NTNU
Norwegian University of
Science and Technology

Utilization of excess nutrients from land-based aquaculture facilities by *Hediste diversicolor* (O.F. Müller, 1776)

Production of polychaete biomass and its
potential use in fish feed

Inka Seekamp

Marine Coastal Development
Submission date: July 2017
Supervisor: Kjell Inge Reitan, IBI
Co-supervisor: Aleksander Handå, SINTEF

Norwegian University of Science and Technology
Department of Biology

Acknowledgements

This thesis was performed as part of the SINTEF Fisheries and Aquaculture "LOWTROPHIC" project on biomass production from lower trophic levels. The practical work was conducted at NTNU Department of Biology (Centre for Fisheries and Aquaculture) and at SINTEF Fisheries and Aquaculture.

First, I would like to thank my supervisor Kjell Inge Reitan at NTNU Department of Biology and my co-supervisor Aleksander Handå at SINTEF Fisheries and Aquaculture as well as Arne Malzahn and Andreas Hagemann. Thank you for your help and guidance throughout the planning and execution of my experiments and the constructive feedback in the writing process of my thesis. My sincere appreciation also goes to Haiqing Wang, PhD candidate at NTNU Department of Biology and SINTEF Fisheries and Aquaculture. Thank you for working with me in every step of this project, for sharing my commitment to "our babies" and for putting so much effort into our work being successful. You are a great friend and colleague and I could not have done it without you.

To everyone who has contributed to the experimental work: Åsmund Johansen, Emili Aas Stavnesli, Kjersti Rennan Dahl, Karina Carvajal, Rasa Slizyte' and Marte Schei – thank you.

I would like to express my gratitude to my family and, above all, to my parents who have always supported me in everything I do. I am thankful for my fellow students at NTNU Sealab for creating a pleasant working and social environment as well as my friends all over the world for moral support, uplifting words, lots of laughs and just the right amount of distraction. Finally, I want to thank my wonderful boyfriend Cyril for his never-ending and unconditional patience, encouragement and support, especially throughout the final stages of my thesis.

Trondheim, July 2017 - Inka Seekamp

Abstract

Farming of Atlantic salmon (*Salmo salar*) is highly dependent on marine resources such as fish meal and fish oil. In Norway, supply of high quality raw materials and control of waste discharges from aquaculture are factors potentially limiting industry growth. A strategy to increase resource efficiency in salmon aquaculture is utilizing nutrient-rich waste streams for production of additional biomass in integrated multi-trophic aquaculture (IMTA) systems. This biomass constitutes a marine resource that could in turn be used in fish feed.

The objective of this thesis was to assess the potential of using *Hediste diversicolor* for recycling of nutrients contained in wastes from land-based aquaculture facilities. Moreover, the suitability of *H. diversicolor*, fed with these wastes, as a feed resource for carnivorous species was evaluated. A series of cultivation experiments using wild-caught polychaetes were performed in the laboratory to determine the impact of different diets on growth, mortality and biochemical composition of *H. diversicolor*. The results indicated that the species can successfully be cultivated on waste sludge as a sole feed source even though elevated ammonia levels restricted survival. Feeding of various, iso-carbonic diets resulted in different specific growth rates; fish feed constituted a more energy-dense diet than waste sludge and microalgae and thus lead to significantly higher growth. Different cultivation substrates had limited effect on growth and mortality, and mortality was not affected by different feed sources. Further, the biochemical composition of supplied diets did not significantly impact polychaete composition. Conclusively, it was shown that *H. diversicolor* can effectively utilize and incorporate nutrients contained in wastes from land-based aquaculture and subsequently function as a dietary resource for fish feed as it is high in protein and moreover contains for fish essential fatty acids and amino acids.

Keywords: *Hediste diversicolor*, polychaete, nutrient recycling, IMTA

Table of contents

Abbreviations

ANOVA	Analysis of Variance
ARA	Arachidonic acid, C20:4 n-6
AS-C	Aquarium sand - Coarse (1.2-3.0 mm)
AS-F	Aquarium sand - Fine (0.4-0.8 mm)
AS-M	Aquarium sand - Medium (1.0 mm)
CHA	Chamotte
DHA	Docosahexaenoic acid, C22:6 n-3
DW	Dry weight
E1	Experiment 1 – Evaluation of culture sediment
E2	Experiment 2 – Trial with cleaner fish waste
E3	Experiment 3 – Trial with post-smolt waste sludge
E4	Experiment 4 – 30-day trial with smolt waste sludge
EAA	Essential amino acid/s
EFA	Essential fatty acid/s
EPA	Eicosapentaenoic acid, C20:5 n-3
FA	Fatty acid/s
FCR	Feed conversion rate
FF	Fish feed (treatment group in E1-E4)
FID	Flame ionization detector
GC	Gas chromatograph/y
HOG	Head-on gutted
HPLC	High-performance liquid chromatograph/y
IMTA	Integrated multi-trophic aquaculture
L/D	Light to darkness
Mix	Mix of smolt waste sludge and shellfish diet (ratio 5:1) (treatment group in E4)
MUFA	Monounsaturated fatty acid/s
NEAA	Non-essential amino acid/s
NOK	Norwegian krone/r
PUFA	Polyunsaturated fatty acid/s
RAS	Recirculated aquaculture system/s
SAFA	Saturated fatty acid/s
SD	Standard deviation
SFD	Shellfish diet (treatment group in E4)
SGR	Specific growth rate [d^{-1}]
SWS	Smolt waste sludge (treatment group in E4)
TAN	Total ammonia nitrogen
TOM	Total organic matter
WW	Wet weight

1 Introduction

The increasing world population, estimated to reach 9.8 billion by 2050, along with a doubling in average income per capita will globally result in a 59-98% greater food demand (Grafton et al., 2015, UN DESA - Population Division, 2017). However, growth in agricultural production is highly limited by yield growth and fresh water availability and yields from the fishing industry have been stagnating since 1990, thus amplifying the importance of aquaculture which is already the fastest growing food-producing sector (OECD/FAO, 2017). Amounting to 73.8 million tonnes (live weight) in 2014 with a value of USD 160.2 billion, aquatic animal production is predicted to contribute over 100 million tonnes per year to the global food supply by 2026 (Figure 1.1) and up to 140 million tonnes by 2050 (FAO, 2016, OECD/FAO, 2017). Almost doubling the global production in an ecologically and economically sustainable way will be a major challenge (Waite et al., 2014). In Norway, the Atlantic salmon (*Salmo salar*) industry is expected to increase its annual production 4-fold by 2050 (Olafsen et al., 2012). A constraint for industry growth will be minimizing waste discharges from aquaculture production as well as supply of high quality raw materials, particularly marine-derived resources for feed production (Olafsen et al., 2012, Tacon et al., 2011).

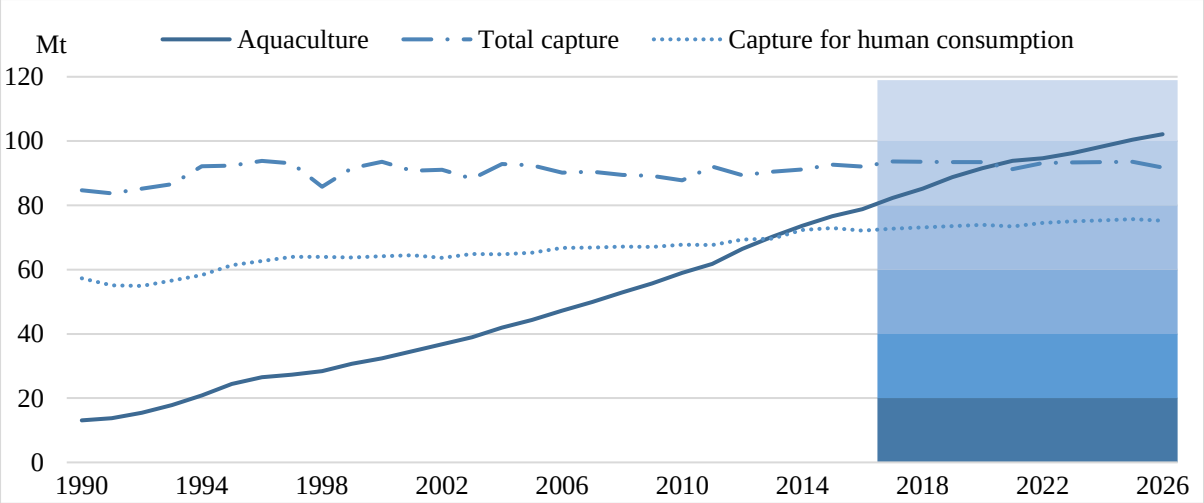

Figure 1.1 – Aquatic animal capture and production 1990-2016; highlighted in blue are projected values until 2026 (adapted from OECD/FAO 2017).

1.1 Environmental aspects of aquaculture in Norway

Even though Norwegian salmon aquaculture has a low carbon footprint and is an efficient food production sector when compared to other livestock (Hognes et al., 2011), the majority of resources contained in salmon feed are not utilized. Studies found that salmon only retains a fraction of added nutrients; approximately 62% of feed C, 57% of feed N and 76% of feed P are not incorporated the fish but lost to the environment through feed wastage, excretion and feces production (Porter et al., 1987, Wang et al., 2013, Wang et al., 2012, Wu, 1995). Utilizing these waste discharges that are otherwise lost and can potentially be detrimental to the marine environment becomes indispensable for sustainable industry growth, especially when considering that feed accounts for 94% of greenhouse gas emissions in salmon production globally (Pelletier et al., 2009).

Based on a production of 1.3 million tons of salmon (Statistisk sentralbyrå - Statistics Norway, 2016), an FCR of 1.15 (Marine Harvest, 2017b, Wang et al., 2012) as well as 49% C, 6% N and 1% P in feed (Olsen et al., 2008), the theoretical nutrient dispersal from sea-based salmon production in 2015 amounted to 454,200 tonnes C, 51,100 tonnes N and 11,400 tonnes P (calculation adapted from Handå et al. (2012a)). Correspondingly, wastes from land-based aquaculture show a large potential for increasing sustainability of Norwegian aquaculture by utilizing resources more efficiently. Currently, there are over 200 land-based farms for juvenile salmon and 20 post-smolt production facilities in Norway at which salmon is grown up to a size of 250 g before being set out in the sea; in individual cases production up to 1 kg is possible (Fiskeridirektoratet, 2015, Fiskeridirektoratet, 2017a). Moreover, 30 farms for cleaner fish production are planned and an increase of sea-based closed containment systems is projected. As the trend in land-based production goes from flow-through to recirculated aquaculture systems (RAS) and the size of salmon that is transferred to the sea rises, volumes of emerging waste sludge containing valuable nutrients are steadily increasing (Drengstig et al., 2011).

1.2 Integrated multi-trophic aquaculture (IMTA)

Increasing feed and resource efficiency while at the same time diminishing environmental effects of aquaculture could be achieved by concurrent cultivation of organisms in integrated multi-trophic aquaculture (IMTA) systems (Buschmann et al., 2009, Chopin et al., 2001, Troell et al., 2009). In Asia, a more balanced approach to aquaculture in form of polycultures has been practiced since the 10th century (Hao-Ren, 1982). In the Western world, however, the concept of IMTA is comparatively new and much research has been conducted since interest first aroused (Blancheton et al., 2009, Ridler et al., 2007). In IMTA systems, excess nutrients are put to use to diversify the system's products, thus providing a more efficient handling of resources. A basic model combines intensively raised organisms (e.g. fish or shrimp) with organic extractive species (e.g. shellfish, polychaetes) and/or inorganic extractive species (e.g. macroalgae). As a result, excrements of one resource consumer become a resource for the other; dissolved inorganic as well as particulate and suspended organic nutrients are taken up and converted into biomass, thus resembling a natural, balanced ecosystem (Chopin et al., 2001, Neori et al., 2004, Reid et al., 2008).

IMTA can be implemented in both open sea systems and closed land-based production facilities. In current salmon production practices, the largest fraction of wastes is created during the ongrowing stage at sea and thus difficult to attain. Dissolved inorganic and resuspended organic nutrients released into ecosystems are spread and diluted quickly by strong water currents (Pearson and Black, 2000, Wang et al., 2012) whereas particulate wastes were shown sink quickly to the seafloor and build up below cages (Mente et al., 2006). Though volumes of particulate waste output from land-based RAS facilities are much smaller than those during ongrowing, they are easier to obtain and logistically distribute (Campo et al., 2010). The waste sludge moreover emerges from a controlled environment and is treatable which ensures biosecurity and makes it a safe feed source for extractive organisms (Shpigel and Neori, 1996).

1.3 Species description Hediste diversicolor

Figure 1.2 – *Hediste diversicolor* in its natural habitat (Schutzstation Wattenmeer, 2017).

Phylum: Annelida

Class: Polychaeta

Order: Phyllodocida

Family: Nereididae

Genus: *Hediste*

The common ragworm *Hediste diversicolor* (O.F. Müller, 1776) is a polychaete species that inhabits shallow marine and brackish waters under tidal influence along the temperate coast of the North Atlantic (Smith, 1977). It lives in burrows in sandy mud, gravel and clay and is able to tolerate unstable temperatures of 4-25 °C (Hartmann-Schröder, 1996). Being euryoecious and euryhaline, it is able to adapt to a wide range of environmental conditions, including hypoxia and low salinities. However, individuals cannot reproduce below salinities of 5 ppt (Scaps, 2002). Natural population densities can be as high 3000 individuals m^{-2} (Riisgård, 1994).

H. diversicolor can adjust its feeding behavior depending on environmental conditions, seasonality and primary production (Sturdivant et al., 2015). It changes between three different feeding modes. Filter feeding is triggered when the water column presents a high concentration of phytoplankton (Vedel and Riisgård, 1993). *H. diversicolor* excretes a mucus net and creates an irrigation current in its burrows with undulated body movements; suspended particles are retained in the net and the net is subsequently ingested (Riisgård, 1991). Deposit feeding on the sediment surface has been observed in the wild as well as under laboratory conditions and

may be facilitated by the absence of predators (Fidalgo e Costa et al., 2000, Fidalgo e Costa et al., 2006b). More seldom, *H. diversicolor* may act as a carnivorous predator and actively feed on different bottom fauna species (Rönn et al., 1988).

Individuals grow up to 20 cm in length, comprising a maximum of 120 segments. Maturation in this gonochoristic species occurs after 1-3 years; warmer temperatures accelerate development. Upon maturation, a color change from reddish brown to bright green (males) and dark green (females) can be observed. *H. diversicolor* is monotelic, meaning that reproduction is followed by death (Hartmann-Schröder, 1996, Scaps, 2002).

1.4 Potential for *H. diversicolor* in IMTA

To present, no work involving *H. diversicolor* utilizing wastes from Atlantic salmon aquaculture has been published. While many studies focused on exploiting dissolved nutrients discharged by salmon farms using macroalgae (Broch et al., 2013, Handå et al., 2013, Reid et al., 2013, Troell et al., 1997, Wang et al., 2014), only few have focused on recycling of particulate wastes. Uptake of particulate organic matter from fish farms by blue mussels (*Mytilus edulis*) was evaluated in research projects carried out by SINTEF in collaboration with NTNU (Handå et al., 2012a, Handå et al., 2012b).

Polychaetes, as extractive organisms, could potentially be beneficial in IMTA systems. Being able to adapt their feeding mode, depending on food availability and source (Vedel and Riisgård, 1993), they were shown to accumulate beneath fish farms (Salvo et al., 2015). Further, they improve sediment quality as they break down toxic metabolites to subtoxic levels in addition to bioturbating activities that enhance organic matter mineralization and recycling (Heilskov et al., 2006, Papaspyrou et al., 2010). Integration with land-based smolt production would give the chance to utilize very concentrated waste sludge containing not only fish faeces but also uneaten feed and bacterial biofilms (Bischoff, 2007). Moreover, polychaetes could

serve as a dietary source of protein and lipid as well as essential marine fatty acids and amino acids in feed for carnivorous fish and crustaceans, thus constituting a feed loop (Bischoff et al., 2009, Fidalgo e Costa and Cancela da Fonseca, 1998, Luis and Passos, 1995).

Supplying *H. diversicolor* and the closely related *Nereis virens* with waste outputs from production of various fish species, either by feeding of sludge or direct waste supply in integrated cultivation systems, has led to promising results in previous studies (Bischoff, 2007, Bischoff et al., 2009, Bischoff et al., 2010, Brown et al., 2011, Pajand et al., 2017, Suckow, 2010). Nutrients contained in wastes were efficiently incorporated in polychaetes and utilized to grow valuable biomass with great market demand (Fidalgo e Costa et al., 2006a).

Next to its bioremediation potential, large scale production of *H. diversicolor* is economically of interest; enquiries for polychaetes both for use as fishing bait and food source in aquaculture has been increasing over the past years and natural supplies cannot meet European market demands (Nesto et al., 2012, Olive, 1999).

1.5 Utilization of *H. diversicolor* as a resource for fish feed

Atlantic salmon are carnivorous fish depending on essential fatty acids and amino acids in their diet (Cowey and Sargent, 1972). To increase sustainability, fractions of fish meal and fish oil from forage fisheries have largely been replaced by vegetable ingredients, thus lowering the percentage of marine ingredients in salmon feed from 90% in 1990 to 30% in 2013 (Ytrestøyl et al., 2015). However, inclusion of these marine ingredients will remain vital, as salmon requires long-chain n-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for optimal growth and healthy development (Rosenlund et al., 2016). Fully exploited wild stocks make finding alternative sources for marine ingredients fundamental for industry growth (Huntington and Hasan, 2009). As stated before, polychaete biomass could serve as a potential raw material for fish feed.

1.6 Aims and hypotheses of the study

The overall aim of the present study was to investigate the potential of the polychaete species *H. diversicolor* to utilize wastes (fish faeces, uneaten feed and bacterial biofilms) from land-based aquaculture for polychaete biomass production and application. The assessment was performed based on two objectives:

(1) Investigation of the influence of different feed sources on growth, mortality and biochemical composition of polychaetes.
(2) Evaluation of suitability of polychaetes fed on wastes from land-based aquaculture as a resource for fish feed.

Basic cultivation experiments assessing the effect of substrate and different diets on growth and mortality were completed to determine culture conditions and estimate suitable levels of feed supply.

Subsequently, the hypotheses for a bigger trial evaluating growth, mortality and biochemical composition were formulated and tested.

(1) Wastes from salmon aquaculture (smolt production) constitute an appropriate nutrition for growth of *H. diversicolor*.
(2) Feeding of different diets will affect polychaete growth and composition.
(3) Adding microalgae paste to waste sludge will give better results than mono-feeding since microalgae are part of polychaetes' natural diet and will enhance the nutritional value of waste sludge.
(4) Biochemical composition of *H. diversicolor* fed with waste sludge can meet the nutritional requirements for fish feed.

2 Materials and Methods

2.1 Overview

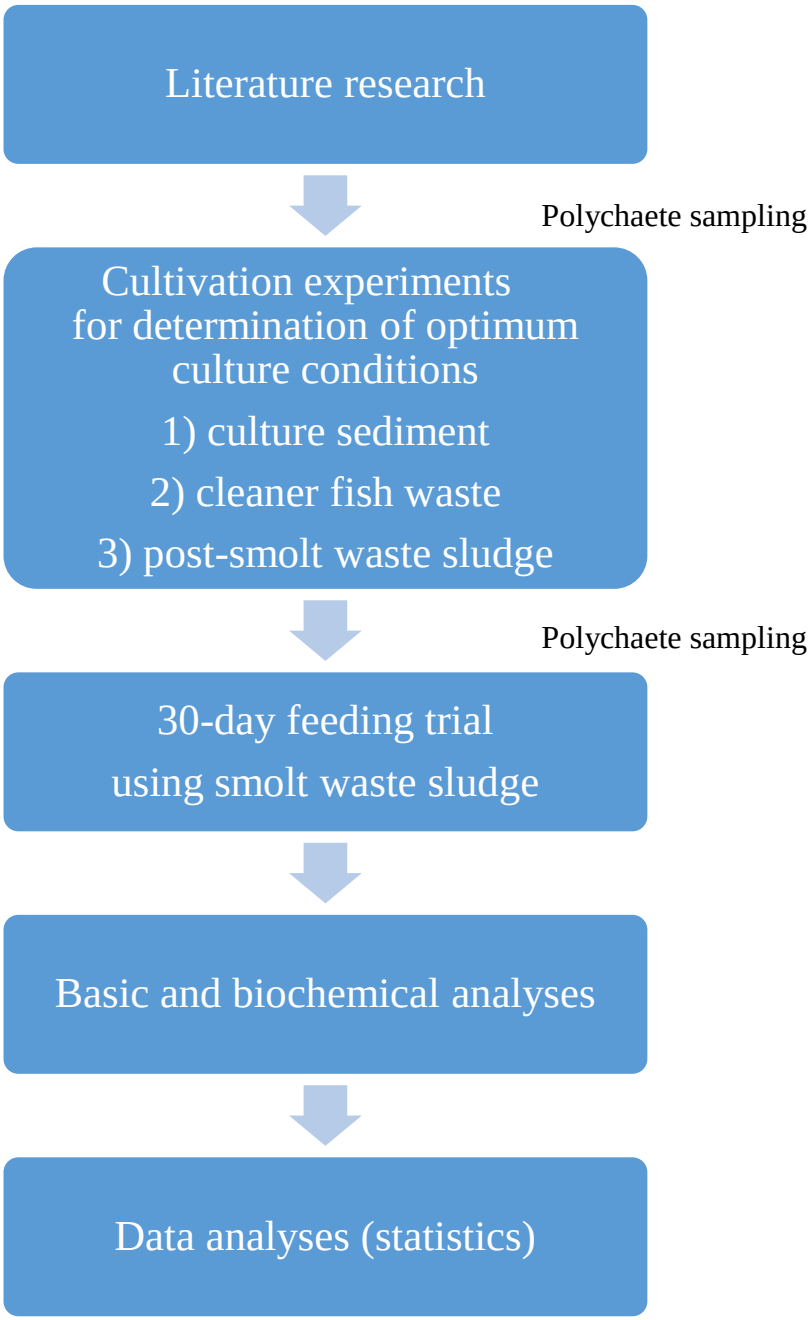

Figure 2.1 – Overview of performed work.

All described work was conducted in collaboration with Haiqing Wang, PhD candidate at NTNU Department of Biology and SINTEF Fisheries and Aquaculture.

2.2 Polychaete sampling

For all experiments, wild-caught polychaetes of the species *Hediste diversicolor* were used. Prior to each experiment, the desired number of individuals was retrieved at low tide from Leangenbukta, a bay east of Trondheim (63.439775 N, 10.474126 E) as shown in Table 2.1 and Figure 2.2. The polychaetes were dug up from loamy sediment at a depth of 30-40 cm using spades and digging forks. In order to minimize stress, natural substrate was included both for transportation and first cultivation in the laboratory. During the adaption period, before the experiments, the polychaetes were cultivated in a flow-through system with big trays containing natural sediment (10-15cm sediment height) at a temperature of 10 °C. The water had a salinity of 35 ppt, the water exchange amounted to 100% daily and the light to darkness ratio in the experimental room was set to L/D = 5.5/18.5 h. Feeding during the adaption period occurred five times weekly with microalgae (*Rhodomonas* sp.). *Rhodomonas* was obtained from another project within SINTEF. Dead polychaetes were removed from the sediment surface daily to avoid negative impacts on water quality.

Table 2.1 – Overview sampling of wild polychaetes *H. diversicolor* to be used in the experiments.

Sampling	Date	Number of individuals	Experiment
I	01.04.2016	300	E1: Evaluation of culture sediment
II	24.05.2016	200	E2: Trial with cleaner fish waste
III	01.07.2016	400	E3: Trial with post-smolt waste sludge
IV	21.10.2016	1700	E4: Trial with smolt waste sludge

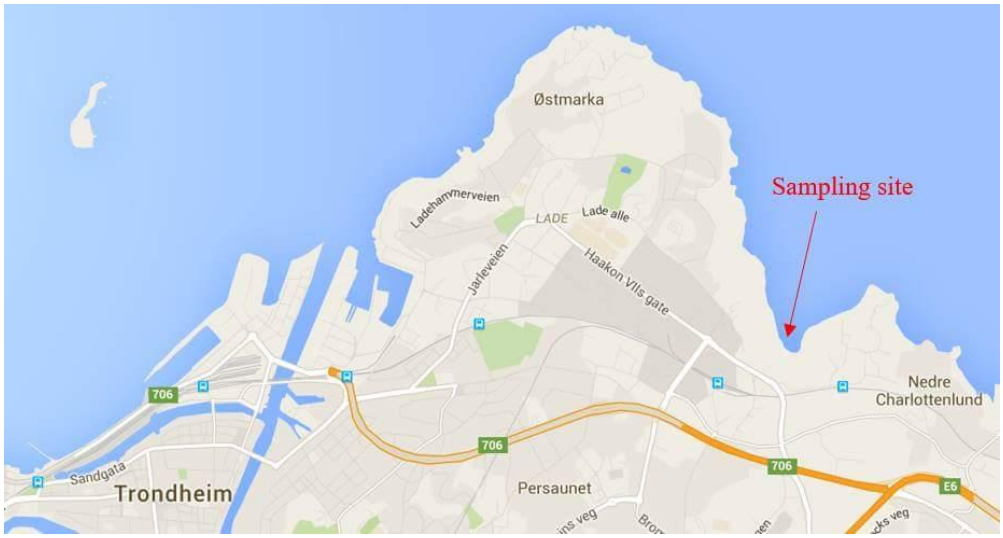

Figure 2.2 – Sampling site for *H. diversicolor* used in all experiments (Google Maps, 2017).

2.3 Cultivation experiments

2.3.1 Evaluation of culture sediment (E1)

The first experiment was conducted in order to determine a suitable culture sediment for subsequent experiments. Evaluation of the sediments was based on survival and growth. Wild-caught polychaetes (Sampling I, Table 2.1) were cultivated for 15 days in glass tanks (LxHxW 95xHx145 mm), feeding on commercial fish feed (GEMMA DIAMOND 1.0, Skretting AS, Norway). The glass tanks were part of a flow-through system with a daily water exchange that ensured high oxygen saturation (100% daily water exchange). Natural seawater was supplied to the laboratory from the Trondheimsfjord (10.2±0.2 °C, 35 ppt). The L/D ratio was set to 8/16 h. Four different sediments with various grain sizes were tested: (1) AS-Fine (AS-F): aquarium sand (0.4-0.8 mm), (2) AS-Medium (AS-M): aquarium sand (1.0 mm), (3) AS-Coarse (AS-C): aquarium sand (1.2-3.0 mm), and (4) CHA: chamotte (varied grain sizes up to 25 mm). For each substrate, four replicate tanks, containing five polychaetes each, as well one control tank, only containing substrate for observing undisturbed suspension of feed, were tested. The average biomass per tank amounted to 682±116 mg (WW). The dimension of the tanks gave a stocking density of 363 individuals m^{-2}. Polychaetes were weighed in groups,

placed into the tanks and starved for 24 h before the experiment started. Dead polychaetes found in this period were removed and replaced. For 15 days, the polychaetes were fed 20 pellets of fish feed (≈ 0.582 mg/pellet; Ø 0.8mm) daily, corresponding to 1.71% of the average polychaete biomass (WW). Before each feeding, remaining feed pellets from the preceding feeding were removed to ensure consistent feed supply and water quality. Dead polychaetes found over the course of the experiment were removed, but not replaced. The amount of pellets was not altered accordingly, leading to an increased feed supply in some cases.

2.3.2 Trial with cleaner fish waste (E2)

E2 was performed to evaluate the influence of feeding wild-caught *H. diversicolor* (Sampling II, Table 2.1) with cleaner fish (lumpsucker, cyclopteridae) waste in order to determine if fish excrements in general are a diet that *H. diversicolor* can thrive on. Using the same flow-through systems as in E1, the experiment was run for 23 days. The impact of three different levels of cleaner fish waste on growth and survival were tested: 0.5%, 1.5%, and 2.5% of polychaete biomass (WW), respectively. The feed was supplied in form of dried waste sludge from cleaner fish production; it was provided by Flatanger Settefisk AS. As a control, one group was fed with commercial fish feed (GEMMA DIAMOND 1.0, Skretting AS, Norway) at a level of 1.5% of polychaete biomass (WW). Fish feed pellets were ground prior to feeding. For each group, four replicates of five polychaetes per replicate were used, the average polychaete biomass (WW) accounted for 736±93 mg; all tanks were filled with 5-6 cm of chamotte and 6-7 cm of seawater above. Weighing of individuals prior to the experiment occurred in groups rather than individually, giving a mean value for the weight of each individual. Stocking density and system parameters as well as L/D ratio resembled the preceding experiment (363 individuals m^{-2}, 100% daily water exchange, T = 10.5±0.4 °C, 35 ppt, L/D = 8/16 h). Following a starvation period of 24 h in which any dead individuals were replaced, feeding occurred 17

times over the course of 23 days (five times per week). Dead polychaetes discovered in that period were removed, but not replaced, again leading to an increased feed supply in some cases.

2.3.3 Trial with post-smolt waste sludge (E3)

A third trial was conducted in order to evaluate the impact of feeding wild-caught *H. diversicolor* (Sampling III, Table 2.1) with different levels of post-smolt waste sludge. Aim of the experiment was to study growth and survival. Polychaetes fed on different diets were cultivated in a flow-through system (see previous experiments) for 28 days. Post-smolt waste sludge was provided by the Njord Salmon AS post-smolt facility in Tjeldbergodden and fed to the polychaetes in three different quantities: 0.5%, 1.5% and 2.5% of polychaete wet weight respectively. Commercial fish feed (GEMMA DIAMOND 1.0, Skretting AS, Norway) served as a control; 1.5% of polychaete biomass (WW) were fed in form of ground fish feed pellets. Each group consisted of four replicate glass tanks containing five individuals each; the total polychaete biomass (WW) amounted to 1255±215 mg. 5-6 cm of chamotte were used as substrate, overlying was a 6-7 cm seawater layer. Polychaete density, system parameters and L/D ratio were in accordance with the prior experiments (363 individuals m^{-2}, 100% daily water exchange, T = 10.8±0.4 °C, 35 ppt, L/D = 8/16 h). The combined weight of polychaetes was measured for each group and they were starved 24 h before the experiment. Any dead individuals found before the experiment start were replaced. Feeding occurred five times a week for 28 days. During the experimental period, dead individuals were removed but not replaced, thus partly causing elevated feed supply.

2.4 30-day trial with smolt waste sludge (E4)

Based on evaluation of results from the preceding experiments, a 30-day trial using smolt waste sludge as well as fish feed and a diet consisting of different algae was planned. The objective was to evaluate the influence of different diets on growth and biochemical composition in terms of CN-content, total organic matter (TOM), carbohydrates, total lipid and total fatty acid content as well as the fatty acid and amino acid profile of polychaetes *H. diversicolor* (Sampling IV, Table 2.1). Throughout the trial, the polychaetes were cultivated in a fully automated XR3 cultivation system (Aquatic habitats, Pentair plc, USA) as shown in Figure 2.3.

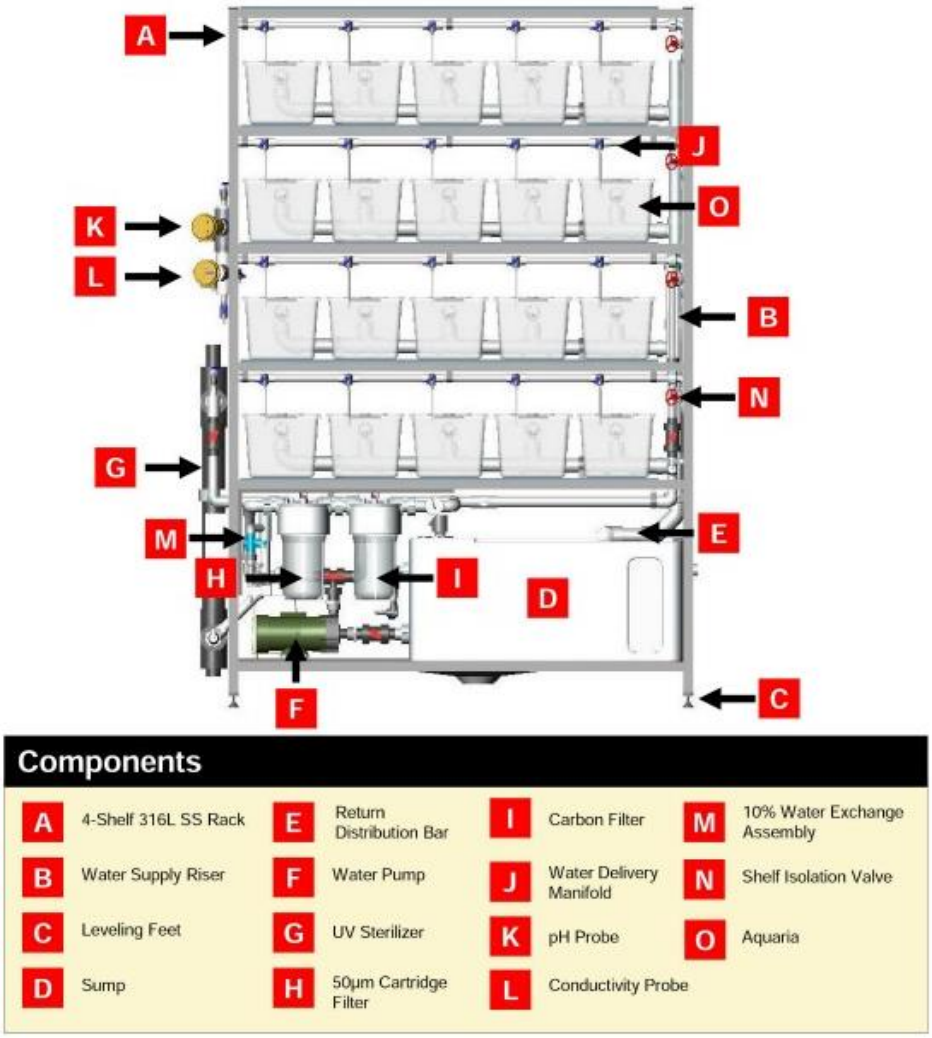

Figure 2.3 – XR3 cultivation system (Pentair plc, USA Aquatic habitats) (MBKI Installations Ltd - Pentair, 2017).

The system was initially constructed as a recirculated system with different filtration units for water treatment including mechanical (filter pads, cartridge filter), biological (moving bed bio filter) and chemical filtration (activated carbon filter), along with disinfection (ultraviolet sterilizer). However, in the original set-up, cell sizes of diet components were small enough to pass through the filtration system, thus leading to an undesired mixing of diets within the cultivation system. Therefore, constructional alterations were made in order to be able to run the cultivation rig as a flow-through system. An overview of the tank set-up can be found in Table 2.2.

Table 2.2 – Overview tank set-up E4 (30-day trial with smolt waste sludge).

Row	Number of tanks	Volume per tank	Treatment
1 (top)	5	16 L	(1) FF: Commercial fish feed
2	5	16 L	(2) SWS: Smolt waste sludge
3	5	16 L	(3) SFD: Shellfish Diet 1800 ®
4 (bottom)	5	16 L	(4) Mix: Smolt waste sludge/ shellfish diet (ratio 5:1)

The water flow in all tanks was set to ensure a daily water exchange of 100%. Natural seawater was supplied from the Trondheimsfjord and heated to a temperature of 19.5±0.3 °C (34.5±0.5 ppt) prior to entering the tanks. The L/D ratio in the room was set to 16/8 h. Four different diets were tested: (1) FF: commercial fish feed (GEMMA DIAMOND 1.0, Skretting AS, Norway), (2) SWS: smolt waste sludge provided by SalMar, (3) SFD: Instant Algae ® Shellfish Diet 1800 ® (Reed Mariculture, USA), and (4) Mix: a mix of smolt waste sludge and shellfish diet in the ratio 5:1.

Prior to the trial, carbon contents of the different diets were determined using an ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., USA). Quantities of each diet were calculated to achieve a carbon content in each treatment which was equivalent to the carbon content in the amount of fish feed that corresponds to 3% of polychaete biomass (WW). Therefore, the diets in the different treatment groups were iso-carbonic.

The following will give a more detailed overview of the different diets and their treatment prior to the trial.

(1) FF: commercial fish feed (GEMMA DIAMOND 1.0, Skretting AS, Norway)

To achieve a smaller particle size, the fish feed was ground using a mortar. During feeding, it was suspended in seawater.

(2) SWS: Smolt waste sludge provided by SalMar

Upon arrival at SINTEF, smolt waste sludge was drained and centrifuged for 15 min at 3000 rpm using a KR22i centrifuge (Jouan SA/Thermo Fisher Scientific, USA) to decrease the water content. Subsequently, it was blended to ensure homogenous composition. Small portions were frozen and defrosted a day prior to feeding. For feeding, the smolt waste sludge was re-suspended in seawater and then supplied to the culture tanks.

(3) SFD: Instant Algae ® Shellfish Diet 1800 ® (Reed Mariculture, USA)

Following manufacturer specifications, the liquid shellfish diet paste was stored in the fridge (3 °C) (Reed Mariculture, 2015). During feeding, it was mixed with seawater to increase the volume supplied to the tanks and ensure an even distribution. Instant Algae ® Shellfish Diet 1800 ® was chosen since it constitutes a nutritious mixture of microalgae (Table 2.3) and most importantly does not contain *Rhodomonas* sp., which was the feed supplied in the adaption period prior to the 30-day trial and polychaetes could have therefore accustomed to it.

Table 2.3 – Composition of SFD: Instant Algae ® Shellfish Diet 1800 ® (Reed Mariculture, 2015).

Component (microalgae)	**Percentage (DW)**
Tisochrysis lutea	40%
Pavlova sp.	15%
Tetraselmis sp.	25%
Thalassiosira pseudonana	20%
Thalassiosira weissflogii	Traces
Chaetoceros calcitrans	Traces

Table 2.4 – Overview of the biomass per tank and treatment at the beginning of E4 (30-day trial with smolt waste sludge). FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1). Same superscripts indicate non-significant differences ($P \geq 0.05$).

Treatment		**FF [g]**	**SWS [g]**	**SFD [g]**	**Mix**
Tank	1	5.68	5.63	6.60	6.04
	2	6.17	6.17	6.82	5.26
	3	5.95	5.44	6.05	6.10
	4	6.03	6.19	5.25	5.99
	5	6.03	6.21	6.12	7.03
Mean±SD		5.97±0.18[a]	5.93±0.37[a]	6.17±0.61[a]	6.08±0.28[a]

For each diet, five replicate tanks with 30 individuals of *H. diversicolor* per tank (LxHxW 473x260x178 mm, total available tank volume V_T =16L) were used, corresponding to a stocking density of 275 individuals m^{-2}. The average biomass per tank and treatment were as shown in Table 2.4. The used culture sediment was chamotte. It was washed prior to the experiment in order to rinse out the smallest particles. The sediment height was set to 8 cm, leaving enough space for 6.5 cm (= 8 L) of seawater above the sediment.

Polychaetes for the experiment were obtained from holding tanks after an adaption period of three weeks. The L/D ratio was gradually increased throughout the adaption period (from L/D

= 5.5/18.5 h to 16/8 h) to ensure adjustment of the individuals to experimental conditions. They were carefully retrieved from the natural sediment in the holding tanks, washed with seawater, superficially dried, individually weighed (Collection Science Education scale, SE 622, VWR International, USA) and then given into the culture tanks in the experimental rig. Following a starvation period of 24 h in which any dead individuals were replaced, feeding occurred daily over the course of the next 30 days at 16:00. For this purpose, the "feeding" function of the system was used, allowing even the lightest diet components to settle and thus making them available to the polychaetes. This function disables water flow for a desired amount of time (16 h in this trial) while maintaining aeration of the tanks. Water flow subsequently re-started at 08.00 h the following morning. Dead polychaetes discovered in the experimental period were removed twice a day, but not replaced, leading to an increased feed supply in some cases.

Following 30 days of feeding, no feed was supplied for 36 h to avoid gut contents of polychaetes impacting the results of chemical analyses. Afterwards, all polychaetes were retrieved from the sediment and surviving individuals were placed in sea water to promote clearing of the guts. Preceding experiments showed that after two hours, no further emptying of the guts takes place. Therefore, polychaetes were taken out of the seawater after two hours; adherent water was removed and individuals were weighed (Collection Science Education scale, SE 622, VWR International, USA). Then, polychaetes were rinsed with freshwater, pooled in treatment groups, given into 50 mL Falcon™ tubes and frozen at -80 °C.

The frozen polychaete samples as well as samples of the used diets were freeze-dried for later chemical analysis.

2.5 Basic analyses

2.5.1 Water quality parameters

Water temperature, pH values, and dissolved oxygen levels were measured and recorded throughout all experiments using a Professional Plus Multiparameter instrument (YSI Incorporated, USA) for E1-E3 and an YSI ProDSS Multiparameter Water Quality Meter (YSI Incorporated, USA) for E4.

2.5.2 Growth, specific growth rate and mortality

Table 2.5 – Overview of basic analyses carried out in E1 (Evaluation of culture sediment), E2 (Trial with cleaner fish waste), E3 (Trial with post-smolt waste sludge) and E4 (30-day trial with smolt waste sludge).

Analyses	Unit	Method	Number of samples (n)			
			E1	E2	E3	E4
Mean weight	mg	Weighing	4x4	4x4	4x4	4x5
Specific growth rate	% d^{-1}	Calculation	4x4	4x4	4x4	4x5
Mortality	%	Calculation	4x4	4x4	4x4	4x5

2.5.2.1 Growth

Prior to all experiments, polychaetes were retrieved from the holding tanks and adherent substrate was removed by washing; the individuals were then placed on tissue paper for drying and subsequently weighed using a VWR Collection Science Education scale (SE 622, VWR International, USA). At the end of the experiment, the procedure was repeated with the surviving polychaetes. Mean weight (±SD) before and after the experiment were registered. Since individual polychaetes were weighed in the 30-day trial, weight distributions corresponding to weight classes were compiled.

2.5.2.2 Specific growth rate

The specific growth rate μ of the different treatments was calculating using the following formula (Jørgensen, 1990):

$$\mu = \frac{\ln(W_t) - \ln(W_0)}{t}$$

With μ = specific growth rate [d^{-1}]

W_0 = average biomass (WW) per polychaete prior to the experiment [g]

W_t = average bio mass (WW) per polychaete after the experiment [g]

t = time [d]

The percentage growth per day P [% d^{-1}] was calculated by:

$$P = 100 * (\exp(\mu) - 1)$$

2.5.2.3 Mortality

The relative mortality resulted from the difference between polychaetes used in the experiment and the surviving individuals, and was calculated using the following formula:

$$Mort = \left(1 - \frac{N_t}{N_0}\right) * 100\%$$

With Mort = mortality [%]

N_0 = number of polychaetes used in the experiment [-]

N_t = number of surviving polychaetes [-]

2.5.3 Dissolved inorganic nutrients

Randomly picked water samples were taken from each treatment and analyzed using Hach reagent sets for photometry (Hach Lange GmbH, Germany). Concentrations were measured with a DR/890 portable colorimeter (Hach Lange GmbH, Germany). Prior to analysis, samples were vacuum-filtered using 25 mm GF/F filters (0.7 μm, Whatman plc, UK). An overview of dissolved nutrient analyses can be found in Table 2.6.

Table 2.6 – Overview of dissolved inorganic nutrient analyses.

Experiment	**Ammonia NH_3-N (n)**	**Nitrate NO_3^--N (n)**	**Phosphate PO_4^{3-} (n)**
E1: Evaluation of culture sediment	-	-	-
E2: Cleaner fish waste trial	2x4x4	-	-
E3: Post-smolt waste sludge trial	4x4x4	-	-
E4: 30-day trial with smolt waste sludge	7x4x3	7x4x3	7x4x3

Nitrogen, ammonia NH_3-N

Measurement of ammonia was performed following the salicylate method (Hach powder pillow method 8155 for 0-0.50 mg L^{-1} $N\text{-}NH_3$, Hach Lange GmbH, Germany). It involves a three-step reaction sequence. In the first step, ammonia reacts with chlorine to monochloramine. 5-aminosalicyate is formed by reaction of monochloramine with salicylate. Lastly, 5-aminosalicyate is oxidized with sodium nitroferricyanide and the extinction of the blue-green colored solution is measured at 610 nm using a colorimeter (Hach Company/Hach Lange GmbH, 2015b).

Nitrate NO_3^--N

Measurement of nitrate was performed according to the cadmium reduction method (Hach powder pillow method 8192 for 0-0.50mg L^{-1} NO_3^--N (LR), Hach Lange GmbH, Germany). Nitrate is reduced to nitrite by addition of cadmium. Nitrite ions react in an acidic medium with

sulfanilic acid to form an intermediate diazonium salt. Due to coupling of the salt with chromotropic acid, the solution turns pink; the extinction is measured at 520 nm (Hach Company/Hach Lange GmbH, 2015a).

Phosphate, reactive (Orthophosphate) PO_4^{3-}

Measurement of phosphate was performed following the ascorbic acid method (Hach powder pillow method 8048 for 0-2.50 mg L^{-1} PO_4^{3-}, Hach Lange GmbH, Germany). Phosphate reacts in an acidic medium with molybdate to produce a mixed phosphate/molybdate complex. The solution obtains an intense molybdenum blue color due to reduction of the complex with ascorbic acid; the extinction is measured with a colorimeter at 610 nm (Hach Company/Hach Lange GmbH, 2017).

2.6 Chemical analyses of diets and polychaetes (E4)

Table 2.7 – Overview of analyses carried out for the diets used in E4 (30-day trial with smolt waste sludge). Values for TOM, protein, total lipid and carbohydrate content of shellfish diet were obtained from the manufacturer and are marked with (M).

Analyses (diets)	Unit	Method	Number of samples (n)		
			Fish feed	Smolt waste sludge	Shellfish diet
TOM content	%	Combustion	5	5	(M)
Protein content	mg g^{-1} DW	Calculation	4	4	(M)
C and N content	[%]	CHNSO analyzer	4	4	4
C:N ratio	-	Calculation	4	4	4
Amino acids (relative)	%	HPLC	2	2	2
Total lipid	mg g^{-1} DW	Extraction	2	2	(M)
Total fatty acids	mg g^{-1} DW	Extraction	2	2	3
Fatty acids (relative)	%	GC	2	2	3
Carbohydrate content	mg g^{-1} DW	Calculation	1	1	(M)

Table 2.8 - Overview of analyses carried out in E4 (30-day trial with smolt waste sludge). *H. diversicolor* under different food regimes. Wild - natural field; Initial – *Rhodomonas* sp.; FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1).

Analyses (polychaetes)	Unit	Method	Number of samples (n)					
			Wild	Initial	FF	SWS	SFD	Mix
TOM content	%	Combustion	-	6	5	5	5	5
Protein content	mg g^{-1} DW	Calculation	3	3	5	5	5	5
C and N content	%	CHNSO analyzer	3	3	5	5	5	5
Carbon:Nitrogen ratio	-	Calculation	3	3	5	5	5	5
Amino acids (relative)	%	HPLC	1	2	5	5	5	5
Total lipid	mg g^{-1} DW	Extraction	2	3	5	5	5	5
Total fatty acids	mg g^{-1} DW	Extraction	2	3	5	5	5	5
Fatty acids (relative)	%	GC	2	3	5	5	5	5
Carbohydrate content	mg g^{-1} DW	Calculation	-	1	1	1	1	1
TOM content sediment	%	Combustion	-	-	5	5	5	5

2.6.1 Total organic matter

For determination of the total organic matter content of polychaetes and diets, sub-samples of dried polychaetes and diets were used. The samples were weighed using an analytic scale (AG204 DeltaRange®, Mettler-Toledo, USA) and then placed in a muffle furnace (Hagan Elektroovner AS, Norway) for five hours at 450 °C. Afterwards, the remaining ash was weighed using the same scale.

Total organic matter was calculated using the following formula:

$$TOM = \frac{(m_{sample+crucible,bef.} - m_{crucible}) - (m_{sample+crucible,aft.} - m_{crucible})}{(m_{sample+crucible,\ bef.} - m_{crucible})} * 100\%$$

With TOM = Total organic matter [%]

$m_{sample+crucible,bef.}$ = mass of crucible + sample before combustion [g]

$m_{crucible}$ = mass of the crucible [g]

$m_{sample+crucible,aft.}$ = mass of crucible + sample (ash) after combustion [g]

For determination of TOM content in the sediment, after the trial substrate samples were taken from each tank within the different treatments and dried at 60 °C for 24 h (TS 8000 Series, Termaks AS, Norway). Afterwards they were combusted using a muffle furnace (Hagan Elektroovner AS, Norway) as described above. For calculation of TOM content, the same formula as for polychaetes and diets was used.

2.6.2 Carbon and nitrogen determination

Carbon (C) and nitrogen (N) analyses of diets and polychaetes were conducted by SINTEF Fisheries and Aquaculture (Marte Schei) using an ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., USA). Samples of freeze-dried and ground diets as well as polychaetes were weighed in tin capsules using an ultra-micro scale (UM3, Mettler-Toledo, USA). The tin capsules containing samples were placed in the combustion chamber where they

reacted with oxygen and were combusted at a temperature of 1700-1800 °C; thereby, the samples were broken down into its elements. Combustion gases were separated in a gas chromatograph (GC) and detected by a thermal conductivity detector. A signal proportional to each element in the sample was produced and evaluated by comparing the peak to a known standard using the element analysis software. The percentage of each element was calculated based on the initial sample weight (Costech Analytical Technologies, 2017). The C:N ratio was calculated by dividing carbon by nitrogen content.

2.6.3 Protein content

The relative protein content was determined by multiplication of the nitrogen content with a factor of 6.25, assuming that protein contains 16% nitrogen (Jones, 1941).

2.6.4 Amino acid analysis

Protein-bound amino acids were analyzed by SINTEF Aquaculture and Fisheries (Ana Karina Carvajal, Rasa Slizyte') by means of high-performance liquid chromatography (HPLC) and following the manufacturer's handbook (Pinnacle PCX, Pickering laboratories, USA). 0.05-0.10 g of freeze-dried and ground diet and polychaete samples were weighed in a test tube and hydrolyzed for 24h at 110 °C using 6 M HCL containing 0.4% mercaptoethanol which served as a protecting group against destruction of amino acids. Subsequently, samples were filtered and the pH was adjusted to 2.2 before being analyzed by a HPLC system (Agilent Infinity 1260, Agilent Technologies, USA) coupled to an online post-column derivatization module (Pinnacle PCX, Pickering laboratories, USA) using ninhydrin (trione) as a derivatizing reagent and as well as a Na^+-ion exchange column. Using this method, a total of 18 amino acids as well as taurine were quantified.

2.6.5 Total lipid content and fatty acid analysis

Lipids in polychaetes, fish feed and smolt waste sludge were extracted according to Bligh&Dyer (1959). 20-30 mg of freeze-dried and ground polychaete and diet aliquots were weighed in 10mL test tubes with tight screw caps. To avoid degradation of lipids, the test tubes were kept in ice throughout the whole extraction. 0.8 mL deionized water, 2.0 mL methanol and 3.0 mL chloroform containing 40 ng μL^{-1} of internal standard (C23:0, Nu Chek Prep, Inc., USA) for latter fatty acid analysis were added and the sample was homogenized for one minute using an IKA® Ultra-Turrax® T10 disperser (Sigma-Aldrich Co. LLC, USA). 1.0 mL of chloroform was added, the sample was again homogenized for 20 s; afterwards 1.0 mL of deionized water was added and the sample was homogenized for another 20 s, moving the test tube up and down. The samples were then further mixed using a vortex mixer (Reax top, Heidolph instruments GmbH & Co. KG, Germany). Throughout this process, lipids were solved in chloroform. In order to separate the chloroform phase from the aqueous phase, the test tubes were centrifuged for 10 min at 4000 rpm and 5 °C using a Hettich Universal 32R centrifuge (Andreas Hettich GmbH & Co.KG, Germany). Due to its higher density of 1.49g cm^{-3}, chloroform containing lipids aggregated on the bottom of the test tube. It was retrieved using a glass pipet and transferred into a 20 mL glass bottle. 0.5 mL of the solution were then added into pre-weighed brown glass vials and evaporated using nitrogen. The remaining lipid was weighed. 1.0 mL were used for fatty acid analysis (Bligh and Dyer, 1959).

Lipid extraction for subsequent fatty acid extraction of shellfish diet required an extra step for breaking down cell walls of the plant cells and followed the method of Jakobsen et al. (2008).

20-25 mg of freeze-dried shellfish diet were suspended in 0.7 mL 0.1 M Tris HCl buffer (pH 7.5 at 100 °C) in a 50 mL test tube with a tight screw cap and heated in a drying oven (T9053, Termaks AS, Norway) at 98 °C. Afterwards, the sample was cooled down to 50 °C using ice. 0.1 mL of freshly made protease (*Streptomyces griseus*) solution containing 10 mg protease/mL

Tris HCl buffer was added to the sample and the test tube was incubated for 1 h at 50 °C (Jakobsen et al., 2008). Subsequently, 2.0 mL methanol and 3.0 mL chloroform containing 40 ng μL^{-1} of internal standard (C23:0) were added for later fatty acid analysis and lipid extraction was performed as described above.

Following the total lipid extraction, fatty acids in the lipid extract were esterified to fatty acid methyl esters by formal condensation with methanol following Metcalfe et al. (1966). As a first step, 1.0 mL of lipid extract were transferred into 10 mL test tubes with tight screw caps and 1.0 mL of 0.5 N NaOH-methanol were added. After each step, samples were mixed using a vortex mixer (Reax top, Heidolph instruments Gmbh & Co. KG, Germany). The test tubes were placed into a block heater (Dri-Block® DB-3D, Techne Ltd., UK) and heated for 15 min at 100 °C followed by cooling of the samples in ice water. Then, 2.0 mL of BF_3-methanol were added for esterification and the samples were placed into the block heater for another 5 min at 100 °C. After cooling down in ice water, 1.0 mL of isooctane was added to the samples and the test tubes were put into the block heater for 1 min at 100 °C. Subsequent to a final cooling, 3.0 mL of saturated NaCl solution as well as 0.5 mL of isooctane were added to the samples which were then mixed on the vortex mixer and centrifuged for 3 min at 4000 rpm (Universal 32R centrifuge **(**Andreas Hettich GmbH & Co.KG, Germany). After centrifugation, fatty acid methyl esters could be found in the isooctane phase which was the upper of the two resulting layers. The isooctane phase was transferred into a 20 mL glass bottle with a glass pipet, and another 0.5 mL of isooctane were added to the sample. Mixing, centrifugation and transfer of the isooctane phase were conducted as before and the process was repeated another time using 0.5 mL isooctane, resulting in a total of three separating processes (Metcalfe et al., 1966). The resulting solution was transferred into a GC-vial (Teknolab AS, Norway) and sealed.

Fatty acid methyl esters were separated using a gas chromatograph **(**7890B GC, Agilent Technologies, USA) with helium carrier, a WCOT fused-silica capillary column coated with

CP-wax 52CB (Holger CP7713) and a flame ionization detector (FID) using the program presented in Table 2.9.

Table 2.9 – Measurement program gas chromatograph used for fatty acid analysis in E42 (30-day trial with smolt waste sludge).

	Rate [° C/min]	Temperature [° C]	Holding time [min]	Run time [min]
(Initial)		90	2	2
Ramp 1	30	150	0	4
Ramp 2	2.5	230	0	36
Ramp 3	10	240	23	60

An auto-sampler (7693A Automatic Liquid Sampler, Agilent Technologies, USA) was used for injection of external standard and samples. Isooctane was used to wash needle and separation column before and between analyses of samples. Fatty acids were qualified using an external standard 68D (Nu Chek Prep, Inc., USA); it was measured prior to the samples. Retention times of fatty acids in the standard were compared to those in the samples by the OpenLAB CDS Software 2.1 (Agilent Technologies, USA) for final identification. The same software quantified fatty acids by integrating peak areas and relating them to the peak area of the internal standard C23:0 (Nu Chek Prep, Inc., USA) as well as to the initial sample mass.

$$Conc._{FA} = \frac{m_{ISTD} * A_{FA}}{A_{ISTD} * m_{sample} * DF}$$

With $Conc._{FA}$ = fatty acid content [mg g^{-1} DW]

m_{ISTD}. = mass of the internal standard [mg]

A_{ISTD} = area of the internal standard [mm^2]

A_{FA} = area of the fatty acid [mm^2]

m_{sample} = mass of the sample [g]

DF = dilution factor [-]

2.6.6 Carbohydrates

The carbohydrate content of polychaetes and diets was calculated by subtracting values of protein and total lipid from total organic matter (TOM).

2.7 Statistics

Statistical analyses were carried out using SigmaPlot® for Windows Version 13.0 (SigmaPlot, Systat Software Inc., USA). Tables were made in Microsoft® Office Professional Plus Excel and Word 2013 for Windows (Microsoft Corporation, USA). Graphs were realized in SigmaPlot ® 13.0.

Normal distribution of data was tested using Shapiro-Wilk tests; equality of variance was analyzed by the Brown-Forsythe test.

Water quality parameters, mean weights of polychaetes, specific growth rates, mortality as well as nutritional composition (TOM, protein content, C:N ratio, amino acids, lipid content and fatty acids) of diets and polychaetes were tested for significant differences. Statistical analysis was performed at the 95% confidence level ($P < 0.05$).

Unpaired t-tests and Mann-Whitney rank sum tests were conducted when comparing only two groups (initial and final biomass; some diet analyses) of normally and non-normally distributed data, respectively. One way analysis of variance (ANOVA) was carried out to compare means of normally distributed numerical data; Kruskall-Wallis one way ANOVA on ranks was used for non-normally distributed numerical data. Pairwise comparisons following one way ANOVA were made using Holm-Sidak tests; one way ANOVA on ranks were followed by Tukey and Dunn's post-hoc tests, the latter in case of unequal treatment groups.

3 Results

3.1 Evaluation of culture sediment (E1)

Table 3.1 – Parameters (Mean±SD, 2x4x4n) of the cultivation system during the culture sediment evaluation experiment (E1). AS-F – 0.4-0.8mm aquarium sand; AS-M – 1mm aquarium sand; AS-C – 1.2-3 mm aquarium sand; CHA – Chamotte. Different superscripts indicate significant differences ($P < 0.05$).

Parameters	**AS-F**	**AS-M**	**AS-C**	**CHA**
Temperature [°C]	10.1 ± 0.0^{bc}	10.3 ± 0.2^{ab}	10.1 ± 0.1^{c}	10.4 ± 0.1^{a}
pH	8.2 ± 0.0^{a}	8.2 ± 0.0^{a}	8.2 ± 0.0^{a}	8.2 ± 0.01^{a}
Dissolved oxygen [mg L^{-1}]	8.9 ± 0.1^{a}	8.9 ± 0.1^{a}	9.0 ± 0.0^{a}	8.9 ± 0.1^{a}

A summary of basic parameters during E1 – evaluation of culture sediment is presented in Table 3.1. Water quality parameters in tanks with four different substrates were monitored for comparison between treatments and in order to ensure appropriate culture conditions throughout the experiment. All measurements were within the suitable range for *H. diversicolor* (Hartmann-Schröder, 1996). Temperatures were measured to be 10.0-10.5 °C in the cultivation system and were significantly different between treatments. Values for pH were around 8.2 and dissolved oxygen levels for all treatments were high (≥8.9 mg L^{-1}). With regard to pH and dissolved oxygen levels, there was no statistical difference between treatments.

Figure 3.1 describes initial and final mean weight (WW) of polychaetes in E1. The average initial wet weight of polychaetes cultured in different substrate was not significantly different between groups (one way ANOVA on ranks) and varied between 130.0±22.9 mg (AS-M) and 142.5±17.7 mg (CHA). The final individual biomass (WW) was between approximately 126 mg (AS-F, AS-M and AS-C) and 159.1±43.6 mg (CHA) per polychaete. Both, the differences between initial and final value within a treatment (t-tests) and the final values between treatments (one way ANOVA) showed no statistical significance, hence indicating no quantifiable growth. Consequently, specific growth rates [d^{-1}] (Figure 3.2) were close to zero

for polychaetes in all substrates. Polychaetes cultured in chamotte showed slightly positive growth whereas in all other treatments specific growth rates were negative; however, differences were not significant (one way ANOVA).

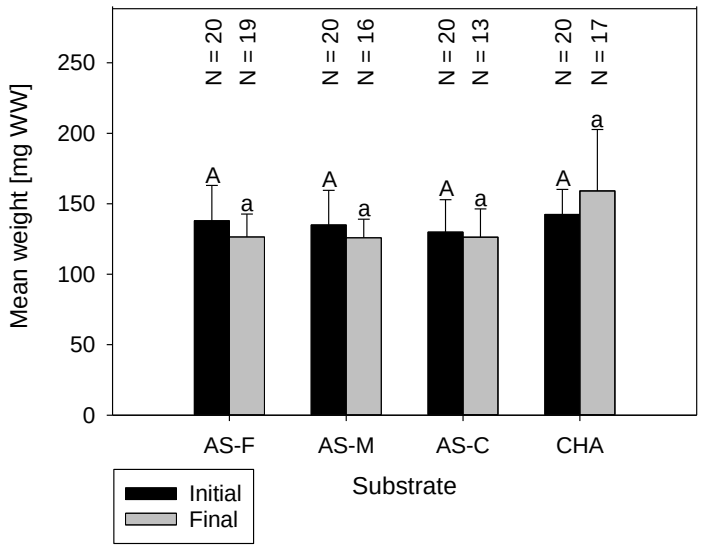

Figure 3.1 – Mean weight (±SD) of *H. diversicolor* cultivated in different substrates (4x4n). AS-F – 0.4-0.8mm aquarium sand; AS-M – 1mm aquarium sand; AS-C – 1.2-3 mm aquarium sand; CHA – Chamotte. N equals the number of weighed polychaetes per group. Same uppercase superscripts indicate non-significant difference between initial values. Same lowercase superscripts indicate non-significant differences between final values ($P \geq 0.05$).

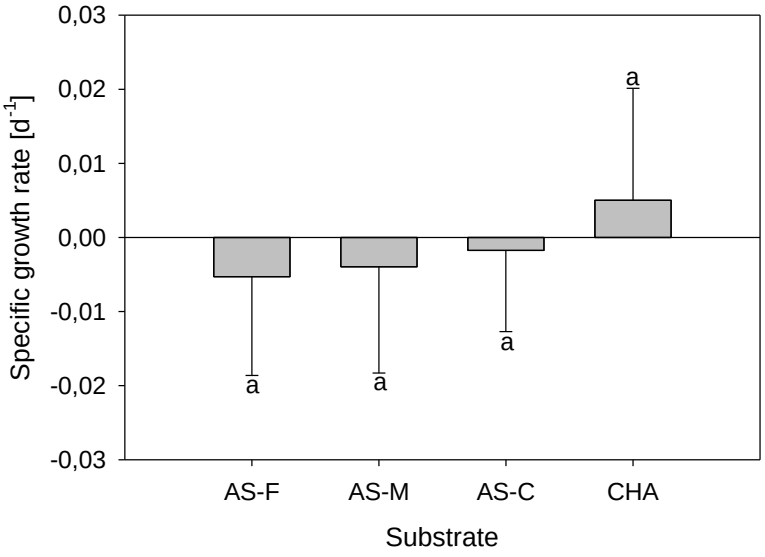

Figure 3.2 – Specific growth rate (Mean±SD) of *H. diversicolor* cultivated in different substrates (4x4n). AS-F – 0.4-0.8 mm aquarium sand; AS-M – 1.0 mm aquarium sand; AS-C – 1.2-3 mm aquarium sand; CHA – Chamotte. Same superscripts indicate non-significant differences ($P \geq 0.05$).

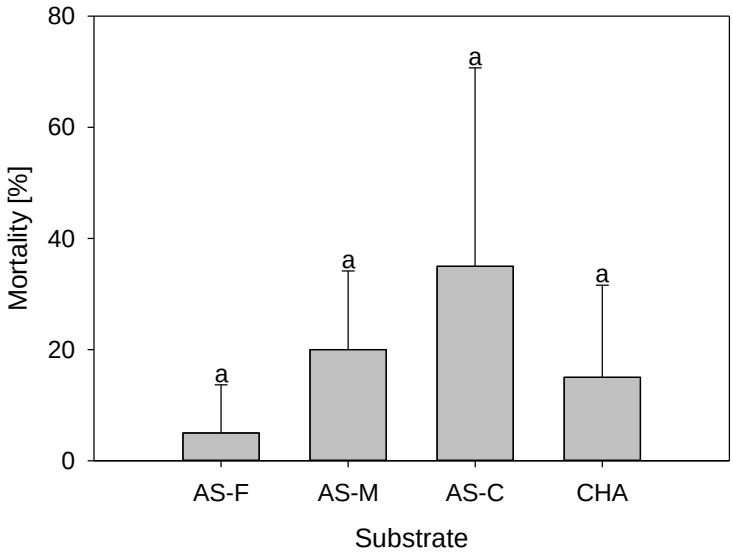

Figure 3.3 – Mortality (Mean±SD) of *H. diversicolor* cultivated in different substrates (4x4n). AS-F – 0.4-0.8mm aquarium sand; AS-M – 1mm aquarium sand; AS-C – 1.2-3 mm aquarium sand; CHA – Chamotte. Same superscripts indicate non-significant differences ($P \geq 0.05$).

As seen in Figure 3.3, mortality in all treatments was low to moderate, ranging from 5.0±8.7% (AS-F) to 35.0±35.7% (AS-C). One way ANOVA on ranks concluded no significant difference between culture sediments.

3.2 Trial with cleaner fish waste (E2)

Table 3.2 – Parameters (Mean±SD, 2x4x4n) of the cultivation system during the cleaner fish waste trial (E2). 1.5% FF – 1.5% fish feed; 0.5% CFW – 0.5% Cleaner fish waste; 1.5% CFW – 1.5% Cleaner fish waste; 2.5% CFW – 2.5% Cleaner fish waste. The percentages correspond to the initial weight (WW) of the polychaetes. Different superscripts indicate significant differences ($P < 0.05$).

Parameters	**1.5% FF**	**0.5% CFW**	**1.5% CFW**	**2.5% CFW**
Temperature [°C]	10.8 ± 0.4^{a}	10.6 ± 0.3^{ab}	10.3 ± 0.2^{b}	10.5 ± 0.4^{ab}
pH	8.2 ± 0.1^{a}	8.2 ± 0.1^{a}	8.2 ± 0.1^{a}	8.3 ± 0.0^{a}
Dissolved oxygen [mg L^{-1}]	8.4 ± 0.8^{a}	8.6 ± 0.1^{a}	8.2 ± 0.1^{a}	8.6 ± 0.1^{a}
NH_3 – N [mg L^{-1}]	0.05 ± 0.03^{a}	0.05 ± 0.03^{a}	0.06 ± 0.04^{a}	0.03 ± 0.02^{a}

Table 3.2 displays an overview of basic experimental parameters during E2 – trial with cleaner fish waste. In all treatments, measured water quality parameters and ammonia concentrations were within optimum range for *H. diversicolor* (Hartmann-Schröder, 1996). Mean temperatures were 10.1-11.0 °C, being significantly different between treatments. Dissolved oxygen levels remained stable in all tanks and were not significantly different between treatments; ammonia concentrations were low in all treatments and did not differ significantly (< 0.1 mg L^{-1}).

Figure 3.4 describes initial and final mean weight of polychaetes in E2 fed with four different diets. Values for the initial mean wet weight were similar and not significantly different between treatments (one way ANOVA), ranging from 135.0±13.6 mg (2.5% CFW) to 159.0±19.9 mg (1.5% FF). Final mean values for polychaetes biomass were between 117.4±24.0 mg (0.5% CFW) and 173.8±48.5 mg (1.5% FF). Significant differences were not detected, neither between final mean weight of the polychaetes fed with different diets (one way ANOVA), nor between initial and final values within treatments (t-tests). As a result, specific growth rates (Figure 3.5) were low, varying between -0.0081±0.0049 d^{-1} (0.5% CFW) and 0.0026±0.0104 d^{-1} (1.5% FF). One way ANOVA on ranks concluded that differences in

growth of polychaetes fed with different diets were not great enough to exclude the possibility that the differences were due to random sampling variability.

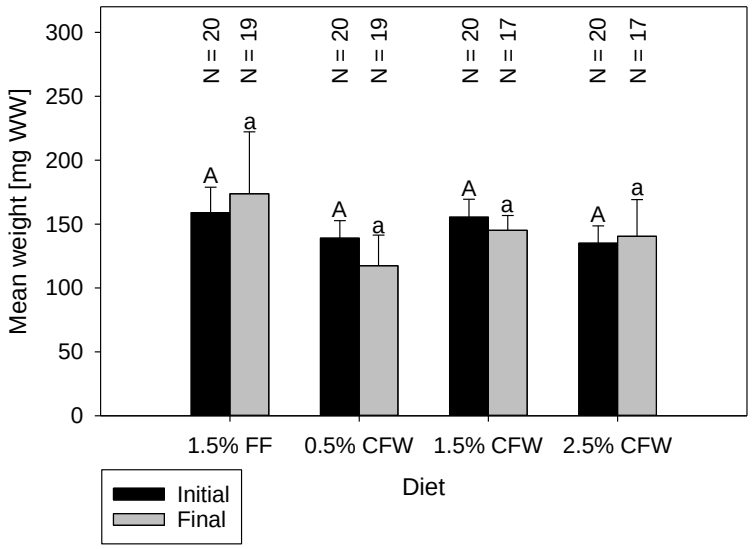

Figure 3.4 – Mean weight (±SD) of *H. diversicolor* (before and after the trial) fed on various diets (4x4n). 1.5% FF – 1.5% fish feed; 0.5% CFW – 0.5% Cleaner fish waste; 1.5% CFW – 1.5% Cleaner fish waste; 2.5% CFW – 2.5% Cleaner fish waste. The percentages correspond to the initial weight (WW) of the polychaetes. N equals the number of weighed polychaetes per group. Same uppercase superscripts indicate non-significant difference between initial values. Same lowercase superscripts indicate non-significant differences between final values ($P \geq 0.05$).

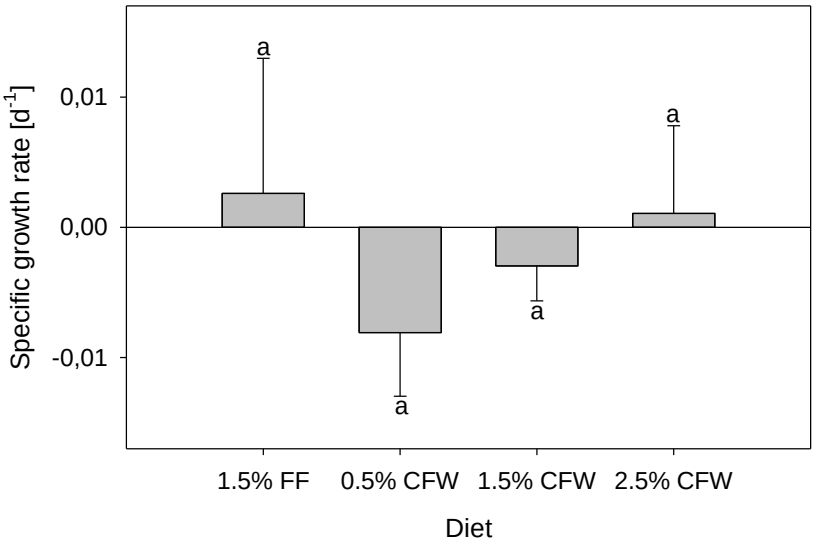

Figure 3.5 – Specific growth rate (Mean±SD) of *H. diversicolor* fed on various diets (4x4n). 1.5% FF – 1.5% fish feed; 0.5% CFW – 0.5% Cleaner fish waste; 1.5% CFW – 1.5% Cleaner fish waste; 2.5% CFW – 2.5% Cleaner fish waste. The percentages correspond to the initial weight (WW) of the polychaetes. Same superscripts indicate non-significant differences ($P \geq 0.05$).

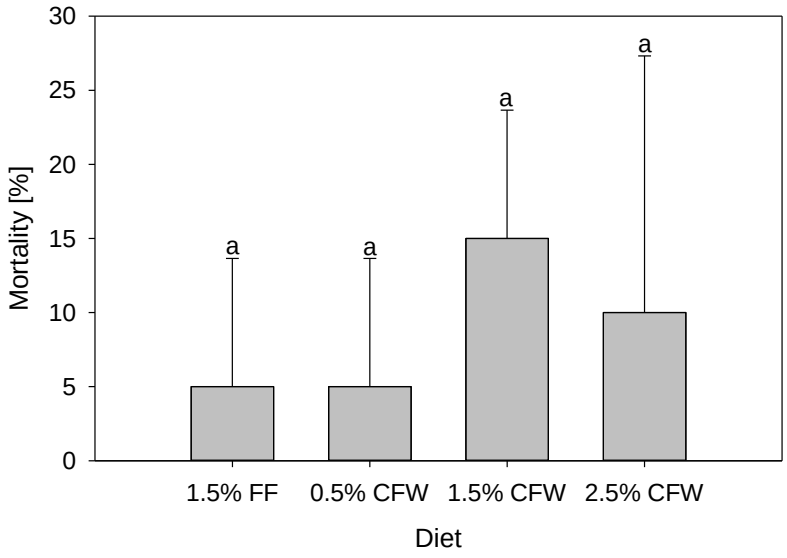

Figure 3.6 – Mortality (Mean±SD) of *H. diversicolor* fed on various diets (4x4n). 1.5% FF – 1.5% fish feed; 0.5% CFW – 0.5% Cleaner fish waste; 1.5% CFW – 1.5% Cleaner fish waste; 2.5% CFW – 2.5% Cleaner fish waste. The percentages correspond to the initial weight (WW) of the polychaetes. Same superscripts indicate non-significant differences ($P \geq 0.05$).

Mortality (Figure 3.6) during the cleaner fish waste trial was very low, ranging between 5.0±8.7% (1.5% FF and 0.5% CFW) and 15.0±8.7% (1.5% CFW). There were no significant differences between treatments (one way ANOVA in ranks).

3.3 Trial with post-smolt waste (E3)

Table 3.3 – Parameters (Mean±SD, 4x4x4n) of the cultivation system during post-smolt waste sludge trial (E3). 1.5% FF – 1.5% fish feed; 0.5% PSWS – 0.5% Post-smolt waste sludge; 1.5% PSWS – 1.5% Post-smolt waste sludge; 2.5% PSWS – 2.5% Post-smolt waste sludge. The percentages correspond to the initial weight (WW) of the polychaetes. Same superscripts indicate non-significant differences ($P \geq 0.05$).

Parameters	**1.5% FF**	**0.5% PSWS**	**1.5% PSWS**	**2.5% PSWS**
Temperature [°C]	11.0±0.4[a]	10.9±0.4[a]	10.7±0.4[a]	10.8±0.4[a]
pH	8.0±0.1[a]	8.1±0.1[a]	8.1±0.1[a]	8.1±0.1[a]
Dissolved oxygen [mg L^{-1}]	8.2±0.3[a]	8.4±0.2[a]	8.4±0.3[a]	8.4±0.3[a]
NH_3 – N [mg L^{-1}]	0.08±0.13[a]	0.04±0.08[a]	0.02±0.03[a]	0.03±0.03[a]

A summary of experimental parameters in E3 – trial with post-smolt waste is shown above (Table 3.3). Monitored water parameters and ammonia concentrations were within the suitable range for *H. diversicolor* (Hartmann-Schröder, 1996). Temperatures and pH values in all tanks remained stable throughout the trial, fluctuating only marginally between tanks and measurements (10.4-11.6 °C and 7.9-8.1, respectively). Dissolved oxygen levels were high and ensured sufficiently high oxygen saturation in each treatment. There were slight differences in ammonia concentrations between treatments, though all values remained under 0.14 mg L^{-1}. No statistical differences between treatments for neither of the measured parameters could be concluded.

Figure 3.7 describes initial and final mean weight of polychaetes in E3. The initial wet weight of polychaetes was not significantly different between feeding regimes (one way ANOVA) and had values from 224.0±16.1 mg (1.5% PSWS) to 265.5±22.0 mg (0.5% PSWS). The final individual biomass (WW) amounted to values between 220.8±6.6 mg (1.5% PSWS) and 279.8±32.3 mg (1.5% FF). Data analyses showed no significantly differences between the final mean weights (WW) of polychaetes (one way ANOVA on ranks). Furthermore, no significant differences were found between initial and final mean values for polychaete biomass within

treatment groups (t-tests). Specific growth rates [d^{-1}] (Figure 3.8) were negative in all groups fed with post-smolt waste sludge and only slightly positive in the polychaete group fed with fish feed, though not significantly different between treatments (one way ANOVA).

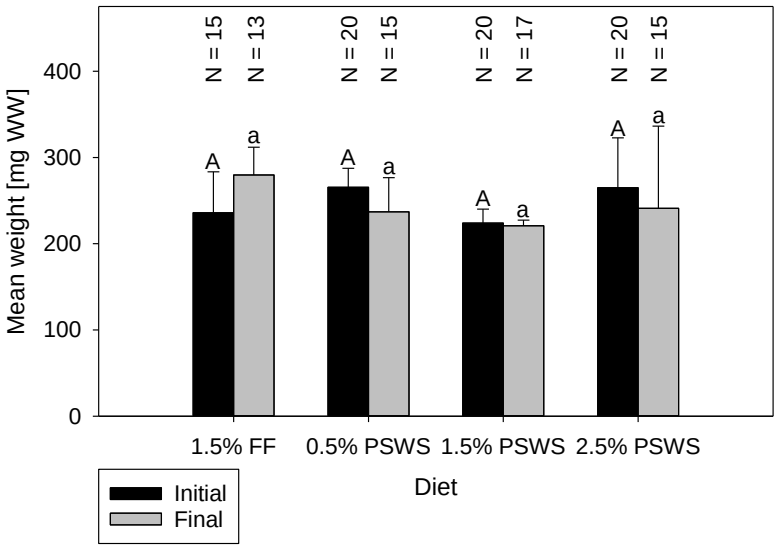

Figure 3.7 – Mean weight (±SD) of *H. diversicolor* (before and after the trial) fed on various diets (4x4n). 1.5% FF – 1.5% fish feed; 0.5% PSWS – 0.5% Post-smolt waste sludge; 1.5% PSWS – 1.5% Post-smolt waste sludge; 2.5% PSWS – 2.5% Post-smolt waste sludge. The percentages correspond to the initial weight (WW) of the polychaetes. N equals the number of weighed polychaetes per group. Same uppercase superscripts indicate non-significant difference between initial values. Same lowercase superscripts indicate non-significant differences between final values ($P \geq 0.05$).

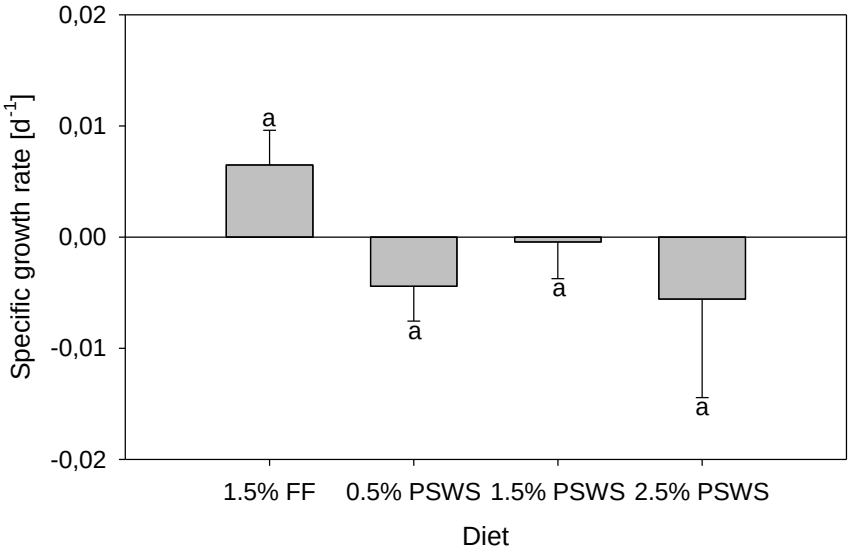

Figure 3.8 – Specific growth rate (Mean±SD) of *H. diversicolor* (before and after the trial) fed on various diets (4x4n). 1.5% FF – 1.5% fish feed; 0.5% PSWS – 0.5% Post-smolt waste sludge; 1.5% PSWS – 1.5% Post-smolt waste sludge; 2.5% PSWS – 2.5% Post-smolt waste sludge. The percentages correspond to the initial weight (WW) of the polychaetes. Same superscripts indicate non-significant differences ($P \geq 0.05$).

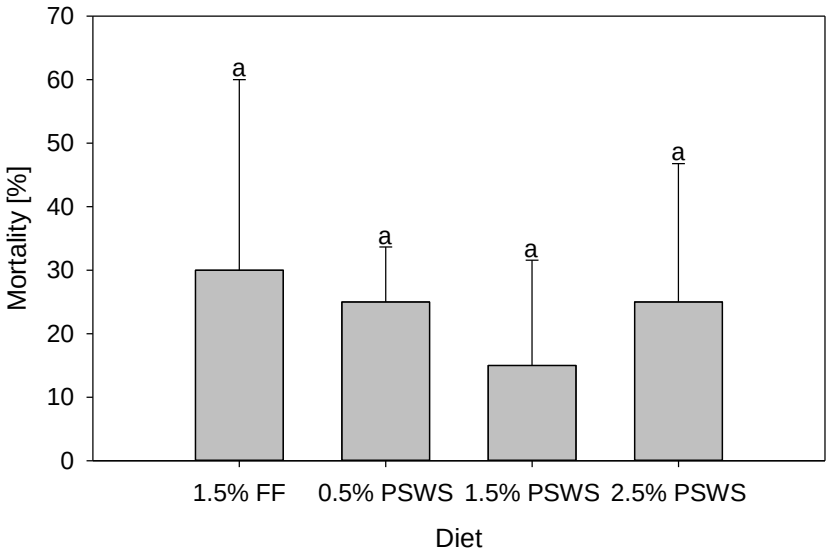

Figure 3.9 – Mortality (Mean±SD) of *H. diversicolor* fed on various diets (4x4n). 1.5% FF – 1.5% fish feed; 0.5% PSWS – 0.5% Post-smolt waste sludge; 1.5% PSWS – 1.5% Post-smolt waste sludge; 2.5% PSWS – 2.5% Post-smolt waste sludge. The percentages correspond to the initial weight (WW) of the polychaetes. Same superscripts indicate non-significant differences ($P \geq 0.05$).

With values ranging from 15.0±16.6% (1.5% PSWS) to 30.0±30.0% (1.5% FF), polychaetes showed moderate mortality in the trial evaluating suitability of post-smolt waste as a diet for *H. diversicolor* (Figure 3.9). Statistical analyses showed survival rates to be independent of the treatment (one way ANOVA).

3.4 30-day trial with smolt waste sludge (E4)

3.4.1 Water quality parameters

Table 3.4 gives an overview of the water parameters in the 30-day trial, all of which were in the appropriate range for *H. diversicolor* (Hartmann-Schröder, 1996). Water temperatures were measured to be between 19.3-20.0 °C, differing significantly between tanks with fish feed (FF) and tanks with a mix of smolt waste sludge and shellfish diet (Mix). Values for salinity and pH were constant and similar between treatments; oxygen saturation was high and remained stable throughout the trial. No significant differences for oxygen saturation and salinity were detected between treatments.

Table 3.4 – Summary of technical and biological parameters (Mean±SD, 6x4x3n) of the four different treatments during E4 (30-day trial with smolt waste sludge). FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1). Different superscripts indicate significant differences ($P < 0.05$).

Parameters	**FF**	**SWS**	**SFD**	**Mix**
Temperature [°C]	19.5 ± 0.3^{b}	19.6 ± 0.3^{b}	19.7 ± 0.2^{ab}	19.8 ± 0.2^{a}
pH	7.8 ± 0.1^{b}	7.8 ± 0.1^{b}	7.9 ± 0.1^{a}	7.9 ± 0.1^{a}
Oxygen saturation [%]	95.2 ± 3.6^{a}	95.3 ± 3.5^{a}	96.5 ± 2.7^{a}	95.9 ± 2.9^{a}
Salinity [ppt]	34.3 ± 0.5^{a}	34.5 ± 0.6^{a}	34.8 ± 0.7^{a}	34.5 ± 0.4^{a}

3.4.2 Growth

Figure 3.10 describes the mean weight (WW) of individual polychaetes in E4, both before and after the trial. The initial wet weight was approximately 200 mg per individual and did not differ between treatments (one way ANOVA). Final weights were significantly higher than initial values in the groups fed with fish feed, smolt waste sludge and shellfish diet (t-tests). Polychaetes fed with a mix of smolt waste sludge and shellfish diet did not have a significantly higher final than initial weight. There were significant differences in final weight between groups (one way ANOVA); specimen fed with fish feed had the highest final weight (424.2±27.4 mg) whereas polychaetes fed with the mix had the lowest final value (218.1±15.8 mg). With final mean weights of 275.8±26.7 mg, respectively, 314.7±42.4 mg, polychaetes in the groups fed with smolt waste sludge and shellfish diet were positioned between the other two.

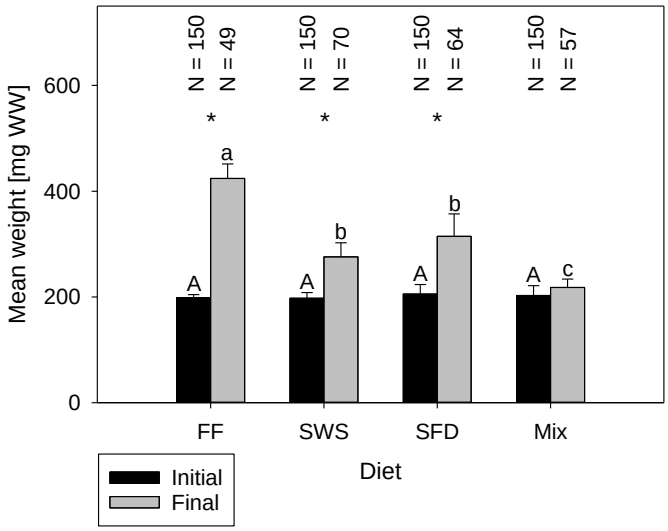

Figure 3.10 – Mean weight (±SD) of *H. diversicolor* (before and after the trial) fed on various diets (4x5n). FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1). N equals the number of weighed polychaetes per group. Significant differences between initial and final weight within treatments are marked with a star. Same uppercase superscripts indicate non-significant difference between initial values ($P \geq 0.05$). Different lowercase superscripts indicate significant differences between final values ($P < 0.05$).

Specific growth rates (Figure 3.11) are reflecting the change of polychaete mean weights. With 0.025±0.002 d^{-1} (2.5±0.2% d^{-1}), the specific growth rate was highest for polychaetes fed with fish feed; polychaetes fed with smolt waste sludge and shellfish diet had significantly lower specific growth rates, amounting to 0.011±0.003 d^{-1} (1.1±0.3% d^{-1}) and 0.014±0.003 d^{-1} (1.4±0.3% d^{-1}), respectively. Polychaetes fed with a mix of waste sludge and shellfish diet showed the least growth, their specific growth rate came to 0.002±0.005 d^{-1} (0.2±0.5% d^{-1}).

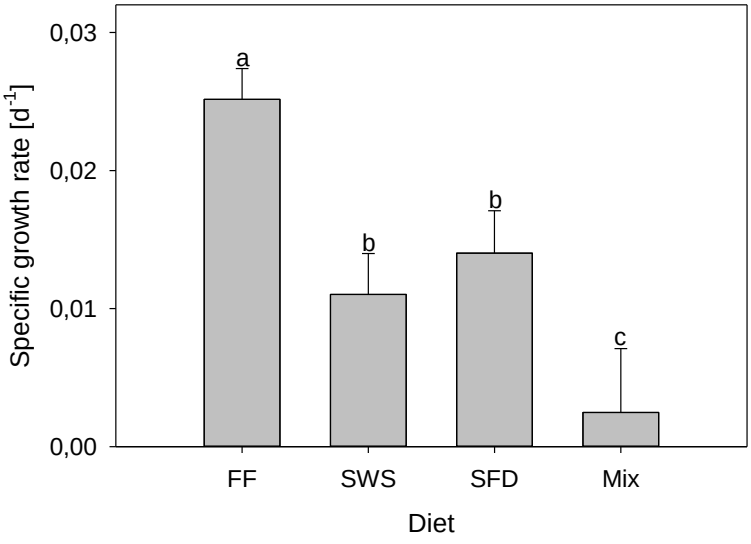

Figure 3.11 – Specific growth rate (Mean±SD) of *H. diversicolor* fed on various diets (4x5n). FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1). Different superscripts indicate significant differences ($P < 0.05$).

In order to determine changes in weight distribution of polychaetes before and after the trial depending on diet, weight classes ranging from <0.10 g to >0.45 g were attributed to individually weighed specimens (Figure 3.12). The proportion of polychaetes in different weight classes resembled the results comparing initial and final mean weight as well as specific growth rates. The graphs indicate a shift from smaller weight classes to higher ones in all treatment groups, except for the group fed with the mix. This shift is especially noticeable for polychaetes fed with fish feed. The weight distribution in the group fed with the mix remained approximately the same.

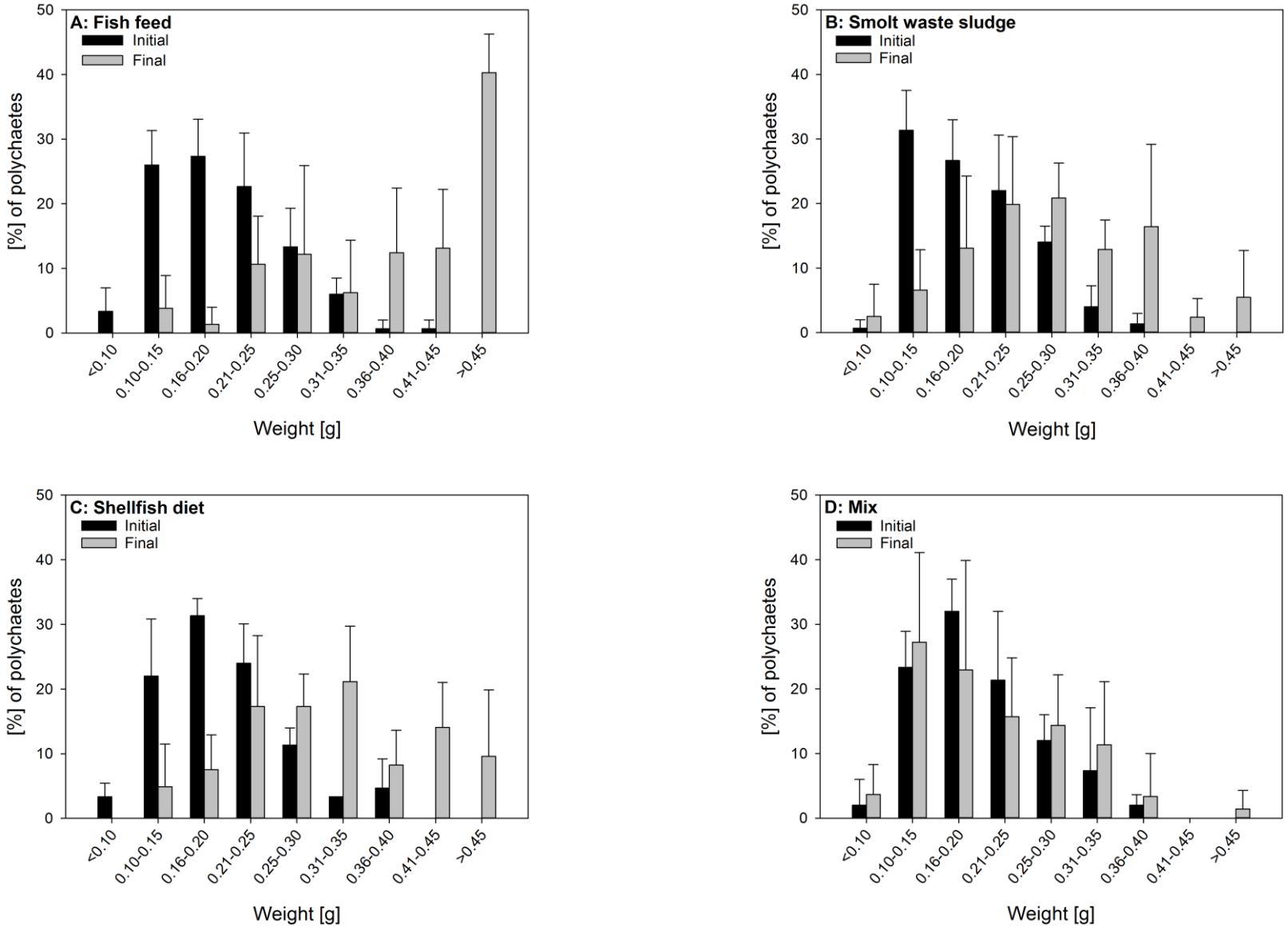

Figure 3.12 – Weight distribution (Mean±SD) of *H. diversicolor* (before and after E4) fed on various diets. A: Fish feed; B: Smolt waste sludge; C: Shellfish diet; D: Mix of smolt waste sludge and shellfish diet (ratio 5:1).

3.4.3 Mortality

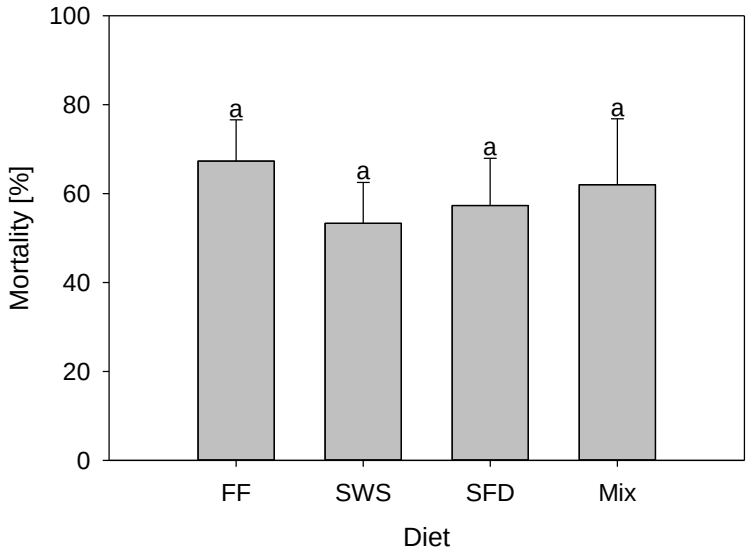

Figure 3.13 – Mortality (Mean±SD) of *H. diversicolor* fed on various diets (4x5n). FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1). Same superscripts indicate non-significant differences ($P \geq 0.05$).

As pictured in Figure 3.13, mortality during the 30-day trial was relatively high for all treatments. Average values ranged from 53.3±9.2% (SWS) over 57.3±10.6% (SFD) and 62.0±14.8% (Mix) to 67.3±9.3% (FF). Statistical analyses concluded no significant differences in mortality between groups of polychaetes fed with different diets (one way ANVOA). Figure 3.14 displays the observed mortality over the course of the experiment. While overall mortality was high, observed daily mean mortalities were low for all treatments, stretching from 0-1.4 dead individuals per day; the largest share in all treatment groups was made up by polychaetes that could not be found or retrieved alive from the sediment at the end of the trial (Unknown). When adding up all dead individuals that were retrieved and comparing them with the unknown group, the number of unknown deaths is a multiple of the known ones. Factors ranged from 2.6 (FF) to 6.8 (SFD). The graphs describing mortality of polychaetes fed with fish feed, smolt waste sludge and a mix of smolt waste sludge and shellfish diet (Figure 3.17 A, B, D) display a slight trend of increased mortalities towards the end of the experiment. However, a substantial increase in mortality over time could not be detected for any of the treatment groups.

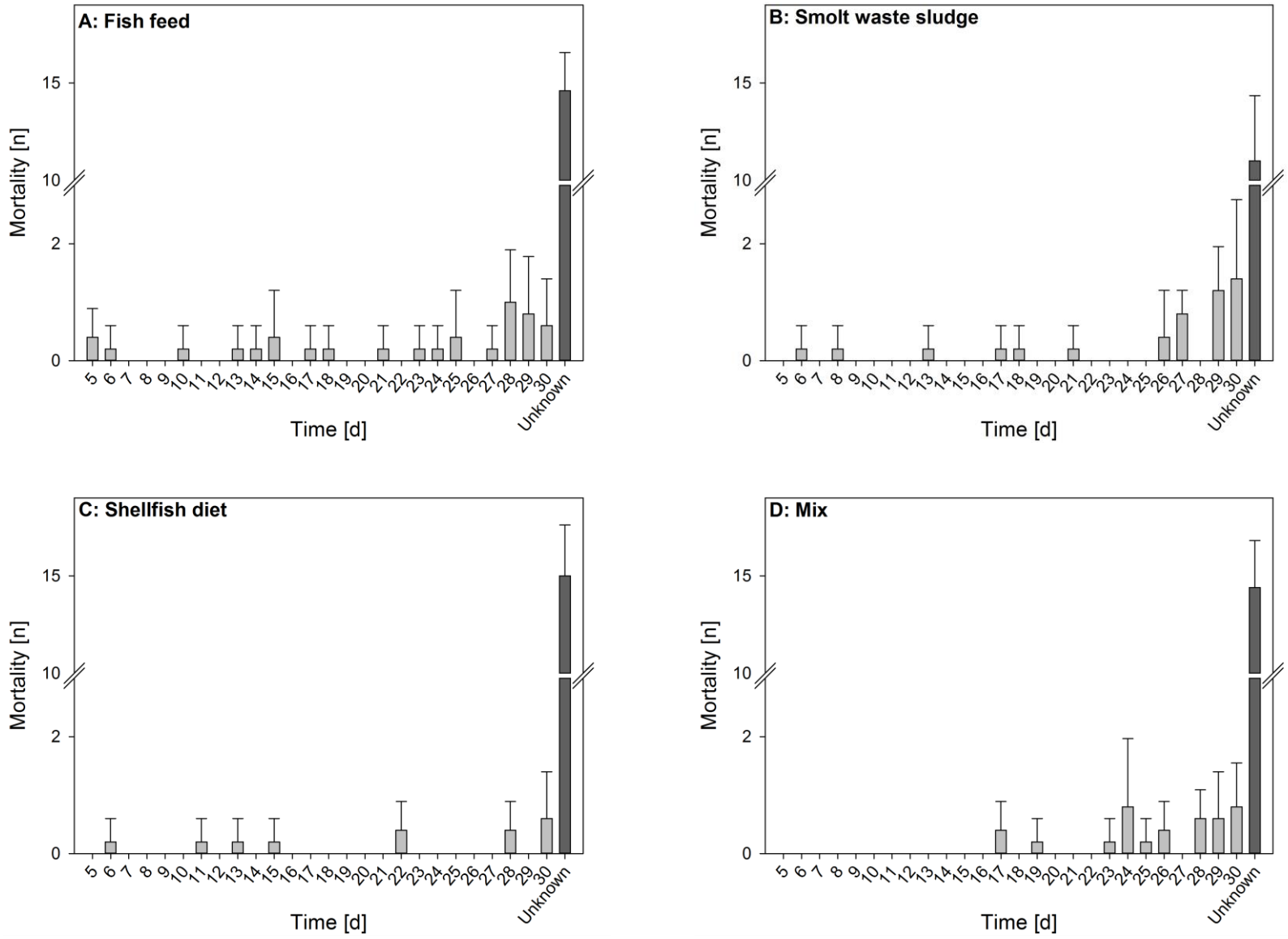

Figure 3.14 – Mortality (Mean±SD) of *H. diversicolor* fed on various diets over the course of E4 (30-day trial with smolt waste sludge). A: Fish feed; B: Smolt waste sludge; C: Shellfish diet; D: Mix of smolt waste sludge and shellfish diet (ratio 5:1).

3.4.4 Dissolved inorganic nutrients

Dissolved inorganic nutrients were measured seven times over the course of the trial; in three random tanks per treatment. The concentration of ammonia, nitrate and phosphate can be seen in Figure 3.15-3.17, respectively.

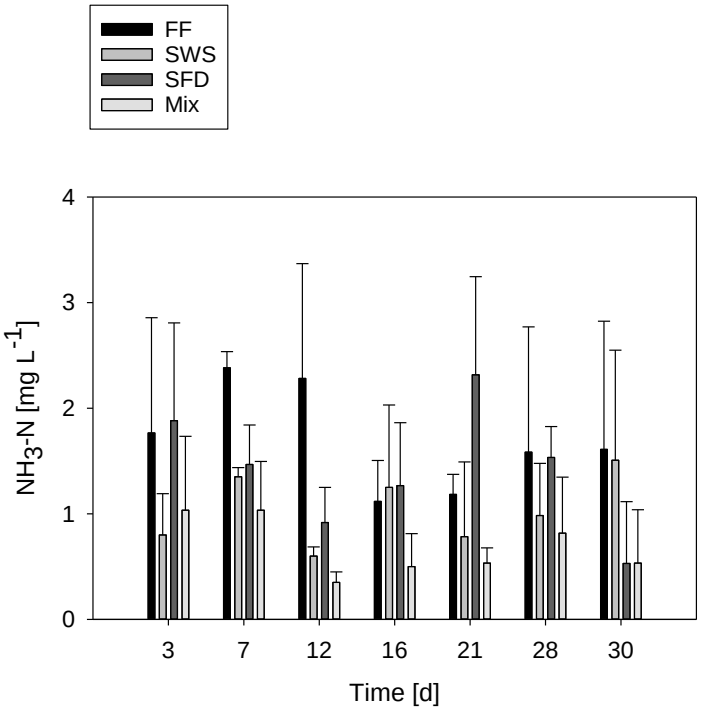

Figure 3.15 – Ammonia concentrations measured in the seawater during E4 (30-day trial with smolt waste sludge) (Mean±SD, 7x4x3n). FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1).

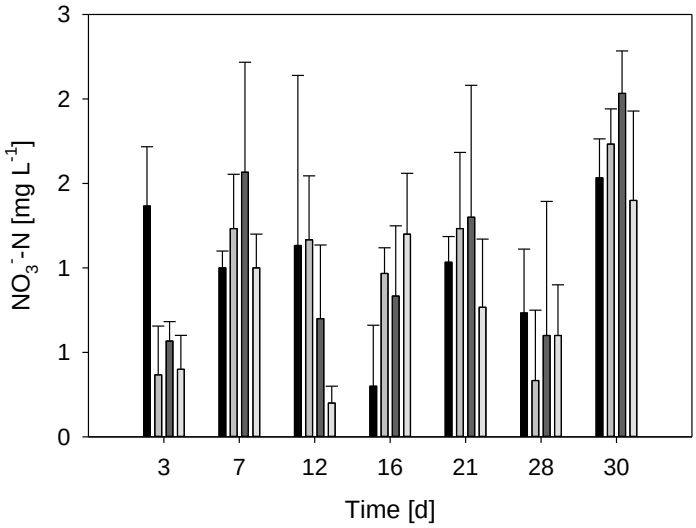

Figure 3.16 – Nitrate concentrations measured in the seawater during E4 (30-day trial with smolt waste sludge) (Mean±SD, 7x4x3n). FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1).

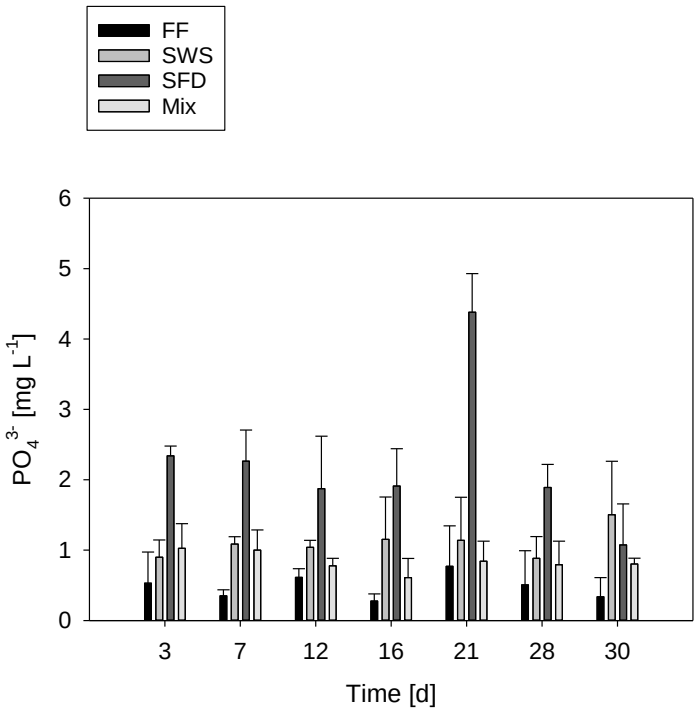

Figure 3.17 – Phosphate concentrations measured in the seawater during E4 (30-day trial with smolt waste sludge) (Mean±SD, 7x4x3n). FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1).

Mean ammonia concentrations in the water of FF tanks were highest in all but one measurements, fluctuating between 1.1±0.4 mg L^{-1} and 2.4±0.2 mg L^{-1}. At levels from 0.9±0.3 mg L^{-1} to 2.3±0.9 mg L^{-1}, samples taken from the SFD tanks on average had slightly lesser ammonia concentrations. Ammonia levels in SFD and Mix tanks were 0.6±0.1 to 1.5±1.0 mg L^{-1} and 0.4±0.3 to 1.0±0.7 mg L^{-1}, respectively and hence lowest of all four treatments. Nitrate concentration were fluctuating strongly between 0.2±0.1 mg L^{-1} and 2.0±0.3 mg L^{-1} in all treatment groups. None of the groups could be concluded to have the highest or lowest overall nitrate level. Phosphate concentrations were highest in SFD tanks (1.1±0.6-4.4±0.5 mg L^{-1}); levels of phosphate in Mix and SWS tanks were similar and remained stable with average values of 0.8±0.1 mg L^{-1} and 1.1±0.2 mg L^{-1}, respectively. The average phosphate concentration in FF tanks was slightly lower (0.5±0.2 mg L^{-1}). An increase in dissolved nutrient concentrations over the course of the experiment could not be detected for any treatment or measured nutrient.

3.4.5 Total organic matter (TOM)

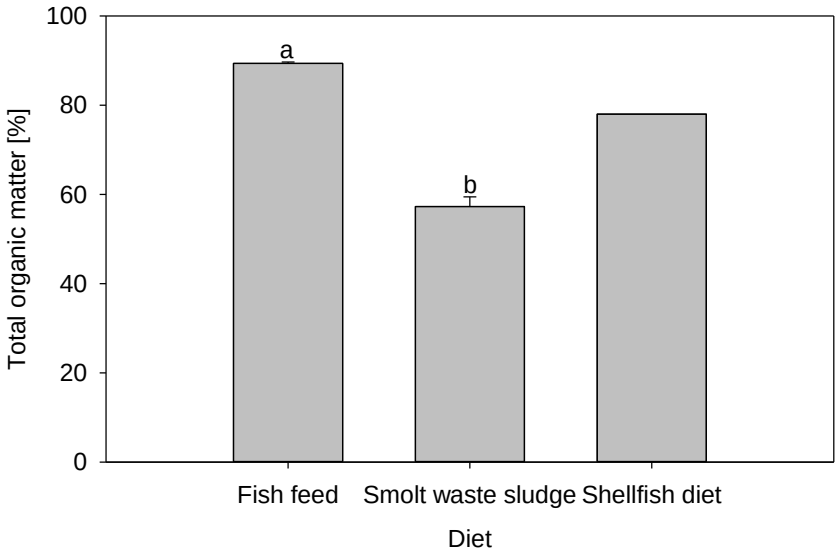

Figure 3.18 – Total organic matter (TOM) content of the different diets (2x5n) used in the trial (Mean±SD). Different superscripts indicate significant differences ($P < 0.05$).

Displayed in Figure 3.18 is the percentage of total organic matter in the diets used in E4. The mean value for total organic matter measured in fish feed (89.4±0.3%) was significantly higher than that in smolt waste sludge shellfish diet (57.3±2.2%) (rank sum test). According to manufacturer's information, shellfish diet consisted of 78% organic matter (Reed Mariculture, 2015).

Total organic matter in polychaetes (Figure 3.19) was measured both before and after the feeding trial. All groups had mean values between 86.2±2.3% (SFD) and 89.1±5.7% (Initial) and did not differ significantly from each other (one way ANOVA).

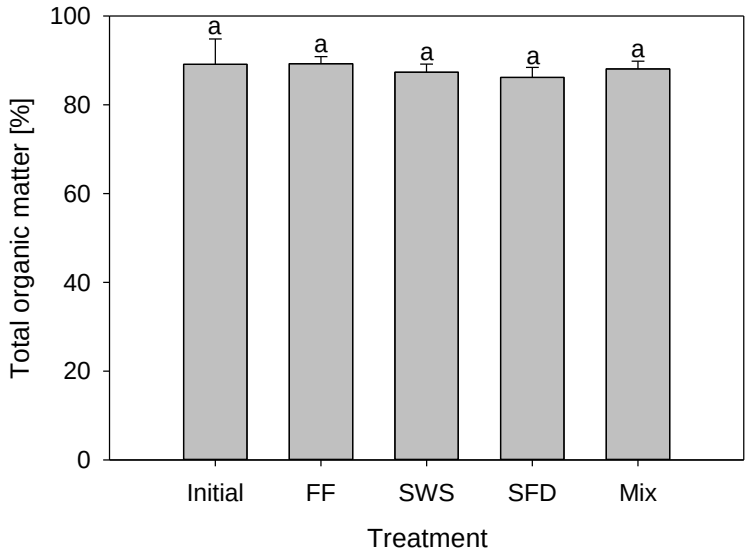

Figure 3.19 – Total organic matter (TOM) content of *H. diversicolor* fed on various diets (Mean±SD). Initial – *Rhodomonas* sp.(6n), FF – fish feed (5n); SWS – smolt waste sludge (5n); SFD – shellfish diet (5n); Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1) (5n). Same superscripts indicate non-significant differences ($P \geq 0.05$).

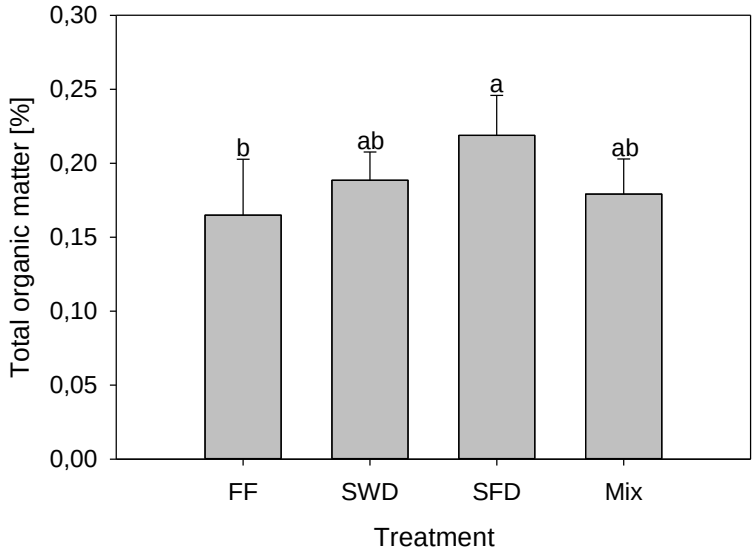

Figure 3.20 – Total organic matter (TOM) content in the sediment (4x5n) of the different treatments (Mean±SD). FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1). Different superscripts indicate significant differences ($P < 0.05$).

Proportions of total organic matter in the sediment of the different treatment groups are presented in Figure 3.20. Overall TOM in the substrate was low and differed significantly between sediment exposed to fish feed (0.16±0.04%) and shellfish diet (0.22±0.03%). The sediment in tanks affected by smolt waste sludge (0.19±0.02%) and the mixed diet (0.18±0.02%) were ranked between the two other groups and not significantly different from any treatment (one way ANOVA).

3.4.6 Carbon and Nitrogen

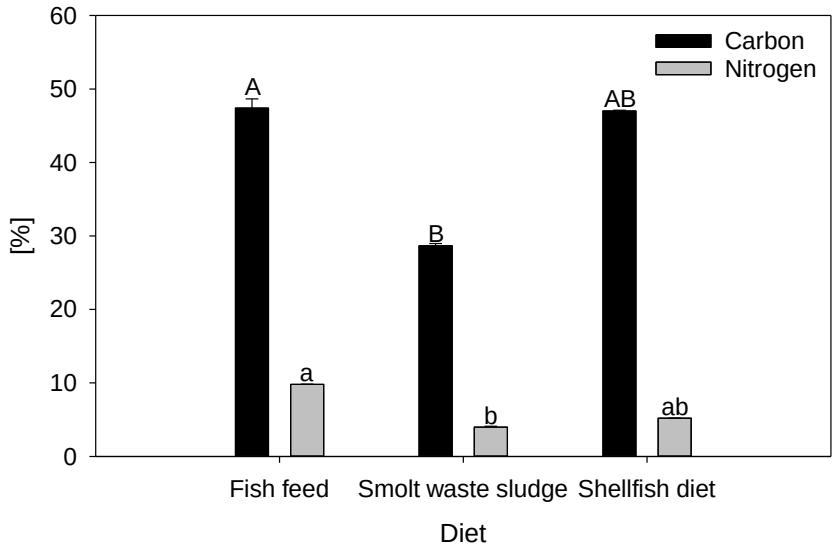

Figure 3.21 – Carbon and nitrogen (Mean±SD) in the different diets (3x4n) used in E4 (30-day trial with smolt waste sludge). Different uppercase superscripts indicate significant differences in carbon percentages. Different lowercase superscripts indicate significant differences in nitrogen percentages ($P < 0.05$).

Percentages of carbon and nitrogen in the different diets are displayed in Figure 3.21. Fish feed contained 47.4±1.3% carbon and 9.8±0.0% nitrogen. Shellfish diet had a similar value for carbon (47.0±0.1) but a lower nitrogen content (5.2±0.0). Smolt waste sludge contained 28.7±0.3% carbon and 4.0±0.1% nitrogen; one way ANOVA on ranks concluded significant differences between carbon and nitrogen percentages of fish feed and shellfish diet.

C:N ratios in diets, pictured in Figure 3.22, were significantly different (one way ANOVA). Shellfish diet had the highest value (9.03±0.04), followed by smolt waste sludge (7.20±0.16) and fish feed (4.84±0.14).

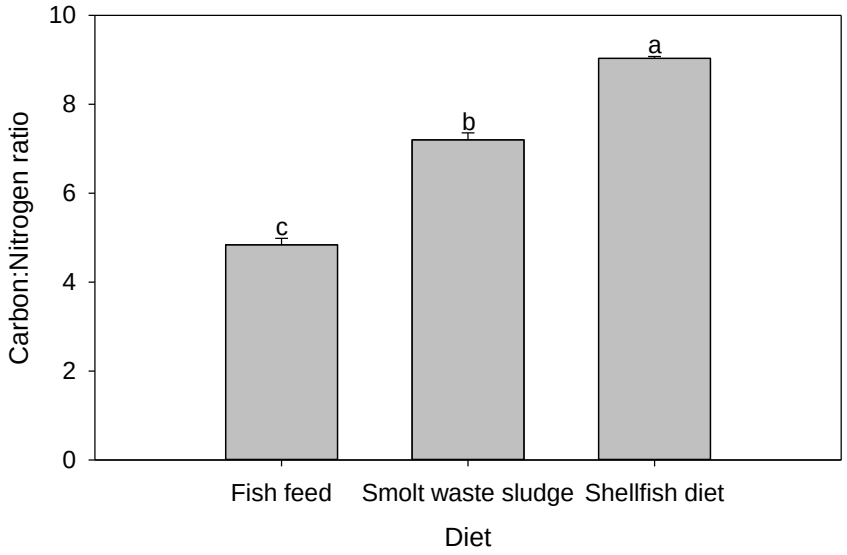

Figure 3.22 – Carbon:Nitrogen ratio (Mean±SD) of the different diets (3x4n) used in E4 (30-day trial with smolt waste sludge). Different superscripts indicate significant differences ($P < 0.05$).

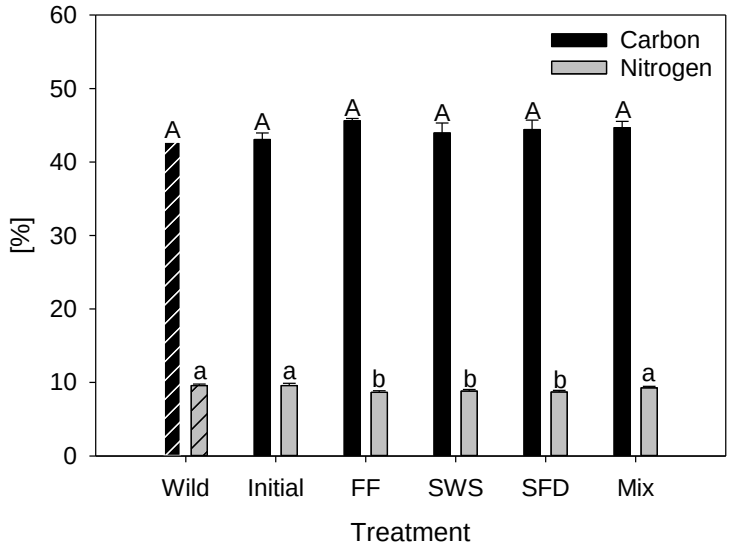

Figure 3.23 – Carbon and nitrogen (Mean±SD) in *H. diversicolor* under different food regimes. Wild - natural field (3n); Initial – *Rhodomonas* sp. (3n); FF – fish feed (5n); SWS – smolt waste sludge (5n); SFD – shellfish diet (5n); Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1) (5n). Same uppercase superscripts indicate non-significant difference in carbon percentages ($P \geq 0.05$). Different lowercase superscripts indicate significant differences in nitrogen percentages ($P < 0.05$).

Figure 3.23 shows the percentages of carbon and nitrogen in polychaetes. Carbon did not differ significantly between groups and ranged from 43-46%. Nitrogen was significantly higher in wild polychaetes, the initial group and the group fed with the mix (~9.5%) than in the other three groups (~8.7%).

The C:N ratio of polychaetes (Figure 3.24) cultured on different diets during the 30-day trial was significantly higher than that of polychaetes before the feeding trial (one way ANOVA). With 5.32±0.08, polychaetes fed with fish feed had the highest C:N ratio, followed by shellfish diet (5.10±0.10), smolt waste sludge (4.98±0.17) and the mix (4.83±0.20). Wild polychaetes and polychaetes immediately before the trial (Initial) had C:N ratios of 4.46±0.13 and 4.50±0.06, respectively.

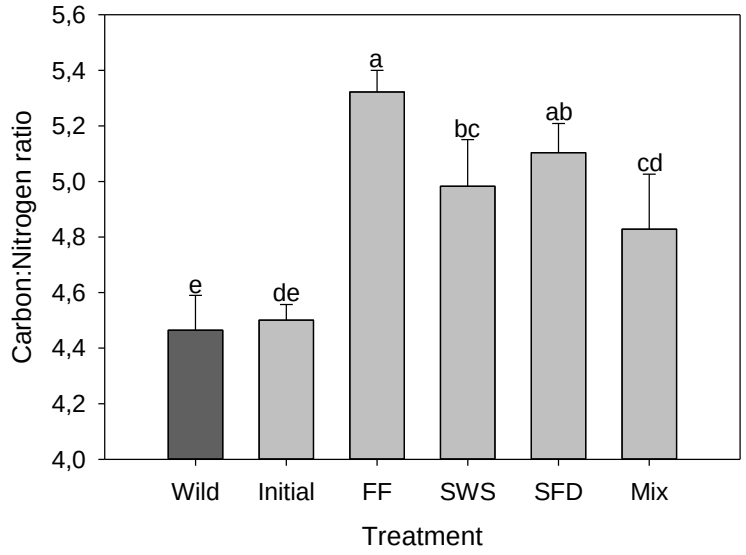

Figure 3.24 – Carbon:Nitrogen ratio (Mean±SD) of *H. diversicolor* under different food regimes. Wild - natural field (3n); Initial – *Rhodomonas* sp. (3n); FF – fish feed (5n); SWS – smolt waste sludge (5n); SFD – shellfish diet (5n); Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1) (5n). Different superscripts indicate significant differences ($P < 0.05$).

3.4.7 Protein

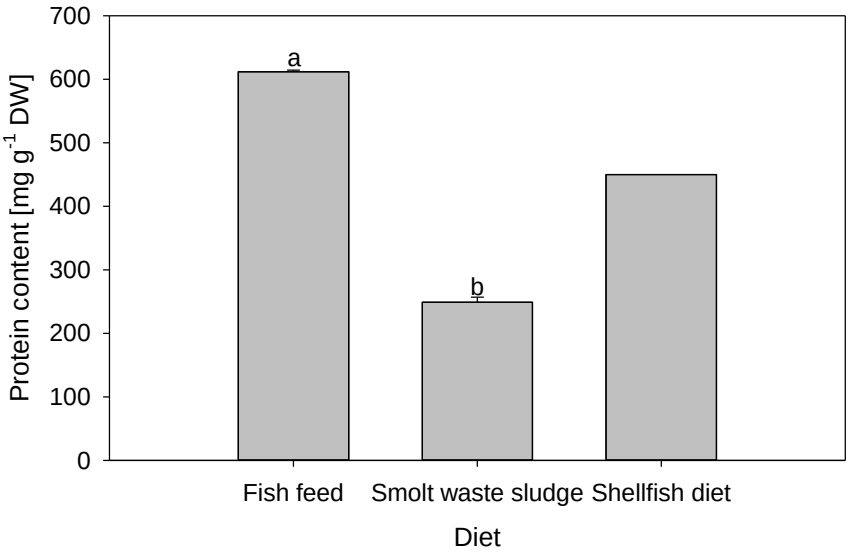

Figure 3.25 – Protein content (Mean±SD) of the different diets (2x4n) used in E4 (30-day trial with smolt waste sludge). Different superscripts indicate significant differences ($P < 0.05$).

Protein contents of fish feed, smolt waste sludge and polychaetes were calculated based on nitrogen contents and are displayed in Figure 3.25 and 3.26, respectively. Of the different diets used in the experiment, fish feed had the highest protein content (611.8±2.6 mg g^{-1} DW) which was significantly higher than that of smolt waste sludge (248.9±8.0 mg g^{-1} DW). Shellfish diet was indicated to have a protein content of 450 mg g^{-1} DW (Reed Mariculture, 2015).

Polychaetes before the feeding trial were significantly higher in protein than the groups fed with different diets for 30 days. Both wild polychaetes from a natural feeding regime and polychaetes fed with *Rhodomonas* sp. (Initial) had a protein content of about 598 mg g^{-1} DW. Of the polychaetes fed with different diets, individuals fed with a mix of smolt waste sludge and shellfish diet (Mix) had the highest protein content (578.8±13.5 mg g^{-1} DW). With values below 551.5 mg^{-1} DW, the other three treatment groups were significantly lower in protein (one way ANOVA on ranks).

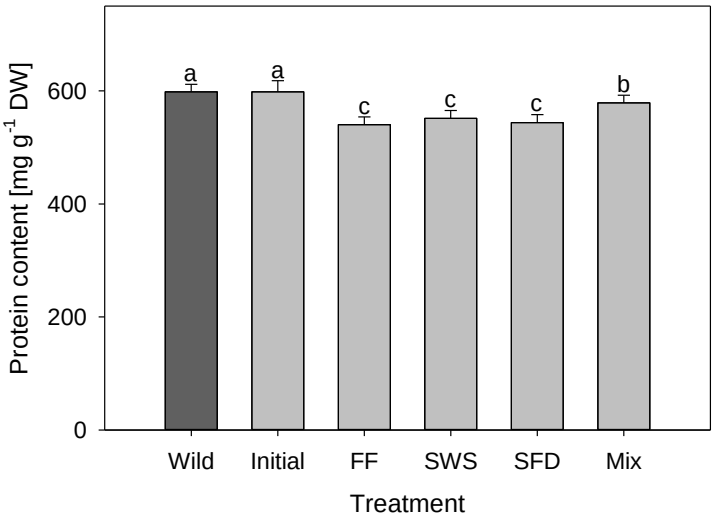

Figure 3.26 – Protein content (Mean±SD) of *H. diversicolor* under different food regimes. Wild – natural field (3n); Initial – *Rhodomonas* sp. (3n); FF – fish feed (5n); SWS – smolt waste sludge (5n); SFD – shellfish diet (5n); Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1) (5n). Different superscripts indicate significant differences ($P < 0.05$).

3.4.8 Amino Acids

The diet amino acid (AA) compositions are displayed in Table 3.5. One way ANOVA on ranks concluded no significant differences for the summed total AA [mg g^{-1} DW] even though fish feed had a content of 358.8±7.9 mg g^{-1} DW which was much higher than that of smolt waste sludge and shellfish diet. Moreover, one way ANOVA on ranks concluded no significant differences for essential (EEA) and non-essential amino acids (NEAA) [%; mg g^{-1} DW] as well as for individual AA [%]. With 164.8±6.0 mg g^{-1} DW, respectively, 194.1±1.9 mg g^{-1} DW of EAA and NEAA, the content in fish feed was a multiple of that in the other diets. EAA and NEAA that could be detected with the used method were there same in the diets as in the polychaetes. The percentage of EAA was ~45-47.5% in all diets. Correspondingly, NEAA amounted to ~52.5-55%.

Of the EAA, leucine (Leu) and lysine (Lys) had the highest percentages in all diets. Shellfish diet had a slightly higher percentage of leucine (9.6±0.1%) than the two other groups (~8.5%) while fish feed was higher in lysine (8.4±0.3%) than shellfish diet (6.3±0.1%) and smolt waste sludge (6.2±0.4%). Histidine (His), methionine (Met) and tryptophan (Trp) were the EAA with the lowest proportion of total AA.

Glutamine/glutamic acid (Gln/Glu) was the most prominent AA in all diets, ranging from 11.2±0.2% in shellfish diet to 15.7±0.4% in fish feed. Other NEAA that had high proportions were asparagine/aspartic acid (Asn/Asp) and proline (Pro).

Table 3.6 shows the result of polychaete AA analysis. The total AA [mg g^{-1} DW] decreased significantly in all treatments over the course of the experiment. Looking at individual AA, none showed significant differences between all treatments as overlapping between groups was frequent. The summed percentage of EAA increased significantly while the total quantifiable content of EAA [mg g^{-1} DW] decreased during the feeding trial. However, this decrease was

only significant for polychaetes fed with fish feed. Both, the percentage of NEAA and the total NEAA [mg g^{-1} DW] decreased throughout the trial in all treatments.

Polychaetes in all groups contained all EAA for marine fish, Arginine (Arg), Histidine (His), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Threonine (Thr), Tryptophan (Trp) and Valine (Val). With values from 8.2% to 10.0±0.7% of the total AA, lysine was the EAA with the highest proportion in all treatments. It increased non-significantly in polychaetes fed with fish feed (FF) and smolt waste sludge (SWS) while it decreased in groups fed with shellfish diet (SFD) and the mix. Other prominent EAA in all treatments were leucine (~7%), arginine (~5-6%) and isoleucine (~5%). Methionine (~1.5%) and tryptophan (<1%) showed the lowest percentages.

NEAA that could be detected in all polychaete groups were Alanine (Ala), Asparagine/Aspartic acid (Asn/Asp), Cysteine (Cys), Glutamine/Glutamic acid (Glu/Gln), Glycine (Gly), Proline (Pro), Serine (Ser) and Tyrosine (Tyr). Even though not an AA by definition, traces of Taurine (Tau) were also measured. Proline and glutamine/glutamic acid were the most prominent AA among the non-essential ones, ranking from ~10% to ~13%. They were followed by asparagine/aspartic acid (~7.5%) and alanine. However, with ~10% in wild and initial polychaetes and ~7% in polychaetes after the trial, the percentage of alanine decreased significantly in all treatment groups. Cysteine had the lowest percentage of NEAA in all treatments.

Table 3.5 – Amino acid composition (Mean±SD) of the different diets used in the trial. Same superscripts indicate non-significant differences ($P \geq 0.05$). Fish feed (2n), smolt waste sludge (2n) and shellfish diet (3n).

Essential amino acids – diets			
[%]	**Fish feed**	**Smolt waste sludge**	**Shellfish diet**
Arg	6.3±0.0[a]	5.6±0.3[a]	4.4±0.0[a]
His	2.7±0.0[a]	2.4±0.4[a]	1.9±0.0[a]
Ile	4.9±0.1[a]	5.6±0.6[a]	5.4±0.1[a]
Leu	8.5±0.1[a]	8.4±0.3[a]	9.6±0.1[a]
Lys	8.4±0.3[a]	6.2±0.4[a]	6.3±0.1[a]
Met	2.1±0.1[a]	1.8±0.6[a]	2.0±0.1[a]
Phe	4.6±0.1[a]	6.4±0.6[a]	6.1±0.1[a]
Thr	3.3±0.0[a]	4.4±1.0[a]	3.7±0.1[a]
Trp	0.4±0.1[a]	1.3±1.4[a]	0.5±0.1[a]
Val	4.6±0.1[a]	5.4±0.8[a]	5.4±0.1[a]
Total EAA	45.9±0.7[a]	47.5±3.0[a]	45.3±0.3[a]
in [mg g^{-1} DW]	164.8±6.0[a]	55.6±1.4[a]	76.5±1.3[a]
Non-essential amino acids – diets			
Asn/Asp	7.5±0.2[a]	8.1±1.1[a]	8.8±0.0[a]
Cys	-	1.7±0.6[a]	2.1±0.1[a]
Gln/Glu	15.7±0.4[a]	12.4±2.5[a]	11.2±0.2[a]
Gly	5.5±0.1[a]	6.3±0.6[a]	5.5±0.0[a]
Pro	8.6±0.1[a]	5.5±1.2[a]	7.6±0.2[a]
Ser	6.0±0.0[a]	6.8±0.7[a]	6.2±0.3[a]
Tau	0.7±0.1[a]	-	0.2±0.3[a]
Tyr	3.2±0.2[a]	3.7±0.1[a]	3.5±0.1[a]
Total NEAA	54.1±0.7[a]	52.5±3.0[a]	54.7±0.3[a]
in [mg g^{-1} DW]	194.1±1.9[a]	61.7±5.9[a]	92.5±2.8[a]
Total AA [mg g^{-1} DW]	**358.8±7.9[a]**	**117.2±4.5[a]**	**169.0±4.0[a]**

Table 3.6 – Amino acid composition (Mean±SD) of *H. diversicolor* under different food regimes. Wild - natural field (1n); Initial – *Rhodomonas* sp. (2n); FF – fish feed (5n); SWS – smolt waste sludge (5n); SFD – shellfish diet (5n); Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1) (5n). Different superscripts indicate significant differences ($P < 0.05$).

Essential amino acids – polychaetes						
[%]	**Wild**	**Initial**	**Treatment**			
			FF	**SWS**	**SFD**	**Mix**
Arg	6.0	6.2±0.0^{a}	5.5±0.2^{c}	5.9±0.1^{b}	5.8±0.1^{b}	6.1±0.1^{a}
His	2.6	2.5±0.0^{b}	2.6±0.1^{b}	2.8±0.1^{a}	2.6±0.0^{ab}	2.7±0.1^{ab}
Ile	4.6	4.3±0.0^{d}	5.2±0.2^{a}	4.8±0.1^{bc}	4.9±0.1^{b}	4.6±0.2^{cd}
Leu	7.3	7.3±0.0^{c}	7.8±0.2^{a}	7.5±0.1^{abc}	7.7±0.2^{a}	7.4±0.2^{bc}
Lys	8.2	8.4±0.1^{ab}	10.0±0.7^{a}	8.5±0.2^{ab}	8.3±0.2^{b}	8.3±0.2^{b}
Met	1.6	1.6±0.1^{a}	1.6±0.1^{a}	1.6±0.1^{a}	1.5±0.1^{a}	1.7±0.1^{a}
Phe	3.9	3.9±0.0^{b}	4.2±0.1^{ab}	4.3±0.1^{a}	4.2±0.1^{ab}	4.2±0.2^{ab}
Thr	3.1	2.9±0.2^{b}	3.1±0.1^{b}	4.0±0.3^{a}	3.2±0.1^{b}	3.3±0.4^{b}
Trp	0.4	0.6±0.0^{a}	0.3±0.2^{ab}	0.4±0.1^{ab}	0.4±0.1^{ab}	0.2±0.1^{b}
Val	4.0	3.9±0.0^{c}	4.5±0.1^{a}	4.4±0.2^{ab}	4.5±0.1^{a}	4.2±0.2^{bc}
Total EAA [%]	41.8	41.6±0.2^{d}	44.9±0.8^{a}	44.3±0.8^{ab}	43.1±0.7^{bc}	42.7±1.1^{bc}
in [mg g^{-1} DW]	149.0	148.3±5.3^{a}	128.0±5.0^{b}	137.5±3.3^{ab}	135.5±3.7^{ab}	141.6±4.3^{a}
Non-essential amino acids – polychaetes						
Ala	10.0	10.5±0.2^{a}	6.9±0.6^{b}	6.9±0.3^{b}	7.3±0.2^{b}	7.6±0.3^{b}
Asn/Asp	7.9	7.5±0.2^{a}	7.3±0.3^{a}	7.3±0.4^{a}	7.4±0.4^{a}	7.7±0.4^{a}
Cys	2.5	2.6±0.1^{a}	1.9±0.3^{ab}	1.0±0.8^{b}	1.8±0.1^{ab}	1.8±0.7^{ab}
Gln/Glu	12.5	11.8±0.1^{ab}	11.2±0.5^{b}	13.2±0.7^{a}	12.1±0.5^{ab}	12.6±0.6^{a}
Gly	5.8	5.7±0.0^{a}	4.9±0.2^{b}	6.0±0.2^{a}	5.8±0.3^{a}	6.0±0.4^{a}
Pro	9.7	10.4±0.3^{b}	13.1±0.4^{a}	11.5±0.8^{ab}	12.2±0.2^{ab}	11.3±0.5^{b}
Ser	6.0	5.8±0.3^{a}	4.7±0.4^{b}	5.2±0.3^{ab}	5.7±0.1^{a}	5.5±0.5^{a}
Tau	1.0	1.2±0.2^{a}	1.0±0.3^{a}	1.2±0.2^{a}	1.0±0.1^{a}	1.2±0.23^{a}
Tyr	2.9	3.0±0.2^{c}	4.1±0.4^{a}	3.4±0.2^{bc}	3.7±0.2^{b}	3.6±0.2^{b}
Total NEAA [%]	58.3	58.4±0.2^{a}	55.1±0.8^{c}	55.8±0.8^{bc}	56.9±0.7^{ab}	57.3±1.1^{ab}
in [mg g^{-1} DW]	207.8	208.5±6.1^{a}	157.3±7.4^{d}	173.3±4.9^{c}	178.9±8.8^{c}	189.9±7.6^{b}
Total AA [mg g^{-1} DW]	**356.8**	**356.7±11.4^{a}**	**285.3±11.5^{d}**	**310.8±6.6^{c}**	**314.5±11.2^{c}**	**331.4±9.2^{b}**

3.4.9 Lipids and fatty acids

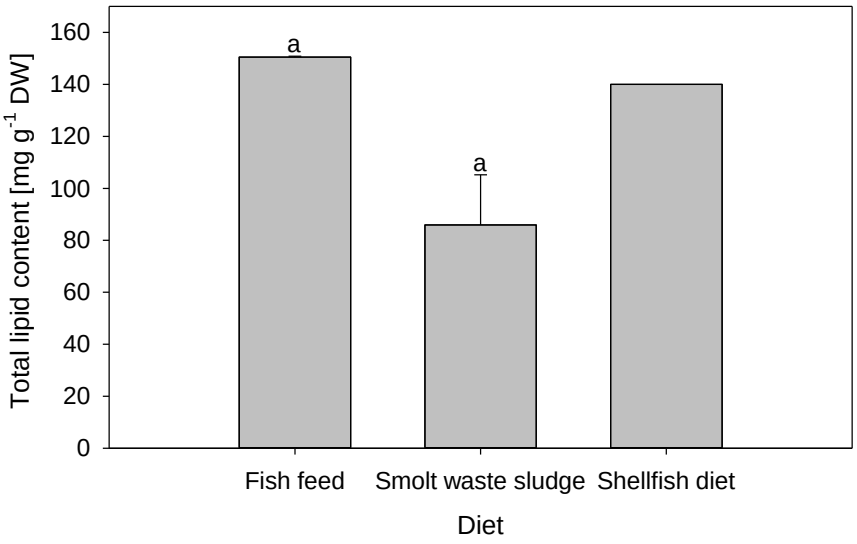

Figure 3.27 – Total lipid content (Mean±SD) of the different diets (2x2n) used in E4 (30-day trial with smolt waste sludge). Same superscripts indicate non-significant differences ($P \geq 0.05$).

Figure 3.27 shows that there were no significant differences in total lipid content between fish feed (150.5±0.4 mg g^{-1} DW) and smolt waste sludge (85.9±19.3 mg g^{-1} DW; one way ANOVA on ranks). According to Reed Mariculture (2015), shellfish diet contained 140 mg g^{-1} DW lipid.

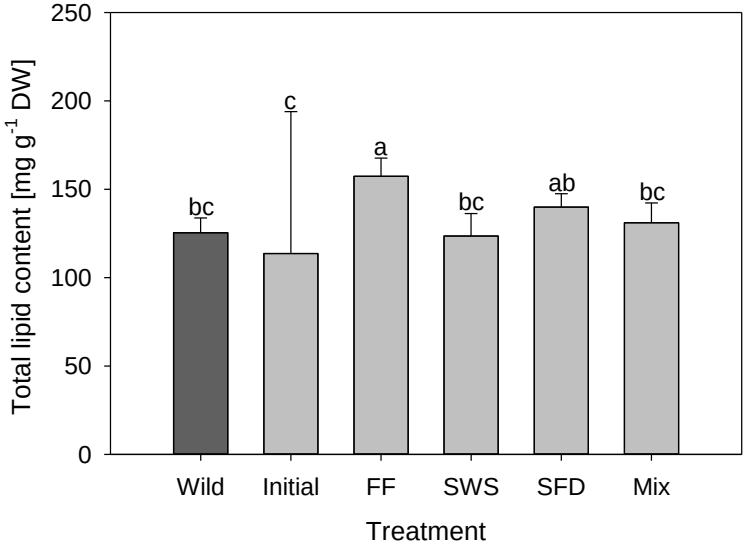

Figure 3.28 – Total lipid content (Mean±SD) of *H. diversicolor* under different food regimes. Wild - natural field (2n); Initial – *Rhodomonas* sp. (3n); FF – fish feed (5n); SWS – smolt waste sludge (5n); SFD – shellfish diet (5n); Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1) (5n). Different superscripts indicate significant differences ($P < 0.05$).

Total lipid content in polychaetes ranged from 113.7±80.3 mg g^{-1} DW (Initial) to 157.4±10.3 mg g^{-1} DW (FF) and differed significantly between treatments as displayed in Figure 3.28. Compared to the initial group, total lipid contents increased in all four treatment groups.

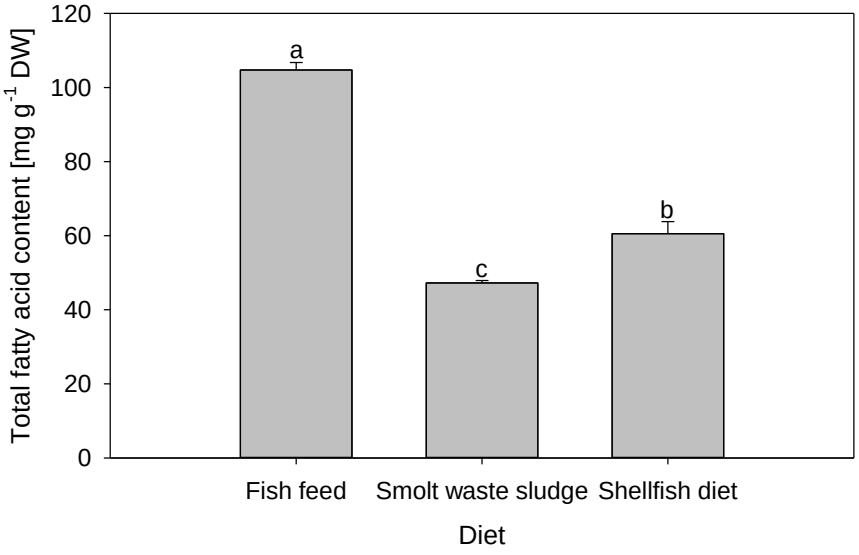

Figure 3.29 – Total fatty acid content (Mean±SD) of the different diets used in E4 (30-day trial with smolt waste sludge). Fish feed (2n), smolt waste sludge (2n) and shellfish diet (3n). Different superscripts indicate significant differences ($P < 0.05$).

The used diets showed significant differences in fatty acid (FA) content (one way ANOVA). As seen in Figure 3.29, fish feed had the highest proportion of FA (104.7±2.0 mg g^{-1} DW). Shellfish diet and smolt waste sludge and had FA contents of 60.6±3.3 mg g^{-1} DW and 47.2±0.6 mg g^{-1} DW, respectively.

The total summed FA content (Figure 3.30) differed significantly between groups (one way ANOVA). It was highest in polychaetes fed with fish feed (73.7±5.6 mg g^{-1} DW). On average, individuals had a higher proportion of FA after the trial than before. Polychaetes fed with shellfish diet, smolt waste sludge and a mix of those diets had total FA content of 56.8±2.9 mg g^{-1} DW, 56.7±7.6 mg g^{-1} DW and 53.7±6.1 mg g^{-1} DW, respectively. At 41.6±0.4 mg g^{-1} DW and 41.2±0.5 mg g^{-1} DW, wild polychaetes and those immediately before the experiment (initial) had the lowest FA content.

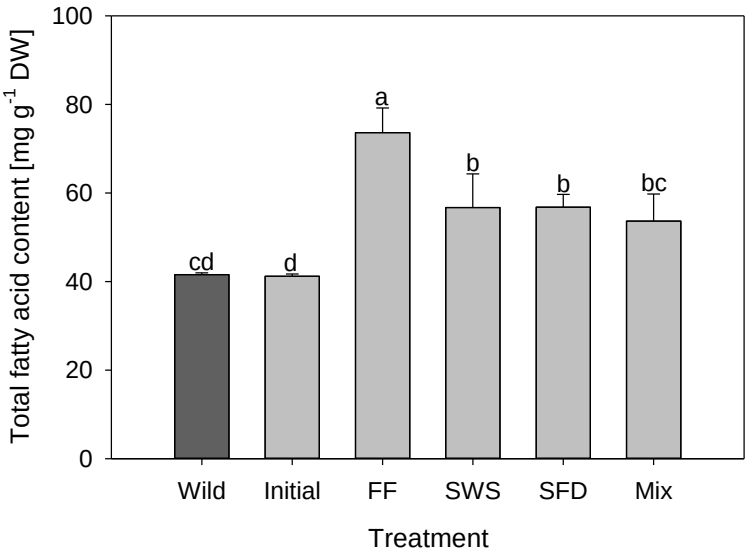

Figure 3.30 – Total fatty acid content (Mean±SD) of *H. diversicolor* under different food regimes. Wild - natural field; Initial – *Rhodomonas* sp.; FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1). Different superscripts indicate significant differences ($P < 0.05$).

The results from fatty acid analyses for diets and polychaetes are displayed in Table 3.7 and 3.8, respectively. Fatty acids are presented as percentage of the total know fatty acids.

Statistical analyses in form of one way ANOVA on ranks concluded no significant differences for FA in diets even though the calculated proportions varied greatly between groups (Table 3.7). In fish feed, the proportion of polyunsaturated fatty acids (PUFA) was only slightly higher (37.8±0.2%) than that of saturated (SAFA) and monounsaturated fatty acids (MUFA). In smolt waste sludge, SAFA constituted the largest proportion (40.9±0.2%) while PUFA had the smallest share. Only nine FA could be detected in shellfish diet, over 50% of those were polyunsaturated. Palmitic acid (C16:0) had the highest percentage of all FA in fish feed and smolt waste sludge, followed by oleic acid **(**C18:1 n-9). In shellfish diet, eicosapentaenoic acid (EPA, C20:5 n-3) (20.6±0.1%) made up the largest proportion. In fish feed, it amounted to 11.2±0.1% while being much lower in smolt waste sludge (3.8±0.0%). Docosahexaenoic acid (DHA, C22:6 n-3) was highest in fish feed (11.0±0.0%) and lower in the other groups. Proportions amounted to 8.8±0.1% and 6.2±0.0% in shellfish diet and smolt waste sludge,

respectively. Corresponding DHA:EPA ratios were between 1.0±0.0 in fish feed, 1.6±0.0% in smolt waste sludge and 1.8±0.0% in shellfish diet.

In all polychaete groups, PUFA constituted the largest share with 45-50%; however, the percentage of PUFA decreased significantly over the course of the experiment (Table 3.8). Polyunsaturated n-3 FA made up about a third of all FA while n-6 fatty acids had a share of ~10-14.5%. The percentage of MUFA increased significantly in the FF, SWS and mix group, shifting from 22.6±0.1% in the initial group to ~25% after the trial. The proportion of SAFA increased significantly in polychaetes fed with shellfish diet and the mix as well as non-significantly in polychaetes fed with fish feed and smolt waste sludge. EPA had the highest percentage of all FA in wild polychaetes and polychaetes before the feeding trial (Initial). Its proportion decreased significantly over the course of the experiment in all treatment groups. In polychaetes after the trial, palmitic acid (C16:0) had the highest percentage. Compared to the initial group, it was higher in all treatment groups; differences were significant for polychaetes fed with smolt waste sludge, shellfish diet and the mix. Oleic acid (C18:1 n-9), and vaccenic acid (C18:1 n-7) were the MUFA with the highest percentages. While the proportion of oleic acid increased during the feeding trial, percentages of vaccenic acid did not change significantly. Having the second highest percentage among PUFA in the initial group (5.8±0.7%), the proportion of linoleic acid (C18:2 n-6) decreased in polychaetes fed with shellfish diet and the mix while it increased in polychaetes fed with fish feed and smolt waste sludge. DHA amounted to 1.5±0.1% before the trial and increased in all treatments. With a value of 7.8±1.1%, polychaetes fed with fish feed registered the strongest increase. While still being very low, DHA:EPA ratios increased correspondingly over the course of the experiment.

Table 3.7 – Fatty acid composition (Mean±SD) of the different diets used in E4 (30-day trial with smolt waste sludge). Fish feed (2n), smolt waste sludge (2n) and shellfish diet (3n). Same superscripts indicate non-significant differences ($P \geq 0.05$).

	Fish feed	**Smolt waste sludge**	**Shellfish diet**
Total FA [mg g^{-1} DW]	**104.7±2.0^{a}**	**47.2±0.6^{c}**	**60.6±3.28^{b}**
% of total FA			
C12:0	0.1±0.0	-	-
C14:0	6.3±0.0^{a}	5.9±0.0^{a}	9.9±0.1^{a}
C15:0	0.5±0.0^{a}	0.5±0.0^{a}	-
C16:0	22.7±0.1^{a}	27.0±0.1^{a}	16.9±0.1^{a}
C17:0	0.4±0.0^{a}	0.4±0.0^{a}	-
C18:0	3.9±0.0^{a}	5.8±0.0^{a}	-
C20:0	0.3±0.0^{a}	0.8±0.0^{a}	-
C22:0	0.2±0.0^{a}	0.4±0.0^{a}	-
C24:0	0.2±0.0	-	-
ΣSAFA	**34.7±0.2^{a}**	**40.9±0.2^{a}**	**26.8±0.2^{a}**
C16:1 n-7	6.9±0.0^{a}	4.8±0.0^{a}	14.3±0.0^{a}
C18:1 n-9	14.4±0.0^{a}	21.1±0.0^{a}	6.4±0.0^{a}
C18:1 n-7	3.3±0.0^{a}	3.7±0.0^{a}	-
C20:1 n-9	2.2±0.0^{a}	4.9±0.0^{a}	-
C22:1 n-9	0.3±0.0^{a}	0.9±0.0^{a}	-
C24:1	0.6±0.1^{a}	1.3±0.0^{a}	-
ΣMUFA	**27.5±0.0^{a}**	**36.9±0.0^{a}**	**20.7±0.1^{a}**
C18:2 n-6	9.3±0.0^{a}	7.9±0.1^{a}	5.0±0.0^{a}
C18:3 n-3	1.9±0.0^{a}	2.1±0.0^{a}	5.4±0.0^{a}
C18:4 n-3	1.6±0.0^{a}	1.2±0.0^{a}	12.7±0.0^{a}
C20:2 n-6	0.3±0.0^{a}	0.1±0.2^{a}	-
C20:4 n-6	1.2±0.0^{a}	0.5±0.0^{a}	-
C20:3 n-3	0.1±0.1^{a}	-	-
C20:5 n-3	11.2±0.1^{a}	3.8±0.0^{a}	20.6±0.1^{a}
C22:5 n-3	1.2±0.0^{a}	0.6±0.0^{a}	-
C22:6 n-3	11.0±0.0^{a}	6.2±0.0^{a}	8.8±0.1^{a}
ΣPUFA	**37.8±0.2^{a}**	**22.3±0.1^{a}**	**52.5±0.3^{a}**
Σn-3	27.0±0.2^{a}	13.8±0.0^{a}	34.4±0.3^{a}
Σn-6	11.9±0.0^{a}	8.3±0.0^{a}	6.4±0.0^{a}
DHA:EPA	1.0±0.0^{a}	1.6±0.0^{a}	1.8±0.0^{a}

Table 3.8 – Fatty acid composition (Mean±SD) of *H. diversicolor* under different food regimes. Wild - natural field (2n); Initial – *Rhodomonas* sp. (3n); FF – fish feed (5n); SWS – smolt waste sludge (5n); SFD – shellfish diet (5n); Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1) (5n). Different superscripts indicate significant differences ($P < 0.05$).

	Wild	Initial	Treatments			
			FF	SWS	SFD	Mix
Total FA	**41.6±0.4^{cd}**	**41.2±0.5^{d}**	**73.7±5.6^{a}**	**56.7±7.6^{b}**	**56.8±2.9^{b}**	**53.7±6.1^{bc}**
[mg g^{-1} DW]						
% of total FA						
C14:0	1.9±0.7^{bc}	1.2±0.0^{c}	2.4±0.5^{b}	1.7±0.6^{bc}	3.7±0.3^{a}	1.7±0.2^{bc}
C15:0	0.6±0.0^{bc}	0.6±0.0^{bc}	0.5±0.1^{c}	0.6±0.1^{bc}	0.9±0.1^{a}	0.7±0.1^{b}
C16:0	20.0±1.0^{bc}	18.6±0.3^{c}	20.1c±1.0^{bc}	20.8±0.9^{b}	23.0±0.8^{a}	21.1±0.2^{b}
C17:0	1.0±0.0^{ab}	1.0±0.0^{a}	0.7±0.1^{b}	0.9±0.1^{ab}	1.0±0.1^{ab}	1.0±0.0^{ab}
C18:0	6.0±0.0^{a}	5.7±0.1^{ab}	4.1±0.6^{b}	5.0±0.7 ^{ab}	4.6±0.1^{ab}	5.4±0.1^{ab}
C22:0	-	-	-	0.3±0.1	-	-
ΣSAFA	**29.4±1.8^{bc}**	**27.2±0.5^{c}**	**27.8±1.3^{bc}**	**29.3±1.3^{bc}**	**33.2±1.0^{a}**	**29.8±0.3^{b}**
C16:1 n-7	4.7±0.1^{ab}	4.4±0.3^{ab}	5.3±0.7^{ab}	4.1±1.0^{b}	5.7±0.2^{a}	4.2±0.6^{b}
C18:1 n-9	9.3±0.4^{bc}	8.8±0.4^{c}	11.5±0.3^{a}	10.5±0.8^{b}	7.1±0.4^{d}	9.5±0.4^{bc}
C18:1 n-7	5.6±0.2^{ab}	5.4±0.1^{ab}	5.3±0.4^{ab}	5.7±0.2^{a}	4.9±0.2^{b}	5.4±0.4^{ab}
C20:1 n-9	3.7±0.0^{ab}	3.5±0.2^{ab}	2.6±0.6^{b}	4.1±0.9^{ab}	3.5±0.3^{ab}	4.5±0.2^{a}
C22:1 n-9	1.0±0.3^{a}	0.5±0.8^{a}	0.3±0.1^{a}	0.8±1.0^{a}	0.4±0.3^{a}	1.0±0.8^{a}
C24:1	-	-	0.3±0.1^{a}	0.4±0.1^{a}	-	0.2±0.2^{a}
ΣMUFA	**24.4±0.4^{a}**	**22.6±0.1^{b}**	**25.3±0.8^{a}**	**25.5±0.7 ^{a}**	**21.5±0.3^{b}**	**24.8±0.6^{a}**
C18:2 n-6	5.3±0.3^{c}	5.8±0.7^{bc}	6.9±0.3^{a}	6.5±0.3^{ab}	3.6±0.1^{d}	5.3±0.4^{c}
C18:3 n-3	3.4±0.1^{a}	3.4±0.1^{a}	2.0±0.1^{d}	2.1±0.2^{cd}	2.6±0.0^{b}	2.3±0.2c
C18:4 n-3	0.6±0.0^{ab}	0.5±0.0^{ab}	0.8±0.2^{ab}	0.4±0.3^{b}	1.3±0.1^{a}	0.6±0.2^{ab}
C20:2 n-6	4.6±0.2^{a}	4.7±0.2^{a}	4.9±0.4^{a}	4.9±0.4^{a}	4.3±0.2^{a}	5.0±0.2^{a}
C20:4 n-6	4.0±0.1^{a}	3.7±0.2^{ab}	2.2±0.5^{b}	3.3±0.6^{ab}	2.3±0.1^{ab}	3.1±0.1^{ab}
C20:3 n-3	0.7±0.0^{ab}	0.8±0.0^{a}	0.5±0.1^{b}	0.6±0.1^{ab}	0.4±0.3^{bc}	0.6±0.1^{ab}
C20:5 n-3	22.8±0.6^{b}	26.1±0.4^{a}	19.0±0.9^{d}	19.2±0.7^{d}	22.6±0.5^{b}	20.6±0.9^{c}
C22:5 n-3	3.4±0.1^{ab}	3.8±0.1^{a}	2.9±0.1^{d}	2.9±0.2^{cd}	3.2±0.2^{bcd}	3.2±0.2^{bc}
C22:6 n-3	1.4±0.1^{b}	1.5±0.1^{b}	7.8±1.1^{a}	5.4±1.3^{ab}	5.0±0.2^{ab}	4.6±0.2^{b}
ΣPUFA	**46.2±1.4^{b}**	**50.3±0.6^{a}**	**46.9±1.6^{b}**	**45.2±1.4^{b}**	**45.3±0.9^{b}**	**45.3±0.8^{b}**
Σn-3	32.3±0.8^{ab}	36.0±0.2^{a}	32.9±2.1^{ab}	30.6±2.0^{b}	35.1±0.9^{a}	32.0±0.8^{b}
Σn-6	13.9±0.6^{ab}	14.3±0.6^{ab}	14.0±0.6^{ab}	14.6±0.7^{a}	10.2±0.1^{c}	13.4±0.3^{b}
DHA:EPA	0.1±0.0^{b}	0.1±0.0^{b}	0.4±0.0^{a}	0.3±0.1^{ab}	0.2±0.0^{ab}	0.2±0.0^{ab}

3.4.10 Carbohydrates

Carbohydrate contents of diets and polychaetes are presented in Figure 3.31 and 3.32, respectively. Fish feed had the lowest carbohydrate content (131.6 mg g^{-1} DW) while smolt waste sludge and shellfish diet contained 238.1 mg g^{-1} DW and 190 mg g^{-1} DW (Reed Mariculture, 2015). Carbohydrates in polychaetes differed significantly between the Mix and the FF group and ranged from 171.0±20.5 mg g^{-1} DW (Mix) to 201.5±8.5 mg g^{-1} DW (FF).

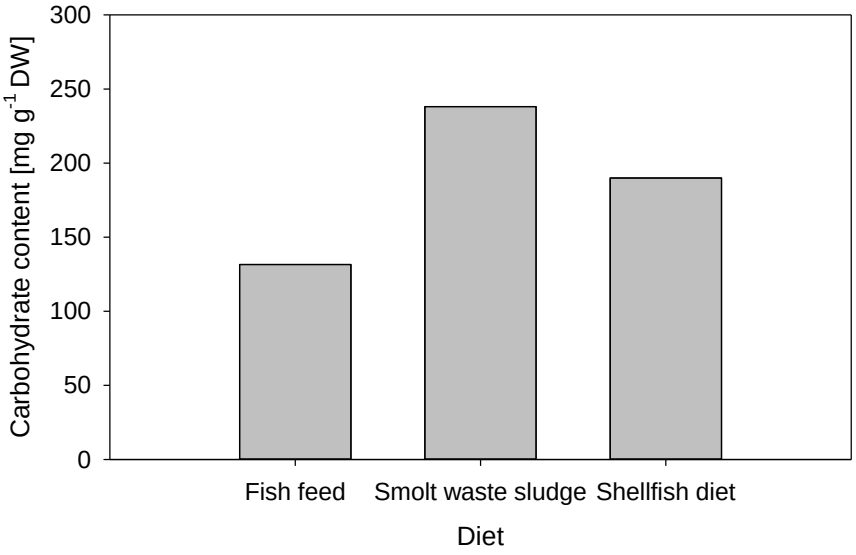

Figure 3.31 – Carbohydrate content of the different diets used in E4 (30-day trial with smolt waste sludge).

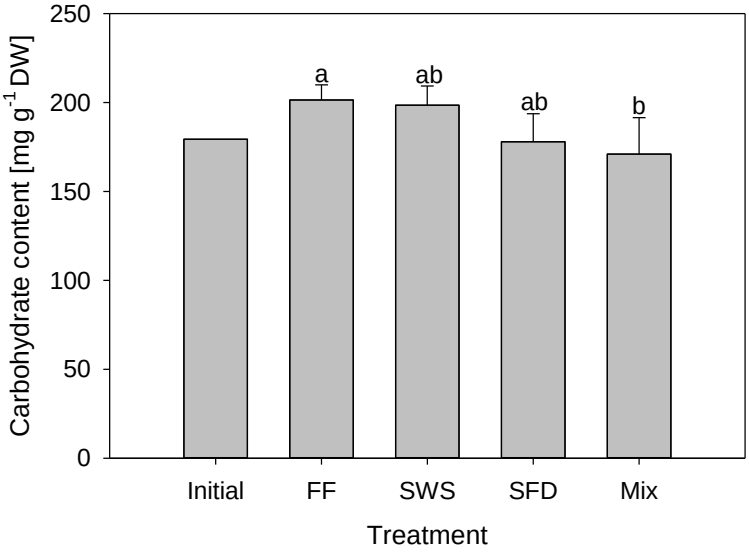

Figure 3.32 – Carbohydrate content (Mean±SD) of *H. diversicolor* fed on various diets. Initial – *Rhodomonas* sp.*;* FF – fish feed (5n); SWS – smolt waste sludge (5n); SFD – shellfish diet (5n); Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1) (5n). Different superscripts indicate significant differences ($P < 0.05$).

4 Discussion

In the present study, the effect of feeding different diets on growth, mortality and biochemical composition of *H. diversicolor* was tested. The main objective was to increase resource utilization in land-based aquaculture by converting wastes from smolt/post-smolt/cleaner fish production into polychaete biomass. The subsequent goal was to create a high-value product that can be further used as a resource for fish feed. It was shown that polychaetes could survive and grow when fed on smolt waste sludge as a single food source. Moreover, they were able to efficiently incorporate lipid and protein as well as fatty and amino acids from their diets.

4.1 Mortality

When comparing mortalities in all carried out experiments, it becomes evident that higher ammonia concentration correlated with lower survival rates. In E4, measured ammonia concentrations were as high as 2.4 mg L^{-1}. Correspondingly, recorded mortalities were considerably higher than in E2 and E3. These were carried out in the same flow-through system and recorded ammonia concentrations were very low, never exceeding 0.10 mg L^{-}1, respectively, 0.14 ml L^{-1}. In E1, ammonia was not measured. Feed supply was increased between the cultivation experiments E1-E3 and E4, thus potentially causing increased leaching of ammonia from diets into the water and leading to higher concentrations.

When comparing to studies with similar set-ups, mortality in E4 was much higher (Bischoff, 2007, Brown et al., 2011, Nesto et al., 2012, Pajand et al., 2017, Santos et al., 2016, Suckow, 2010). Similarly high mortalities of *H. diversicolor* in feeding trials could only be observed when total ammonia nitrogen (TAN) concentrations were exceeding 10 mg L^{-1} (Bischoff, 2007). Bischoff (2007) recommended TAN concentrations below 8 mg L^{-1} and concluded that other dissolved inorganic nutrients such as nitrate and phosphate do not affect survival of *H. diversicolor*. Since only ammonia and not TAN concentrations were measured in E4, it is likely

that TAN concentrations in E4 were actually close to the critical levels suggested by literature. Moreover, water flow was stopped for 16 h after feeding in E4, meaning the highest dissolved nutrient concentrations were to be expected before the re-start of water flow. Since water samples for later measurement were taken approximately two hours after the water flow in the system was re-started, ammonia concentrations may have been estimated too low. Another hypothesis, assuming higher than measured ammonia concentrations in the pore water of the polychaetes' burrows can however be neglected as they were shown to act as a biodiffusers that considerably reduce TAN concentrations in its burrows by creating irrigation currents with undulated body movements (Kristensen and Hansen, 1999, Riisgård et al., 1992).

Critical thresholds for negative effects of nitrate and phosphate on polychaetes could not be found. Generally, they are only known to be toxic in marine environments at very high concentrations (Fösel, 2007, Guderian and Gunkel, 2013, Suckow, 2010). Since concentrations of both nitrate and phosphate were moderate in E4, it was assumed that they had no impact on mortality.

In all experiments that tested different diets and feeding levels (E2-E4), no significant differences in mortality between treatments could be detected, therefore indicating that these had no effect on mortality of *H. diversicolor*; previous studies came to the same conclusion (Bischoff, 2003, Bischoff, 2007, Santos et al., 2016, Suckow, 2010).

The possible impact of substrate on mortality was tested in E1. No significant differences between substrates could be detected, consequently rejecting the theory that chamotte as a substrate in E4 could have led to high mortalities. Moreover, it was concluded to be a suitable substrate in E2 and E3 as well as in other cultivation experiments at SINTEF Aquaculture and Fisheries (pers. comm. A. Mahlzahn). Delays in delivery made it not possible to add chamotte to the cultivation system used in E4 an appropriate amount before the trial. In former studies,

culture sediment was added to the systems 2-4 week prior to experiments to allow for biofilm formation (Bischoff, 2007, Suckow, 2010); in E4, culture sediment was added one day before polychaetes and two days before first feeding, hence eliminating the chance of microbial growth before the experiment. Lacking of opportunistic bacteria may have influenced mortality as they facilitate nitrification and thus lower ammonia levels (Bischoff, 2007, Olivier et al., 1995). Moreover, other trials with polychaetes have used natural sediment partly from which organic matter was removed (Batista et al., 2003, Christensen et al., 2000, Lindqvist et al., 2013) or u-shaped glass tubes (Nielsen et al., 1995, Vedel et al., 1994, Vedel and Riisgård, 1993). Using these instead of artificial substrate could minimize chances of the sediment influencing mortality of *H. diversicolor*.

Cannibalism as suggested in literature (Batista et al., 2003, Fidalgo e Costa et al., 2006b, Goerke, 1971) as a reason for high mortality can be precluded because feed supply was sufficient and the chosen polychaete stocking density was equal or lower than in former studies (Batista et al., 2003, Bischoff, 2007, Nesto et al., 2012, Suckow, 2010) and less than half than in natural environments (Olivier et al., 1995, Olivier et al., 1997, Riisgård, 1994)

Since no accumulated mortality could be observed in E4 over the first few days, mortality due to stress during transition into the new cultivation system can be excluded.

4.2 Growth

Specific growth rates in E1, E2 and E3 were negative or just above zero. Likely, the supplied amount of feed was not sufficient and was correspondingly altered prior to E4.

In E4, polychaetes fed with different diets showed significant increase in mean weights and had positive SGR. Growth in all treatment groups except for the polychaetes fed with a mix of smolt waste sludge and shellfish diet was higher than in natural populations (Chambers and Milne, 1975, Kristensen, 1984, Vedel and Riisgård, 1993). The highest growth rate was

achieved in polychaetes fed with fish feed. With 2.5% d^{-1} it was significantly higher than that of polychaetes fed with smolt waste sludge (1.1% d^{-1}). Polychaetes fed with shellfish diet had a growth of 1.4% d^{-1} whereas those fed with the mix showed very low growth (0.2% d^{-1}). The differences in growth likely draw back to size and compositions of the used diets (Brown et al., 2011, Suckow, 2010). Fish feed, even though ground, had a bigger grain size than the other diets. Polychaetes fed on ground pellets consequently needed to ingest fewer feed particles than the other groups to consume the same amount nutrients and therefore had to spend less energy on feeding (Goerke, 1971, Suckow, 2010). While micronutrients were not analyzed, analyses of macronutrients concluded significant differences between diets. Total organic matter, protein and lipid were highest in fish feed, valuing at 89%, 61% and 15% of dry weight, respectively. With 78% TOM, 45% protein and 14% lipid the values for shellfish diet obtained by the manufacturer (Reed Mariculture, 2015) were slighter lower. Smolt waste sludge had the lowest TOM (57%), protein (25%) and lipid (10%) content.

A potential explanation for the low growth in the mix group could be selective feeding on only the shellfish diet as suspension feeding in *H. diversicolor* is triggered and occurs when an algal cells are present in the water (Riisgård, 1991, Vedel and Riisgård, 1993). The growth rate of 0.2% d^{-1} is approximately one sixth of that of polychaetes that solely fed on shellfish diet thus reflecting the proportion of shellfish diet in the mix. Feeding on only microalgae would have led to limited food supply on one hand and increased energy use for feed search on the other, both factors which slow down growth. However, the possibility of selective filter-feeding on the shellfish diet as opposed to deposit feeding on smolt waste sludge needs to be further evaluated and reinforced.

As in Bischoff et al. (2009), correlating growth to feed consumption by analysis of total organic matter in the culture sediment of the different treatments was not successful. While leftover feed, polychaete faeces and dead polychaetes could have contributed to an increase in TOM,

only quantities of the fed diets appeared to have an influence. The results concluded little TOM in all treatments; tanks with fish feed had the lowest TOM content (0.16%) and those with shellfish diet had the highest (0.22%), which reflects the weight of supplied feed: 0.18 g d^{-1} of fish feed as opposed to 2.33 g d^{-1} of shellfish diet (Appendix I).

Suckow (2010) fed the closely related *Nereis virens* with different energy levels of fish feed and European bass (*Dicentrarchus labrax*) faeces for six weeks. In his trial, individuals fed with fish feed reached growth rates of ~1.5% d^{-1}, those fed with faeces grew ~0.9% d^{-1}. The higher growth rates of polychaetes in E4 might be attributed to higher cultivation temperatures and a longer photoperiod which are both linked with higher feeding and associated growth in Nereididae (Arias and Drake, 1995, Ivleva, 1970, Lambert et al., 1992, Last and Olive, 1999, Olive, 1999, Olivier et al., 1997, Rees and Olive, 1999). Long day lengths increase growth in polychaetes by suppressing gametogenesis. In Suckow's trial the significantly higher initial weight of polychaetes cultivated at an L/D ratio of 9/15 h may have further slowed down growth. The used individuals had reached the critical weight for sexual maturation which in combination with light periods under twelve hours made is possible that they invested energy for production of reproductive cells rather than growth (Last and Olive, 1999, Olive et al., 1997, Olive, 1999, Pellerin-Massicotte et al., 1994, Snow and Marsden, 1974, Suckow, 2010).

Another study carried out over 80 days with *N. virens* fed with fecal waste and uneaten feed pellets of halibut attained the same SGR as in E4 when fed with fish feed. Feeding of fecal waste resulted in a higher growth rate 2.1% d^{-1} (Brown et al., 2011). Since culture conditions were similar, this almost twice as high growth can be attributed to a better composition of fecal wastes, particularly a higher protein content (Nesto et al., 2012) which was twice as high in fecal wastes of halibut than in smolt waste sludge. Additionally, halibut faeces contained 14% more lipid, thus constituting a more energy-dense diet in general.

Bischoff (2007) conducted a large-scale experiment with *H. diversicolor* feeding on solid wastes in an integrated system with Gilt-head bream (*Sparus aurata*) in which polychaetes reached growth rates of 2.5% d^{-1}. Thereby he showed that even higher growth of *H. diversicolor* over long periods (200 days) at high densities (1000 individuals m^{-2}) can be reached in integrated systems. Factors that may have enhanced growth in Bischoff's trial could be continuous feed supply and a lower salinity of 25 ppt (Nielsen et al., 1995). At a stocking density of 2000 individuals m^{-2}, a salinity of 1 ppt and 23 °C, *H. diversicolor* integrated with European sturgeons (*Huso huso*) reached a maximum growth rate 3.40% d^{-1}, thus further substantiating the potential of integrated aquaculture with polychaetes (Pajand et al., 2017).

An impact of the culture sediment on growth as suggested by Bischoff (2007) could limitedly be confirmed in E1 – Evaluation of culture sediment. While coarse culture sediment was said to increase oxygen saturation and lower ammonia concentrations, it requires more mucus for stabilization of burrows. Since mucus production is energy demanding and can slow down growth, fine sediments were recommended for cultivation of polychaetes. In E1, polychaetes showed negative growth rates for all aquarium sands. Chamotte, which has a texture similar to natural sediment when wet, was the only sediment in which *H. diversicolor* had positive growth. In E4, the before mentioned lacking biofilm in the sediment may have had a negative effect on growth in all treatments as bacterial biomass could serve as an additional food source for *H. diversicolor* (Batista et al., 2003, Bischoff, 2007, Suckow, 2010).

4.3 Biochemical composition

Total organic matter was high in all polychaete groups, both before and after the trial. The measured values of ~85% of DW resembled those found by Suckow (2010). The proportion of carbon ranged from 43-36% of DW whereas nitrogen was amounted to ~9% of DW. While the percentage of carbon was the same as found in Suckow (2010), nitrogen was slightly higher in this trial. As a result, C:N ratios were lower: 4.8 (Mix) to 5.3 (FF) in comparison to 5.4-6.1 (Suckow, 2010). The C:N ratio of polychaetes before the trial was significantly lower than in all treatments after the trial except for the mix. Since the percentage of carbon did not significantly change, this can be attributed to a decrease in nitrogen in polychaetes fed with fish feed (FF), smolt waste sludge (SWS) and shellfish diet (SFD).

Protein had the largest share of the organic material in all polychaetes; values were similar to other published data (Luis and Passos, 1995, Pajand et al., 2017, Suckow, 2010). Corresponding to the decrease in nitrogen, protein contents decreased from 600 mg g^{-1} to <550 mg g^{-1} DW in FF, SWS and SFD polychaetes over the course of the experiment. A similar decrease was found for *N. virens* in Suckow (2010). The reason for higher protein contents in wild and initial polychaetes as well as in the group fed with the mix, could be limited food supply. While polychaetes fed on fish feed, smolt waste sludge and shellfish diet supposedly had sufficient/excess food supply, polychaetes in the other groups had to invest more energy on search for food. A consequent higher muscle stress could lead to formation of more muscle tissue which consists to a large degree of proteins (Klinke et al., 2009, Schmidt-Nielsen, 1997, Suckow, 2010).

The lipid content of 114-157 mg g^{-1} DW in *H. diversicolor* resembles the values found in Pajand et al. (2017) and is within the natural range of *H. diversicolor* in summer/fall (García-Alonso et al., 2008, Luis and Passos, 1995). In other studies, however, lipid contents in polychaetes were higher (Brown et al., 2011, García-Alonso et al., 2008, Suckow, 2010).

Throughout the trial, values increased in accordance with lipid contents in the fed diets (Santos et al., 2016). Polychaetes fed with fish feed (containing 150 mg lipid g^{-1} DW) showed the biggest increase of 40 mg g^{-1} DW whereas those fed with smolt waste sludge (containing 86 mg lipid g^{-1} DW) non-significantly increased by 10 mg g^{-1}. The general increase in all treatments may also be attributed to excess supply of diets that are relatively high in lipids. In previous studies, polychaetes were shown to store lipids in times of high food supply before winter and sexual maturation (García-Alonso et al., 2008, Luis and Passos, 1995).

The proportion of carbohydrates in all polychaetes was similar and did not reflect the different carbohydrate contents of diets. No previous studies on carbohydrates in Nereididae could be found for comparison.

Similar as in Suckow's trial (2010) composition in terms of carbon, nitrogen, protein and lipid of polychaetes fed with fish feed (FF), smolt waste sludge (SWS) and shellfish diet (SFD) showed almost invariably no significant differences. Therefore, it can be assumed that polychaetes in these groups received more feed than needed as they could not utilize the excess nutrients.

Amino acids

A significant decrease in summed total amino acids was registered in all polychaete groups when compared with the initial group. This loss was highest for polychaetes fed with fish feed, whose total AA content (mg g^{-1} DW) was more than two times higher than in the other diets, indicating that the AA in fish feed could not be effectively processed. In another study, where *H. diversicolor* was reared on *D. labrax* waste, the sum of AA increased (Bischoff et al., 2010).

Resembling Suckow (2010), proportions of AA were similar in all polychaete groups and there were only few significant changes from the initial to the final compositions. However, these shifts were then noticeably in all groups, regardless of diet AA composition. All groups showed

the same major AA: Proline, glutamine/glutamic acid, lysine, leucine and asparagine/aspartic acid; the low percentages of tryptophan, methionine and cysteine are attributed to destruction during hydrolysis (Darragh et al., 1996, Fountoulakis and Lahm, 1998). Moreover, all ten EAA for fish could be detected in all polychaete groups. Since AA composition is controlled by genes rather than by diet and excess AA which are not used for protein synthesis are catabolized for energy production (Owen et al., 1979), an impact of diets on AA composition of *H. diversicolor* could not be concluded. Therefore, smolt waste sludge can be assumed to be an equally as qualitative feed source for polychaetes as fish feed in terms of amino acid composition.

In fish, AA play an important role as major components of proteins and polypeptides, as well as in regulating metabolic pathways responsible for growth, reproduction and immunity (Schmidt-Nielsen, 1997, Wu, 2009). Deficiency in individual EAA can lead to increased mortality and abnormalities such as scoliosis/lordosis (tryptophan), fin erosion (lysine, tryptophan) and cataract (methionine) (Lall and Tibbetts, 2009, Tacon, 1987). On the contrary, elevated levels of AA and their products are pathogenic factors which is why a balanced AA profile of feed is of high importance (Takeuchi, 2007, Wu, 2009).

A direct comparison of the EAA profile of fish feed and polychaetes fed with smolt waste sludge (SWS and Mix) to demonstrate their suitability as a feed resource is displayed in Figure 4.1. While total EAA (mg g^{-1} DW) in fish feed was higher than in polychaetes fed with waste sludge, proportions of essential amino acids (EFA) were similar in all three groups. Statistical analysis of differences between groups (one way ANOVA) for individual amino acids concluded that only leucine (Leu) and methionine (Met) were significantly higher in fish feed than in SWS and Mix polychaetes; arginine (Arg) showed significant differences between all groups; threonine (Thr) was significantly higher in polychaetes and no significant differences

between groups could be for the remaining EAA, thus making polychaetes a source for AA that is similar to fish feed.

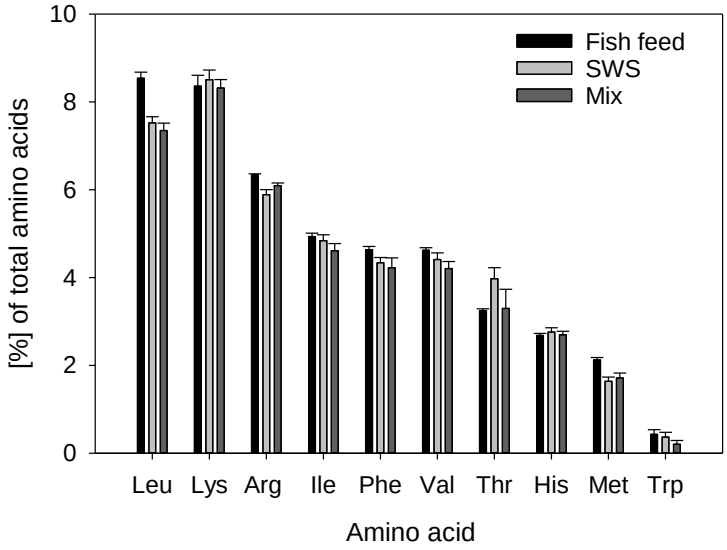

Figure 4.1 – Essential amino acid composition (Mean±SD) of fish feed (2n) and *H. diversicolor* fed with different diets; SWS – smolt waste sludge (5n) and Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1) (5n).

Fatty acids

Like in earlier studies, the proportion of total FA increased significantly in all treatments throughout E4 (Bischoff, 2007, Bischoff et al., 2009, Suckow, 2010). Hereby, the increase in FA was similar to the increase in lipids as well as to the proportions of FA in diets. Even though smolt waste sludge and shellfish diet had significantly differing total FA contents (mg g^{-1} DW), the increase of FA in polychaetes fed with these diets was the same, which could indicate more efficient processing of FA from smolt waste sludge (Suckow, 2010).

The major FA in polychaetes were the same as found in literature: EPA, palmitic acid (C16:0), oleic acid (C18:1 n-9) and linoleic acid (C18:2 n-6). Moreover, DHA and ARA (arachidonic acid, C20:4 n-6) were found in similar proportions as in previous work (Bischoff, 2007, Bischoff et al., 2009, Brown et al., 2011, Fidalgo e Costa et al., 2000, García-Alonso et al., 2008, Luis and Passos, 1995, Pajand et al., 2017, Santos et al., 2016, Suckow, 2010).

EPA, DHA and ARA are essential in marine fish; they must be supplied with the food as they cannot or only in very low quantities be biosynthesized (Sargent et al., 1999). EPA is a precursor of prostaglandins and thromboxane as well as a major component of fish oil (García-Alonso et al., 2008). DHA is another major component of fish oil; it is essential for gonad maturation and effective in increasing tolerance to stress in fish larvae (García-Alonso et al., 2008, Sahu et al., 2017). ARA is the primary precursor for eicosanoids and plays a role in regulating the ionic balance in fish as well as in the immune response (Glencross, 2009, Koven et al., 2001). Palmitic acid, the first metabolite in FA synthesis, is a precursor of many types of molecules of physiological relevance such as membrane lipids, fats and waxes (Nelson et al., 2008). Oleic acid and linoleic acids are precursors of long chain n-3 and n-6 PUFA (Dalsgaard et al., 2003, Suckow, 2010).

According to literature, the FA composition of marine animals reflects the lipid and FA composition of their diets as well as show biosynthetically derived FA. Moreover, *H. diversicolor* is described to biosynthesize EPA and DHA if it is low or absent in its diet (Cowey and Sargent, 1972, Fidalgo e Costa et al., 2000, Luis and Passos, 1995).

Changes in polychaete FA composition throughout this trial mostly followed the FA composition of the fed diets and were similar to changes observed in previous studies (Bischoff, 2007, Bischoff et al., 2009, Suckow, 2010). The proportion of DHA increased in all treatments because the fed diets were considerably higher in DHA than the initial polychaetes. ARA was low in fish feed and smolt waste sludge, even lacking in shellfish diet and correspondingly decreased in all polychaete treatment groups. For some FA, there appears no clear correlation. The proportion of palmitic acid increased in all treatments with the highest increase in SFD polychaetes even though palmitic acid accounted for a much smaller fraction in shellfish diet than in fish feed and smolt waste sludge, therefore indicating selective enrichment. The relative proportions of EPA decreased in all treatments; polychaetes fed with fish feed and smolt waste

sludge showed the same decrease even though the percentage of EPA in fish feed was three times higher than that in smolt waste sludge. Polychaetes after the trial showed a significantly lower proportion of PUFA than the initial group. This decrease appeared independent of the PUFA content in the fed diet since all polychaete groups showed the same decrease (~5%) even though proportions of PUFA in the diets were greatly different. DHA:EPA ratios were below one in all polychaetes. They resemble those found in previous studies and may be attributed to omnivorous feeding behavior of *H. diversicolor* (Brown et al., 2011, Fidalgo e Costa et al., 2000, Santos et al., 2016).

The FA composition of polychaetes fed with smolt waste sludge and the mix are very similar; however, the FA profile of polychaetes fed with shellfish diet deviates from the others which is a contradiction to the hypothesis of *H. diversicolor* selectively filter-feeding on shellfish diet in the mix.

As an overall result and similar to preceding studies, polychaetes could successfully incorporate FA from their diets (Bischoff, 2007, Bischoff et al., 2009, Luis and Passos, 1995, Suckow, 2010). Compared to the initial group, the FA profiles of *H. diversicolor* did not change substantially and polychaetes fed with all diets increased their total FA content (mg g^{-1} DW). Based on this, cultivation of polychaetes fed on smolt waste sludge can be regarded as an efficient way of utilizing FA that are still contained in wastes from land-based aquaculture.

Figure 4.2 compares FA profiles of fish feed and polychaetes fed with smolt waste sludge (SWS and Mix) to establish their suitability as a feed resource. Polychaetes contained 21 of the 24 FA that could be detected in fish feed, 15 of them in an equal or larger relative proportion. Moreover, the major FA were identical. Statistical analysis of differences between the three groups (one way ANOVA) concluded that percentages of PUFA were significantly higher in polychaetes than in fish feed; subsequently, fish feed was higher in SAFA and MUFA.

Proportions of myristic acid (C14:0), palmitoleic acid (C16:1 n-7), oleic acid (C18:1 n-9), linoleic acid (C18:2 n-6), stearidonic acid (C18:4 n-3) and DHA were significantly higher in fish feed than in both polychaete groups. There were no significant differences for palmitic acid (C16:0), stearic acid (C18:0), behenic acid (C22:0), nervonic acid (C24:1) and α-linoleic acid (C18:3 n-3). The percentage of all remaining FA, including EPA, was significantly higher in SWS and Mix polychaetes than in fish feed, thus making polychaetes a source for FA that is similar to fish feed.

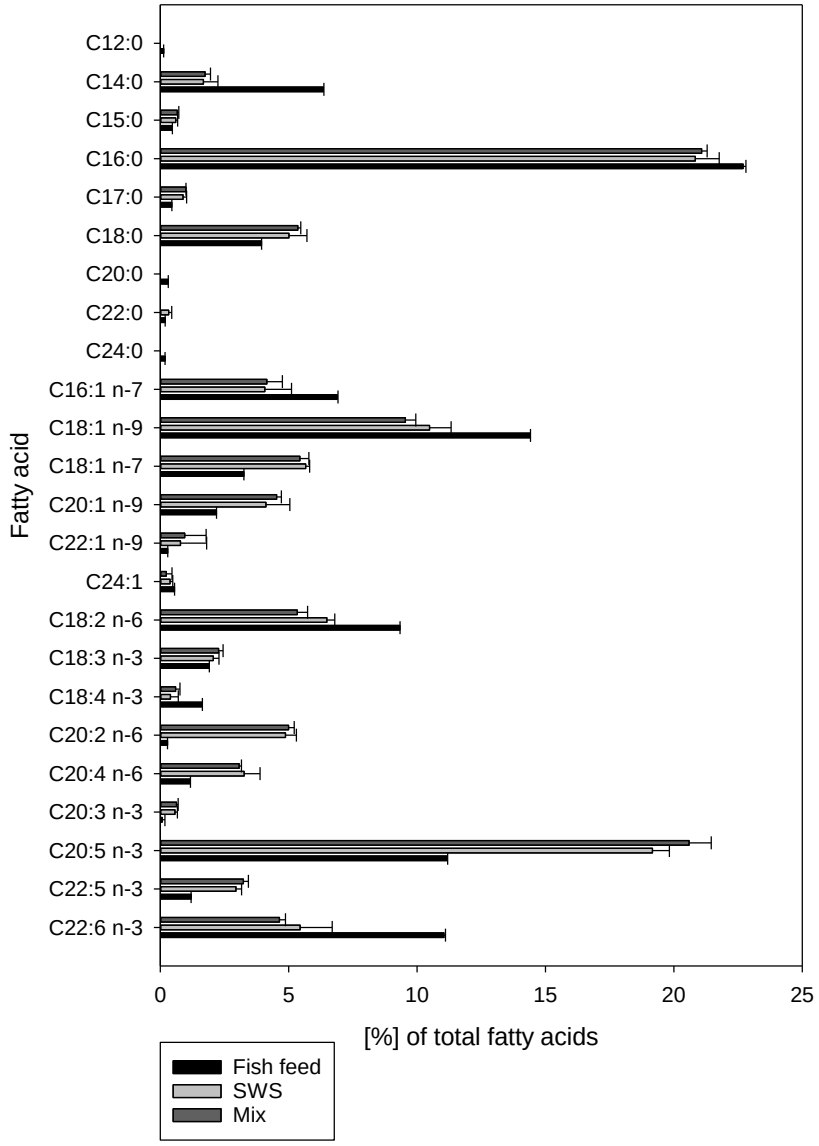

Figure 4.2 – Fatty acid composition (Mean±SD) of fish feed (2n) and *H. diversicolor* fed with different diets. SWS – smolt waste sludge (5n) and Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1) (5n).

4.4 Suitability of *H. diversicolor* reared on waste sludge as a feed resource

Utilizing polychaete biomass as a resource for fish feed for salmon as well as other carnivorous fish and crustacean species could help reduce pressure on resources such as fish oil and fish meal from wild fish (FAO, 2016).

Protein and lipid contents in polychaetes was comparable to those of fish feed and well within the recommended values for marine fish (Creswell, 1993, National Research Council, 2011).

No minimum dietary requirement for carbohydrates has been demonstrated in fish; however, if carbohydrates are not provided in the diet, a higher percentage of protein and lipid are catabolized for energy (Lall and Tibbetts, 2009). Creswell (1993) suggests a maximum of 15-25% of carbohydrates in the diet of carnivorous fish which was not exceeded in polychaetes.

As previously established, a balanced amino acid profile of feed is essential for fish (Takeuchi, 2007, Wu, 2009). In this trial, *H. diversicolor* was shown to largely fulfill these requirements.

Polychaetes fed on waste sludge showed to be a good source of fatty acids as they had the same major fatty acids as fish feed as well as contained the essential fatty acids EPA, DHA and ARA and were rich in PUFA which are important for growth and development in fish (Rosenlund et al., 2016, Sargent et al., 1995). By upscaling production in integrated systems, large amounts of fatty acids could be generated as shown by Bischoff (2007) and Pajand (2017).

Minerals and vitamins were not assessed in this study, however adequate supply via feed is indispensable as dearth leads to deficiencies and ultimately to death (Lall and Dumas, 2015).

In conclusion, polychaetes reared on waste sludge from land-based aquaculture can be considered a suitable feed resource for carnivores. Aspects concerning large scale production and harvest as well as legal and economic considerations need to be further evaluated.

5 Conclusion

The results of this study indicate that *H. diversicolor* can successfully be reared on waste sludge from land-based aquaculture (smolt production) as a sole food source. It was shown that polychaetes utilize and incorporate excess nutrients contained in sludge that are normally lost and therefore have the potential to increase sustainability of current practices by more efficient use of resources.

Feeding of different diets resulted in significantly different growth rates in E4. Fish feed, which was used as a control in this study, constituted a more energy-dense diet than waste sludge and microalgae, thus leading to higher growth in *H. diversicolor*. In general, mortality in polychaetes was linked to ammonia concentrations in the water and could not be correlated with fed diets or cultivation substrates. An effect of diets on biochemical composition of *H. diversicolor* could not be concluded. Polychaetes fed with fish feed, smolt waste sludge and shellfish diet showed similar proportions of carbon, nitrogen, protein, lipids and carbohydrates. Chosen culture conditions were suitable and within the natural range of the species. However, comparison with other studies showed, that there is potential for improvement and altered culture conditions may lead to better results regarding growth and mortality.

Contrary to expectations, adding microalgae paste to waste sludge did not give better results than mono-feeding; polychaetes fed with the mix showed very little growth.

Comparison of biochemical composition including fatty acid and amino acid profiles of polychaetes reared on waste sludge with those of fish feed concluded that *H. diversicolor* fed with excess nutrients from land-based aquaculture can be assumed a suitable feed resource for carnivorous fish and crustacean species. By serving as a marine raw material in feed it could contribute to mitigate pressure on wild fish stocks that are used for production of fish meal and fish oil.

6 Recommendations and future prospects

Even though conclusive findings were made in this thesis, there are certain aspects than can be improved in future studies. The treatment set-up in E4 was not randomized, meaning the treatments in the upper tanks (FF and SWS) received more light. A potential impact of irradiance as well as other culture conditions such as temperature and salinity should be evaluated in further experiments to attain optimum culture conditions as well as delay maturation since *H. diversicolor* dies after reproduction (Santos et al., 2016). In order to reach maximum growth, different levels of waste sludge as feed should be tested. Moreover, an own broodstock should be used to minimize influence of biological variety (Santos et al., 2016). To prevent cross-species transmission and spreading of diseases, biosecure systems as used in shrimp aquaculture should be developed, thus ensuring pathogen-free broodstocks (Bischoff et al., 2009, Browdy and Bratvold, 1998, Lightner, 2005, Moss et al., 1998). Recirculating systems would be recommended for cultivation to avoid long hours of no water flow and high TAN concentrations as well as ensure stable culture conditions.

It would be useful to carry out more accurate analyses for proteins and carbohydrates since the ones used in this thesis only gave approximate values. Of interest could moreover be analysis of lipid classes (Luis and Passos, 1995, Pocock et al., 1971) as dietary lipid composition affects quality and fatty acid composition of sperm and eggs in fish (Lall and Dumas, 2015). Levels of micronutrients such as minerals and vitamins should further be examined to verify suitability of polychaetes as a feed resource.

Eventually, experiments should be carried out over a longer period in large-scale systems. Direct integration of *H. diversicolor* could lead to a more efficient waste reduction and increased polychaete growth (Bischoff, 2007, Pajand et al., 2017). The potential of polychaete aquaculture is illustrated in Figure 6.1.

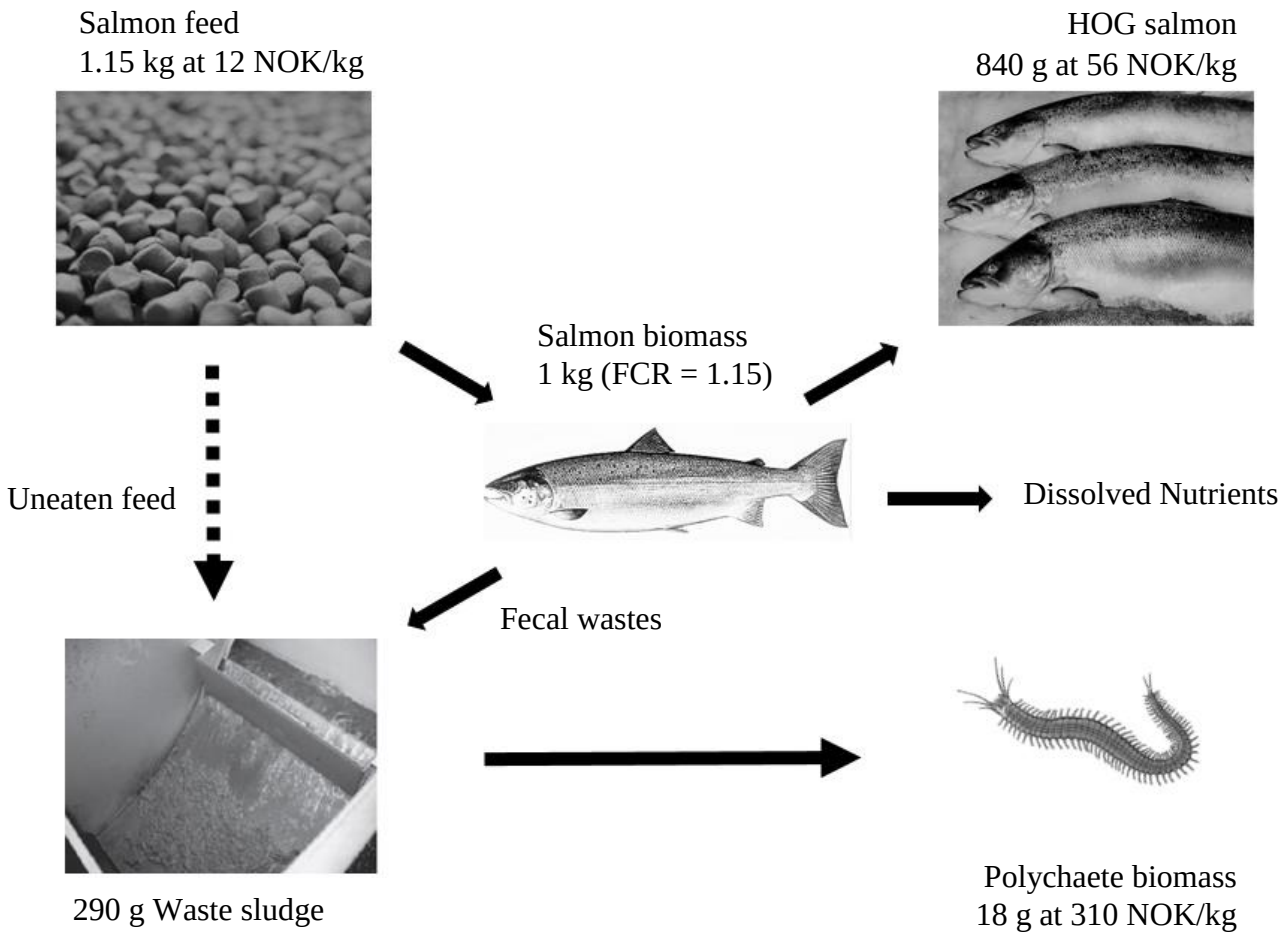

Figure 6.1 – Conversion of feed into salmon and polychaete biomass (Brown et al., 2011, Eatglobe, 2015, Fisheries and Oceans Canada, 2017, Institute of Oceanology, 2017, Marine Harvest, 2017b, Stavis Seafoods, 2017, Wang et al., 2012)

Adapted from Brown (2011) and supported by literature, data collected in this trial allowed for an estimate of prospective polychaetes biomass produced by utilizing waste sludge from RAS.

Records from salmon production indicate a FCR of 1.15 for salmon, meaning that for every 1 kg of salmon produced, 1.15 kg of feed are used (Marine Harvest, 2017a, Wang et al., 2012). While FCR for smolts are lower (0.7-0.95), the overall FCR for salmon was used in this estimation to highlight the additional value created when looking at the final product, head-on gutted (HOG) salmon. The conversion factor from salmon at harvest to HOG salmon is 1.19, resulting 840 g of HOG salmon per 1 kg of salmon at harvest (Fiskeridirektoratet, 2017b).

In smolt production, roughly 25% of feed are converted into sludge which contains fecal waste, bacterial biofilms and uneaten feed (Campo et al., 2010). Thus, per 1kg of salmon produced, ~290 g of sludge remain which could be converted in 18 g of polychaete biomass (not taking into account mortality).

At a current market price of 310 NOK/kg for *H. diversicolor* and 67 NOK/kg for HOG salmon, the respective values created in an integrated system would be 5.6 NOK and 56 NOK (Online Baits UK, 2017, The NASDAQ OMX Group, 2017).

Since this is only a rough estimate based on the results obtained in E4, and inputs of labor, facilities and energy costs for growing polychaetes are not accounted for, the potential increase in productive value appear small. However, when considering the estimated 1,600 tonnes of sludge annually emerging from Norwegian smolt production which correspond to a prospective production of 100 tonnes of polychaetes, the potential of utilizing smolt waste sludge becomes more obvious (Campo et al., 2010). As the share of smolt production facilities using RAS continuously grows, so does sludge production. By developing integrated systems with smolt and polychaetes, this ever-growing sludge creation could be put to valuable use.

References

ARIAS, A. & DRAKE, P. 1995. Distribution and production of the polychaete *Nereis diversicolor* in a shallow coastal lagoon in the Bay of Cadiz (SW Spain). *Cahiers de biologie marine*.

BATISTA, F., FIDALGO E COSTA, P., MATIAS, D., JOAQUIM, S., MASSAPINA, C., PASSOS, A., FERREIRA, P. P. & CANCELA DA FONSECA, L. 2003. Preliminary results on the growth and survival of the polychaete *Nereis diversicolor* (OF Muller, 1776), when fed with faeces from the carpet shell clam Ruditapes decussatus (L., 1758). *BOLETIN-INSTITUTO ESPANOL DE OCEANOGRAFIA,* 19**,** 443.

BISCHOFF, A. 2003. Growth and mortality of the polychaete *Nereis diversicolor* under experimental rearing conditions. *Institute of Marine Research & Department of Animal Sciences, Chairgroup of Fish Culture and Fisheries: 103pp*.

BISCHOFF, A. 2007. *Solid waste reduction of closed recirculated aquaculture systems by secondary culture of detritivorous organisms.* Christian-Albrechts-Universität zu Kiel.

BISCHOFF, 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.

BISCHOFF, A., WECKER, B., KOCH, B. & BUCK, B. H. 2010. Investigating *Nereis diversicolor* (OF Mueller, 1776) as a Candidate for Integrated Aquaculture from an Amino Acid Perspective.

BLANCHETON, J.-P., BOSC, P., HUSSENOT, J., ROQUE D'ORBCASTEL, E. & ROMAIN, D. 2009. The 'new' European fish culture systems: Recirculating systems, offshore cages, integrated systems. *Cahiers Agricultures,* 18**,** 227-234.

BLIGH, E. G. & DYER, W. J. 1959. A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology,* 37**,** 911-917.

BROCH, O. J., ELLINGSEN, I. H., FORBORD, S., WANG, X., VOLENT, Z., ALVER, M. O., HANDÅ, A., ANDRESEN, K., SLAGSTAD, D. & REITAN, K. I. 2013. Modelling the cultivation and bioremediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in Norway. *Aquaculture Environment Interactions,* 4**,** 187-206.

BROWDY, C. L. & BRATVOLD, D. Preliminary development of a biosecure shrimp production system. Proceedings of the US Marine Shrimp Farming Program Biosecurity Workshop, 1998. 19-38.

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.

BUSCHMANN, A. H., VARELA, D. A., HERNÁNDEZ-GONZÁLEZ, M. C. & HUOVINEN, P. Opportunities and challenges for the development of an integrated seaweed-based aquaculture activity in Chile: determining the physiological capabilities of *Macrocystis* and *Gracilaria* as biofilters. Nineteenth International Seaweed Symposium, 2009. Springer, 121-127.

CAMPO, L. M. D., IBARRA, P., GUTIÉRREZ, X. & TAKLE, H. 2010. Utilization of sludge from recirculation aquaculture. *Nofima Marin AS report, Innovation Norway*.

CHAMBERS, M. R. & MILNE, H. 1975. Life cycle and production of *Nereis diversicolor* O. F. Müller in the Ythan Estuary, Scotland. *Estuarine and Coastal Marine Science,* 3**,** 133-144.

CHOPIN, T., BUSCHMANN, A. H., HALLING, C., TROELL, M., KAUTSKY, N., NEORI, A., KRAEMER, G. P., ZERTUCHE-GONZÁLEZ, J. A., YARISH, C. & NEEFUS, C.

2001. Integrating seaweeds into marine aquaculture systems: a key toward sustainability. *Journal of Phycology,* 37**,** 975-986.
CHRISTENSEN, B., VEDEL, A. & KRISTENSEN, E. 2000. Carbon and nitrogen fluxes in sediment inhabited by suspension-feeding *(Nereis diversicolor*) and non-suspension-feeding (*N. virens*) polychaetes. *Marine Ecology Progress Series***,** 203-217.
COSTECH ANALYTICAL TECHNOLOGIES. 2017. *ECS 4010 NITROGEN / PROTEIN ANALYZER - OPERATING PRINCIPLE* [Online]. Available: http://www.costechanalytical.com/products/ecs4010_principle.aspx [Accessed 05.05.2017].
COWEY, C. & SARGENT, J. 1972. Fish nutrition. *Advances in marine biology,* 10**,** 383-494.
CRESWELL, R. L. 1993. Feeding and Nutrition. *Aquaculture Desk Reference.* Boston, MA: Springer US.
DALSGAARD, J., JOHN, M. S., KATTNER, G., MÜLLER-NAVARRA, D. & HAGEN, W. 2003. Fatty acid trophic markers in the pelagic marine environment. *Advances in marine biology,* 46**,** 225-340.
DARRAGH, A. J., GARRICK, D. J., MOUGHAN, P. J. & HENDRIKS, W. H. 1996. Correction for Amino Acid Loss during Acid Hydrolysis of a Purified Protein. *Analytical Biochemistry,* 236**,** 199-207.
DRENGSTIG, A., ULGENES, Y., LILTVED, H. & BERGHEIM, A. 2011. Recent RAS trends of commercial salmon smolt farming in Norway. *Aquaculture America*.
EATGLOBE. 2015. *Cargill Buys Salmon Feed Producer* [Online]. Available: http://www.eatglobe.com/news/fishing/307-cargill-buys-salmon-feed-producer.html [Accessed 07.07.2017].
FAO 2016. The State of World Fisheries and Aquaculture 2016 - Contributing to food security and nutrition for all.
FIDALGO E COSTA, P. & CANCELA DA FONSECA, L. 1998. *First Data on Nereis (Hediste) Diversicolor OF Müller, 1776 (Annelida, Polychaeta) in the Estuary of the Aljezur River (SW Coast of Portugal)*, Museu Bocage.
FIDALGO E COSTA, P., GIL, J., PASSOS, A. M., PEREIRA, P., MELO, P., BATISTA, F. & CANCELA DA FONSECA, L. 2006a. The market features of imported non-indigenous polychaetes in Portugal and consequent ecological concerns. *Scientia Marina,* 70**,** 787-792.
FIDALGO E COSTA, P., NARCISO, L. & CANCELA DA FONSECA, L. 2000. Growth, survival and fatty acid profile of *Nereis diversicolor* (OF Müller, 1776) fed on six different diets. *Bulletin of marine science,* 67**,** 337-343.
FIDALGO E COSTA, P., OLIVEIRA, R. F. & CANCELA DA FONSECA, L. 2006b. Feeding ecology of *Nereis diversicolor* (OF Müller)(Annelida, Polychaeta) on estuarine and lagoon environments in the southwest coast of Portugal. *Pan-American Journal of Aquatic Sciences***,** 104-113.
FISHERIES AND OCEANS CANADA. 2017. *Atlantic Salmon Watch Program* [Online]. Available: http://www.pac.dfo-mpo.gc.ca/science/aquaculture/aswp/index-eng.html [Accessed 07.07.2017].
FISKERIDIREKTORATET. 2015. *Settefisk* [Online]. Available: https://www.fiskeridir.no/Akvakultur/Tildeling-og-tillatelser/Kommersielle-tillatelser/Laks-oerret-og-regnbueoerret/Settefisk [Accessed 15.06.2017].
FISKERIDIREKTORATET 2017a. Laks, regnbueørret og ørret - Settefiskproduksjon.
FISKERIDIREKTORATET 2017b. Tabell over offisielle norske omregningsfaktorer for fisk.
FOUNTOULAKIS, M. & LAHM, H.-W. 1998. Hydrolysis and amino acid composition analysis of proteins. *Journal of Chromatography A,* 826**,** 109-134.

FÖSEL, B. U. 2007. *Mikrobiologie der Stickstoffentfernung in den Biofiltern einer marinen Aquakultur mit geschlossenem Wasserkreislauf.*

GARCÍA-ALONSO, J., MÜLLER, C. T. & HARDEGE, J. 2008. Influence of food regimes and seasonality on fatty acid composition in the ragworm. *Aquatic Biology,* 4**,** 7-13.

GLENCROSS, B. D. 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. *Reviews in Aquaculture,* 1**,** 71-124.

GOERKE, H. 1971. Die Ernährungsweise der *Nereis*-Arten (Polychaeta, Nereidae) der deutschen Küsten. *Veröff. Inst. Meeresforsch. Bremerh,* 13**,** 1-50.

GOOGLE MAPS. 2017. *Leangbukta, Trondheim* [Online]. Available: https://www.google.no/maps/place/Leangbukta/@63.4423509,10.455274,14z/data=!4m5!3m4!1s0x466d30f9799e5327:0x393b907bd6ce653!8m2!3d63.4390601!4d10.4736096?hl=en [Accessed 15.06.2017].

GRAFTON, R. Q., WILLIAMS, J. & JIANG, Q. 2015. Food and water gaps to 2050: preliminary results from the global food and water system (GFWS) platform. *Food Security,* 7**,** 209-220.

GUDERIAN, R. & GUNKEL, G. 2013. *Handbuch der Umweltveränderungen und Ökotoxikologie: Band 3A: Aquatische Systeme: Grundlagen-Physikalische Belastungsfaktoren-Anorganische Stoffeinträge*, Springer-Verlag.

HACH COMPANY/HACH LANGE GMBH 2015a. Nitrate - Cadmium Reduction Method (Method 8192).

HACH COMPANY/HACH LANGE GMBH 2015b. Nitrogen, Ammonia - Salicylate Method (Method 8155).

HACH COMPANY/HACH LANGE GMBH 2017. Phosphorus, Reactive (Orthophosphate) - USEPA PhosVer® (Ascorbic Acid) Method (Method 8048).

HANDÅ, A., FORBORD, S., WANG, X., BROCH, O. J., DAHLE, S. W., STØRSETH, T. R., REITAN, K. I., OLSEN, Y. & SKJERMO, J. 2013. Seasonal-and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in Norway. *Aquaculture,* 414**,** 191-201.

HANDÅ, A., MIN, H., WANG, X., BROCH, O. J., REITAN, K. I., REINERTSEN, H. & OLSEN, Y. 2012a. Incorporation of fish feed and growth of blue mussels (*Mytilus edulis*) in close proximity to salmon (*Salmo salar*) aquaculture: implications for integrated multi-trophic aquaculture in Norwegian coastal waters. *Aquaculture,* 356**,** 328-341.

HANDÅ, A., RANHEIM, A., OLSEN, A. J., ALTIN, D., REITAN, K. I., OLSEN, Y. & REINERTSEN, H. 2012b. Incorporation of salmon fish feed and feces components in mussels (*Mytilus edulis*): implications for integrated multi-trophic aquaculture in cool-temperate North Atlantic waters. *Aquaculture,* 370**,** 40-53.

HAO-REN, L. 1982. Polycultural System of Freshwater Fish in China. *Canadian Journal of Fisheries and Aquatic Sciences,* 39**,** 143-150.

HARTMANN-SCHRÖDER, G. 1996. Annelida, Borstenwürmer, Polychaeta. Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und ihrer Lebensweise, Tiel 58. F. Dahl and H. Schumann, eds. Gustav Fischer Verlag, Jena.

HEILSKOV, A. C., ALPERIN, M. & HOLMER, M. 2006. Benthic fauna bio-irrigation effects on nutrient regeneration in fish farm sediments. *Journal of Experimental Marine Biology and Ecology,* 339**,** 204-225.

HOGNES, E., ZIEGLER, F. & SUND, V. 2011. Carbon footprint and area use of farmed Norwegian salmon. *SINTEF Fisheries and Aquaculture***,** 1-30.

HUNTINGTON, T. & HASAN, M. R. 2009. Fish as feed inputs for aquaculture–practices, sustainability and implications: a global synthesis. *Fish as feed inputs for aquaculture: practices, sustainability and implications.* FAO Rome.

INSTITUTE OF OCEANOLOGY, P. A. O. S. 2017. *Annelida: Hediste diversicolor* [Online]. Available: http://www.iopan.gda.pl/projects/puckbay/html/embs_6/614_nereis.html [Accessed 07.07.2017].

IVLEVA, I. 1970. The influence of temperature on the transformation of matter in marine invertebrates. *Marine food chains***,** 96-112.

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 O2 limitation. *Applied microbiology and biotechnology,* 80**,** 297.

JONES, D. B. 1941. *Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins*, US Department of Agriculture Washington, DC.

JØRGENSEN, C. B. 1990. *Bivalve filter feeding: hydrodynamics, bioenergetics, physiology and ecology*, Olsen & Olsen.

KLINKE, R., PAPE, H.-C., KURTZ, A. & SILBERNAGL, S. 2009. *Physiologie*, Georg Thieme Verlag.

KOVEN, W., BARR, Y., LUTZKY, S., BEN-ATIA, I., WEISS, R., HAREL, M., BEHRENS, P. & TANDLER, A. 2001. The effect of dietary arachidonic acid (20: 4n− 6) on growth, survival and resistance to handling stress in gilthead seabream (Sparus aurata) larvae. *Aquaculture,* 193**,** 107-122.

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

KRISTENSEN, K. & HANSEN, K. 1999. Transport of carbon dioxide and ammonium in bioturbated (*Nereis diversicolor*) coastal, marine sediments. *Biogeochemistry,* 45**,** 147-168.

LALL, S. P. & DUMAS, A. 2015. Nutritional requirements of cultured fish: Formulating nutritionally adequate feeds *In:* DAVIS, D. A. (ed.) *Feed and Feeding Practices in Aquaculture.* Oxford: Woodhead Publishing.

LALL, S. P. & TIBBETTS, S. M. 2009. Nutrition, Feeding, and Behavior of Fish. *Veterinary Clinics of North America: Exotic Animal Practice,* 12**,** 361-372.

LAMBERT, R., DESROSIERS, G., RETIÈRE, C. & MIRON, G. 1992. Activité de prospection de son aire d'alimentation par la polychète *Nereis diversicolor* (OF Müller): données préliminaires. *Cahiers de biologie marine,* 33**,** 43-54.

LAST, K. S. & OLIVE, P. J. W. 1999. Photoperiodic control of growth and segment proliferation by *Nereis (Neanthes) virens* in relation to state of maturity and season. *Marine Biology,* 134**,** 191-199.

LIGHTNER, D. V. 2005. Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. *Journal of the World Aquaculture Society,* 36**,** 229-248.

LINDQVIST, S., GILBERT, F., ERIKSSON, S. P. & HULTH, S. 2013. Activities by *Hediste diversicolor* under different light regimes: Experimental quantification of particle reworking using time-resolved imaging. *Journal of experimental marine biology and ecology,* 448**,** 240-249.

LUIS, O. J. & PASSOS, A. M. 1995. Seasonal changes in lipid content and composition of the polychaete *Nereis (Hediste) diversicolor*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,* 111**,** 579-586.

MARINE HARVEST 2017a. Integrated Annual Report 2016. *Board of Directors Report.*

MARINE HARVEST 2017b. Salmon Farming Industry Handbook 2017.

MBKI INSTALLATIONS LTD - PENTAIR. 2017. *Aquatic Habitats X-Hab Systems XR3* [Online]. Available: http://mbki.com/aquatic-x-hab-system/ [Accessed 02.11.2016].

MENTE, E., PIERCE, G. J., SANTOS, M. B. & NEOFITOU, C. 2006. Effect of feed and feeding in the culture of salmonids on the marine aquatic environment: a synthesis for European aquaculture. *Aquaculture International,* 14**,** 499-522.

METCALFE, L., SCHMITZ, A. A. & PELKA, J. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Analytical chemistry,* 38**,** 514-515.

MOSS, S. M., REYNOLDS, W. J. & MAHLER, L. E. Design and economic analysis of a prototype biosecure shrimp growout facility. Proceedings of the US Marine Shrimp Farming Program Biosecurity Workshop, 1998. Hawaii, The Oceanic Institute, USA, 5-14.

NATIONAL RESEARCH COUNCIL 2011. *Nutrient Requirements of Fish and Shrimp,* Washington, DC, The National Academies Press.

NELSON, D. L., LEHNINGER, A. L. & COX, M. M. 2008. *Lehninger principles of biochemistry,* Macmillan.

NEORI, A., CHOPIN, T., TROELL, M., BUSCHMANN, A. H., KRAEMER, G. P., HALLING, C., SHPIGEL, M. & YARISH, C. 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture,* 231**,** 361-391.

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. & RIISGÅRD, H. U. 1995. Feeding, growth and respiration in the polychaetes *Nereis diversicolor* (facultative filter-feeder) and *N. virens* (omnivorous) - a comparative study. *Marine Ecology Progress Series***,** 149-158.

OECD/FAO 2017. *OECD-FAO Agricultural Outlook 2017-2026,* OECD Publishing.

OLAFSEN, T., WINTHER, U., OLSEN, Y. & SKJERMO, J. 2012. Value created from productive oceans in 2050. *Report of a working group appointed by the Royal Norwegian Society of Sciences (DKNVS) and the Norwegian Academy of Technological Sciences (NTVA).*

OLIVE, P., FLETCHER, J., REES, S. & DESROSIERS, G. 1997. Interactions of environmental temperature with photoperiod in determining age at maturity in a semelparous polychaete Nereis (Neanthes) virens Sars. *Journal of thermal biology,* 22**,** 489-497.

OLIVE, P. J. 1999. Polychaete aquaculture and polychaete science: a mutual synergism. *Hydrobiologia,* 402**,** 175-183.

OLIVIER, M., DESROSIERS, G., CARON, A., RETIERE, C. & CAILLOU, A. 1995. Behavioral responses of *Nereis diversicolor* (OF Muller) and *Nereis virens* (Sars)(Polychaeta) to food stimuli-Use of specific organic matter (algae and halophytes). *Canadian journal of zoology - revue canadienne de zoologie,* 73**,** 2307-2317.

OLIVIER, M., DESROSIERS, G., CARON, A., RETIÈRE, C. & CAILLOU, A. 1997. Juvenile growth of *Nereis diversicolor* (OF Müller) feeding on a range of marine vascular and macroalgal plant sources under experimental conditions. *Journal of Experimental Marine Biology and Ecology,* 208**,** 1-12.

OLSEN, L. M., HOLMER, M. & OLSEN, Y. 2008. Perspectives of nutrient emission from fish aquaculture in coastal waters. *Final report, The Fishery and Aquaculture Industry Research Fund.*

ONLINE BAITS UK. 2017. *Ragworm Live 1 Kg* [Online]. Available: http://www.onlinebaitsuk.co.uk/1-Kg-Live-Ragworm-(approx-150-worms)-105 [Accessed 06.06.2017].

OWEN, O., REICHARD JR, G., PATEL, M. & BODEN, G. 1979. Energy metabolism in feasting and fasting. *Adv Exp Med Biol,* 111**,** 169-188.

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). *Aquaculture Research*.

PAPASPYROU, S., THESSALOU-LEGAKI, M. & KRISTENSEN, E. 2010. The influence of infaunal (*Nereis diversicolor*) abundance on degradation of organic matter in sandy sediments. *Journal of Experimental Marine Biology and Ecology,* 393**,** 148-157.

PEARSON, T. & BLACK, K. 2000. The environmental impacts of marine fish cage culture. *Environmental impacts of aquaculture***,** 1-31.

PELLERIN-MASSICOTTE, J., MARTINEU, P., DESROSIERS, G., CARON, A. & SCAPS, P. 1994. Seasonal variability in biochemical composition of the polychaete *Nereis virens* (Sars) in two tidal flats with different geographic orientations. *Comparative Biochemistry and Physiology Part A: Physiology,* 107**,** 509-516.

PELLETIER, N., TYEDMERS, P., SONESSON, U., SCHOLZ, A., ZIEGLER, F., FLYSJO, A., KRUSE, S., CANCINO, B. & SILVERMAN, H. 2009. Not all salmon are created equal: life cycle assessment (LCA) of global salmon farming systems. *Environmental Science & Technology,* 43**,** 8730-8736.

POCOCK, D. M.-E., MARSDEN, J. R. & HAMILTON, J. 1971. Lipids in an intertidal polychaete and their relation to maturation of the worm. *Comparative Biochemistry and Physiology Part A: Physiology,* 39**,** 683IN1689-688IN6697.

PORTER, C., KROM, M., ROBBINS, M., BRICKELL, L. & DAVIDSON, A. 1987. Ammonia excretion and total N budget for gilthead seabream (Sparus aurata) and its effect on water quality conditions. *Aquaculture,* 66**,** 287-297.

REED MARICULTURE 2015. Shellfish diet ® Technical Data.

REES, S. W. & OLIVE, P. J. 1999. Photoperiodic changes influence the incorporation of vitellin yolk protein by oocytes of the semelparous polychaete Nereis (Neanthes) virens. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 123**,** 213-220.

REID, G., CHOPIN, T., ROBINSON, S., AZEVEDO, P., QUINTON, M. & BELYEA, E. 2013. Weight ratios of the kelps, *Alaria esculenta* and *Saccharina latissima*, required to sequester dissolved inorganic nutrients and supply oxygen for Atlantic salmon, *Salmo salar*, in integrated multi-trophic aquaculture systems. *Aquaculture,* 408**,** 34-46.

REID, G. K., ROBINSON, S. M., CHOPIN, T., MULLEN, J., LANDER, T., SAWHNEY, M., MACDONALD, B., HAYA, K., BURRIDGE, L. & PAGE, F. 2008. Recent developments and challenges for open-water, Integrated Multi-Trophic Aquaculture (IMTA) in the Bay of Fundy, Canada. *Aquaculture Canada 2007. Proceedings of Contributed Papers*.

RIDLER, N., WOWCHUK, M., ROBINSON, B., BARRINGTON, K., CHOPIN, T., ROBINSON, S., PAGE, F., REID, G., SZEMERDA, M. & SEWUSTER, J. 2007. Integrated Multi− Trophic Aquaculture (IMTA): A Potential Strategic Choice for Farmers. *Aquaculture Economics & Management,* 11**,** 99-110.

RIISGÅRD, H. U. 1991. Suspension feeding in the polychaete *Nereis diversicolor*. *Marine Ecology Progress Series***,** 29-37.

RIISGÅRD, H. U. 1994. Filter-feeding in the polychaete *Nereis diversicolor*: a review. *Aquatic Ecology,* 28**,** 453-458.

RIISGÅRD, H. U., VEDEL, A., BOYE, H. & LARSEN, P. S. 1992. Filter-net structure and pumping activity in the polychaete *Nereis diversicolor*: effects of temperature and pump-modelling. *Marine Ecology Progress Series***,** 79-89.

ROSENLUND, G., TORSTENSEN, B. E., STUBHAUG, I., USMAN, N. & SISSENER, N. H. 2016. Atlantic salmon require long-chain n-3 fatty acids for optimal growth throughout the seawater period. *Journal of Nutritional Science,* 5**,** e19.

RÖNN, C., BONSDORFF, E. & NELSON, W. G. 1988. Predation as a mechanism of interference within infauna in shallow brackish water soft bottoms; experiments with an infauna predator, Nereis diversicolor OF Müller. *Journal of Experimental Marine Biology and Ecology,* 116**,** 143-157.

SAHU, S. K., SINGH, R., MURUGESAN, P., MUTHUVELU, S. & KATHIRESAN, K. 2017. Biochemical studies on the live feed polychaete, *Nereis* sp., in relation to maturity stages.

SALVO, F., HAMOUTENE, D. & DUFOUR, S. C. 2015. Trophic analyses of opportunistic polychaetes (Ophryotrocha cyclops) at salmonid aquaculture sites. *Journal of the Marine Biological Association of the United Kingdom,* 95**,** 713-722.

SANTOS, A., GRANADA, L., BAPTISTA, T., ANJOS, C., SIMÕES, T., TECELÃO, C., FIDALGO E COSTA, P., 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.

SARGENT, J., BELL, G., MCEVOY, L., TOCHER, D. & ESTEVEZ, A. 1999. Recent developments in the essential fatty acid nutrition of fish. *Aquaculture,* 177**,** 191-199.

SARGENT, J., BELL, J., BELL, M., HENDERSON, R. & TOCHER, D. 1995. Requirement criteria for essential fatty acids. *Journal of applied Ichthyology,* 11**,** 183-198.

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.

SCHMIDT-NIELSEN, K. 1997. *Animal physiology: adaptation and environment*, Cambridge University Press.

SCHUTZSTATION WATTENMEER. 2017. *Almindelig frynseorm (Hediste diversicolor)* [Online]. Available: https://www.beachexplorer.org/dk/arter/hediste-diversicolor/kendetegn [Accessed 10.07.2017].

SHPIGEL, M. & NEORI, A. 1996. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: I. Proportions of size and projected revenues. *Aquacultural Engineering,* 15**,** 313-326.

SMITH, R. 1977. Physiological and reproductive adaptations of *Nereis diversicolor* to life in the Baltic Sea and adjacent waters. *Essays on polychaetous annelids in memory of Dr. Olga Hartman*.

SNOW, D. R. & MARSDEN, J. R. 1974. Life cycle, weight and possible age distribution in a population of *Nereis virens* (Sars) from New Brunswick. *Journal of Natural History,* 8**,** 513-527.

STATISTISK SENTRALBYRÅ - STATISTICS NORWAY. 2016. *Aquaculture. Final figures (2015)* [Online]. Available: https://www.ssb.no/en/jord-skog-jakt-og-fiskeri/statistikker/fiskeoppdrett/aar [Accessed 07.01.2017].

STAVIS SEAFOODS. 2017. *HEAD-ON, GUTTED NORWEGIAN SALMON* [Online]. Available: http://www.stavis.com/products/head-gutted-norwegian-salmon [Accessed 06.07.2017].

STURDIVANT, S. K., PERCHIK, M., BRILL, R. W. & BUSHNELL, P. G. 2015. Metabolic responses of the Nereid polychaete, Alitta succinea, to hypoxia at two different temperatures. *Journal of Experimental Marine Biology and Ecology,* 473**,** 161-168.

SUCKOW, B. 2010. *Untersuchungen zur integrierten Aquakultur mittels detrivorer Organismen als Verwerter der Feststoffe in der marinen Aquakultur*. Carl von Ossietzky Universität Oldenburg.

TACON, A. G. 1987. The nutrition and feeding of farmed fish and shrimp; a training manual. 1: The essential nutrients.

TACON, A. G., METIAN, M. R., TACON, M. A. G., HASAN, M. R. & METIAN, M. 2011. *Demand and supply of feed ingredients for farmed fish and crustaceans: trends and prospects*.

TAKEUCHI, T. 2007. Amino Acids, Peptides. *In:* NAKAGAWA, H., SATO, M. & GATLIN, D. M. (eds.) *Dietary supplements for the health and quality of cultured fish.*

THE NASDAQ OMX GROUP, I. 2017. Nasdaq Salmon Index 2017 Week 1-27.

TROELL, M., HALLING, C., NILSSON, A., BUSCHMANN, A., KAUTSKY, N. & KAUTSKY, L. 1997. Integrated marine cultivation of *Gracilaria chilensis* (Gracilariales, Rhodophyta) and salmon cages for reduced environmental impact and increased economic output. *Aquaculture,* 156**,** 45-61.

TROELL, M., JOYCE, A., CHOPIN, T., NEORI, A., BUSCHMANN, A. H. & FANG, J.-G. 2009. Ecological engineering in aquaculture - Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture,* 297**,** 1-9.

UN DESA - POPULATION DIVISION 2017. World Population Prospects: The 2017 Revision.

VEDEL, A., ANDERSEN, B. B. & RIISGÅRD, H. U. 1994. Field investigations of pumping activity of the facultatively filter-feeding polychaete *Nereis diversicolor* using an improved infrared phototransducer system. *Marine Ecology Progress Series***,** 91-101.

VEDEL, A. & RIISGÅRD, H. U. 1993. Filter-feeding in the polychaete *Nereis diversicolor*: growth and bioenergetics. *Marine Ecology Progress Series,* 100**,** 145-145.

WAITE, R., BEVERIDGE, M., BRUMMETT, R., CASTINE, S., CHAIYAWANNAKARN, N., KAUSHIK, S., MUNGKUNG, R., NAWAPAKPILAI, S. & PHILLIPS, M. 2014. *Improving productivity and environmental performance of aquaculture,* WorldFish.

WANG, X., ANDRESEN, K., HANDA, A., JENSEN, B., REITAN, K. I. & OLSEN, Y. 2013. Chemical composition and release rate of waste discharge from an Atlantic salmon farm with an evaluation of IMTA feasibility. *Aquaculture Environment Interactions,* 4**,** 147-162.

WANG, X., BROCH, O. J., FORBORD, S., HANDÅ, A., SKJERMO, J., REITAN, K. I., VADSTEIN, O. & OLSEN, Y. 2014. Assimilation of inorganic nutrients from salmon (*Salmo salar*) farming by the macroalgae (*Saccharina latissima*) in an exposed coastal environment: implications for integrated multi-trophic aquaculture. *Journal of applied phycology,* 26**,** 1869-1878.

WANG, 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. *Aquaculture Environment Interactions,* 2**,** 267-283.

WU, G. 2009. Amino acids: metabolism, functions, and nutrition. *Amino Acids,* 37**,** 1-17.

WU, R. 1995. The environmental impact of marine fish culture: towards a sustainable future. *Marine pollution bulletin,* 31**,** 159-166.

YTRESTØYL, T., AAS, T. S. & ÅSGÅRD, T. 2015. Utilisation of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway. *Aquaculture,* 448**,** 365-374.

Appendix I

Table A.1 – Overview of calculated daily feeding amounts in E4 (30-day trial with smolt waste sludge). FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1).

Treatment		**FF [mg d^{-1}]**	**SWS [mg d^{-1}]**	**SFD [mg d^{-1}]**	**Mix (SWS/SFD) [mg d^{-1}]**
Tank	1	170.4	1202.5	2495.2	1075.1/ 380.6
	2	185.1	1317.8	2578.4	936.2/331.4
	3	178.5	1161.9	2287.3	1085.7/384.4
	4	180.9	1322.1	1984.8	1066.2/377.4
	5	180.9	1326.4	2313.8	1251.3/443.0
Mean±SD		179.2±5.4	1266.1±78.0	2331.9±229.2	1082.9±112.0/383.4±39.7

Appendix II

Biochemical compositions of diets and polychaetes (E4).

Table A.2 – Biochemical composition of the different diets used in E4 (30-day trial with smolt waste sludge). Different superscripts indicate significant differences ($P < 0.05$). Values for TOM, protein, total lipid and carbohydrates of shellfish diet were obtained from the manufacturer (Reed Mariculture, 2015)

[g g^{-1} DW]	Fish feed	Smolt waste sludge	Shellfish diet
Ash	0.11 ± 0.00^{a}	0.43 ± 0.02^{ab}	0.22
Carbon	0.46 ± 0.47^{a}	0.29 ± 0.00^{b}	0.47 ± 0.00^{ab}
Nitrogen	0.10 ± 0.00^{a}	0.04 ± 0.00^{b}	0.05 ± 0.00^{ab}
C:N	4.84 ± 0.14^{c}	7.20 ± 0.16^{b}	9.03 ± 0.04^{a}
Protein	0.61 ± 0.00^{a}	0.25 ± 0.01^{ab}	0.45
Lipids	0.15 ± 0.00^{a}	0.09 ± 0.02^{a}	0.14
Carbohydrates	0.13	0.24	0.1

Table A.3 – Biochemical composition of *H. diversicolor* under different food regimes (Mean±SD). Wild - natural field; Initial – *Rhodomonas* sp.; FF – fish feed; SWS – smolt waste sludge; SFD – shellfish diet; Mix – mix of smolt waste sludge and shellfish diet (ratio 5:1). Different superscripts indicate significant differences ($P < 0.05$).

[g g^{-1} DW]	Wild	Initial	Treatment			
			FF	SWS	SFD	Mix
Ash	-	0.11 ± 0.06^{a}	0.11 ± 0.02^{a}	0.13 ± 0.02^{a}	0.14 ± 0.02^{a}	0.12 ± 0.02^{a}
Carbon	0.43 ± 0.00^{a}	0.43 ± 0.01^{a}	0.46 ± 0.00^{a}	0.44 ± 0.01^{a}	0.44 ± 0.01^{a}	0.45 ± 0.01^{a}
Nitrogen	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.09 ± 0.00^{b}	0.09 ± 0.00^{b}	0.09 ± 0.00^{b}	0.09 ± 0.00^{ab}
C:N	4.46 ± 0.13^{e}	4.50 ± 0.06^{de}	5.32 ± 0.08^{a}	4.98 ± 0.17^{bc}	5.1 ± 0.10^{ab}	4.83 ± 0.20^{cd}
Protein	0.60 ± 0.01^{a}	0.60 ± 0.02^{a}	0.54 ± 0.01^{c}	0.55 ± 0.01^{c}	0.54 ± 0.01^{c}	0.58 ± 0.01^{b}
Lipids	0.13 ± 0.01^{bc}	0.11 ± 0.08^{c}	0.16 ± 0.01^{a}	0.12 ± 0.01^{bc}	0.14 ± 0.01^{ab}	0.13 ± 0.01^{bc}
Carbohydrates	-	0.18	0.2 ± 0.01^{a}	0.20 ± 0.01^{ab}	0.18 ± 0.02^{ab}	0.17 ± 0.02^{b}