., McNiven, M.A., 1993. Quantitative dietary lysine requirement of Atlantic salmon (*Salmo salar*) fingerlings. Can. J. Fish. Aquat. Sci. 50, 316–322.

Barata, E.N., Velez, Z., Hubbard, P.C., Hardege, J., Canário, A.V., 2007. The contribution of amino acids to the odour of a prey species in the Senegalese sole (*Solea senegalensis*). Aquaculture 265, 336–342.

Batista, F., E Costa, P.F., Matias, D., Joaquim, S., Massapina, C., Passos, A., Ferreira, P.P., da Fonseca, L.C., 2003. Preliminary results on the growth and survival of the polychaete *Nereis diversicolor* (OF Müller, 1776), when fed with faeces from the carpet shell clam *Ruditapes decussatus* (L., 1758). Bol. Inst. Espanol De Oceanogr. 19, 443–446.

Berge, G.E., Sveier, H., Lied, E., 1998. Nutrition of Atlantic salmon (*Salmo salar*): the requirement and metabolic effect of lysine. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 120, 477–485.

Bischoff, A.A., 2007. Solid Waste Reduction of Closed Recirculated Aquaculture Systems by Secondary Culture of Detritivorous Organisms. Christian-Albrechts-Universität, Kiel, Germany.

Bischoff, A.A., Fink, P., Waller, U., 2009. The fatty acid composition of *Nereis diversicolor* cultured in an integrated recirculated system: possible implications for aquaculture. Aquaculture 296, 271–276.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Bradshaw, S.A., O'Hara, S.C., Corner, E.D., Eglinton, G., 1990. Dietary lipid changes during herbivory and coprophagy by the marine invertebrate *Nereis diversicolor*. J. Mar. Biol. Assoc. U. K. 70, 771–787.

Brown, N., Eddy, S., Plaud, S., 2011. Utilization of waste from a marine recirculating fish culture system as a feed source for the polychaete worm, *Nereis virens*. Aquaculture 322, 177–183.

Carr, W.E., Netherton, I., James, C., Gleeson, R.A., Derby, C.D., 1996. Stimulants of feeding behavior in fish: analyses of tissues of diverse marine organisms. Biol. Bull. 190, 149–160.

Cashion, T., Le Manach, F., Zeller, D., Pauly, D., 2017. Most fish destined for fishmeal production are food-grade fish. Fish Fish. 18, 837–844.

Devic, E., Leschen, W., Murray, F., Little, D.C., 2018. Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets containing Black Soldier Fly (*Hermetia illucens*) larvae meal. Aquac. Nutr. 24, 416–423.

Eckelbarger, K.J., 2005. Oogenesis and oocytes. Hydrobiologia 535, 179–198.

Espe, M., Hevrøy, E.M., Liaset, B., Lemme, A., El-Mowafi, A., 2008. Methionine intake affect hepatic sulphur metabolism in Atlantic salmon, *Salmo salar*. Aquaculture 274, 132–141.

Fang, J.H., Zhang, J.H., Jiang, Z.J., Du, M.R., Liu, Y., Mao, Y.Z., Gao, Y.P., Fang, J.G., 2016. Environmental remediation potential of *Perinereis aibuhitensis* (Polychaeta) based on the effects of temperature and feed types on its carbon and nitrogen budgets. Mar. Biol. Res. 12, 583–594.

FAO, 2017. The State of the World Fisheries and Aquaculture 2016. FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome.

Fournier, V., Huelvan, C., Desbruyeres, E., 2004. Incorporation of a mixture of plant feedstuffs as substitute for fish meal in diets of juvenile turbot (*Psetta maxima*). Aquaculture 236, 451–465.

Francis, G., Makkar, H.P., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197–227.

Ghirardini, A.V., Cavallini, L., Delaney, E., Tagliapietra, D., Ghetti, P., Bettiol, C., Argese, E., 1999. *H. diversicolor*, *N. succinea* and *P. cultrifera* (Polychaeta: Nereididae) as bioaccumulators of cadmium and zinc from sediments: preliminary results in the Venetian lagoon (Italy). Toxicol. Environ. Chem. 71, 457–474.

Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquac. 1, 71–124.

Gomes, T., Gonzalez-Rey, M., Rodríguez-Romero, A., Trombini, C., Riba, I., Blasco, J., Bebianno, M.J., 2013. Biomarkers in *Nereis diversicolor* (Polychaeta: Nereididae) as management tools for environmental assessment on the southwest Iberian coast. Sci. Mar. 77, 69–78.

Halver, J.E., Hardy, R.W., 2002. Fish Nutrition. Academic Press, Amsterdam (an imprint of Elsevier Science).

Heip, C., Herman, R., 1979. Production of *Nereis diversicolor* O. F. Müller (Polychaeta) in a shallow brackish-water pond. Estuar. Coast. Mar. Sci. 8, 297–305. http://www.fao.org/docrep/field/003/ab470e/AB470E02.htm#ch2.4.

Jakobsen, A.N., Aasen, I.M., Josefsen, K.D., Strøm, A.R., 2008. Accumulation of docosahexaenoic acid-rich lipid in thraustochytrid *Aurantiochytrium* sp. strain T66: effects of N and P starvation and O_2 limitation. Appl. Microbiol. Biotechnol. 80, 297–306.

Jones, D.B., 1941. Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Proteins. US Department of Agriculture, Washington, DC.

Kaushik, S.J., Seiliez, I., 2010. Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs. Aquac. Res. 41, 322–332.

Kristensen, E., 1984. Life cycle, growth and production in estuarine populations of the polychaetes *Nereis virens* and *N. diversicolor*. Ecography 7, 249–250.

Last, K., Olive, P., 1999. Photoperiodic control of growth and segment proliferation by *Nereis* (Neanthes) *virens* in relation to state of maturity and season. Mar. Biol. 134, 191–199.

Leelatanawit, R., Uawisetwathana, U., Khudet, J., Klanchui, A., Phomklad, S., Wongtripop, S., Angthoung, P., Jiravanichpaisal, P., Karoonuthaisiri, N., 2014. Effects of polychaetes (*Perinereis nuntia*) on sperm performance of the domesticated black tiger shrimp (*Penaeus monodon*). Aquaculture 433, 266–275.

Li, P., Mai, K., Trushenski, J., Wu, G., 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids 37, 43–53.

Li, K., Kjørsvik, E., Bergvik, M., Olsen, Y., 2015. Manipulation of the fatty acid composition of phosphatidylcholine and phosphatidylethanolamine in rotifers *Brachionus* Nevada and *Brachionus* Cayman. Aquac. Nutr. 21, 85–97.

Marques, B., Calado, R., Lillebø, A.I., 2017. New species for the biomitigation of a super-intensive marine fish farm effluent: combined use of polychaete-assisted sand filters and halophyte aquaponics. Sci. Total Environ. 599, 1922–1928.

Metcalfe, L., Schmitz, A.A., Pelka, J., 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38, 514–515.

Miron, G., Desrosiers, G., Retier, C., 1992. Organization of fighting in the polychaete *Nereis virens* (Sars) and the effects of residency and orientation. Behaviour 121, 20–34.

Montero, D., Robaina, L., Caballero, M., Ginés, R., Izquierdo, M., 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: a time-course study on the effect of a re-feeding period with a 100% fish oil diet. Aquaculture 248, 121–134.

Narciso, L., da Fonseca, L.C., 2000. Growth, survival and fatty acid profile of *Nereis diversicolor* (OF Müller, 1776) fed on six different diets. Bull. Mar. Sci. 67, 337–343.

Nesto, N., Simonini, R., Prevedelli, D., Da Ros, L., 2012. Effects of diet and density on growth, survival and gametogenesis of *Hediste diversicolor* (OF Müller, 1776) (Nereididae, Polychaeta). Aquaculture 362, 1–9.

Nielsen, A.M., Eriksen, N.T., Iversen, J.J.L., Riisgard, H.U., 1995. Feeding, growth and respiration in the polychaetes *Nereis diversicolor* (facultative filter-feeder) and *N. virens* (omnivorous) - a comparative study. Mar. Ecol. Prog. Ser. 125, 149–158.

Norambuena, F., Estevez, A., Bell, G., Carazo, I., Duncan, N., 2012. Proximate and fatty acid compositions in muscle, liver and gonads of wild versus cultured broodstock of Senegalese sole (*Solea senegalensis*). Aquaculture 356, 176–185.

Norkko, A., Villnäs, A., Norkko, J., Valanko, S., Pilditch, C., 2013. Size matters: implications of the loss of large individuals for ecosystem function. Sci. Rep. 3, 2646.
Nunes, A.J., Sá, M.V., Andriola-Neto, F.F., Lemos, D., 2006. Behavioral response to selected feed attractants and stimulants in Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 260, 244–254.
Nunes, A.J., Sá, M.V., Browdy, C.L., Vazquez-Anon, M., 2014. Practical supplementation of shrimp and fish feeds with crystalline amino acids. Aquaculture 431, 20–27.
Olive, P.J.W., 1999. Polychaete aquaculture and polychaete science: a mutual synergism. Hydrobiologia 402, 175–183.
Olivier, M., Desrosiers, G., Caron, A., Retiere, C., 1996. Juvenile growth of the polychaete *Nereis virens* feeding on a range of marine vascular and macroalgal plant sources. Mar. Biol. 125, 693–699.
Oxtoby, L., Budge, S., Iken, K., Brien, D.O., Wooller, M., 2016. Feeding ecologies of key bivalve and polychaete species in the Bering Sea as elucidated by fatty acid and compound-specific stable isotope analyses. Mar. Ecol. Prog. Ser. 557, 161–175.
Pajand, Z.O., Soltani, M., Bahmani, M., Kamali, A., 2017. The role of polychaete *Nereis diversicolor* in bioremediation of wastewater and its growth performance and fatty acid composition in an integrated culture system with *Huso huso* (Linnaeus, 1758). Aquac. Res. 48, 5271–5279.
Palmer, P., 2008. Polychaete-Assisted Sand Filters–Prawn Farm Wastewater Remediation Trial. National landcare programme innovation grant No. 60945, Technical report.
Palmer, P.J., Wang, S., Houlihan, A., Brock, I., 2014. Nutritional status of a nereidid polychaete cultured in sand filters of mariculture wastewater. Aquac. Nutr. 20, 675–691.
Pauly, D., Zeller, D., 2016. Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. Nat. Commun. 7 (ncomms10244).
Reise, K., 1979. Spatial configurations generated by motile benthic polychaetes. Helgoländer Meeresun. 32, 55–72.
Santos, A., Granada, L., Baptista, T., Anjos, C., Simões, T., Tecelão, C., e Costa, P.F., Costa, J.L., Pombo, A., 2016. Effect of three diets on the growth and fatty acid profile of the common ragworm *Hediste diversicolor* (OF Müller, 1776). Aquaculture 465, 37–42.
Scaps, P., 2002. A review of the biology, ecology and potential use of the common ragworm *Hediste diversicolor* (OF Müller) (Annelida: Polychaeta). Hydrobiologia 470, 203–218.
Scaps, P., Retière, C., Desrosiers, G., Miron, G., 1993. Effets de la ration alimentaire, de la densité intraspécifique et des relations entre individus sur la croissance des juvéniles de l'espèce *Nereis diversicolor* (Annelida: Polychaeta). Can. J. Zool. 71, 424–430.
Šližytė, R., Opheim, M., Storrø, I., Sterten, H., 2017. Simple technologies for converting rest raw materials of Atlantic salmon (*Salmo salar*) into high-quality, valuable, and tasty feed ingredients. J. Aquat. Food Product Technol. 26, 604–619.
Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press, United Kingdom.
Tacon, A.G.J., 1987. The Nutrition and Feeding of Farmed Fish and Shrimp-a Training Manual. Food and Agriculture Organization of the United Nations.
Tacon, A.G., Metian, M., 2015. Feed matters: satisfying the feed demand of aquaculture. Rev. Fish. Sci. Aquac. 23, 1–10.
Tocher, D.R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquac. Res. 41, 717–732.
Turchini, G.M., Ng, W.-K., Tocher, D.R., 2010. Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press.
Uttal, L., Buck, K., 1996. Dietary study of the midwater polychaete *Poeobius meseres* in Monterey Bay, California. Oceanogr. Literat. Rev. 125, 333–343.
Velez, Z., Hubbard, P.C., Hardege, J.D., Barata, E.N., Canário, A.V., 2007. The contribution of amino acids to the odour of a prey species in the Senegalese sole (*Solea senegalensis*). Aquaculture 265, 336–342.
Wang, X.X., Olsen, L.M., Reitan, K.I., Olsen, Y., 2012. Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquac. Environ. Interact. 2, 267–283.