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Growth, survival and fatty acids profile of Polychaete, *Nereis diversicolor* (Müller, 1776) cultured using waste water of great sturgeon, *Huso huso* (Linnaeus, 1758), culture at different densities in an integrated farming system

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Abstract

Growth and survival of Polychaete, *Nereis diversicolor* fed on waste water of great sturgeon, *Huso huso* at different densities were studied. The study aimed to assess the efficacy of feeding of *N. diversicolor* with waste water derived from different densities of great sturgeon culture as well as to find a balance between fish density and growth of the worm. The worms (0.2-0.3 kg m^{-2}) with an initial mean weight of 0.05 g were fed for 55 days with waste water of great sturgeon at densities of 2-3, 3-4 and 4-5 kg m^{-2} fish. A group of worms fed with fish feed was considered as the control. The obtained results showed that the biomass and average weight of the harvested worms were significantly higher at the density of 3-4 kg m^{-2} fish than in the other two treatments (p<0.05). Also, worms fed with fish feed (control group) showed higher survival rate and biomass production than the treatments. Some fatty acids were abundant in worms from the treatments, specifically 14:0, 16:0, C18:1n9c, 20:1, 22:1n-9, 18:3n-3, 20:2, 20:5n-3 (EPA) and 22:6n-3 (DHA), but were significantly lower than the control worms. The results demonstrate that production of *N. diversicolor* using fish waste water was highly efficient and can offer a sustainable solution to remove organic load in the aquaculture waste waters. Also, production of *N. diversicolor* via this method as an alternative source of fatty acids and protein for *H. huso* production could achieve multiple aims such as retention of valuable lipids from the *H. huso* waste water.

Keywords: *Huso huso*, *Nereis diversicolor*, Density, Waste water, Integrated aquaculture, Fatty acid

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Introduction

Waste water is one of the main problems in aquaculture sector (Kaspar *et al*., 1988) especially in case of some commercial fish species such as sturgeons. In aquaculture activity all aspects of the nitrogen cycling (Cranford *et al*., 2007) and its impact on the aquatic environment in terms of organic loading and nutrient pollutions are big issues (Kaspar *et al*., 1988). If fish farms effluents is discharged into the environment without treatment, it has undesirable and harmful effects on the environment (Miller and Semmens, 2002; Schulz *et al*., 2003). Thus, nowadays aquaculture is regarded as a potential large-scale polluter of the aquatic environment (Pocklington *et al.,* 1994). However, to date little has been done up to provide a sustainable solution for the treatment of the organic discharge due to the aquaculture activity. Any solution to this problem should ideally convert the original waste into a potentially useful matter. The use of the common rag worm, *Nereis diversicolor*, as a means of wastewater treatment would offer exactly for a reduction of the amounting waste and its conversion into worm biomass.

N. diversicolor feeds on a variety of sources, predominantly grazing algae around the entrance to its burrows (García-Alonso *et al*., 2008) or filter-feeds on suspended organic material (Riisgard, 1994). One of the most important issues that we focused is the cost of doing and to get more income and obtain a valuable by-product simultaneously using fish waste water and uneaten feed by *N. diversicolor* without feed supplement.

N. diversicolor is commonly found in the intertidal zone of estuarine and brackish waters, normally in sediments of medium to high organic content (Kristensen, 1984). Its abundance stretches from the inner Baltic Sea, north-western Europe and north-eastern North America to the Mediterranean, Black and Caspian Sea (Smith, 1977). This widespread abundance in the extreme environment of the intertidal requires *N. diversicolor* to tolerate temperatures ranging from below zero to high summer temperatures, variable salinity from occasionally zero up to hypersaline conditions, and oxygen deficits and presence of sulphide (Smith, 1977). This species has been described as an omnivore (Fidalgo e Costa *et al*., 2000) with high level of adaptability make it a very good candidate to be cultured under laboratory conditions (Smith, 1977). Polychaete worms are highly valuable source as a type of aquaculture feed (Fidalgo e Costa, 2000; Olive, 1999). The importance of *N. diversicolor* in the food chain was demonstrated by recent studies on feeding of *Nereis* to shrimp and fish. The feeding regime resulted in an increased number of eggs per spawn for each female, and increased eggs viability and larval survival in shrimp (Briggs *et al.,* 1993), maturation in cultured shrimps (Cruz *et al.,* 1988) and spawning in hatchery-reared species, e.g. *Solea senegalensis* (Dinis, 1992), *Penaeus kerathurus* (Luis, 1989) and *Penaeus vannani* (Lytle *et al.,* 1990).

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The reason for rapidly increase of these species is mostly due to its important role as a nutrient stimulating gonad maturation and spawning in hatchery-reared species. It also allows brood-stock conditioning for egg production (Olive, 1994). Nereid worms have the potential to supplement fish oil as sources of essential lipid components of feeds, and they are excellent sources of polyunsaturated fatty acids in the industry (Lytle *et al.*, 1990; Fidalgo e Costa *et al.*, 2000; Olive *et al.*, 2000). These fatty acids play an important role in determining brood-stock and larval performance in both cultured marine fish and shrimp (Izquierdo *et al.*, 2001; Wouters *et al.*, 2001).

The great sturgeon, *Huso huso*, is one of the most important species of sturgeons for aquaculture in some regions such as Russia and Iran (Vaciliva *et al*., 2000; Falahatkar *et al*., 2014). The present study was carried out in order to evaluate the survival, growth and feed utilization of *N. diversicolor* juveniles fed with waste water obtained from cultured *H. huso* under different densities for reaching to the best density of *H. huso* farming for creating the equivalence and providing the optimum levels of minerals required for rearing of *N. diversicolor*.

Materials and methods

Animals

N. diversicolor worms reared at the International Sturgeon Research Institute, Rasht. north Iran were used in this experiment. Juvenile worms of a total biomass of 65 g per m^{-2} (mean initial weight, 0.03±0.005 g/ per individual, (Mean±SD) were stocked in each tank (approximately 2000 worms per m^{-2}). Worms were kept into twelve 40 L tanks and bottom of the tanks were covered by an 8-10 cm layer of substrate sediment by sand (50-100 μm), which was rinsed several times with fresh water and then was sundried. Four treatment were assigned each in three replicates. The sediments were collected from the Caspian Sea with grain size in the range up to 2 mm provided a habitat for the worms in which they burrowed tunnels. No additional food was administered to the Polychaetes during the entire course of the experiment.

Fingerlings of *H. huso* (mean initial weight, 27.78±0.14 g N=420) hatched and reared from wild breeders reproduced artificially at the Shahid Beheshti Sturgeon Hatchery Center, Rasht, Iran, were transported to the laboratory (International Sturgeon Research Institute). They were placed into nine 80-L fiberglass tanks, (60 cm diameter with 40 cm water).

System design

Four trials including NWD_1 (*Nereis* fed with waste water of density 2-3 kg m^{-2} fish), NWD_2 (*Nereis* fed with waste water of density 3-4 kg m^{-2} fish), NWD_3 (*Nereis* fed with waste water of densities 4-5 kg m^{-2} fish) and NFF (*Nereis* fed with fish feed) as a control each in 3 replicates (Fig. 1). For all treatments the sand bed was used as a sandy filter in the integrated culture system of *H. huso*. Fish were fed on a commercial sturgeon feeds Biomar Ecostart (pellet size 1.5 mm) containing

[DOR: 20.1001.1.15622916.2020.19.1.18.0]

42.0% crude protein, 22.0% crude lipid, 15.0% carbohydrates, 3.3% fiber, 8.0% ash, 1.1% total phosphorous and 21.5 Mj Kg^{-1} energy according to the manufacturer on a ration equivalent to 3% of their body weight four times daily (08:00, 12:00, 16:00 and 20:00 h) (Mohseni *et al*., 2006). The experiment was run for 55 days.

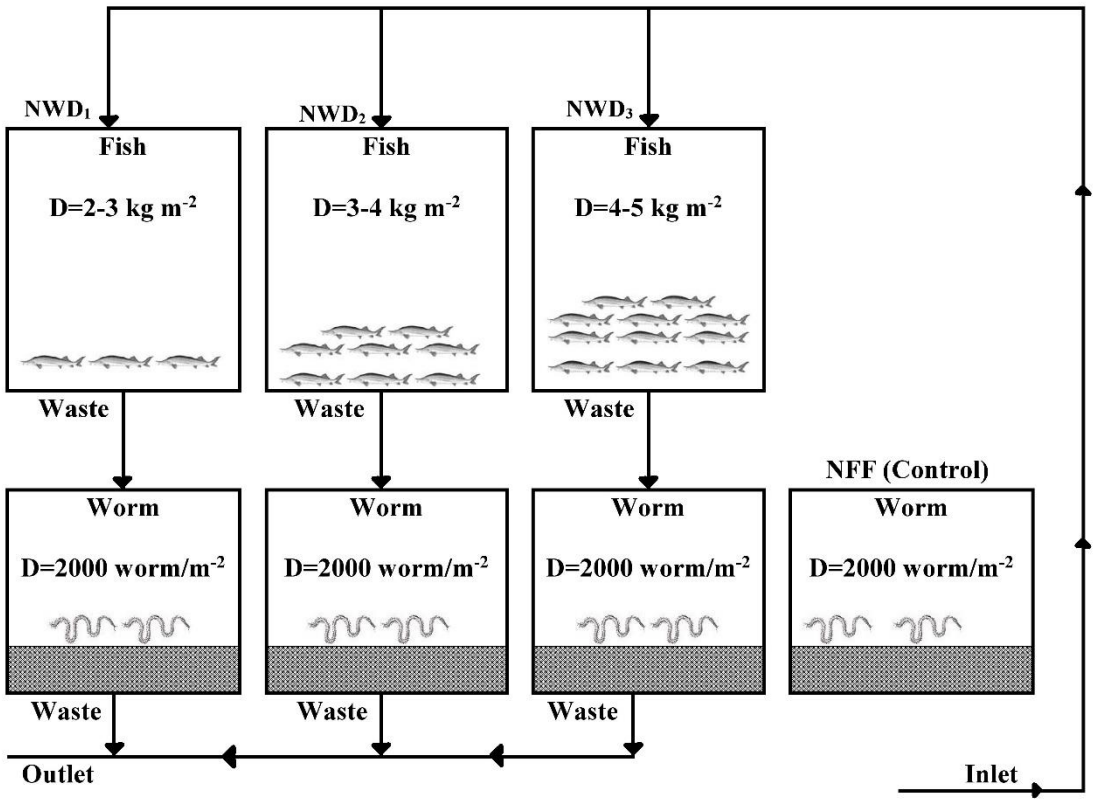

Figure 1: Schematic representation of nutrient flow using wastes from a *Huso huso* production system to grow *Nereis diversicolor*.

Water quality condition

Water supply to the fish tanks was maintained at the rate of 1.5-1.8 L min^{-1}, and water was well aerated in each tank. Water temperature (°C), dissolved oxygen (mg L^{-1}) and pH values were measured using a Hach Multi-parameter liquid analyzer (HQ 40d, Hach-Lange Company, USA) daily. The water temperature, salinity and EC at inlet water were 23.2±0.7°C, l±0.2 ‰ and 1060±40 µS, respectively. Also, dissolved oxygen and pH in the outlet water of tanks were 7.36±0.39 mg L^{-1} and 7.31±0.18, respectively.

Growth performance analysis

Fish were starved for 24 h before each biometry to avoid the interfering of the ingested feed in the fish weight as well as decreasing of stress condition. The biometry of fish was undertaken every week interval, and feeding rate was adjusted accordingly (Ahmed, 2014).

H. huso and worms were counted and weighed to get the initial number and weight for each treatment before their stocking and also at the end of the experiment to get the number of survival and the final body weight in the culture system. The effect of feed and waste water on the growth

[DOR: 20.1001.1.15622916.2020.19.1.18.0]

performance of fish and worms was assessed by measuring the following parameters: Specific growth rate (SGR) = (Ln final weight of fish or worm - Ln initial weight of fish or worm) days^{-1}×100; Weight gain (WG) (%)=((Final body weight of fish or worm -Initial body weight of fish or worm)/ Initial body weight of fish or worm))×100; Survival rate (%)=(number of fish or worm in each group remaining on day 55) (initial number of fish or worm)$^{-1}$×100; Biomass gain (g)=Final biomass (g)−Initial biomass (g); FCR=F/(Wf-Wo), where FCR denotes feed conversion rate, F is the weight of food (g) supplied to fish or worm during the study period, Wo is the live weight of fish or worm at the beginning of the study period and Wf is the live weight of fish or worm at the end of the study period (Ahmed, 2014).

Total protein assay

Total protein in samples was assessed using N-Kjeldhal x 6.25) according to the standard procedures, association of official analytical chemists (AOAC, 1995).

Lipid extraction and fatty acid analysis

Total lipid was extracted by the method Folch *et al*. (1957), and was measured based on the method described by Barnes and BlackStock (1973).

Fatty acid methyl esters (FAMEs) were separated and quantified by gas–liquid chromatography on a Young Lin 6500 GC (Korea), equipped with a Supercowax-10 fused silica wall coated 60m×0.22mm×0.22μm capillary column (Teknokroma, TR-CN100, Spain). Programmed temperature was at 90-170°C for 25°C min^{-1} then at 190°C for 2°C min^{-1} and a holding time of 4 min, then at 210°C for 3°C min^{-1} and finally at 220°C for 5°C min^{-1} with a holding time of 5 min^{-1}. Injector and flame ionization detector temperatures were programmed at 240ºC and 280ºC, respectively. Helium was used as the carrier gas in form of constant flow. Preparation of FAMEs was according to Morrison and Smith (1964). Identification of the FAMEs was based on comparison of retention times to those of reference compounds (Sigma-Aldrich, Shanghai, China). The amount of individual fatty acids was given as percent of the total amount of all detected fatty acids.

Statistical analysis

All data were first tested for normality and homogeneity of variance using the Shapiro-Wilk test. Data from growth performance and survival rate of *N. diversicolor* and *H. huso* test and groups of different types of fatty acids (saturated, monounsaturated, PUFA and ω-3 PUFA) of *N. diversicolor* grown in the culture system with *H. huso* waste water were analyzed by one-way ANOVA. Statistical significance differences among treatments (p<0.05) with 3 replicates were evaluated by the Duncan's Multiple Range Test using SPSS 20 software. All data are presented as mean ±SD.

Results

Growth performance of fish

Table 1 displays the results of growth performance of fish. No significant

[DOR: 20.1001.1.15622916.2020.19.1.18.0]

differences of initial weight, length and biomass was observed among the treatments. Two-fold and three-fold increase in live body weight were detected in the NWD_3 and NWD_1 treatments, respectively at the end of eight weeks of experiment. No mortality was seen in all treatments during the trial. Also, no significant difference was seen in the final weight, weight gain and final biomass among treatments (Table 1), but the SGR in for NWD_1 treatment was higher than other treatments and that of NWD_2 was higher than NWD_3 trail (p<0.05).

Table 1: Growth performance of juvenile *Huso huso* in different densities fed after 8 weeks.

	Huso huso		
Parameters	**NWD_1**	**NWD_2**	**NWD_3**
Final length (cm)	26.5 ±1.2 [b]	25.83±0.56 [ab]	23.65±0.97 [a]
Final weight (g gr.)	89.33±7.02 [c]	66.66±5.03 [b]	45.38±3.95 [a]
Final biomass (g gr.m^{-2})	2233.33 ±175.59 [a]	3333.33±251.66 [b]	4538.66±395.97 [c]
Biomass gain (g gr. m^{-2})	1538.16 ±171.8	1944.16±234.7	1775.66±416.49
Final density (n m^{-2})	25±0.00	50±0.00	100±0.00
Survival rate (%)	100±0.00	100±0.00	100±0.00
SGR (% day^{-1})	2.11±0.13 [c]	1.59±0.11 [b]	0.89±0.17 [a]
Weight gain (%)	221.18 ± 23.64 [c]	139.82±15.15 [b]	64.34±15.54 [a]
Total feed consumed (g.)	310.37 ± 5.77	489.66±18.81	655.94±25.16
Feed conversion ratio (FCR)	1.01±0.08 [a]	1.26±0.05 [a]	1.91±0.47 [b]
Condition factor (CF)	0.49±0.08 [b]	0.39±0.01 [ab]	0.34±0.06 [a]

Note: Initial body weight: 27.78±0.28 g. Initial length: 19.54±0.14 cm. SGR: specific growth rate; Values are means±SD of three replicates. Different superscripts indicate significant differences (p<0.05).

Growth performance of worm

Results of growth performance and survival rate of *N. diversicolor* are showed in Table 2. Results indicated that differences in final biomass and weight gain of NFF treatment were significantly higher than three treatments (NWDs). The results showed that there were no significant differences in final biomass and weight gain among the treatments, but the highest and the lowest values of these parameters were recorded in NWD_2 and NWD_3 treatments, respectively (p<0.05). There were significant differences in growth parameters between the control and treatments (p<0.05). No significant differences was seen in survival rate between NWD_1 and NWD_2 treatments, but the highest and the lowest survival rates were seen in control (84.4±3.02%) and NWD_3 treatment (55.6±7.94%), respectively.

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Table 2: Growth performance of *Nereis diversicolor* fed from fish waste water in different densities (NWDs) and *Nereis* fed with fish feed (NFF).

	Nereis diversicolor			
Parameters	**NWD_1**	**NWD_2**	**NWD_3**	**NFF**
Final weight (g)	0.122 ± 0.01	0.154 ± 0.01	0.151 ± 0.03	0.232 ± 0.02
Final biomass (g m^{-2})	203.33± 16.07 ^{a}	265 ± 26.45 ^{a}	206.51 ± 22.22 ^{a}	488.83 ± 56.79 ^{b}
Biomass gain (g m^{-2})	118.33 ± 10.58 ^{a}	183.58 ± 27.06 ^{b}	124.26 ± 10.03 ^{ab}	409.08 ± 56.07 ^{c}
Final density (n m^{-2})	1656 ± 111 ^{b}	1715 ± 140 ^{b}	1390 ± 198 ^{a}	2110 ± 75 ^{c}
Survival rate (%)	66.26 ± 4.47 ^{b}	68.6 ± 5.6 ^{b}	55.6 ± 7.94 ^{a}	84.4 ± 3.02 ^{c}
SGR (% day^{-1})	2.34 ± 0.27 ^{a}	2.84 ± 0.39 ^{ab}	2.76 ± 0.28 ^{ab}	3.04 ± 0.21 ^{b}
Weight gain (%)	266.36 ± 52.93 ^{a}	385.73 ± 100.59 ^{a}	360.42 ± 68.79 ^{a}	627.9 ± 97.92 ^{b}

Initial body weight: 0.03±0.005 g gr. Values are means ± SD of three replicates. Different superscripts indicate significant difference (ANOVA, p<0.05).

Lipid and fatty acids analysis

The profile of fatty acids of *N. diversicolor* are showed in Table 3. The fatty acids profile revealed a maximum of 22 fatty acids. Fatty acids of 14:0, 16:0, SFA, C18:1n9c, 20:1, 22:1n-9, MUFA, 18:3n-3, 20:2, 20:5n-3, EPA, 22:6n-3 and DHA were nominally similar among the NWD_1, NWD_2 and NWD_3 treatments, but significantly lower than in the control. Palmitic acid (C16:0) was the major saturated fatty acid in all analyzed samples with an average content of 29.9±2.64 mg g^{-1} for the *Nereis* fed with fish feed and 14.27±1.83 mg g^{-1} for *Nereis* fed with waste water of density 3-4 kg m^{-2} fish (NWD_2), as a highest and lowest fatty acid among treatments. The monounsaturated fatty acids were dominated by the C18:1ω9 group. The average content of C18:1 ranged from 25.27±4.37 mg g^{-1} to almost 67.39±7.52 mg g^{-1} in all samples. The level of 18:1ω9 was significantly higher in the NFF treatment than the other treatments (p<0.05).

Table 3: Fatty acid (FA) profiles (mean±SD ; mg FA g^{-1} dry wt) of *Nereis diversicolor* fed from fish waste water in different densities (NWDs) and *Nereis* fed with fish feed (NFF).

	NWD1	**NWD2**	**NWD3**	**NFF (control)**
Saturates				
C8:0	1.3±0.1^{a}	1.3±0.2^{a}	1.4±0.3^{a}	1.5±0.2^{a}
C10:0	0.08±0.01^{ab}	0.09±0.02 ^{ab}	0.06±0.01 ^{a}	0.11±0.03 ^{b}
C12:0	1.2±0.3 ^{a}	1.7±0.4 ^{a}	2.3±0.7 ^{ab}	3.1±0.9 ^{b}
C14:0	1.41±0.24 ^{a}	1.97±0.51 ^{ab}	1.3±0.27 ^{a}	2.3±0.36 ^{b}
C15:0	0.12±0.02 ^{a}	0.17±0.04 ^{a}	0.15±0.03 ^{a}	0.19±0.05 ^{a}
C16:0	16.82±2.14 ^{ab}	14.27±1.83 ^{a}	21.3±3.28 ^{b}	29.9±2.64 ^{c}
C17:0	0.03±0.01^{a}	0.05±0.01 ^{ab}	0.06±0.01 ^{bc}	0.08±0.02 ^{c}
C18:0	1.14±0.23 ^{a}	1.81±0.23 ^{ab}	2.43±0.71 ^{b}	1.87±0.45 ^{ab}
C20:0	0.5±0.12 ^{a}	0.56±0.15 ^{a}	0.62±0.18 ^{a}	0.75±0.15 ^{a}
C22:0	3.87±1.74 ^{ab}	2.24±1.12 ^{a}	4.38±1.34 ^{ab}	6.21±2.81 ^{b}
Monounsaturate				
C14:1	0.12±0.03 ^{a}	0.15±0.02 ^{a}	0.2±0.07 ^{a}	0.14±0.03 ^{a}
C16:1	3.67±1.72 ^{ab}	2.46±0.86 ^{a}	5.81±1.28 ^{bc}	6.34±1.19 ^{c}
C18:1n9c	25.27±4.37 ^{a}	31.28±6.94 ^{a}	46.82±5.42 ^{b}	67.39±7.52 ^{c}
C18:1n9t	0.34±0.12 ^{a}	0. 28±0.15 ^{a}	0.24±0.09 ^{a}	0. 19±0.07 ^{a}
C20:1	1.37±0.23 ^{a}	1.81±0.61 ^{ab}	2.46±0.95 ^{ab}	3.19±1.04 ^{c}
C22:1n9	1.2±0.21 ^{ab}	1.8±0.49 ^{ab}	0.9±0.27 ^{a}	2.34±1.16 ^{b}
Polyunsaturate (PUFA)				
C18:2n6c	18.25±3.61^{a}	24.69±5.76 ^{ab}	25.47±4.58 ^{ab}	34.58±6.69 ^{b}

[DOR: 20.1001.1.15622916.2020.19.1.18.0]

Table 3 continued:				
C18:2n6t	0.41±0.03 [a]	0.61±0.15 [b]	0.55±0.05 [ab]	0.49±0.08 [ab]
C18:3n3	0.87±0.27 [a]	1.05±0.15 [a]	1.18±0.49 [a]	3.28±1.62 [b]
C20:2	2.26±0.75 [a]	4.63±0.75 [b]	3.52±0.74 [ab]	6.92±1.06 [c]
C20:5n3(EPA)	2.74±0.69 [ab]	2.83±0.69 [ab]	2.43±0.07 [a]	3.82±0.67 [b]
C22:6n3 (DHA)	1.16±0.21 [ab]	1.14±0.49 [ab]	0.94±0.17 [a]	2.16±0.86 [b]

Note: Means in the same row with different letters are significantly different (Duncan test, $p<0.05$); * Statistical significance at the level of $p<0.05$; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

The values of 22:1ω9, 18:2ω6, 20:5ω3 (EPA) and 22:6ω6 (DHA) were higher in NWD_2 treatment than in NWD_1 and NWD_3 treatments, while there were significantly lower than NFF treatment ($p<0.05$). The ratios of saturated/unsaturated, ω3/ω6 and DHA/EPA of *N. diversicolor* are represented in Fig. 2.

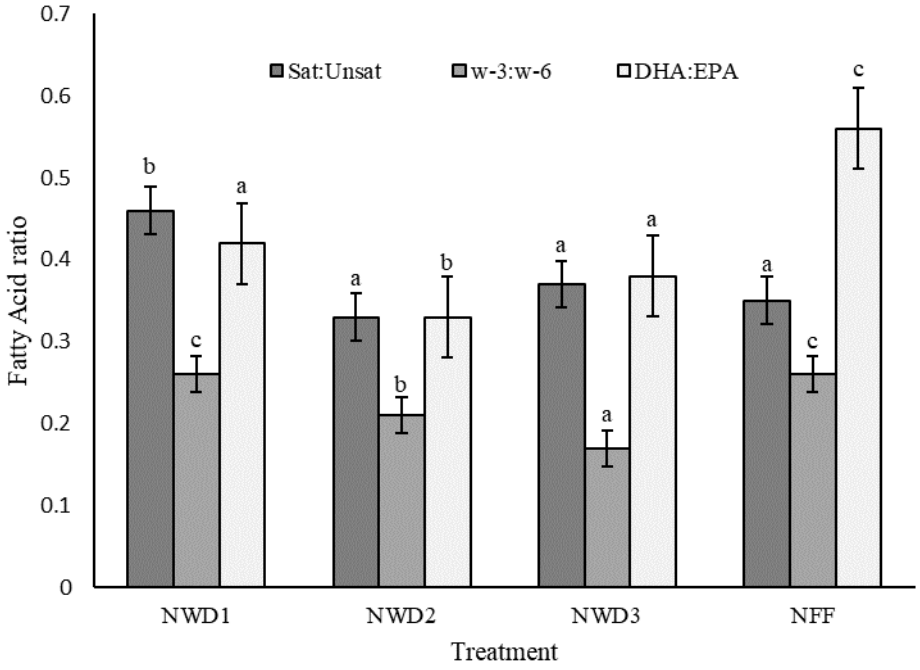

Figure 2: saturated/unsaturated, ω-3/ω-6 and DHA/EPA ratio of *Nereis diversicolor* fed from fish waste water in different densities (NWDs) and *Nereis* fed with fish feed (NFF).

The saturated/unsaturated ratio decreased considerably in *Nereis* fed with *H. huso* waste water at higher density ($p<0.05$). The results revealed a reduction of the ω-3: ω-6 ratio in NWD_3 treatment was highly significant and there was a significant increase in those fed with *H. huso* waste water in low densities ($p<0.05$). No significant differences in the percentage of total protein, total lipid and moisture were found among NWDs treatments, while the total protein and lipid highly indicated significant differences between NWDs and NFF treatments (Table 4).

[DOR: 20.1001.1.15622916.2020.19.1.18.0]

Table 4: Chemical composition of *Nereis diversicolor* fed from fish waste water at different densities (NWDs) and *Nereis* fed with fish feed (NFF).

	NWD_1	NWD_2	NWD_3	NFF (control)
Total lipid % dry weight	12.39±2.11[a]	11.55±1.91[a]	14.26±1.31[ab]	16.84±1.78[b]
Total protein % dry weight	49.34±2.62[a]	51.34±2.46[ab]	50.82±2.29[ab]	55.46±2.12[b]
moisture	85.20±0.87[a]	49.34±2.62[a]	49.34±2.62[a]	49.34±2.62[a]

Note: Means in the same row with different letters indicate significant differences within the same row (Duncan test, $p<0.05$); Values are mean and standard deviation of 3 samples.

Discussion

Results of the present study demonstrated that *N. diversicolor* possess the ability to a better growth in NFF treatment. We obtained the highest survival rate and the highest biomass gain in NFF treatment and was significantly different from treatments NWD_1, NWD_2 and NWD_3. This is probably due to cannibalism lack for worms and the higher protein content found in the sturgeon feed. The highest biomass gain in NWD_2 treatment and the lowest one in NWD_1 treatment was due to the lower weight growth found in the lower density of *H. huso* culture.

The specific growth rates were close to 3% day^{-1} for *N. virens* fed on waste halibut pellets faeces (Brown *at el.*, 2011), and was between 0.45 and 1.66% day^{-1} for *P. nuntia vallata* fed flounder faeces and 3.23% day^{-1} for worms fed the diet formulated for Polychaetes over a 15 days period (Honda and Kikuchi, 2002). In the present study, specific growth rates over the course of 55 days were 2.34 % day^{-1} for *Nereis* fed with waste water of density 2-3 kg m^{-2} *H. huso* and 3.04 % day^{-1} for worms fed the sturgeon feed. This was similar favorably with the SGR reported for *N. virens* by Brown *at el.* (2011). After the experiments on treatment NWD_3 it became clear that the quality of the interstitial water in the sediment layer has a crucial impact on the survival of *N. diversicolor*.

Accordingly, further studies are needed to declare the ability or role of *N. diversicolor* in its growth performance in large scale and in a commercial fish farms considering the more aspects such as energy and labor costs. The results of our study were supported by the results of Bischoff (2007) and Bischoff *et al*. (2009) where *N. diversicolor* uses particulate waste as its primary food source and showed considerable growth on an exclusive diet of particulate waste as valuable nutrients, such as fatty acids from an integrated culture system.

In the present study it was found that *N. diversicolor* uses particulate waste as its primary food source, such as fatty acids and fish waste is a commodity which must be recycled if sustainable aquaculture is the target. This study showed that the highest production of worm which fed on fish waste water and uneaten feed in NWD_2, however this production seems economic.

The organic waste water in aquaculture could be used as a high quality diet converted into worm biomass by *N. diversicolor* and would offer a sustainable solution to the existing problem of organic pollution (García-Alonso *et al*., 2008).

[DOR: 20.1001.1.15622916.2020.19.1.18.0]

Total final lipid values and total final protein values found in the worms in our study was similar to other published values for Nereid worms. In our study, the lower fat levels found in the worms in NWDs may be related to the lower average nutrients in this experiment. Promotional literature for Sea bait *N. virens* gives a value of 16% lipid content. Bischoff *et al.* (2009) analyzed *N. diversicolor* fed wastes from a recirculating system holding sea bream and compared them to wild worms and found levels of 27.1 and 17.8 μg mg^{-1} dry weight, respectively.

Many of the fatty acids found in the *N. diversicolor* in this study, in particular 16:0, 16:1; 18:1n9c; 18:2n6c and 20:5n3 (EPA) are commonly abundant in Nereid worms. They have been found in relatively large amounts in *Nereis* fed with fish feed (NFF treatment) and was significantly different with the treatments NWDs. This is probably due to lipid sources in the commercial *H. huso* diet fed by *N. diversicolor*. The results showed that the highest unsaturated fatty acids in NWD_2 treatment and the lowest in NWD_1 treatment, and this is due to the lower lipid found in the waste *H. huso* pellets in lower density of the *H. huso* culture and there are not significantly different among the NWDs treatments. The results of our study were supported by the results of Bischoff (2007) and Bischoff *et al*. (2009) where *N. diversicolor* uses particulate waste as its primary food source and showed considerable growth on an exclusive diet of particulate waste as valuable nutrients, such as fatty acids from an integrated culture system. The presence of highly unsaturated fatty acids ($HUFA_S$) greatly raises the value of these worms as can be recovered from fish wastes and offers a significant benefit of these Polychaetes with sturgeon as an integrated system. Considering the daily amount of food applied to the fish (around 310, 490, and 655 g during the end of the experiment) and *H. huso* growth as well as the fractions of saturated, monounsaturated and polyunsaturated fatty acids leads to the conclusion that the *Nereis* were produced with approximately 97 mg g^{-1} of the fatty acids. Therefore, fatty acids retention is assumed to be lower compared to optimized conditions. These findings confirm the existing reports on lipid contents and metabolism of *N. diversicolor* (Bradshaw *et al*., 1990; Luis and Passos, 1995).

Except NFF treatment (control), levels of EPA, DHA and 18:2n6t in NWD_2 treatment were increased, but reduced DHA relative to EPA was seen when worms were fed with waste water of density 3-4 kg m^{-2} *H. huso*. Polychaetes including *N. diversicolor*, *Glycera dibranchiata* and *N. viridens* that are promoted for feeds in Penaeid hatcheries tend to have much higher EPA ratios (Lytle *et al*., 1990; Bischoff *et al*., 2009). Thus, the production of the worms via such method is a beneficial and cost-effective of aquaculture activity.

Moreover, our results suggest that *N. diversicolor* admitted a diverse of food sources ranging from mostly organic matter (sturgeon waste water) to a

[DOR: 20.1001.1.15622916.2020.19.1.18.0]

balanced fish food diet in the feeding trials where diet could be the main factor influencing lipid composition in *N. diversicolor* that is supported by the results of Bradshaw *et al*. (1990) and Luis and Passos (1995). This study also demonstrated that *N. diversicolor* can grow readily by feeding on waste water of *H. huso* as a food source in a flow through system which can be highly efficient, although they did not grow and survive as much as the worms fed on commercial fish feed. The results of our study were showed the reduction rates from the experiments with *H. huso* waste water are proportional to the population density of 2000 woms per m^{-2}. Also, both *N. diversicolor* showed yearly production level of about 400 g per m^{-2} in sand filtration beds and appear to aid maintaining a flow through the sand filter and the residual organic matters such becomes as a part of their food source.

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