Master's thesis

NTNU
Norwegian University of Science and Technology
Department of Biology

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Biochemical composition of *Hediste diversicolor* cultivated on aquaculture sludge and utilization as a potential fish feed resource

Master's thesis in Ocean Resources
Supervisor: Kjell Inge Reitan
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Abstract

Cultivation of Atlantic salmon (*Salmo salmar*) is entirely dependent on high quality feed resources such as fishmeal and fish oil. Limitations of the availability of these resources forces the industry to look elsewhere for alternative feed resources to maintain growth and development in a sustainable manner. A proposal from the Research Council of Norway (RCN) calls for production of marine species from low-trophic levels with feed potential, as a response to this issue.

The aim of this thesis was to evaluate the potential of the polychaete *Hediste diversicolor* fed on aquaculture sludge as an alternative feed resource for fish feed. The main objectives were to evaluate the suitability of smolt and post-smolt sludge as diets for *H. diversicolor*, determine the influence of different feeding levels on growth and biochemical composition, and establish the potential of *H. diversicolor* as an alternative feed resource.

Wild polychaetes were collected from beaches in Trondheimsfjorden and cultivated in a laboratory at SINTEF Ocean on smolt and post-smolt sludge. The effects of the different sludges and feeding levels in terms of growth, survival and biochemical composition were evaluated.

The results showed that polychaetes had equally successful growth and survival rates when fed on both sludges as a sole food source. Increased amounts of sludge given to the polychaetes correlated with increased growth rate. The results showed no significant changes in protein, amino acids, minerals or vitamins contents of the polychaetes fed on the two types of aquaculture sludges. Significant differences in relative content of total lipid were found within the treatments and compared to the initial polychaetes. The effect of post-smolt sludge was greater compared to smolt sludge in terms of total lipid.

Increased levels of feed given to the polychaetes also resulted in increased levels of essential omega-3 and omega-6 fatty acids in the different treatment groups, providing proof of the ability of *H. diversicolor* to utilize and incorporate valuable excessive nutrients in aquaculture sludge that otherwise would have been lost into the environment. Polychaetes contained high and adequate levels of essential amino acids, fatty acids, proteins, lipids, minerals, and trace levels of several essential vitamins regarding the nutritional requirements of *Salmo salmar*. Thus, *H. diversicolor* fed on aquaculture sludge displays potential to become a sustainable alternative feed resource in future fish feed, potentially replacing parts of other highly valued marine resources such as fishmeal and fish oil.

Keywords:

Hediste diversicolor, polychaete, aquaculture sludge, alternative feed resource

Sammendrag

Kultiveringen av atlantisk laks (*Salmo salmar*) er helt avhengig av marine ressurser av høy kvalitet som fiskemel og fiskeolje. Begrensinger på tilgangen til disse ressursene tvinger industrien til å utnytte alternative fôrressurser for å opprettholde bærekraftig vekst og utvikling. Det har kommet en oppfordring fra forskningsrådet (RCN) om å utvikle nye kultiveringsmetoder for lavtrofiske arter til benyttelse som fremtidige fôrressurser.

Målet med denne oppgaven var å evaluere potensialet til børstemarken *Hediste diversicolor*, som utelukkede er fôret på slam fra akvakulturnæringen, som en alternativ fôrressurs i fiskefôr. Hovedmålene var å vurdere egnetheten til smolt og postsmolt slam som diet for *H. diversicolor*, bestemme graden av innflytelse av ulike fôrnivåer på vekst og den biokjemiske sammensetningen, og utrede potensialet til *H. diversicolor* som en alternativ fôrressurs.

Børstemarkene ble hentet fra flere strender langs Trondheimsfjorden og kultivert i et laboratorium ved SINTEF Ocean på smolt- og postsmolt slam. Effekten av ulike mengder av de to slammene på *H. diversicolor* med hensyn på vekst, overlevelse og biokjemisk sammensetning ble evaluert.

Resultatene viste at både smolt- og postsmolt slam var like effektive med hensyn på vekst og overlevelse. Økt fôring resulterte i korresponderende økt vekst. Resultatene viste ingen signifikante forskjeller i innholdet av protein, aminosyrer, mineraler eller vitaminer i noen av børstemarkene. Det ble derimot observert signifikante forskjeller i totallipidinnholdet mellom de ulike fôringsnivåene, og mellom fôringsnivåene og kontrollgruppen. Effekten av slam på totallipidinnholdet var størst i postsmolt-gruppene.

Økt fôring resulterte også i økende nivåer av essensielle omega-3 og omega-6 fettsyrer i de ulike gruppene, noe som beskriver evnen *H. diversicolor* har til å utnytte og inkorporere verdifulle, gjenværende næringsstoffer i slam fra akvakulturnæringen som ellers ville gått tapt til miljøet. Børstemarkene inneholdt høye og tilstrekkelige nivåer av essensielle aminosyrer, fettsyrer, proteiner, lipider, mineraler og spornivåer av flere essensielle vitaminer i forhold til næringsbehovet til *Salmo salmar*. Resultatene viser at *H. diversicolor* fôret på slam fra akvakulturnæringen har potensiale til å bli en bærekraftig alternativ fôrressurs i fremtidig fiskefôr, og mulig erstatte andeler av andre verdifulle ressurser som fiskemel og fiskeolje.

Nøkkelord:

Hediste diversicolor, børstemark, akvakulturslam, alternative fôrressurser

Acknowledgements

I would like to express my deepest appreciation to my supervisors Kjell Inge Reitan and Inka Anglade for continuous support and guidance throughout this master thesis. I am also deeply indebted to my co-student Bjørn Stian Broberg Kristensen for excellent cooperation, endless coffee breaks and several good times during our joint work within the POLYCHAETE-project (280836). The experiment was carried out within the framework of the research infrastructure Norwegian Center for Plankton Technology (245937/F50). I am extremely grateful to my wife, Kaja Hagby Dahl, for her patience, never-ending support, and motivational speeches to keep me going.

I would like to extend my sincere thanks to Arne Malzahan and Andreas Hagerman at SINTEF Ocean for additional support and advice during the project. I am also grateful to co-students Håkon Ellingsen Sæther and August Nymoen and SINTEF employee Marius Andersen for additional help in collecting the polychaetes.

I would also like to extend my thanks to Reidun Vadla at NTNU, and Merethe Selnes and Antonio Sarno at SINTEF for their contribution to the different chemical analysis.

Contents

Abbreviations

ANOVA	Analysis of Variance
DHA	Docosahexaenoic acid, C22:6 n-3
DW	Dry weight
EAA	Essential amino acids
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid, C20:5 n-3
FA	Fatty acids
HPLC	High-performance liquid chromatography
LC	Long-chain
L/D	Light to darkness
MUFA	Monounsaturated fatty acids
Non-EAA	Non-essential amino acids
PUFA	Polyunsaturated fatty acids
RAS	Recirculating aquaculture system
RCN	Research council Norway
SAFA	Saturated fatty acids
SD	Standard deviation
SGR	Specific growth rate (d^{-1})
TAN	Total ammonia nitrogen
TOM	Total organic matter
Treatment	Smolt or post-smolt sludge-based diet
Treatment groups	Different feeding levels
Treatment group A	Sludge equal to 5% of tot. nitrogen content in polychaetes d^{-1}
Treatment group B	Sludge equal to 10% of tot. nitrogen content in polychaetes d^{-1}
Treatment group C	Sludge equal to 20% of tot. nitrogen content in polychaetes d^{-1}
Treatment group D	Sludge equal to 40% of tot. nitrogen content in polychaetes d^{-1}
WW	Wet weight

1 Introduction

With an annual growth of 80 million people every year, the world population is expected to reach 9 billion people by 2050. To be able to feed a fast-growing population we must increase the global food production by 70% according to the Food and Agriculture Organization of the United Nations (FAO, 2009). Globally, the aquaculture industry is one of the most important and fastest growing food production sectors with an annual growth rate of 6.3%. Further growth is needed to meet the dietary needs of the increasing human population (Wang et al., 2013). Norwegian salmon production has established itself as the biggest Atlantic salmon producer in the world, and produced a record 1.36 billion tons in 2019 worth 68.0 billion NOK (SSB, 2020).

A sustainably managed salmon industry is important for further growth to be possible. An aspect that needs to be taken into consideration is the environmental impact of the industry. It is important to reduce the number of non-renewable resources and capture and recycle highly valuable resources like lipids and proteins (Wang et al., 2013).

Norway's salmon production still has potential for further growth, but is limited by different factors, where one of them is availability of sustainable feed resources. In the early 1990s, the total amount of marine ingredients in salmon feed was equal to 90%. The traditional salmon feed has largely been based on fishmeal (40-60%) and fish oil (20-30%) (Connor, 2000). Although the total amount of fish oil and fishmeal has been heavily reduced (30% in 2013) and replaced by plant material, it is still a vital part of the modern fish feed. Atlantic salmon (*Salmo salar*) is a carnivorous fish which is dependent on marine essential omega-3 fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as amino acids in its diet for healthy development and survival (Ytrestøyl et al., 2015, Connor, 2000).

1.1 Sustainability and Alternative Feed Resources

According to FAO (2009), the aquaculture industry consumes approximately 57% and 87% of the global fish meal and fish oil, respectively. For perspective, between mid-2005 and mid-2008, the prices for fishmeal increased 50% and 130% for fish oil (Naylor et al., 2009). As the industry continues to grow rapidly, the demand for these resources is expected to increase even further. Since several of the world's commercial fishing stocks are over- or fully exploited, a

challenge is that the current fish meal (and oil) production will not be sufficient to satisfy a rapidly growing industry (figure 1.1). The aquaculture industry was per 2016 responsible for 53% of the current seafood production in the world (FAO, 2016). Per 2018, Norway is the second largest exporter of aquaculture seafood products to the global marked (Franz et al., 2018). As most of Norway's exported seafood products is dominated by Atlantic salmon and other farmed organisms, the demand for feed and feed resources is subsequently high.

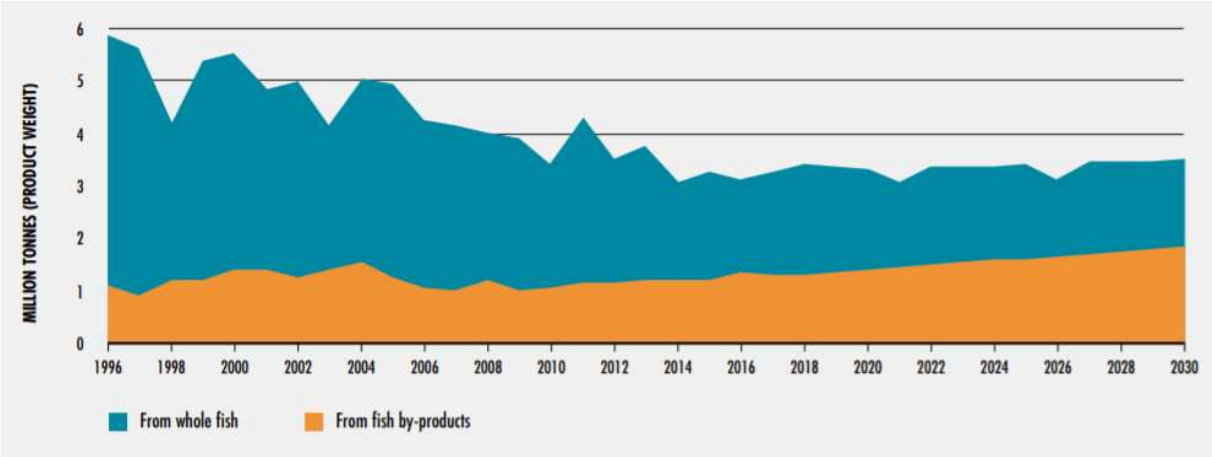

Figure 1.1: Global production of fishmeal from 1996-2030 (FAO, 2018).

Over the last years, there has continuously been put greater emphasis on the sustainability aspect of the aquaculture industry – both by consumers, authorities, and the industry itself. Traditional aquaculture has an immense impact on the environment, and is often relying on dilution in open systems as a response to pollution from sludge, organic emissions and overfeeding (Naylor et al., 1998). Additionally, acquisition of feed and feed resources are other aspects that are pressured by demands of sustainability. The Norwegian aquaculture is largely dependent on acquisition of feed resources from South America (Abualtaher and Bar, 2020). Trade and transportation between South America and Norway results in tremendous energy costs in addition to significant carbon footprints. The supply from the South American regions is also affected by global natural-cycling events like El Niño (Shepherd et al., 2005). Events like El Niño can also influence the economy. In those regards, it will be in the industry's best interest to explore and develop alternative resources and look for new opportunities. Recently, as per January 2021, the entire European salmon sector engaged in a proposition to end deforestation in Amazonas, Brazil, and will not be trading soy grown on deforested land after August 2020. Brazilian soy traders CJ Selecta, Caramuru and Imcopa have all committed to this goal (Reuters, 2021). This action proves the industry's willingness to adapt and puts further

amplification on the importance of sustainability and development of new and sustainable feed resources.

1.2 *Hediste diversicolor*

Figure 1.2: Polychaete species Hediste diversicolor (Kristensen, 2013).

The polychaetes *Hediste diversicolor* (figure 1.2) are small benthic organisms that thrive in muddy sediments where they create small U- and Y-shaped burrows (Scaps, 2002b). *H. diversicolor* is named after its change in colour in relation to maturation. The polychaetes are usually coloured brown or red but change colour to a bright green during maturation. They are distributed all over the Atlantic and thrive in brackish waters and intertidal zones (Smith et al., 1997). Polychaetes have the ability to inhabit several habitats, and they are well known to endure extreme conditions considering e.g., temperature and salinity (Scaps, 2002c). Polychaetes can to grow fast at optimal conditions (Abrantes et al., 1999).

Polychaetes have great value for both the fishing and the aquaculture industry, as bait and feed resources, respectively. They are in high demand because of their high contents of proteins and essential marine fatty acids (Bischoff et al., 2009). Polychaetes are interesting for the aquaculture industry in several regards. In addition to being rich in protein and marine fatty

acids, they have shown tendencies to occur in large densities, up to 3000 individuals per square meter beneath aquaculture facilities (Riisgård, 1994). Polychaetes have the ability to utilize several different feeding modules, depending on what feed is available (Goerke, 1966). They can act as planktonic filter feeders by creating mucus nets to catch prey and organic particles (Goerke, 1966), carnivorous predators or as a decomposers of e.g., sludge and therefore contribute to bioremediation (Riisgård, 1994). Previous studies have shown promising signs that the polychaete *H. diversicolor* can utilize excessive nutrients in sludge to form marine essential fatty acids and high levels of protein (Wang et al., 2019b).

1.3 Aquaculture Sludge

The rapid growing aquaculture industry is very space-demanding and competitive, which are some of the factors that have led parts of the industry to move on to land-based systems. One of the challenges with moving a previous sea-based phase into a land-based aquaculture system, is the large quantities of additional marine waste sludge with a considerable salt content. Traditional uses for freshwater aquaculture sludge are fertilizer for agriculture and production of biogas (Cripps and Bergheim, 2000). Moreover, production of biogas from saline sludge proves to be challenging (in high levels) as it could inhibit the biomethanation process (Letelier-Gordo et al., 2020). Differences in the chemical composition of freshwater and seawater sludge could possibly be a challenge if trying to fit the marine sludge within the same niches. Thus, it is of interest to this project to examine the biochemical differences and quality of both freshwater and brackish sludge to determine if either are appropriate as feed for cultivated polychaetes.

1.4 The POLYCHAETE Project

The POLYCHAETE-project (figure 1.3) is a response to the RCN (Research Council Norway) encouragement to increase the research and development of low-trophic species for feed resource purposes. As polychaetes in wild populations transform organic material from nature and aquaculture facilities into high quality proteins and marine fatty acids, the aim of the project is to recreate this dynamic in intensive fed cultures. The project envisages intensive cultivation of *H. diversicolor* for biological recycling of valuable nutrients which otherwise would have been lost, and produce a raw material for farmed fish. The project is divided into several work

packages and areas of focus. This thesis will be focusing on the biochemical composition of polychaetes fed on smolt and post-smolt sludge and assess the suitability of polychaetes as an alternative feed resource in future fish feed.

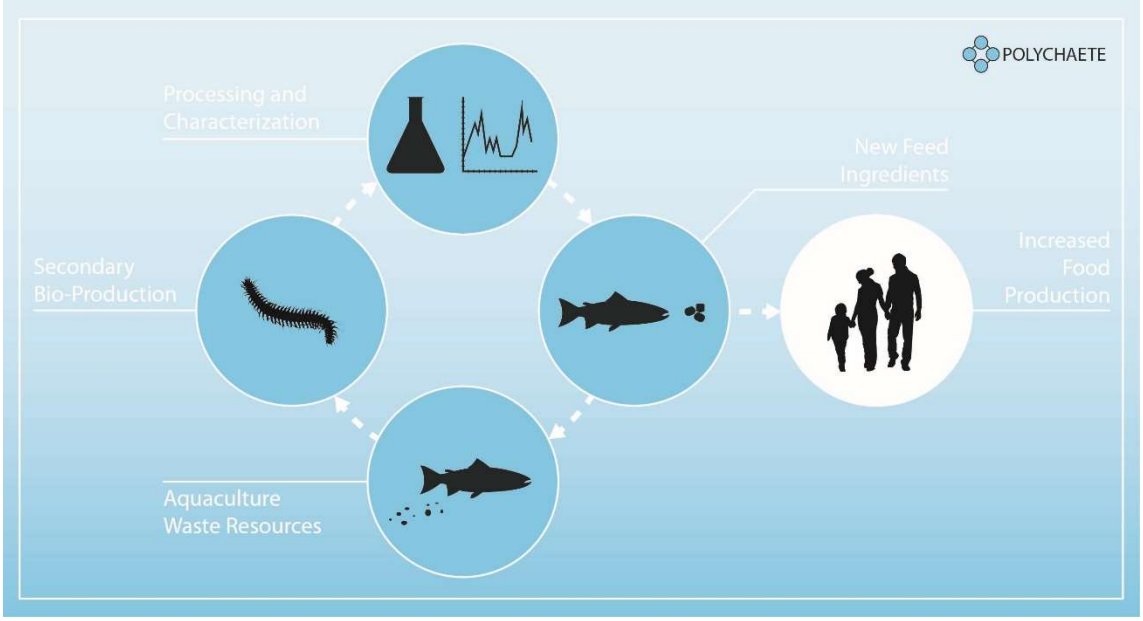

Figure 1.3: The POLYCHAETE project – recycling of highly valuable nutrients to increase the sustainability of intensive fed aquaculture and increase human food production.

1.5 Aims of this Study

The aim of this study is to evaluate the potential of the polychaete species *H. diversicolor*, fed on aquaculture sludge, as an alternative feed resource for fish feed. The main objectives for this study are divided into:

1. Evaluate the suitability of using freshwater (smolt) and marine (post-smolt) aquaculture sludge as diets for cultivation of *H. diversicolor.*
2. Determine the influence of increasing feed levels of smolt and post-smolt sludge on growth and biochemical composition of *H. diversicolor*.
3. Determine the potential of *H. diversicolor* as a potential feed resource with the respect to the nutritional needs of Atlantic salmon (*Salmo salmar*).

Additionally, the assessment of the influence of the different diets on growth and mortality is an objective for the POLYCHAETE project, as well as producing polychaetes in a large scale

exclusively on aquaculture sludge. The secondary objectives for this thesis specifically are to characterize the two sludge types and determine which would be the most appropriate for polychaete production, with respect to their biochemical content as well as their effect on the biochemical composition of polychaetes.

Based on the previous objectives and goals of the experiment, the following hypothesis were formed:

1. The different sludge diets and feed levels (treatment groups) will influence the polychaete growth.
2. The two different types of aquaculture sludge will have significant effects on the biochemical composition of polychaetes (*H. diversicolor*).
3. The biochemical composition of polychaetes fed on aquaculture sludge makes them a suitable resource in future feed for Atlantic salmon (*S. salar*).

2 Materials and Methods

2.1 Collection of Polychaetes

Table 2.1: Overview of the different collection locations and when the collections took place, and approximately how many individuals was collected.

Collection	Date	Location	Number of individuals
I	19.09.21	Spongdal	150
II	01.10.19	Buvika	550
III	07.10.19	Buvika	400

The three polychaete collections took place at two different locations: Spongdal and Buvika, presented in table 2.1. Both locations were well within driving distance from NTNU Sealab (approximately 20 min radius) and the trips themselves took approximately three hours each. The polychaetes, which all were of the species *H. diversicolor*, were collected during the low tides from muddy sediments. The collection was done by hand after digging up parts of the sediment (20-30 cm) with shovels and garden forks. The polychaetes were collected by going through the dug-up sediment by hand and placed into large plastic containers with their natural sediment. Additionally, some seaweed was added prior to transportation to minimize stress during the trip back to the lab.

Between the time of collections and the start of the feeding experiment, the collected polychaetes were stored in several large trays connected to a flow-through system. The flow-through system had a 100% daily water-exchange rate. The trays were also filled with sandbox sand from Byggmax as sediment for the polychaetes. During the adaptation period in the lab, the polychaetes were fed with commercial salmon feed several times a week with assistance from SINTEF employees.

2.2 Obtaining Sludge and Preparation of Diets

The marine post-smolt sludge was shipped to NTNU Sealab from LetSea AS, a smolt facility located in Dønna in Nordland. The freshwater smolt sludge was retrieved from Lerøy's hatchery at Belsvika in Trøndelag. This facility is a recirculating aquaculture system (RAS), and the sludge was collected directly from the drum filter and put into 10-liter plastic tanks.

Both sludge types were then centrifuged (Heraeus, MEGAFUGE 16R, Thermo scientific) (3000 rpm) for 3 minutes in smaller proportions to get rid of water. The centrifuged sludge was then placed into smaller zip-lock bags and stored in a freezer (-20°C) until weighing and feeding. The zip-lock bags were taken out of the freezer a few hours before the diets were prepared so that the sludge could thaw. Additionally, small proportions of both sludge types were weighed and dried in a heating cabinet for 24 hours to determine the water content.

The two sludge types were defined as two separate treatments: smolt and post-smolt. Within each treatment, there were four different treatment groups as presented in table 2.2. The amount of feed was based on the total nitrogen content in polychaetes. Based on a previous similar experiment where the diets were based on carbon content, the results indicated that nitrogen is the limiting nutrient in the polychaetes (Seekamp, 2017). Therefore, the diets in this experiment were based on the nitrogen content in the diets and in the polychaete biomass.

Theoretically, the polychaetes in each treatment group were fed daily with amounts accordingly to table I and II (appendix I). E.g., polychaetes in smolt treatment group A, were fed with a certain amount of smolt sludge corresponding to 5% of the total nitrogen content in polychaetes (*H. diversicolor*) in the tank every day during the feeding experiment. In reality, the polychaetes were fed every other day, as they have shown a tendency to not necessarily eat every day (Seekamp, 2017). The amount of feed was then doubled, so the numbers added up at the end.

Table 2.2: The two treatments (sludge types) and the four treatment groups (diets) within each treatment. The diets in each treatment group were based on the total nitrogen content in polychaetes. Treatment group A = 5% of the total nitrogen content in polychaetes, B = 10%, C = 20% and D = 40%.

Treatment	**Treatment groups**			
Smolt sludge	A	B	C	D
Nitrogen content (% of polychaete biomass)	5%	10%	20%	40%
Post-smolt sludge	A	B	C	D
Nitrogen content (% of polychaete biomass)	5%	10%	20%	40%

2.3 The Experimental Design

The feeding experiment took place at SINTEF Ocean in two fully automated XR3 cultivation systems (Aquatic habitats X-hab system, Pentair plc, USA). The system is illustrated in figure 2.1. Each system contained 20 polycarbonate 16-liter tanks filled with sediment and seawater. The sediment consisted of sandbox sand from Byggmax. The sediment was mixed and washed

thoroughly with seawater before it was added to the cultivation tanks. The cultivations tanks were filled with sediment at approximately 7 cm height. After the sediment was added and the cultivation tanks were placed back into the rig, the water was turned on and the system ran for 24 hours before the polychaetes were added to make sure everything worked as intended in terms of water exchange, temperature, and light regime. Regarding water flow, there was a continuously daily water exchange of 100% with natural seawater supplied from the Trondheimsfjord. The water was then heated to a temperature of 16 ℃ before it entered the tanks. The light/day regime in the room was set at 16/8 hours.

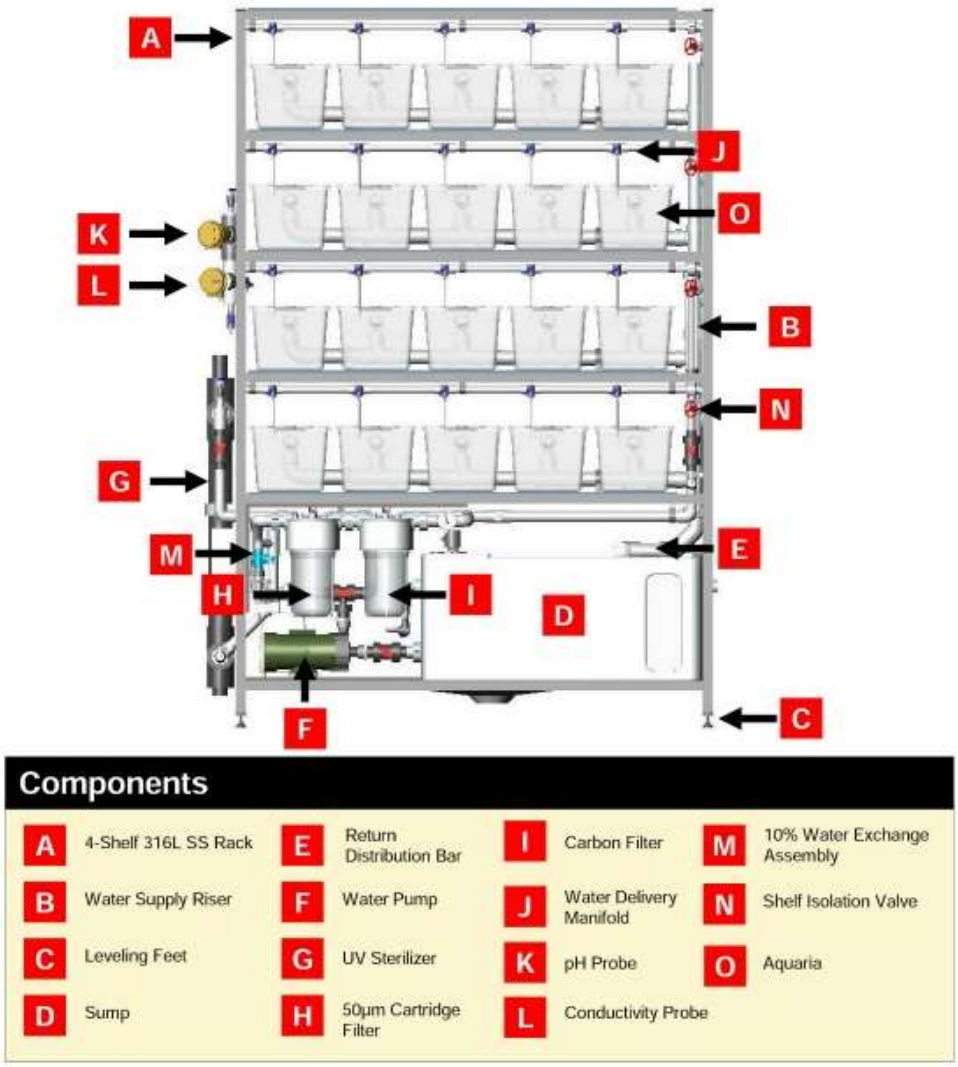

Figure 2.1: Aquatic habitat X-hab tank system (MBKI Installations Ltd – Pentair, 2021).

The smolt and post-smolt treatment groups had each a separate system. For each treatment group, there was four replicate tanks as described in figure 2.2. The placements of the

cultivation tanks were completely random, but the same for both treatments (randomizer function, Microsoft Excel).

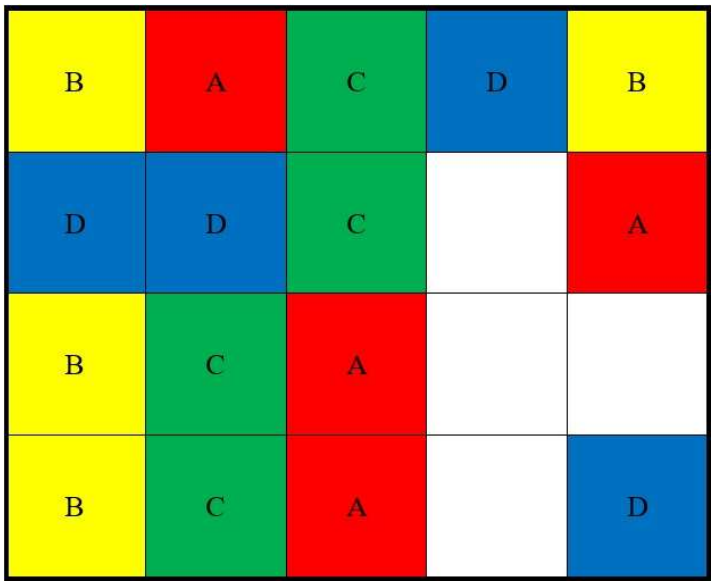

Figure 2.2: Tank overview. Treatment A (5% of the total nitrogen, red), B (10% of the total nitrogen, yellow), C (20% of the total nitrogen, green) and D (40% of the total nitrogen, blue).

2.4 30-day Feeding Experiment

The previously collected polychaetes were dug up from the sediment in the plastic trays where they had been held for the past 4-7 weeks and put into smaller buckets for two hours for them to empty their stomachs. Before weighing, the largest and smallest polychaetes were removed to get the distribution as even as possible. Sexually mature polychaetes (coloured green) were also removed as they are not expected to put any more energy into growth at that point in their life cycle. After two hours, polychaetes in groups of 15 were washed with freshwater to get rid of salt and sediment which could affect the weight. They were then dried on a paper tissue, weighed and put into the Pentair cultivation tanks. The weight of the 15 polychaetes added to each tank respectively are shown in table 2.3.

Table 2.3: The total weight [g] of the 15 polychaetes (H. diversicolor) in each of the tanks at the start of the feeding experiment. The average weight and the standard deviation for each treatment group are also included.

	Smolt treatment			
Replicate	**A [g]**	**B [g]**	**C [g]**	**D [g]**
1	4.29	3.66	3.66	3.01
2	3.99	4.27	2.96	3.63
3	3.64	3.4	4.22	3.46
4	5.96	4.13	4.56	3.32
Mean±SD	4.47±1.03	3.87±0.41	3.85±0.70	3.36±0.26
	Post-smolt treatment			
1	2.89	3.65	5.16	2.86
2	3.85	4.23	3.67	3.52
3	4.67	3.67	2.97	3.34
4	3.20	2.46	3.45	4.25
Mean±SD	3.65±0.79	3.50±0.75	3.81±0.94	3.49±0.58

The first feeding took place when all polychaetes were in place in the cultivation tanks. The feeding was completed in accordance with the method described in section 2.2. As the polychaetes were fed only every other day, the day in between was used to prepare feed portions for the next day using an analytical scale (Mettler Toledo, XA204DR). The exact amounts of feed fed to the polychaetes are presented in table I and II (appendix) for the smolt- and post-smolt treatment, respectively. The numbers are based on a previous, similar experiment presented in table III (appendix) (Seekamp, 2017).

During the cultivation period, different abiotic parameters such as temperature [°C], dissolved O_2-levels [%], salinity [ppt], and pH were measured several times a week (YSI ProDDS multiparameter Water Quality), in accordance with table 2.4, to make sure the conditions were stable. Additionally, as biofilm was starting to accumulate in some of the tanks, all cultivations tanks were cleaned once a week. The first step of the cleaning procedure consisted of stirring the top of the sediment carefully with the oxygen-supplier in the tanks to dissolve any biofilm at the surface of the sediment. The water inlet was then turned off and the tanks were emptied for water with a cut off water hose using hydrostatic pressure. The water coming out from the cultivations tanks was filtered to make sure no polychaetes got lost in the process. The system was turned back on after all the tanks had been emptied. During the feeding experiment, dead polychaetes were also removed regularly after daily inspections.

Table 2.4: Measuring dates of the abiotic factor's temperature, dissolved oxygen, salinity, and pH. Experimental period: week 45-50, 2020.

Week 45	Week 46	Week 47	Week 48	Week 49	Week 50
08.11	12.11	17.11	25.11	02.12	07.12
	15.11	20.11	27.11	05.12	
		22.11	29.11		

At the end of the experiment, the system was turned off and the cultivations tanks were emptied for water using the same method as described above regarding cleaning. Then, each cultivation tank was taken down separately and the sediment was searched very closely by hand to find and capture the remaining polychaetes. Each tank was dedicated its own bucket of sea water for the polychaetes to empty their stomach before weighing. Polychaetes from each tank were dried on a paper tissue and weighed in groups of five or less, but then kept separately in small plastic tubes in the freezer (-20 °C). They were stored in the freezer until further treatment and analysis.

2.5 Growth Rate and Survival

The change in biomass of the polychaetes was determined by comparing the change in individual weight [g] over time, using the initial individual average wet weight compared to the final individual average wet weight. The number of remaining polychaetes was taken into consideration. The individual average change in weight in each tank was calculated using the following equation:

$$Change\ in\ individual\ weight = \frac{Final\ [g]}{q} - \frac{Initial\ [g]}{n}$$

where final [g] is the total wet weight of the remaining polychaetes and q is the number of remaining polychaetes. Initial [g] is the total wet weight of the polychaetes at the start of the experiment and n is the number of polychaetes at the start of the experiment.

The specific growth rate (SGR) of the polychaetes during the experiment was calculated based on the average wet weight of the polychaetes at the start and end of the experiment. The specific growth rate was calculated (Jørgensen, 1990) with respect to the changes in wet weight [g] and the length of the experiment (days).

$$SGR = \frac{\ln(final\ average[g]) - \ln(initial\ average[g])}{t\ (days)}$$

The survival rate of the polychaetes was calculated based on how many of the initial 15 polychaetes in each tank were retrieved at the end of the experiment. The survival rate was calculated based on the following equation:

$$Survival\ rate = \left(\frac{N_s}{N_0}\right) * 100\%$$

where N_0 is the number of polychaetes at the start of the experiment and N_s is the number of surviving polychaetes.

2.6 Chemical Analysis

In preparation for chemical analyses, all polychaetes were freeze-dried (Labconco, FreeZone Benchtop Freeze Dryers). Further, polychaetes from the same tank were pooled together and grinded into a homogenic powder using a pestle and mortar. The polychaete powder was then put into plastic tubes. Nitrogen gas was added to the tubes to prevent degradation of lipids before they were put into another freezer (-80 °C). The same drying and treatment-procedure was applied for both sludge types as well. The freeze-dried sludge and polychaete powder were then analysed as listed in table 2.5 and 2.6, respectively.

Most of the units in the analysis are presented as mg/g DW. The exceptions are minerals [mg/kg DW] and water-soluble vitamins (WS) and fat-soluble vitamins (FS) [ng/mg DW].

Table 2.5: Chemical analysis performed to characterize the smolt and post-smolt diets and number of replicates.

				Sludge types	
Analysis	**Section**	**Unit**	**Method**	**Smolt (n)**	**Post-smolt (n)**
Amino acids	2.6.1	% of total AA/ mg/g DW	HPLC	3	3
Protein	2.6.2	mg/g DW	Calculation	3	3
Total lipid	2.6.3	mg/g DW	Extraction	3	3
Fatty acids	2.6.4	% of total FA/ mg/g DW	Extraction/GC	3	3
Carbohydrates	2.6.5	mg/g DW	Calorimetry	3	3
Minerals	2.6.6	mg/kg DW	Analysis	1	1
WS-vitamins	2.6.7.1	ng/mg DW	LCMS	1	1
FS-vitamins	2.6.7.2	ng/mg DW	LCMS	3	3
Ash content	2.6.8	mg/g DW	Combustion	3	3

Table 2.6: Overview of the different biochemical analysis and the number of samples (n) from each group. The table includes the initial polychaetes (I) as well as the polychaetes from the smolt and post-smolt treatment groups (A-D).

				Polychaete treatment groups				
Analysis	**Section**	**Unit**	**Method**	**I**	**A**	**B**	**C**	**D**
Amino acids	2.6.1	%/mg/ DW	HPLC	1	4	4	4	4
Protein	2.6.2	mg/g DW	Calculation	1	4	4	4	4
Total lipid	2.6.3	mg/g DW	Extraction	1	4	4	4	4
Fatty acids	2.6.4	% of total FA/ mg/g DW	Extraction/GC	1	4	4	4	4
Carbohydrates	2.6.5	mg/g DW	Calorimetry	1	4	4	4	4
Minerals	2.6.6	mg/kg DW	Analysis	1	4	4	4	4
WS-vitamins	2.6.7.1	ng/mg DW	LCMS	1	4	4	4	4
FS-vitamins	2.6.7.2	ng/mg DW	LCMS	1	4	4	4	4
Ash content	2.6.8	mg/g DW	Combustion	1	4	4	4	4

2.6.1 Amino Acids

Amino acids were analysed at SINTEF Ocean. The samples analysed was between 50-100 mg in size. This method was developed by Agilent and Pickering laboratories. The amino acid profile in freeze-dried ground polychaetes and sludge samples were analysed by a HPLC system (Agilent Infinity 1260, Agilent Technologies). An application note provided by Pickering laboratories was followed (Pinnacle PCX, Pickering laboratories, Mountain View, CA, USA). The amino acids, taurine and ammonia was quantified from standard curves.

Prior to the analysis, the samples were hydrolysed in 6 M HCl containing 0.4% merkaptoethanol for 24 h at 110°C (HCl hydrolysis). Glutamine and asparagine were converted to glutamic and aspartic acid, respectively, during the acid hydrolysis. Cysteine was quantified as cystine (Cys-Cys). The pH was adjusted before all samples were filtered and further diluted with a citrate buffer prior to the HPLC analysis. All buffers, reagents, amino acid standards and the column used during the analysis were obtained from Pickering laboratories (Mountain View, CA, USA). HCl, NaOH, taurine and mercapto ethanol were obtained from Sigma-Aldrich. The amino acid tryptophan was not accounted for as it was degraded during this method of analysis.

2.6.2 Protein Content

The protein content was calculated based on the total content of amino acids [mg/g DW]. The basis for this calculation is the fact that amino acids create peptide bonds when they are linked together. The actual binding between single amino acids consists of water (OH – H) (Kierulf, 2019). Assuming that the water molecule is not part of the actual protein, the total protein content was calculated by subtracting the molar mass [M] of water from the molar mass of every single amino acid to determine the dehydrated weight of the amino acid. Then, the dehydrated weight of the amino acid was multiplied by the actual content of the corresponding amino acid [mg/g DW]. The protein content in every amino acid was calculated according to the following equation where x is a specific amino acid:

$$Protein\ content = \frac{Amino\ acid\ x\ [M] - Water\ [M]}{Amino\ acid\ x\ [M]} * Amino\ acid\ x\ \left[\frac{mg}{g} DW\right]$$

2.6.3 Total Lipid

The total lipid extraction was performed at NTNU Sealab. Samples of 15 mg (per replicate) were used in the analysis, both for the polychaete and the sludge samples. The samples were measured using an analytical scale (Mettler Toledo, UMX2). There were two replicates from each cultivation tank, and three replicates from each of the diets – smolt and post-smolt sludge. The total lipid analysis was based on an established extraction method (Bligh and Dyer, 1959). It is a standard procedure often used to isolate the total lipid fractions in biological material. The solvent system it is based on the chemicals chloroform and methanol to extract the lipid from the organic material. 0.5 mL of the lipid extract was dried with nitrogenous gas and used to calculate the total lipid content in the samples [mg/g DW]. The dried lipid extract was then kept in a desiccator for 24 hours and put back into the freezer (-80 °C) afterwards before they were sent to SINTEF for analysis of fat-soluble vitamins.

2.6.4 Fatty Acids

The fatty acids analysis was also performed at NTNU Sealab, but ran at SINTEF. The analysis was based on a procedure from Metcalfe (Metcalfe et al., 1966). 1 mL of the lipid extract from the total lipid extraction (2.6.3) was the base for the fatty acid analysis. An internal standard

(C23:0) was used in the analysis as reference. The method was based on hydrolyzation and esterification of lipid extracts. The fatty acid methyl esters were dissolved in isooctane and analysed through a gas chromatograph (GC).

Prior to analyzation on the GC, the fatty acid samples were all cleaned from contaminants using a thin layer chromatography method (TLC). A solution consisting of hexane:ether:acetic acid (90:10:1) were added to a chroma tank to create the mobile phase. The solution reached approximately 0.5 cm from the bottom of the tank. The mobile phase settled for 30 minutes. While settling another chroma tank containing iodine (s) was prepared by putting it into lukewarm water to create iodine gas within the system.

The samples which previously were dissolved in an organic solvent were evaporated using nitrogen gas. Then, the samples were dissolved in 30 µl hexane and added to silica plates as described in figure 2.3 using a Hamilton syringe. The silica plates were then placed into the chroma tank containing the mobile phase.

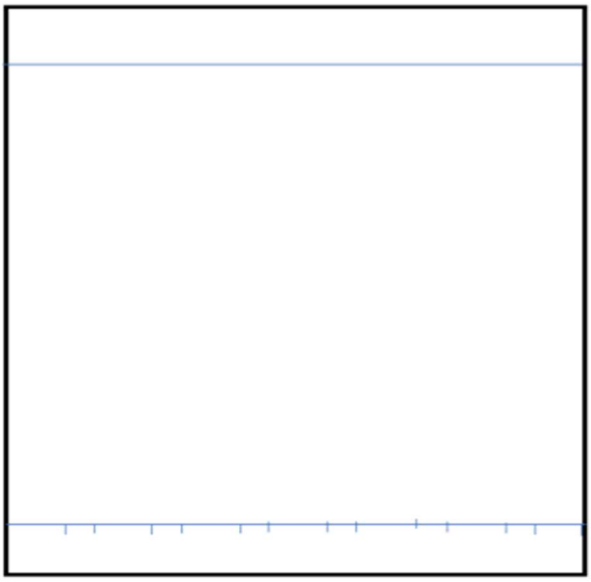

Figure 2.3: Illustration of the silica plates used to separate the methyl ethers from possible contamination. Each plate contained 5-6 samples added in intervals (0.5 cm) with 1 cm between each sample interval. The samples were added 1 cm from the frame to avoid contamination from the mobile phase itself and handling.

After the mobile phase had moved upwards, approximately 1 cm from the upper line, the plates were taken out of the chroma tank, air-dried, and then replaced into the other chroma tank containing iodine (g). As the iodine (g) coloured the methyl ethers yellow, the plates were removed from the tank and the brightest yellow areas containing methyl ethers were marked with a pencil. The marked areas were then scratched off the silica plates into kimax tubes using a scalpel and a funnel.

The kimax tubes containing silica were added 2x2 ml hexane:ether (1:1), mixed with a vortex mixer and centrifuged at 4000 rpm, 4 ℃ and 3 minutes before the liquid were extracted and transferred into another kimax tube. The same procedure was repeated twice, and the organic solvent was at the end evaporated using a gentle nitrogen gas steam. Lastly, the fatty methyl ethers were dissolved in 0.4 ml isooctane and transferred to GC-glasses. The samples were then sent to SINTEF for analysis. After analyzation and ascertaining of the fatty acid peaks, the content of each fatty acid in the samples were determined by integrating the area beneath each peak [mg/g DW].

2.6.5 Carbohydrates

Prior to the analysis, a standard curve was made based on a dilution series consisting of glucose standards. 3 mg of glucoses was dissolved in 50 ml distilled water. 25 ml of the glucose solution was transferred into a new container and added another 25 ml distilled water. The process was repeated 5 times, resulting in 6 solutions of glucoses with different concentrations.

Samples of approximately 200 µg were weighed out for the polychaete analysis and 100 µg for the smolt and post-smolt sludge using an analytic scale (Mettler Toledo, UMX2). The samples were transferred to kimax tubes prior to the analysis. 500 ml of water was added to the samples in addition to 1 ml of a 3% phenol-solution. The samples were mixed with a vortex mixer, sealed, and then rested for 20 minutes in room temperature.

After resting, the samples were moved to an ice-bath before 5 ml concentrated sulphuric acid was added. The samples rested on the ice for a few minutes before the procedure continued. The samples were mixed gently with the vortex mixer before they were centrifuged for 10 minutes (3000 rpm) at 5 °C. After centrifugation, the samples were transferred, one at the time, into a quartz cuvette. The samples were analysed in a calorimeter and the absorbans was read.

Due to unknown reasons, the results from the calorimeter were not reasonable when converted into mg carbohydrates/g DW as the glucose levels were higher than what was physically possible. The results from this analysis will therefore not be included further into this thesis.

2.6.6 Minerals

The element analysis was done by SINTEF Norlab (Mo i Rana). In total, 11 samples were sent to the laboratory. The samples consisted of the initial polychaetes (1), smolt and post-smolt sludge (2) and one sample from each of the different treatment groups (8). The analysis required a sample between 100-300 mg, thus the 4 replicates within each treatment group were pooled together to one common sample (approximately 30 mg from each replicate which equals 120 mg from each treatment group). The analysis was done in accordance with NS-EN ISO 17294-2:2016, which is the Norwegian Standard for determination of selected elements including uranium isotopes.

2.6.7 Vitamins

The analyses of both water- and fat-soluble vitamins were done by Antonio Sarno at SINTEF Ocean. All LCMS (liquid chromatography- mass spectrometry) analyses were performed on an Agilent 1260-series UPLC equipped with a diode array detector coupled to an Agilent 4670 triple quadrupole mass spectrometer equipped with an electrospray ion source.

The mass spectrometer was operated in positive ionization mode for both water- and fat-soluble vitamins, and the mass transitions and their respective collision energies and fragmentor voltages are given in appendix IV.

2.6.7.1 Water Soluble Vitamins

Water-soluble vitamins were homogenized by bead beating, extracted with methanol, and cleaned-up with hexane. Freeze-dried samples of 15 mg were suspended in 1 ml methanol containing 6-10 (BEADS) and homogenized in a Precellys homogenizer for 30 seconds at 6000 rpm. Samples were extracted for 30 min with shaking at 1000 rpm at 4 °C and debris was removed by centrifugation for 15 min at 5000 x g at 4 °C. One volume hexane was added to the supernatants and the mixture was shaken for 15 min at 1000 rpm at 4 °C and centrifuged centrifugation for 15 min at 5000 x g at 4 °C. The bottom (methanol) phase was recovered, dried at room temperature under a gentle nitrogen stream, and reconstituted in 100 µl MilliQ water prior to analysis.

For the water-soluble vitamins, a HILIC column was used (ACE HILIC-A 2.1x100 mm, 1.8 µm particle size) using a flow rate of 300 µl/min and 5 µl injection volume. The mobile phase consisted of (A) 90:10 acetonitrile:water containing 10 mM ammonium formate and 0.1 % formic acid and (B) 100% aqueous 10 mM ammonium formate and 0.1 % formic acid. The 13-minute-high performance liquid chromatography (HPLC) program was as follows: gradient from 100% A to 40% A by 3 min, hold at 40% A until 8 min, 100% A by 8.1 min until 13 min.

2.6.7.2 Fat Soluble Vitamins

Prior to analysis, the lipid extracts were reconstituted in 100 µl 40:60 acetonitrile:water containing 10 mM ammonium formate and 0.1% formic acid. For fat-soluble vitamins, a reverse phase column was used (Supelco Ascentis Express C18 2.1x150 mm, 2.7 µm fused-core particle size) using a flow rate of 300 µl/min and 5 µl injection volume. The mobile phase consisted of (A) 90:10 methanol:water containing 10 mM ammonium formate and 0.1 % formic acid and (B) methanol containing 0.1% formic acid. The 20 min HPLC program was as follows: 100% A for 2 min, 100% B from 2.1 min to 15 min, and 100% A from 15.1 min to 20 min.

2.6.8 Ash

The ash content in the sludge and polychaetes samples were determined by combustion at Trondheim biological station. The sludge and polychaete material were weighed and distributed into smaller pottery and placed into a muffle furnace for six hours at 500 °C. Afterwards, the samples rested in a desiccator overnight before the samples were weighed again to determine the ash content of the samples.

Based on the content of ash, the total organic matter was determined using the following equation:

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

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]

2.7 Statistics

Statistical analyses were carried out using SigmaPlot® for Windows Version 14.0 (SigmaPlot, Systat Software Inc., USA). However, mean and standard deviation (STDEV) were calculated in Microsoft® Excel. Tables were made in Microsoft® Excel and Word 2013 for Windows (Microsoft Corporation, USA). The graphs were made in SigmaPlot ® 14.0.

Normal distribution of data was tested using Shapiro-Wilk tests and equality of variance was analysed by the Brown-Forsythe test. Water quality parameters, mean weights of polychaetes, specific growth rates, mortality as well as nutritional composition (TOM, protein content, 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 ($P < 0.05$).

One way analysis of variance (ANOVA) was used to compare different means of normally distributed data between multiple treatment groups (treatment groups A-D and initial polychaetes). All pairwise multiple comparison procedures following one way ANOVA were carried out using Bonferroni t-test. In cases of non-normally distributed data, Kruskall-Wallis one way ANOVA on ranks was applied.

Comparison of only two groups (sludge diets and corresponding treatment groups across treatments) was performed using unpaired t-test assuming normal distribution; in the case of non-normally distributed data, Mann-Whitney Rank Sum Test was applied to the data. These kinds of tests were used to compare initial and final biomass and corresponding treatment groups across the two main treatments.

2.8 Cooperation

Due to limitations regarding time, competence and economy, the workload was distributed between several NTNU students and SINTEF employees.

Sections 2.1-2.5 were all done in collaboration with master student Bjørn Stian Broberg Kristensen and PhD student Inka Anglade, who are also working within the same project (POLYCHAETE); section 2.6 was done exclusively for this master thesis.

The amino acid analysis described in section 2.6.1 was performed by Rasa Slizyte at SINTEF Ocean. The total lipid (2.6.3) and fatty acid extraction (2.6.4) were performed by the author at NTNU Sealab, but the samples were analysed at SINTEF Ocean by Merethe Selnes. The

carbohydrate analysis (2.6.5) was performed by co-student Bjørn Stian Broberg Kristensen at NTNU Sealab. The element analysis (2.6.6) was performed by SINTEF Norlab. Antonio Sarno performed the water- and fat-soluble vitamin analysis (2.6.7.1 and 2.6.7.2) at SINTEF Ocean. Lastly, Bjørn Stian Broberg Kristensen performed the combustion of the remaining material (2.6.8) to determine ash content and total organic matter (TOM) at Trondheim Biological Station. Despite the joint work on the different performed analysis, the author has been responsible for handling, editing, and performing statistical analysis on all the raw data output.

3 Results

3.1 Water Quality Parameters

Table 3.1: Abiotic factors (Mean±SD) during the 30-day feeding experiment from the different smolt and post-smolt treatment groups.

Parameters / Smolt treatment	**A**	**B**	**C**	**D**
Temperature [°C]	16.3±0.2	16.4±0.2	16.3±0.1	16.2±0.2
Dissolved oxygen [%]	99.2±0.2	99.3±0.2	98.9±0.5	99.0±0.3
Salinity [ppt]	34.4±0.1	34.5±0.2	34.5±0.0	34.5±0.1
pH	8.0±0.1	7.9±0.1	8.0±0.1	8.0±0.0
Parameters / Post-smolt treatment	**A**	**B**	**C**	**D**
Temperature [°C]	16.3±0.0	16.3±0.1	16.3±0.0	16.3±0.0
Dissolved oxygen [%]	99.1±0.3	99.1±0.4	98.8±0.3	98.7±0.3
Salinity [ppt]	34.4±0.1	34.2±0.2	33.7±0.1	34.2±0.0
pH	8.0±0.1	7.9±0.1	8.0±0.0	8.0±0.0

The abiotic parameters in the smolt treatment groups are presented in table 3.1. The temperature in the tanks were relatively stable with small variations reflected in the non-significant differences between the treatment groups. There were no significant differences in either dissolved oxygen or salinity. The pH was stable around 8.0 (± 0.1) which is slightly more basic than acidic.

The abiotic factors for the post-smolt treatment groups are also presented in table 3.1. There were no significant differences between any of the treatment groups in regard of temperature [°C], dissolved oxygen [%], salinity [ppt] or pH. The pH was slightly more basic the acidic.

3.2 Growth

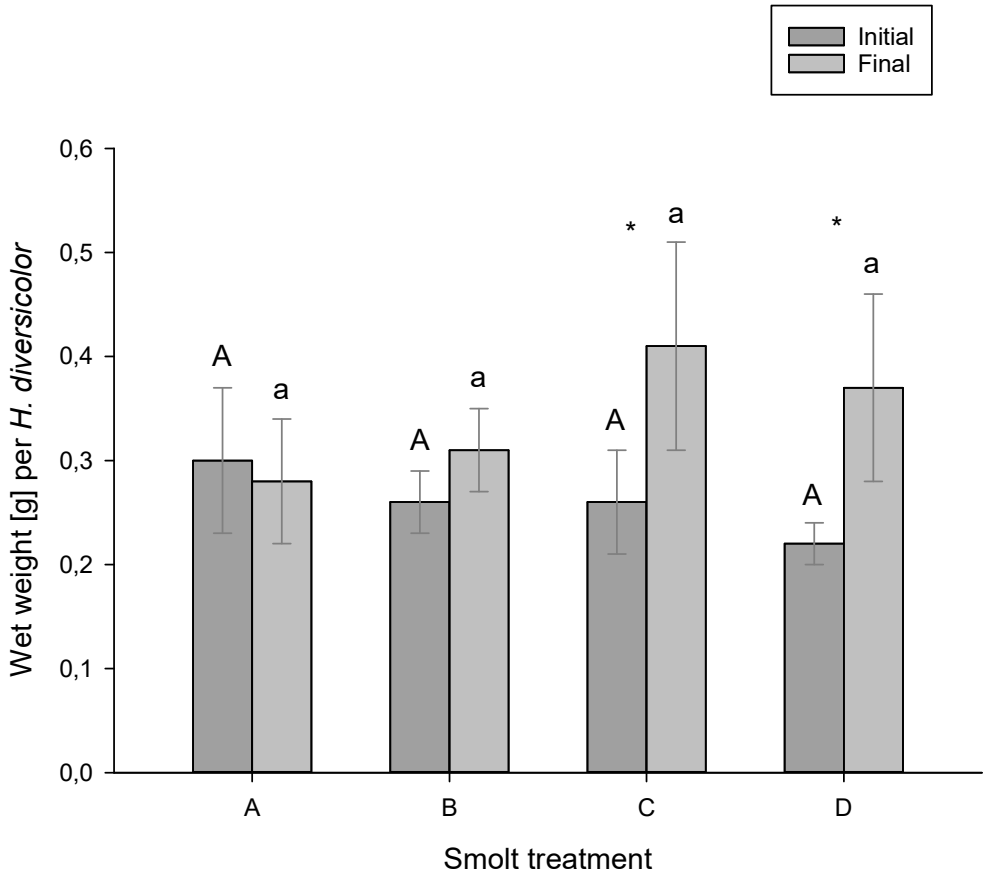

Figure 3.1: Changes in individual wet weight (Mean±SD) at start and end of the experiment for the different treatment groups in the smolt treatment (A – 5% of nitrogen content, B – 10% of nitrogen content, C – 20% of nitrogen content and D – 40% of nitrogen content). Non-significant differences in initial wet weight between the treatment groups are marked with similar uppercase letters. Non-significant differences in final wet weight between the different treatment groups are marked with similar lowercase letters. Statistically significant differences between initial and final wet weight within a treatment group is marked with a star (*).

Figure 3.1 describes the differences in mean wet weight (WW) of individual polychaetes within each smolt treatment group, prior and after the feeding experiment. The individual wet weight at the start of the experiment ranged between 0.22 – 0.30 g between the different treatment groups with no significant differences ($P \geq 0.05$); the final weight ranged between 0.28 – 0.41 g with no significant differences ($P \geq 0.05$). Treatment group B, C and D all had increasing individual wet weight; treatment group A had a decrease in wet weight at the end of the experiment. There was no statistically significant difference in wet weight within treatment groups A and B before and after the experiment ($P \geq 0.05$). There was a significant difference between the wet weight at the start of the experiment and at the end for treatment groups C and D ($P < 0.05$).

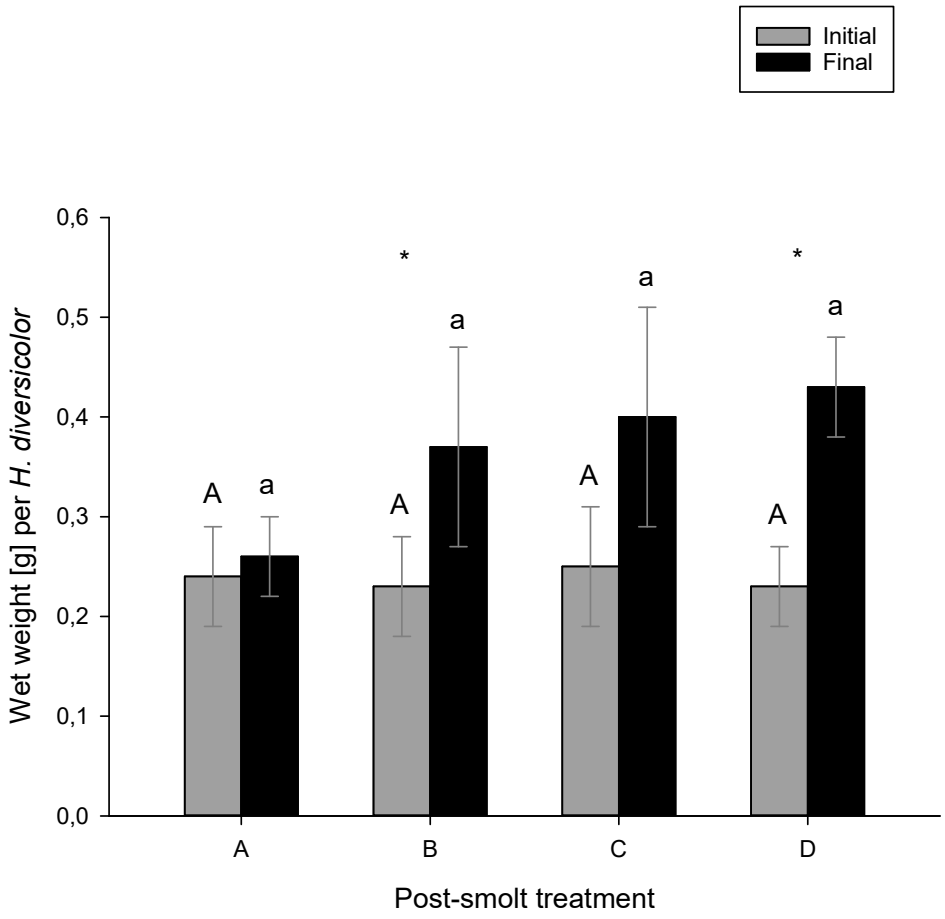

Figure 3.2: Changes in individual wet weight (Mean±SD) at start and end of the experiment for the different treatment groups in the post-smolt treatment (A – 5% of nitrogen content, B – 10% of nitrogen content, C – 20% of nitrogen content and D – 40% of nitrogen content). Non-significant differences in initial wet weight between the treatment groups are marked with similar uppercase letters. Non-significant differences in final wet weight between the different treatment groups are marked with similar lowercase letters. Statistically significant differences between initial and final weight within a treatment group are marked with a star (*).

Figure 3.2 describes the changes in individual wet weight of the polychaetes during the experiment in the post-smolt treatment groups. The individual wet weight increased in all the different treatment groups with a few exceptions within treatment group A. There was no significant difference between any of the treatment groups at the start of the experiment ($P \geq 0.05$) and the average individual wet weight of the polychaetes added to the tanks was between 0.23 – 0.25 g. The final weight ranged between 0.26 – 0.43 g; there was no statistically significant differences in final weights between the different treatment groups. There was no significant difference between the wet weight at the start of the experiment compared to the end for treatment groups A and C ($P \geq 0.05$); there was a significant difference in treatment groups B and D ($P < 0.05$).

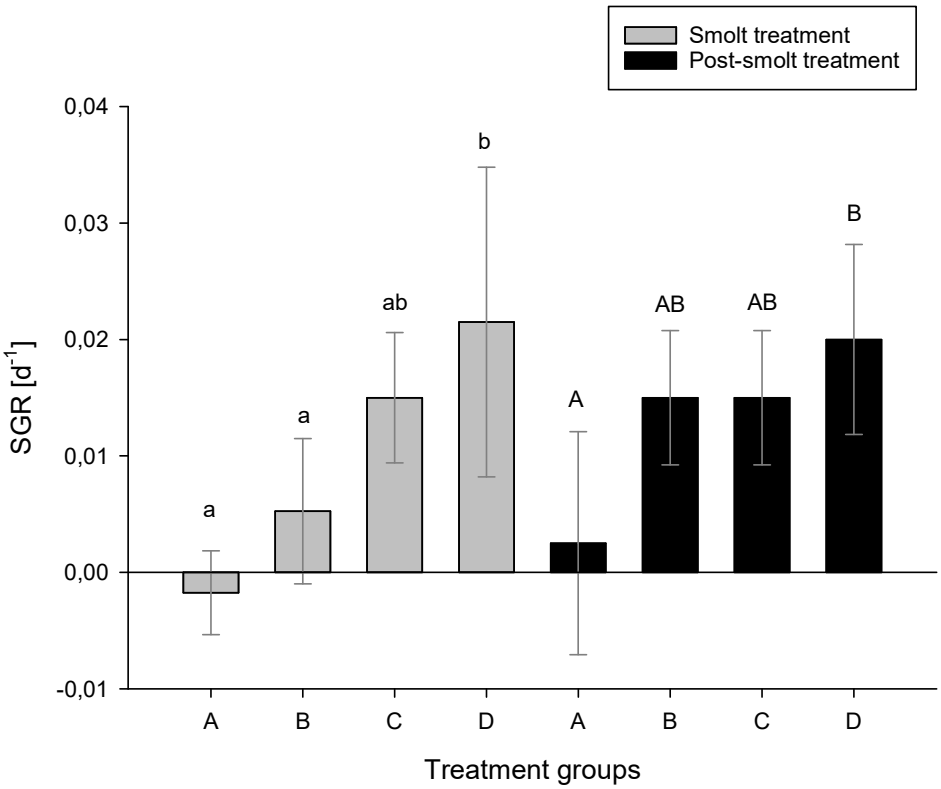

Figure 3.3: Specific growth rate for the smolt and post-smolt treatment groups, respectively (Mean±SD). The standard deviation is also included in the figure. Significant differences within the smolt treatment are marked with different lowercase letters (P<0.05). Significant differences within the post-smolt treatment are marked with uppercase letters (P<0.05). One or more similar letters indicate non-significant differences (P≥0.05).

Figure 3.3 presents the specific growth rates for all treatment groups (A-D) in the smolt- and post-smolt treatments. There was a statistically significant difference between treatment groups A and D in the smolt and post-smolt treatments, respectively ($P<0.05$). Additionally, there was also a significant difference between smolt treatment group B and D ($P<0.05$). No statistically significant difference was found between any of the corresponding treatment groups across the treatments ($P\geq0.05$).

3.3 Survival

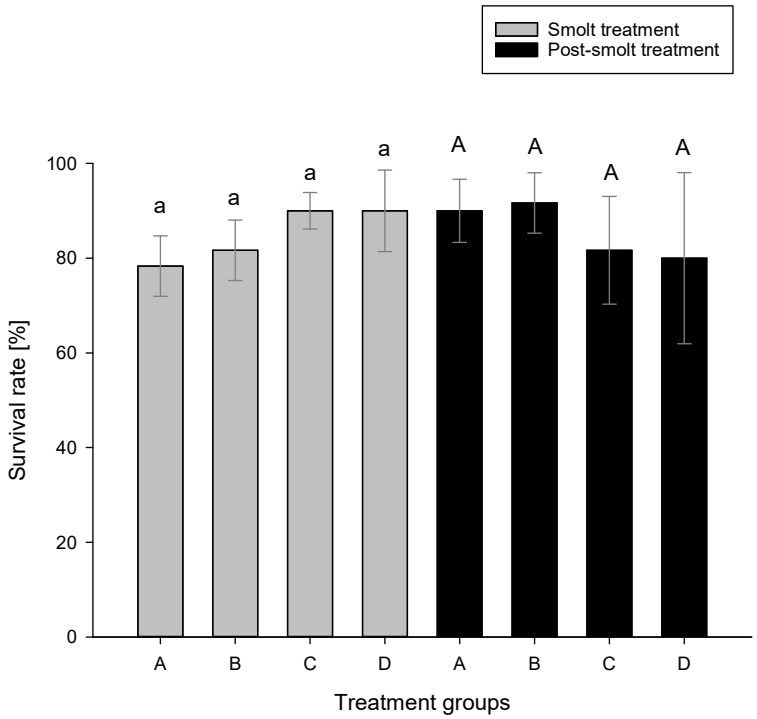

Figure 3.4: Survival rate [%] for all treatment groups (Mean±SD). Smolt treatment groups A, B, C and D followed by the post-smolt treatment groups A, B, C and D. Similar letters indicate non-significant differences within the respective treatment (P≥0.05).

Figure 3.4 presents the survival rate for all treatment groups within each treatment. The mean survival rate within each treatment groups are between 80-90% which corresponds to 12-14 individuals of the initial 15. There were a few exceptions in form of outliers in the post-smolt treatment group C and D, which have one tank each with only 10 and 8 survivors, respectively. There was no statistically significant difference among the treatment groups (P≥0.05).

One of the tanks in the smolt treatment group D were finished early (after 11 days) because of concern of an unknown, red biofilm accumulating and covering parts of the sediment. Despite the biofilm, all 15 individuals were found alive.

3.4 Chemical Composition

3.4.1 Amino Acids

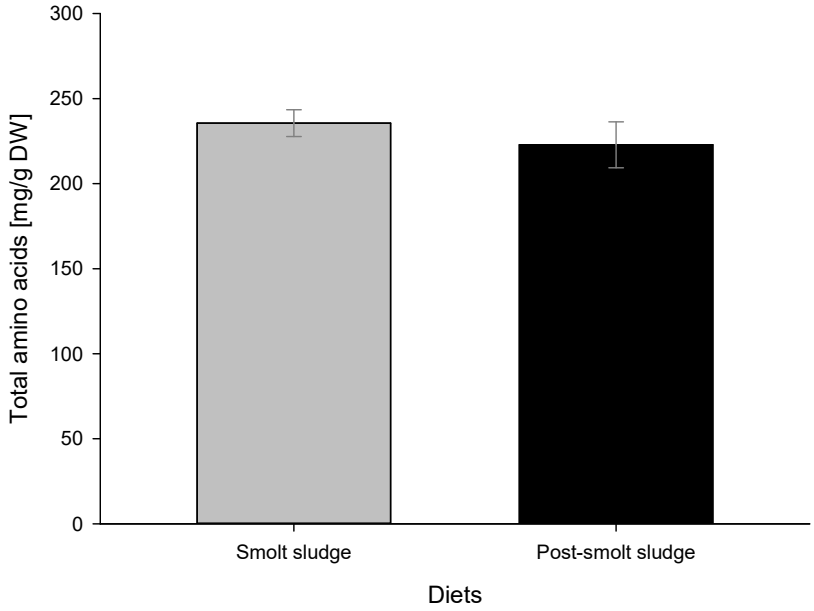

Figure 3.5: Total amino acid content [mg/g DW] in smolt and post-smolt sludge (Mean±SD).

Figure 3.5 presents the total amino acid content in both sludge diets, smolt and post-smolt, respectively. The average amino acid content in the smolt sludge amounted to 236 mg/g DW, in comparison, the average total amino acid content in the post-smolt sludge was 223 mg/g DW. There was no significant difference between the two diets ($P \geq 0.05$).

Table 3.2: Total amino acid content divided into essential (EAA) and non-essential (non-EAA) for both diets – smolt and post-smolt sludge. Similar letters indicate non-significant differences (P≥0.05).

Essential amino acids – sludge		
[%]	**Smolt**	**Post-smolt**
Arginine	3.94±0.51	4.63±0.32
Histidine	2.69±1.24	3.55±1.46
Isoleucine	5.39±0.16	5.40±0.24
Leucine	9.14±0.22	9.47±0.40
Lysine	5.62±0.04	6.28±0.52
Methionine	2.21±0.26	2.64±0.12
Phenylalanine	5.83±0.25	6.08±0.56
Threonine	4.35±0.69	3.67±0.47
Tryptophan	-	-
Valine	6.48±0.11	6.39±0.55
Total EAA [%]	45.64±1.26[a]	48.11±2.33[a]
In [mg/g DW]	107.51±2.87	107.04±1.71
Non-essential amino acids – sludge		
Alanine	6.67±0.82	8.30±0.92
Aspartic acid + asparagine	9.09±0.76	8.79±1.02
Cystine	1.41±1.12	2.80±0.81
Glutamic acid + glutamine	15.32±1.90	11.13±2.31
Glycine	5.47±0.46	5.82±1.02
Proline	6.27±1.08	5.05±0.23
Serine	6.94±0.91	5.66±0.75
Taurine	0.09±0.09	0.15±0.15
Tyrosine	2.92±0.15	3.67±0.76
Methionine sulfoxide	0.02±0.03	0.00±0.00
Hydroxyproline	0.09±0.16	0.29±0.38
Hydroxylysine	0.08±0.07	0.08±0.07
Total non-EAA [%]	54.36±1.26[a]	51.89±2.23[a]
In [mg/g DW]	128.09±5.06	115.83±9.80
Total AA [mg/g DW]	235.61±6.45	222.87±11.03

Table 3.2 presents the total amino acid content divided into essential amino acids (EAA) and non-essential amino acids (non-EAA). The content of essential amino acids in the smolt sludge corresponds to 45.64% of the total amino acid content; the content of essential amino acids in the post-smolt sludge was 48.11% of the total amino acid content. This means that the total content of non-essential amino acids was 54.36% and 51.89% for the smolt- and post-smolt sludge, respectively.

Leucine was the most dominant essential amino acid for both diets with a content of 9.14% and 9.45% for the smolt and post-smolt sludge, respectively. In smolt sludge, valine (6.47%), phenylalanine (5.83%) and lysine (5.62%) were the other most abundant amino acids. In post-smolt sludge, valine (6.40%), lysine (6.26%) and phenylalanine (6.05%) were also the highest occurring essential amino acids after leucine. Methionine (2.21%) and histidine (2.66%) were the lowest scoring essential amino acids in the smolt sludge. A similar pattern was found in the post-smolt sludge with a methionine content of 2.65% and a histidine content of 3.49%. However, tryptophan is valued (-) for both treatments, that was necessarily not the case as the method used for amino acid extraction fails to capture the tryptophan content.

Glutamic acid + glutamine, were the highest occurring non-essential amino acids for both diets with 15.36% and 11.22% for the smolt and the post-smolt sludge, respectively. Regarding the smolt sludge, the combination of aspartic acid + asparagine (9.10%), serine (6.95%) and alanine (6.66%) were other relatively strong contributions to the total content. For the post-smolt sludge, aspartic acid + asparagine (8.80%), alanine (8.27%) and glycine (6.02%) were the most occurring besides glutamic acid + glutamine. Methionine sulfoxide (0.02%) and hydroxylysine (0.10%) were the least occurring amino acids in the smolt treatment. Taurine (0.09%) and hydroxyproline (0.10%) were also lower contributors in the same regard. For the post-smolt sludge, methionine sulfoxide was not present in the sample. Hydroxylysine (0.08%), taurine (0.16%) and hydroxyproline (0.28%) were the least occurring non-essential amino acids in the post-smolt diet.

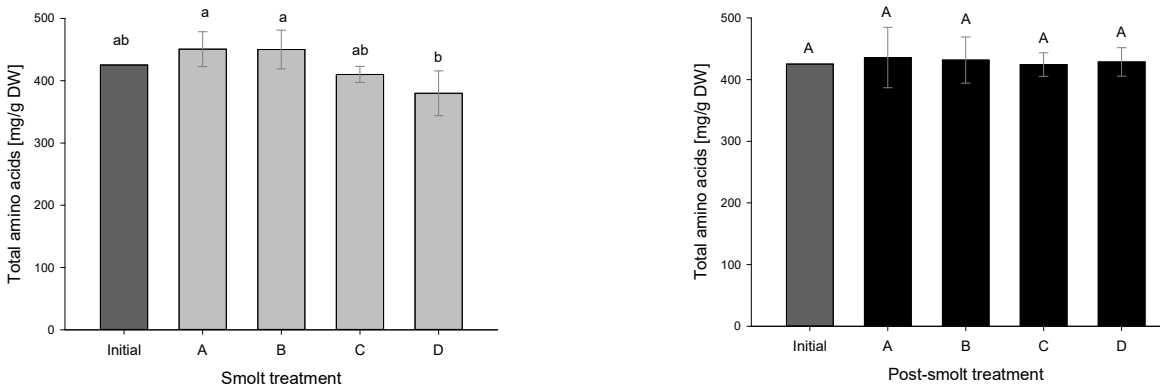

Figure 3.6: Total amino acid content in the initial, untreated polychaetes, compared to the smolt and the post-smolt treated polychaetes (Mean±SD) groups. Significant differences within the smolt treatment are marked with different lowercase letters; one or more similar letter indicate no significant differences. Similar uppercase letters indicate non-statistically significant differences within the post-smolt treatment groups.

Figure 3.6 presents the total amino acid content in all the treatment groups within the smolt and the post-smolt treatments. The amino acid content within the smolt treatment groups ranged between 380 – 451 mg/g DW, with groups A and B at the higher end and group D at the lower end. The initial polychaetes have a total amino acid content of 425 mg/g DW. In comparison, the total amino acid content in the post-smolt treatment groups ranged between 424 – 436 mg/g DW.

There was no significant difference between neither of the smolt treatment groups and the initial worms ($P \geq 0.05$). Although, smolt treatment group D was significantly different from smolt treatment groups A and B ($P < 0.05$). For the post-smolt treatment groups, there was also no significant differences compared to the initial polychaetes ($P \geq 0.05$).

Table 3.3: Total amino acids in the initial polychaetes (I) and the different smolt treatments groups (A, B, C and D). The amino acids are divided into essential and non-essential and are presented as [%] of the total amount of amino acids. The contribution of EAA and non-EAA to the total weight [mg/g DW] are also included. Non-significant differences are marked with similar lowercase letters (P≥0.05).

Essential amino acids – polychaetes

[%]	Initial	Treatment – smolt			
	I	**A**	**B**	**C**	**D**
Arginine	6.17	5.88±0.15	5.83±0.65	5.91±0.21	5.20±0.33
Histidine	3.19	1.69±0.08	1.31±0.88	1.99±0.75	2.15±0.70
Isoleucine	4.50	4.38±0.32	5.01±1.19	4.40±0.35	4.72±0.09
Leucine	7.92	7.51±0.16	6.41±2.64	7.45±0.42	7.74±0.18
Lysine	7.72	7.29±0.07	7.21±0.40	7.33±0.35	7.55±0.23
Methionine	3.25	2.73±0.10	3.10±0.84	2.88±0.16	2.78±0.37
Phenylamine	4.54	4.34±0.34	3.75±1.49	4.40±0.21	4.34±0.06
Threonine	3.47	4.40±0.22	4.07±1.48	3.34±0.30	3.39±0.54
Tryptophan	-	-	-	-	-
Valine	4.54	5.64±0.11	4.86±1.88	5.7±1.03	5.95±0.07
Total EAA [%]	45.29[a]	43.76±0.41[a]	41.56±4.41[a]	43.48±1.15[a]	43.82±0.89[a]
In [mg/g DW]	192.65	193.05±11.21	186.10±9.19	178.29±6.39	166.49±14.61

Non-essential amino acids – polychaetes

Alanine	11.12	7.60±0.86	6.14±3.65	7.48±0.58	7.92±0.14
Asparagine + aspartic acid	7.56	7.67±0.81	7.28±2.69	7.85±0.55	8.15±0.40
Cystine	4.75	2.37±1.18	4.01±4.52	2.52±0.73	2.63±0.11
Glutamine + glutamic acid	12.39	12.86±1.50	12.12±0.81	11.85±0.27	12.00±0.62
Glycine	5.48	6.18±0.53	5.87±0.45	5.91±0.69	5.70±0.37
Proline	4.13	8.76±0.78	8.74±0.68	10.52±1.18	9.61±0.52
Serine	4.76	6.77±0.69	6.61±0.69	5.81±0.92	5.77±0.73
Taurine	1.07	1.03±0.82	0.72±0.75	1.00±0.78	0.77±0.26
Tyrosine	3.44	2.67±0.65	3.00±0.98	3.23±0.92	3.14±0.52
Methionine sulfoxide	0.00	0.04±0.04	0.04±0.05	0.02±0.04	0.03±0.07
Hydroxyproline	0.00	0.28±0.08	2.09±3.15	0.25±0.29	0.35±0.29
Hydroxylysine	0.00	0.03±0.05	1.82±3.62	0.09±0.07	0.10±0.12
Total non EAA [%]	54.71[a]	56.24±0.41[a]	58.44±4.41[a]	56.52±1.15[a]	56.18±0.89[a]
In [mg/g DW]	232.68	253.47±13.11	264.02±33.18	231.79±7.49	213.33±17.12
Total AA [mg/g DW]	425.33	450.72±24.21	450.12±26.87	410.08±11.17	379.83±31.18

Table 3.3 shows the content of the different amino acids in the initial polychaetes, compared to the polychaetes that went through the different smolt treatments (A, B, C and D). The total essential amino acids in the initial polychaetes accounted for 45.29% of the total amino acid content. In comparison, the total EAA content in the smolt treatment groups ranged between 41.56-43.82%. The EAA profile for the initial and the smolt treatment groups are similar. Despite similar profiles, the total content in the smolt treatment groups was lower overall. There was an overall decrease in each EAA within the smolt treatment groups compared to the initial, with a few exceptions.

On the bottom half of the table, the total content of non-EAA for the initial polychaetes was 54.71%. For the smolt treatment groups, the content ranged between 56.18-58.44%. The mostly occurring amino acid in both the initial and the smolt treatment group was glutamine + glutamic acid, with values of 12.39% for the initial and between 11.85-12.86% for the smolt treatment groups, respectively. Alanine was the second most abundant non-EAA in the initial polychaetes (11.12%), which was decreasing, but not significantly in all the smolt treatment groups (6.14-7.92%) ($P \geq 0.05$). There was a significant increase in proline from the initial polychaetes (4.13%) to all the smolt treatment groups (8.74-10.52%) ($P < 0.05$). Despite a slight increase in the serine abundance in all the smolt treatment groups (5.77-6.77%) from the initial (4.76%), there was no significant difference ($P \geq 0.05$). The non-EAA methionine, hydroxyproline and hydroxylysine were not found in the initial polychaetes. However, they were all found in smaller amounts in all the smolt treatment groups; methionine sulfoxide (0.02—0.04%), hydroxyproline (0.25-2.09%) and hydroxylysine (0.03-1.82%). Due to limitation to the method, the essential amino acid tryptophan was lost during the extraction.

Table 3.4: Total amino acid content in the initial (I) and the different post-smolt treatments (A, B, C and D). Non-significant differences are marked with similar uppercase letters (P≥0.05).

Essential amino acids – polychaetes

[%]	Initial	Treatment – post-smolt			
	I	A	B	C	D
Arginine	6.17	5.74±0.65	5.88±0.25	4.97±0.53	5.42±0.46
Histidine	3.19	1.75±0.09	2.11±0.79	2.06±0.83	2.04±0.88
Isoleucine	4.50	4.42±0.20	4.30±0.13	4.59±0.24	4.68±0.19
Leucine	7.92	7.63±0.23	7.47±0.15	7.69±0.21	7.68±0.19
Lysine	7.72	7.28±0.12	7.42±0.24	7.62±0.19	7.49±0.19
Methionine	3.25	2.67±0.24	2.73±0.04	2.85±0.03	2.77±0.08
Phenylamine	4.54	4.23±0.22	4.40±0.15	4.49±0.15	4.32±0.17
Threonine	3.47	4.38±0.43	3.69±0.41	3.68±0.40	4.21±0.52
Tryptophan	-	-	-	-	-
Valine	4.54	5.59±0.26	5.52±0.39	5.65±0.11	5.79±0.27
Total EAA [%]	45.39^{A}	43.69±0.41^{A}	43.52±0.83^{A}	43.60±1.13^{A}	44.37±0.69^{A}
In [mg/g DW]	192.65	190.27±17.24	187.70±11.40	185.06±8.53	190.13±7.11

Non-essential amino acids – polychaetes

Alanine	11.12	7.37±0.06	7.81±0.78	7.76±0.94	8.00±0.86
Asparagine + aspartic acid	7.56	8.56±0.81	8.03±0.81	7.55±0.34	6.91±0.10
Cystine	4.75	1.61±0.70	1.96±0.86	1.83±0.87	1.87±1.27
Glutamine + glutamic acid	12.39	13.20±1.44	12.38±0.45	12.73±0.47	11.47±0.66
Glycine	5.48	6.99±0.51	6.16±0.18	5.90±0.78	5.21±0.41
Proline	4.13	8.47±1.35	9.76±0.65	9.34±0.56	10.63±0.55
Serine	4.76	6.25±1.07	6.22±0.74	6.43±0.96	6.45±1.67
Taurine	1.07	0.78±0.70	0.77±0.63	1.10±0.53	0.98±0.75
Tyrosine	3.44	2.76±0.47	3.07±0.57	3.54±0.24	3.67±0.28
Methionine sulfoxide	0.00	0.03±0.06	0.05±0.04	0.03±0.06	0.06±0.04
Hydroxyproline	0.00	0.22±0.17	0.21±0.17	0.15±0.10	0.31±0.23
Hydroxylysine	0.00	0.06±0.05	0.07±0.08	0.05±0.08	0.05±0.04
Total non-EAA [%]	54.71^{A}	56.31±0.41^{A}	56.48±0.83^{A}	56.40±1.13^{A}	55.63±0.69^{A}
In [mg/g DW]	232.68	245.54±25.21	244.08±20.99	239.39±10.01	238.59±13.21
Total AA [mg/g DW]	425.33	435.83±42.43	431.78±32.37	424.45±16.47	428.72±20.01

Table 3.4 gives an overview of the share of essential amino acids and non-essential amino acids in the initial polychaetes compared to the post-smolt treatment groups. The proportion of EAA in the initial polychaetes equals to 45.29%. In comparison, the proportion of EAA in the post-smolt treatment groups ranged between 43.52-44.37%. The differences in the total content of EAA were non-significant. Leucine and lysine were the most abundant EAA in both the initial polychaetes and in the post-smolt treatment groups. The leucine content in the initial and post-smolt treatment groups were 7.92% and 7.47-7.69% respectively. The lysine content was 7.72% for the initial and ranged between 7.28-7.62% for the post-smolt treatment groups. There were non-significant differences in arginine, histidine and phenylamine across all groups compared to the initial. Despite an increase, there were non-significant differences in threonine and valine across all post-smolt treatment groups.

The total content of non-EAA was 54.71% in the initial polychaetes, and ranged between 55.63-56.48% in the post-smolt groups. Glutamine + glutamic acid was the most abundant non-EAA in the initial polychaetes (12.39%) as well as in all of the post-smolt treatment groups (11.47-13.20%). Alanine (11.12%) was also abundant in the initial but was decreasing non-significantly across the post-smolt treatment groups (7.37-8.00%) ($P \geq 0.05$). Although the cysteine levels were more than halved from the initial (4.75%) to the post-smolt treatment groups (1.61-1.96%), there was no significant difference ($P \geq 0.05$). The proline levels have increased significantly from 4.13% (initial) to 8.47-10.63% (post-smolt treatment groups) ($P \geq 0.05$). Methionine sulfoxide, hydroxyproline and hydroxylysine were likely below the detection limit in the initial samples but were found in smaller levels in the post-smolt treatment groups; methionine sulfoxide (0.03-0.06%), hydroxyproline (0.15-0.31%) and hydroxylysine (0.05-0.07%). In the same regard as with the smolt treatment, tryptophan was not measured due to limitation to the extraction method.

3.4.2 Protein

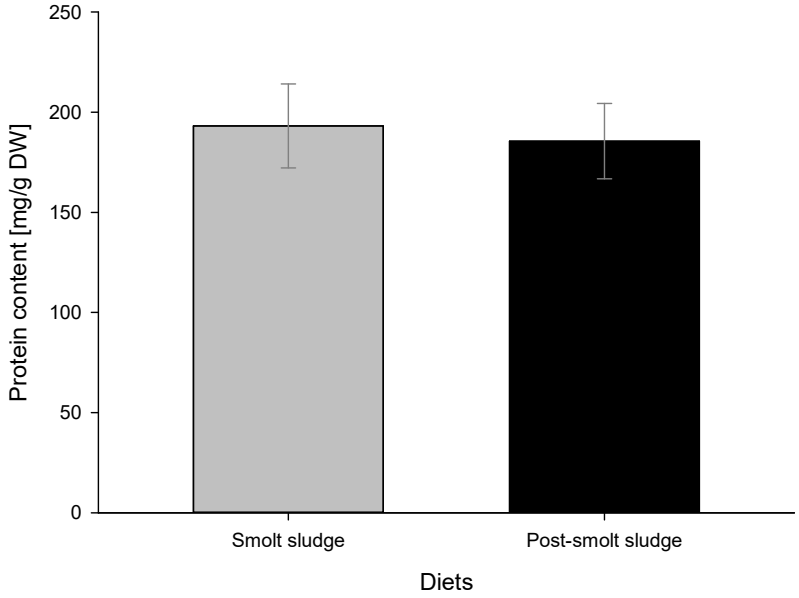

Figure 3.7: Total protein content [mg/g DW] in both diets – smolt sludge and post-smolt sludge (Mean±SD).

The total protein content in the diets is presented in figure 3.7. The total protein content in the smolt sludge was 186 mg/g DW. On the other side, the total protein content in the post-smolt sludge was 193 mg/g DW; however, there was no significant difference in protein content between the two diets (P≥0.05).

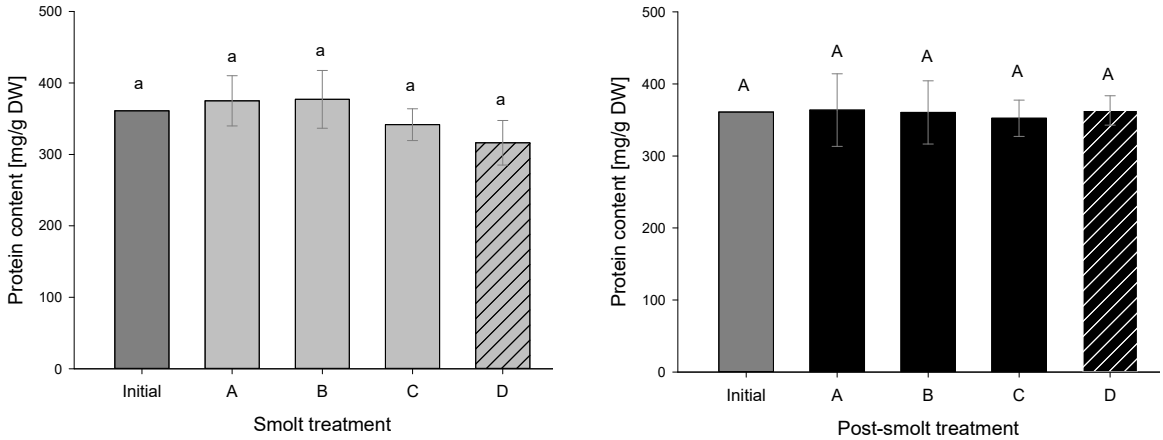

Figure 3.8: Total protein content [mg/g DW] in the initial polychaetes (I) compared to both treatments and treatment groups (Mean±SD). Left-hand side: smolt treatment (A, B, C and D) and right-hand side: post-smolt treatment (A, B, C and D). Standard deviation is included in the figure. There are no statistical differences between the initial polychaetes and any of the treatment groups. Significant differences between corresponding treatment groups across treatments are marked with shades (P<0.05).

The total protein content in the polychaetes is presented in figure 3.8 The total protein content in the initial polychaetes was 364 mg/g DW. There were some variations between the smolt treatment groups as the total protein content ranged from 319-378 mg/g DW. Despite the difference between the treatments, there were no significant differences between the treatment groups and the initial, or within the treatment groups themselves ($P \geq 0.05$). For the post-smolt treatment groups, the total protein content ranged between 356-367 mg/g DW. There were small differences between the treatment groups, but there was no significant difference within the treatment groups or compared to the initial polychaetes ($P \geq 0.05$). For treatment group D, there was a significant difference between the smolt and post-smolt group ($P < 0.05$).

3.4.3 Total Lipid

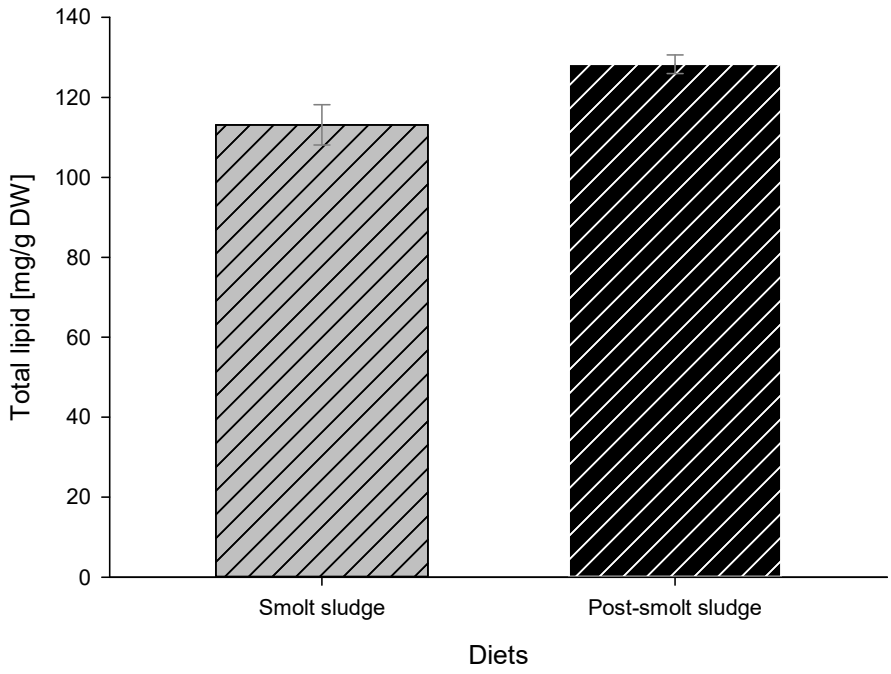

Figure 3.9: Total lipid content [mg/g DW] in both sludge diets (Mean±SD). Significant differences are shaded on the bars.

The total lipid content in the smolt sludge and the post-smolt sludge is presented in figure 3.9. The total lipid content in the smolt sludge was 113 mg/g DW and the total lipid content in the post-smolt was 128 mg/g DW. There was a significant difference between the two diets ($P < 0.05$).

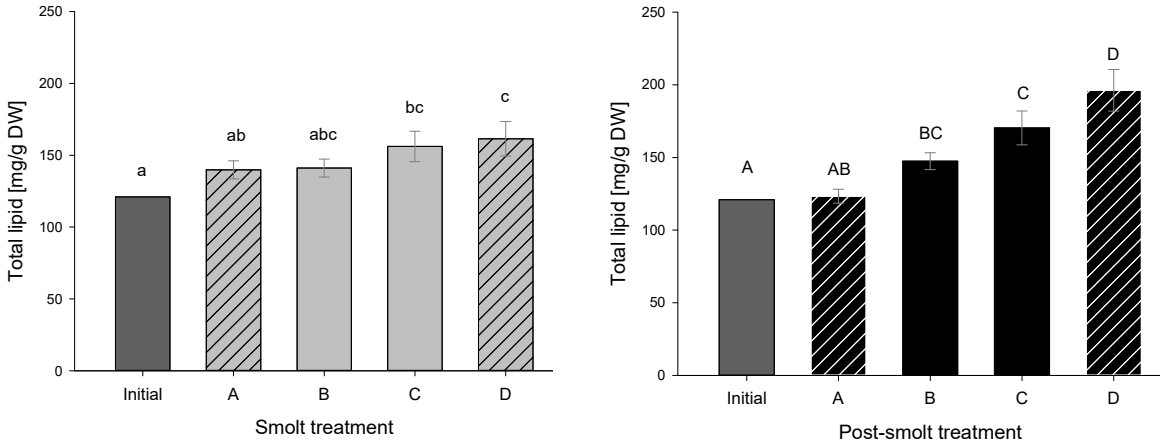

Figure 3.10: Total lipid content [mg/g DW] in the initial polychaetes compared to all the smolt and post-smolt treatment groups (A, B, C and D) (Mean±SD). Significant differences within the smolt treatment groups are marked with different lowercase letters (P<0.05). Statistically significant differences in within the post-smolt treatment groups are marked with different uppercase letters (P<0.05). Non-significant differences are marked with one or more common letter (P≥0.05). Significant differences between corresponding treatment groups across the treatments are marked with shaded bars (P<0.05).

The total lipid content in all treatment groups is presented in figure 3.10. The total lipid content in the initial polychaetes was 121 mg/g DW. In the smolt treatment groups, the total lipid content ranged between 140-162 mg/g DW. There was no significant difference in the lipid content between the initial polychaetes and smolt treatment groups A and B (P≥0.05); there was a significant difference between the initial polychaetes and the smolt C and D treatment groups (P<0.05). There was also a significant difference between treatment group D and treatment group A (P<0.05).

The total lipid content in the post-smolt treatment groups ranged from 123-196 mg/g DW. There was no significant difference in total lipid content between the initial polychaetes and treatment group A (P≥0.05); there was a significant difference between the initial and treatment groups B, C and D (P<0.05). There was also a significant difference between treatment group A and C, and there were significant differences between treatment group D and all the other treatment groups (P<0.05).

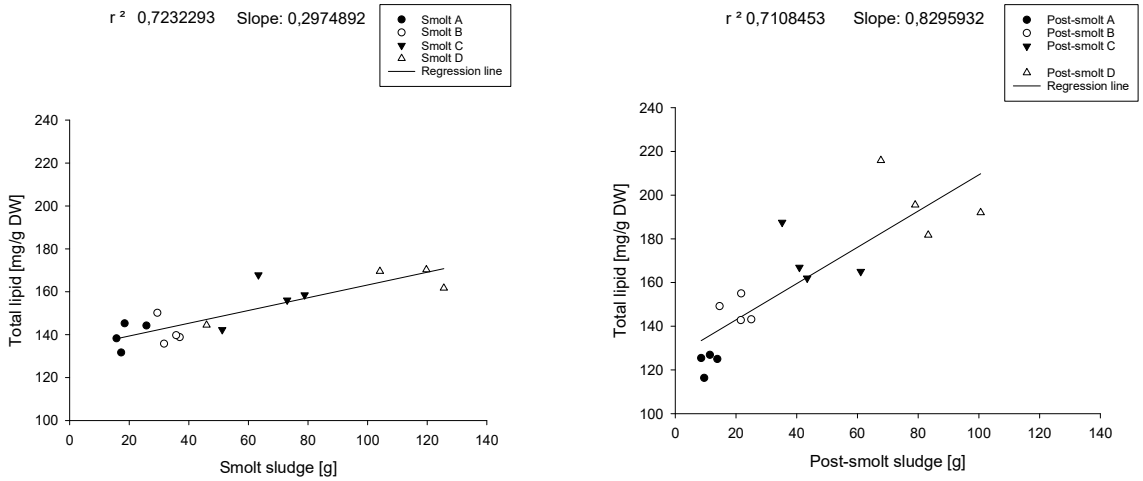

Figure 3.11: Total lipid content [mg/g DW] in the smolt (left figure) and post-smolt (right figure) treatment groups (polychaetes) as a function of the amount of feed (sludge, g) given to the respective treatment groups throughout the experiment.

The total lipid content in the polychaetes from the different treatment groups as a function of the amount of sludge fed are presented in figure 3.11. Based on the respective regression lines in the figures, the slope in the smolt graph was 0.297, which is less steep then the post-smolt slope which corresponds to 0.829. The fit of the regression lines is relatively similar with r^2 -values of 0.72 and 0.71 for the smolt and post-smolt treatment, respectively. There was a significant difference between the two slopes ($P<0.05$).

3.4.4 Fatty Acids

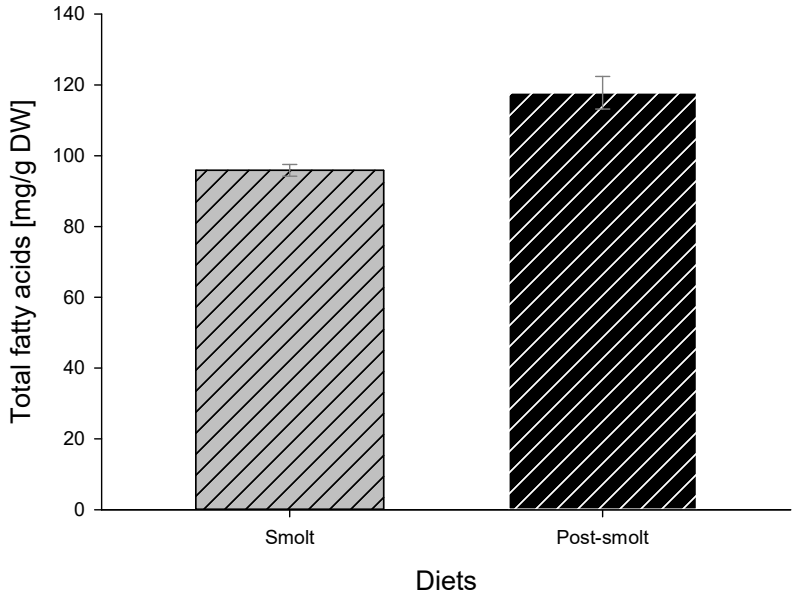

Figure 3.12: Total content [mg/g DW] of fatty acids in the smolt and post-smolt sludge (Mean±SD). Shading indicates significant differences (P<0.05).

The total content of fatty acids in both sludge diets are presented in figure 3.12. The average content of fatty acids in the smolt sludge was 96 mg/g DW; the average content of fatty acids in the post-smolt sludge was 117 mg/g DW. There was a significant difference between the two sludge diets (P<0.05).

Table 3.5: Total content [mg/g DW] of fatty acids in the smolt and post-smolt sludge diets, and the contribution of each fatty acid [%] on the total composition. One or more similar lowercase letter indicates non-significant differences (P≥0.05); no common letters indicate significant differences (P<0.05).

	Sludge diets	
	Smolt sludge	**Post-smolt sludge**
Total FA [mg/g DW]	95.87±1.67[a]	117.79±4.59[b]
% of total FA		
C14:0	4.45±0.22	3.69±0.16
C15:0	0.46±0.03	0.33±0.01
C16:0	17.93±0.76	18.87±0.10
C17:0	0.36±0.01	0.36±0.00
C18:0	3.65±0.15	8.36±0.09
C20:0	0.57±0.02	1.05±0.02
C22:0	0.41±0.00	2.01±0.06
C24:0	0.00±0.00	0.00±0.00
ΣSAFA	27.81±1.18[a]	34.68±0.15[b]
C14:1	0.37±0.02	0.20±0.02
C16:1	3.79±0.13	3.70±0.05
C17:1	0.02±0.00	0.01±0.00
C18:1 n-11	0.00±0.00	0.00±0.00
C18:1 n-9	30.11±0.59	28.17±0.11
C18:1 n-7	2.99±0.04	3.07±0.04
C20:1	5.79±0.04	2.30±0.07
C22:1 n-11	5.57±0.13	1.21±0.04
C22:1 n-9	0.75±0.05	0.37±0.03
C24:1 n-9	0.52±0.01	0.00±0.00
ΣMUFA	49.90±0.50[a]	39.04±0.18[b]
C18:2 n-6	11.28±0.26	14.92±0.04
C18:3 n-6	0.07±0.01	0.12±0.01
C18:3 n-3	3.18±0.21	4.73±0.07
C18:4 n-3	1.05±0.12	0.56±0.01
C20:2 n-6	0.37±0.01	0.31±0.02
C20:3 n-6	0.00±0.00	0.00±0.00
C20:4 n-6	0.30±0.06	0.25±0.00
C20:3 n-3	0.00±0.00	0.00±0.00
C20:4 n-3	0.23±0.04	0.15±0.01
C20:5 n-3 (EPA)	2.71±0.42	3.18±0.14
C22:2	0.00±0.00	0.00±0.00
C22:3	0.00±0.00	0.00±0.00
C22:5 n-3	0.00±0.00	0.28±0.03
C22:6 n-3 (DHA)	3.10±0.65	1.78±0.09
ΣPUFA	22.29±1.61[a]	26.28±0.32[b]
Σn-3	10.27±1.42[a]	10.68±0.32[a]
Σn-6	12.02±0.20[a]	15.60±0.03[b]
DHA:EPA	1.14±0.07[a]	0.56±0.01[b]

The fatty acid content in both sludge diets is presented in table 3.5. The most abundant fatty acid in both sludge diets was oleic acid (C18:1 n-9) with contents of 30% and 28% for smolt and post-smolt sludge, respectively. Furthermore, palmitic acid (C16:0) (18% smolt, 19% post-smolt) and linolelaidic acid (C18:2 n-6) (11% smolt, 15% post-smolt) were other fatty acids with high proportions. The relative content of saturated fatty acids (SAFA) in the smolt sludge was 29% and 35% in the post-smolt sludge; there was a significant difference between the two sludge diets ($P<0.05$). The relative content of monounsaturated fatty acids in the smolt sludge (50%) was significantly higher than the post-smolt sludge (39%). Also, there was a significant difference in the proportion of polyunsaturated fatty acids between the smolt (22%) and the post-smolt sludge (26%) ($P<0.05$). Further, there was no significant differences in the omega-3 proportion of the diets ($P\geq0.05$). The content of omega-6 fatty acids was significantly higher in the post-smolt sludge ($P<0.05$). Moreover, the DHA:EPA ratio was significantly higher in the smolt sludge ($P<0.05$). The content of DHA+EPA in the smolt sludge was accounting for 6% of the total fatty acids, and the content of DHA+EPA in the post-smolt sludge was 5%.

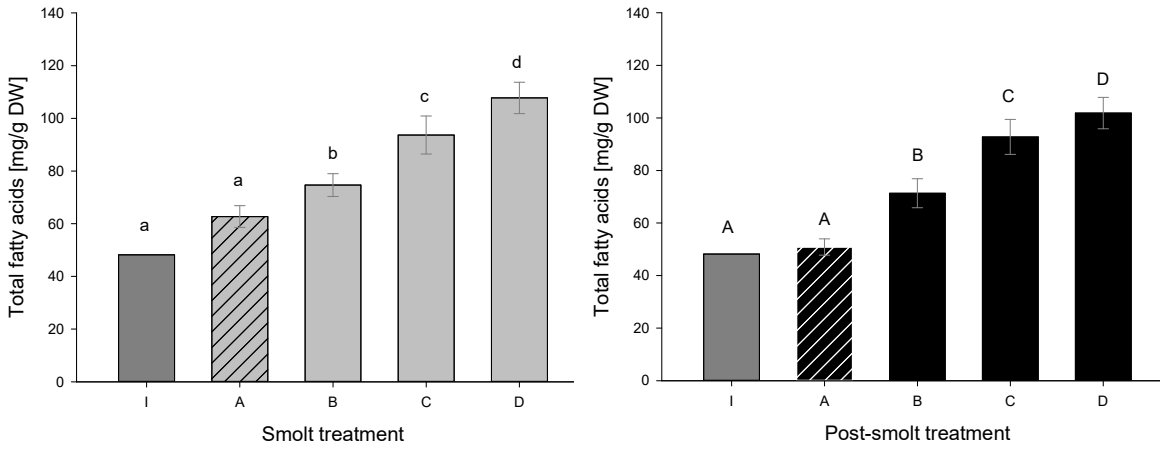

Figure 3.13: Total content of fatty acids [mg/g DW] in the initial polychaetes (I) compared to the smolt and post-smolt treatment groups (A, B, C and D) (Mean±SD). Similar lowercase letters indicate non-significant differences within the smolt treatment groups ($P\geq0.05$); different lowercase letters indicate significant differences ($P<0.05$). Similar uppercase letters indicate non-significant differences within the post-smolt treatment groups ($P\geq0.05$); different letters indicate significant differences ($P<0.05$). Shaded bars mark significant differences between corresponding treatment groups across treatments ($P<0.05$).

The total content of fatty acids in the smolt and post-smolt treatment groups are presented in figure 3.13 together with the initial polychaetes. The content of fatty acids in the initial polychaetes was 48 mg/g DW. The content of fatty acids within the smolt treatment groups ranged between 62-107 mg/g DW. There was not a significant difference between the initial

polychaetes and smolt treatment group A ($P \geq 0.05$), but between the initial polychaetes and treatment groups B, C and D ($P < 0.05$).

The content of fatty acids in the post-smolt treatment groups ranged between 50-102 mg/g DW. There was no significant difference between the initial polychaetes and treatment group A ($P \geq 0.05$); there was a significant difference between the initial polychaetes and the remaining treatment groups B, C and D ($P < 0.05$). Additionally, there was a significant difference between the corresponding A treatment groups across treatments ($P < 0.05$).

Table 3.6: Total content [mg/g DW] of essential fatty acids in the initial polychaetes (I) and the different smolt treatment groups (A, B, C and D), and the contribution of each fatty acid [%] on the total composition. One or more similar lowercase letter indicates non-significant differences (P≥0.05); no common letters indicate significant differences (P<0.05).

	Smolt treatment groups				
	I	**A**	**B**	**C**	**D**
Total FA [mg/g DW]	48.19±3.44[a]	62.72±1.37[a]	74.69±4.32[b]	93.68±7.22[c]	107.76±5.95[d]
% of total FA					
C14:0	1.52±0.02	1.56±0.09	1.58±0.10	1.65±0.11	1.85±0.07
C15:0	1.00±0.02	0.88±0.05	0.74±0.09	0.67±0.04	0.64±0.05
C16:0	24.07±0.28	22.17±0.83	20.67±1.17	18.79±0.86	17.59±0.83
C17:0	1.06±0.02	0.92±0.02	0.82±0.05	0.66±0.06	0.58±0.03
C18:0	6.28±0.18	5.63±0.22	5.24±0.19	4.58±0.27	4.10±0.17
C20:0	0.54±0.03	0.55±0.02	0.53±0.08	0.55±0.03	0.35±0.03
C22:0	0.27±0.02	0.30±0.03	0.31±0.04	0.35±0.02	0.26±0.02
C24:0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
ΣSAFA	34.73±0.08[a]	32.00±0.94[a]	29.89±1.45[c]	27.3±1.1[d]	25.37±1.02[e]
C14:1	0.27±0.01	0.24±0.04	0.22±0.04	0.18±0.03	0.17±0.02
C16:1	3.92±0.14	3.57±0.18	3.33±0.19	3.45±0.27	3.74±0.18
C17:1	0.37±0.14	0.19±0.07	0.17±0.04	0.10±0.03	0.13±0.03
C18:1 n-11	6.27±0.02	4.30±0.35	3.55±0.43	2.72±0.23	2.39±0.16
C18:1 n-9	8.37±0.18	11.72±1.77	14.75±1.44	17.19±.96	20.79±1.18
C18:1 n-7	7.32±0.19	5.55±0.31	5.10±0.25	4.77±0.10	4.89±0.17
C20:1	9.43±0.14	10.82±0.46	11.12±0.29	11.10±0.21	11.16±0.29
C22:1 n-11	1.15±0.07	2.36±0.19	2.79±0.23	3.69±0.40	4.47±0.27
C22:1 n-9	0.00±0.00	0.35±0.07	0.38±0.04	0.51±0.04	0.57±0.05
C24:1 n-9	0.00±0.00	0.00±0.00	0.00±0.00	0.04±0.10	0.34±0.05
ΣMUFA	37.11±0.06[a]	39.10±1.23[a]	41.42±1.33[b]	43.70±1.30[c]	48.66±1.67[d]
C18:2 n-6	3.98±0.21	5.76±0.56	7.02±0.56	7.77±0.31	8.610.14
C18:3 n-6	0.10±0.14	0.09±0.11	0.19±0.03	0.11±0.03	0.11±0.04
C18:3 n-3	4.09±0.05	2.57±0.23	2.44±0.27	2.34±0.07	2.40±0.21
C18:4 n-3	0.69±0.00	0.33±0.09	0.28±0.08	0.29±0.05	0.32±0.07
C20:2 n-6	2.19±0.19	3.81±0.35	4.19±0.17	4.18±0.46	3.57±0.48
C20:3 n-6	0.45±0.06	0.49±0.03	0.61±0.06	0.68±0.12	0.60±0.11
C20:4 n-6	2.05±0.05	1.22±0.13	1.22±0.18	1.14±0.05	0.96±0.08
C20:3 n-3	0.83±0.02	0.76±0.11	0.64±0.06	0.57±0.06	0.47±0.05
C20:4 n-3	0.64±0.02	0.41±0.03	0.36±0.08	0.38±0.03	0.34±0.04
C20:5 n-3 (EPA)	8.07±0.11	8.54±1.08	7.34±1.24	6.85±0.59	5.00±0.85
C22:2	0.27±0.02	0.49±0.06	0.42±0.06	0.40±0.04	0.32±0.06
C22:3	1.78±0.05	1.07±0.12	0.85±0.19	0.71±0.06	0.57±0.08
C22:5 n-3	2.70±0.06	2.08±0.33	1.60±0.43	1.39±0.16	1.01±0.18
C22:6 n-3 (DHA)	0.00±0.00	1.28±0.16	1.53±0.33	2.20±0.18	1.70±0.32
ΣPUFA	28.16±0.14[a]	28.90±1.86[a]	28.68±2.50[a]	29.00±1.00[a]	25.98±1.73[a]
Σn-3	17.01±0.01[a]	15.97±1.98[a]	14.18±2.40[a]	14.0±0.90[a]	11.24±1.50[b]
Σn-6	8.88±0.11[a]	11.38±0.23[b]	13.23±0.51[c]	13.88±0.64[c]	13,85±0.57[c]
DHA:EPA	0.00±0.00[a]	0.15±0.02[b]	0.21±0.03[c]	0.32±0.03[d]	0.34±0.03[d]

The fatty acids content in the smolt treatment groups are presented in table 3.6. The most abundant fatty acids across all smolt treatment groups were palmitic acid (C16:0) (18-22%), elaidic acid (C18:1 n-9) (12-21%) and eicosenoic acid (C20:1) (11%). The same fatty acids were the most abundant in the initial polychaetes as well with values of 24%, 8% and 9%, respectively. The relative amount of saturated fatty acids (SAFA) decreased significantly in all treatment groups except treatment group A during the experiment ($P<0.05$). The proportion of MUFA increased significantly in all treatment groups except treatment group A with respect to the initial polychaetes ($P<0.05$). There were no significant differences in the content of polyunsaturated fatty acids (PUFA).

In terms of omega-3 fatty acids, there were no significant differences in treatment group A, B and C compared to the initial group ($P\geq0.05$); there was a significant difference in treatment group D ($P<0.05$). However, there were significant increases in all treatment groups regarding omega-6 fatty acids compared to the initial group ($P<0.05$). The essential marine fatty acid docosahexaenoic acid (DHA) was absent in the initial group but occurred in all smolt treatment groups. The relative content of DHA was increasing in all treatment groups. Likewise, the DHA:EPA ratio (eicosapentaenoic acid) was increasing significantly throughout all treatment groups ($P<0.05$). The relative content of DHA+EPA of the total fatty acids in the smolt treatment groups ranged between 7-10%.

Table: 3.7 Total content [mg/g DW] of essential fatty acids in the initial polychaetes (I) and the different post-smolt treatment groups (A, B, C and D), and the respective contribution of each fatty acid [%] on the total composition. One or more common uppercase letter indicates non-significant differences (P≥0.05); no common letters indicate significant differences (P<0.05).

	Post-smolt treatment groups				
	I	**A**	**B**	**C**	**D**
Total FA [mg/g DW]	48.19±3.44^{A}	50.84±3.10^{A}	71.36±5.53^{B}	92.80±6.20^{C}	101.8±6.00^{D}
% of total FA					
C14:0	1.52±0.02	1.50±0.11	1.56±0.14	1.63±0.25	1.48±0.08
C15:0	1.00±0.02	0.95±0.10	0.77±0.06	0.67±0.06	0.55±0.04
C16:0	24.07±0.28	28.01±1.46	22.60±1.47	13.74±7.92	15.62±0.81
C17:0	1.06±0.02	1.19±0.06	0.89±0.05	0.71±0.06	0.54±0.04
C18:0	6.28±0.18	8.09±0.25	6.16±0.42	5.30±0.81	4.62±0.29
C20:0	0.54±0.03	0.49±0.03	0.40±0.05	0.41±0.06	0.48±0.02
C22:0	0.27±0.02	0.23±0.03	0.27±0.03	0.28±0.03	0.27±0.03
C24:0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
ΣSAFA	34.73±0.08^{AB}	40.46±1.91^{A}	32.65±1.92^{AB}	22.73±7.05^{B}	23.57±0.91^{B}
C14:1	0.27±0.01	0.35±0.04	0.22±0.05	0.18±0.04	0.11±0.02
C16:1	3.92±0.14	3.39±0.10	3.48±0.28	3.97±0.71	3.89±0.09
C17:1	0.37±0.14	0.54±0.27	0.45±0.09	0.16±0.03	0.06±0.04
C18:1 n-11	6.27±0.02	4.57±0.18	3.49±0.20	2.98±0.25	2.39±0.24
C18:1 n-9	8.37±0.18	15.26±0.91	19.84±0.92	22.04±2.09	21.47±0.53
C18:1 n-7	7.32±0.19	6.12±0.15	5.74±0.21	5.52±0.65	5.13±0.22
C20:1	9.43±0.14	9.51±0.47	9.10±0.23	9.07±0.71	8.35±0.29
C22:1 n-11	1.15±0.07	0.96±0.17	1.13±0.09	1.28±0.09	1.36±0.10
C22:1 n-9	0.00±0.00	0.29±0.07	0.29±0.07	0.31±0.04	0.32±0.02
C24:1 n-9	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
ΣMUFA	37.11±0.06^{A}	41.00±1.80^{A}	43.73±0.87^{A}	45.52±4.18^{A}	43.08±0.73^{A}
C18:2 n-6	3.98±0.21	5.85±0.51	8.64±0.55	11.64±1.29	12.05±0.42
C18:3 n-6	0.10±0.14	0.11±0.01	0.11±0.01	0.16±0.04	0.35±0.06
C18:3 n-3	4.09±0.05	1.96±0.22	2.39±0.17	3.20±0.53	3.27±0.19
C18:4 n-3	0.69±0.00	0.38±0.07	0.35±0.13	0.24±0.06	0.30±0.06
C20:2 n-6	2.19±0.19	2.47±0.41	2.95±0.38	4.15±0.36	4.18±0.29
C20:3 n-6	0.45±0.06	0.26±0.03	0.33±0.04	0.58±0.13	0.71±0.03
C20:4 n-6	2.05±0.05	0.75±0.12	0.80±0.12	0.92±0.07	0.84±0.07
C20:3 n-3	0.83±0.02	0.42±0.10	0.41±0.10	0.53±0.09	0.58±0.05
C20:4 n-3	0.64±0.02	0.21±0.02	0.25±0.03	0.33±0.07	0.33±0.04
C20:5 n-3	8.07±0.11	4.05±0.81	4.70±0.53	6.05±2.28	6.75±0.58
C22:2	0.27±0.02	0.24±0.03	0.33±0.04	0.40±0.06	0.40±0.07
C22:3	1.78±0.05	0.54±0.12	0.59±0.10	0.63±0.07	0.49±0.11
C22:5 n-3	2.70±0.06	0.93±0.28	1.04±0.14	1.41±0.20	1.36±0.21
C22:6 n-3	0.00±0.00	0.38±0.26	0.73±0.23	1.50±0.26	1.73±0.23
ΣPUFA	28.16±0.14^{AB}	18.54±1.74^{B}	23.61±1.99^{C}	31.74±3.72^{AD}	33.34±1.45^{D}
Σn-3	17.01±0.01^{A}	8.32±1.47^{B}	9.86±1.00^{B}	13.26±2.78^{BC}	14.32±1.19^{AC}
Σn-6	8.88±0.11^{A}	9.44±0.53^{A}	12.84±0.92^{B}	17.45±1.77^{C}	18.13±0.59^{C}
DHA:EPA	0.00±0.00^{A}	0.10±0.07^{A}	0.15±0.04^{A}	0.22±0.02^{AB}	0.26±0.03^{B}

The fatty acids in the post-smolt treatment groups are presented in table 3.7. The most abundant fatty acids in the post-smolt treatment groups were palmitic acid (C16:0) (14-28%), elaidic acid (C18:1 n-9) (15-22%) and linolelaidic acid (C18:2 n-6) (6-12%). The relative content of SAFA were decreasing, but non-significantly from the initial polychaetes (ANOVA on ranks, ($P \geq 0.05$)). Although, treatment groups B and D were significantly different from treatment group A ($P<0.05$). There were no significant differences in MUFA abundance in any of the treatment groups compared to the initial group. The relative amount of PUFA in the initial group were significantly higher compared to post-smolt treatment groups A and B ($P<0.05$) but was significantly lower compared to treatment group D ($P<0.05$). The relative content of omega-3 was the lowest in treatment group A, but steadily increased throughout the treatment groups and was the highest in treatment group D. A similar pattern was found within the treatment groups regarding the omega-6 fatty acids and the DHA:EPA ratio. The relative DHA+EPA content within the post-smolt treatment groups ranged between 4-8% of the total fatty acids.

3.4.5 Minerals

Table 3.8: Elements in the smolt and post-smolt sludge [mg/kg DW].

	Element analysis	
[mg/kg DW]	**Smolt sludge**	**Post-smolt sludge**
Magnesium	$2.6*10^3$	$7.4*10^3$
Phosphorus	$19*10^3$	$27*10^3$
Sulphur	$1.5*10^3$	$2.9*10^3$
Potassium	$1.4*10^3$	200
Calcium	$39*10^3$	$54*10^3$
Chromium	2,4	9,2
Manganese	120	77
Iron	630	$1.2*10^3$
Cobalt	0.28	0.25
Nickel	2	2.1
Copper	22	16
Zink	400	690
Arsenic	1.5	1.2
Molybdenum	1.6	1.7
Silver	<0.085	0.2
Cadmium	0.42	0.97
Tin	<3.8	<3.8
Mercury	<0.7	<0.7
Lead	0.67	0.5

The mineral composition in both sludge diets is presented in figure table 3.8. Calcium, phosphorous and magnesium were the most abundant minerals in both sludges. The overall content of minerals is higher in the post-smolt sludge with a few exceptions. Manganese, cobalt, copper, arsenic, and lead all had a greater amount in the smolt sludge in comparison to the post-smolt sludge. The content of tin and mercury is uncertain as they were below the detection limit. A graphic representation of the mineral content in the sludge diets is presented in appendix V.

Table 3.9: Elements in the initial polychaetes (I) compared to the polychaetes from the smolt treatment groups (A-D) [mg/kg DW].

	Element analysis				
[mg/kg DW]	**Initial**	**Smolt treatment**			
	I	**A**	**B**	**C**	**D**
Magnesium	$4.9*10^3$	$4.4*10^3$	$4.2*10^3$	$4.1*10^3$	$3.5*10^3$
Phosphorus	$7.5*10^3$	$7*10^3$	$7.2*10^3$	$7*10^3$	$6.4*10^3$
Sulphur	$8.2*10^3$	$5.5*10^3$	$4.9*10^3$	$5.4*10^3$	$5.2*10^3$
Potassium	$13*10^3$	$12*10^3$	$12*10^3$	$11*10^3$	$11*10^3$
Calcium	$1.2*10^3$	$1.4*10^3$	$1.3*10^3$	$1.4*10^3$	$1.2*10^3$
Chromium	2	1	1.6	1	1.1
Manganese	21	20	24	21	20
Iron	910	580	670	670	810
Cobalt	7.7	6.2	6.4	5.4	5.7
Nickel	2.5	4	5.3	7.1	7.3
Copper	30	25	21	21	21
Zink	110	210	160	170	140
Arsenic	9.4	7.1	6.6	5.9	5.5
Molybdenum	3.9	1.7	1.6	1.5	1.4
Silver	0.8	0.65	1.23	0.19	0.24
Cadmium	0.091	0.14	0.11	0.094	0.074
Tin	<3.8	<3.8	<3.8	<3.8	<3.8
Mercury	<0.7	<0.7	<0.7	<0.7	<0.7
Lead	0.66	0.51	0.64	0.48	0.76

Table 3.10: Elements in the initial polychaetes (I) compared to the polychaetes from the post-smolt treatment groups (A-D) [mg/kg DW].

	Element analysis				
[mg/kg DW]	**Initial**	**Post-smolt treatment**			
	I	**A**	**B**	**C**	**D**
Magnesium	$4.9*10^3$	$4.4*10^3$	$4.8*10^3$	$3.7*10^3$	$3.7*10^3$
Phosphorus	$7.5*10^3$	$7.5*10^3$	$7.8*10^3$	$7.6*10^3$	$7.4*10^3$
Sulphur	$8.2*10^3$	$6.3*10^3$	$5.7*10^3$	$5.5*10^3$	$5.4*10^3$
Potassium	$13*10^3$	$12.0x10^3$	$12*10^3$	$12*10^3$	$12*10^3$
Calcium	$1.2*10^3$	$1.3*10^3$	$1.8*10^3$	$1.4*10^3$	$1.1*10^3$
Chromium	2	1.2	1.3	0.95	0.86
Manganese	21	20	25	20	16
Iron	910	690	810	580	500
Cobalt	7.7	7.6	7.5	6.1	5.1
Nickel	2.5	5.4	5.8	5	3.6
Copper	30	23	20	21	30
Zink	110	190	170	160	160
Arsenic	9.4	7	7	6	5.3
Molybdenum	3.9	1.9	1.9	1.6	1.6
Silver	0.8	0.4	0.34	0.24	0.32
Cadmium	0.091	0.13	0.12	0.1	0.077
Tin	<3.8	<3.8	<3.8	<3.8	<0.38
Mercury	<0.7	<0.7	<0.7	<0.7	<0.7
Lead	0.66	0.54	0.60	0.48	0.41

Table 3.9 and 3.10 presents a general overview of the mineral content in the initial polychaetes compared to the smolt and post-smolt treatments, respectively. The content of silver, molybdenum, and arsenic decreased from the initial polychaetes to both treatments. The contents of tin and mercury was uncertain since the amounts of these elements were beneath the detection limit. A graphic representation of the elemental composition of the polychaetes is presented in appendix VI.

3.4.6 Water-Soluble Vitamins

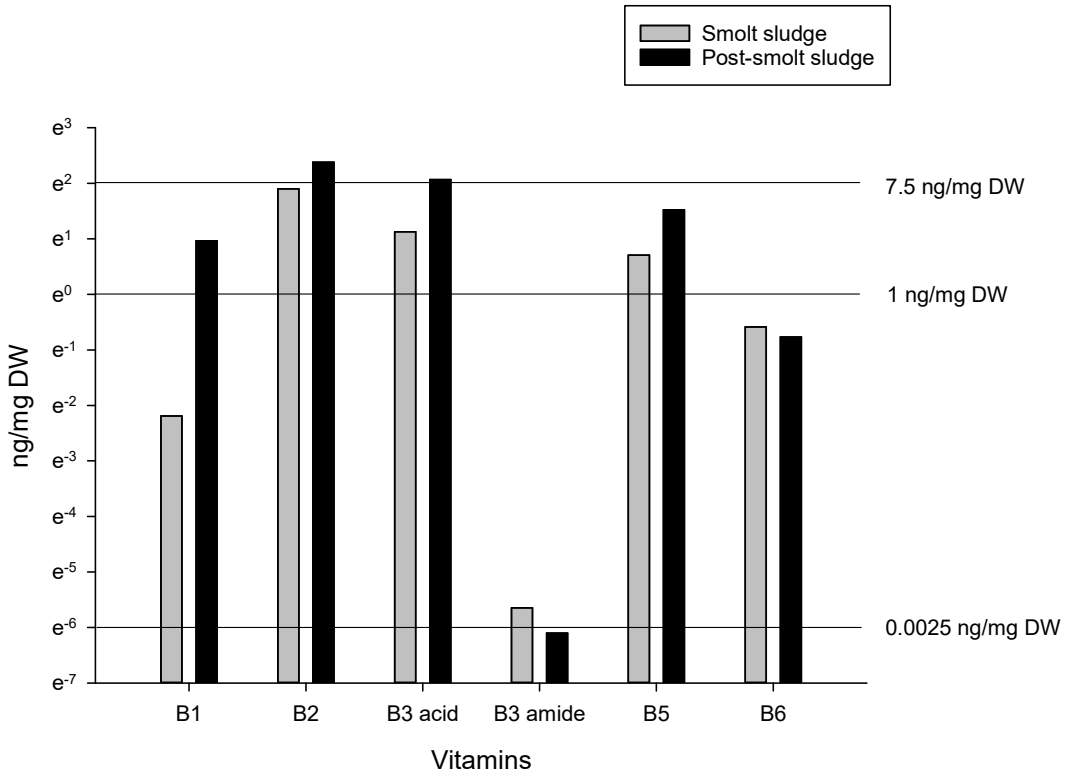

Figure 3.14: Water-soluble vitamin content in smolt and post-smolt sludge [ng/mg DW]. Because of extreme differences in content between the different vitamins, the Y-axis is made of a logarithmic scale. Additionally, some reference lines have been added to clarify the results. Lack of shading indicates no significant differences between the two sludge diets (P≥0.05).

Figure 3.14 presents a graphic overview of the water-soluble vitamin content in both diets – smolt and post-smolt sludge. Overall, the quantity of vitamins was more abundant in the post-smolt sludge. Two exceptions are B3 amide and vitamin B6, which were slightly more abundant in the smolt sludge. Vitamin B2 was the most abundant, while vitamin B3 amide was the least abundant vitamin in both sludges. Although there were some differences in content in the two sludges, there was no significant difference between any of the vitamins in the two sludges.

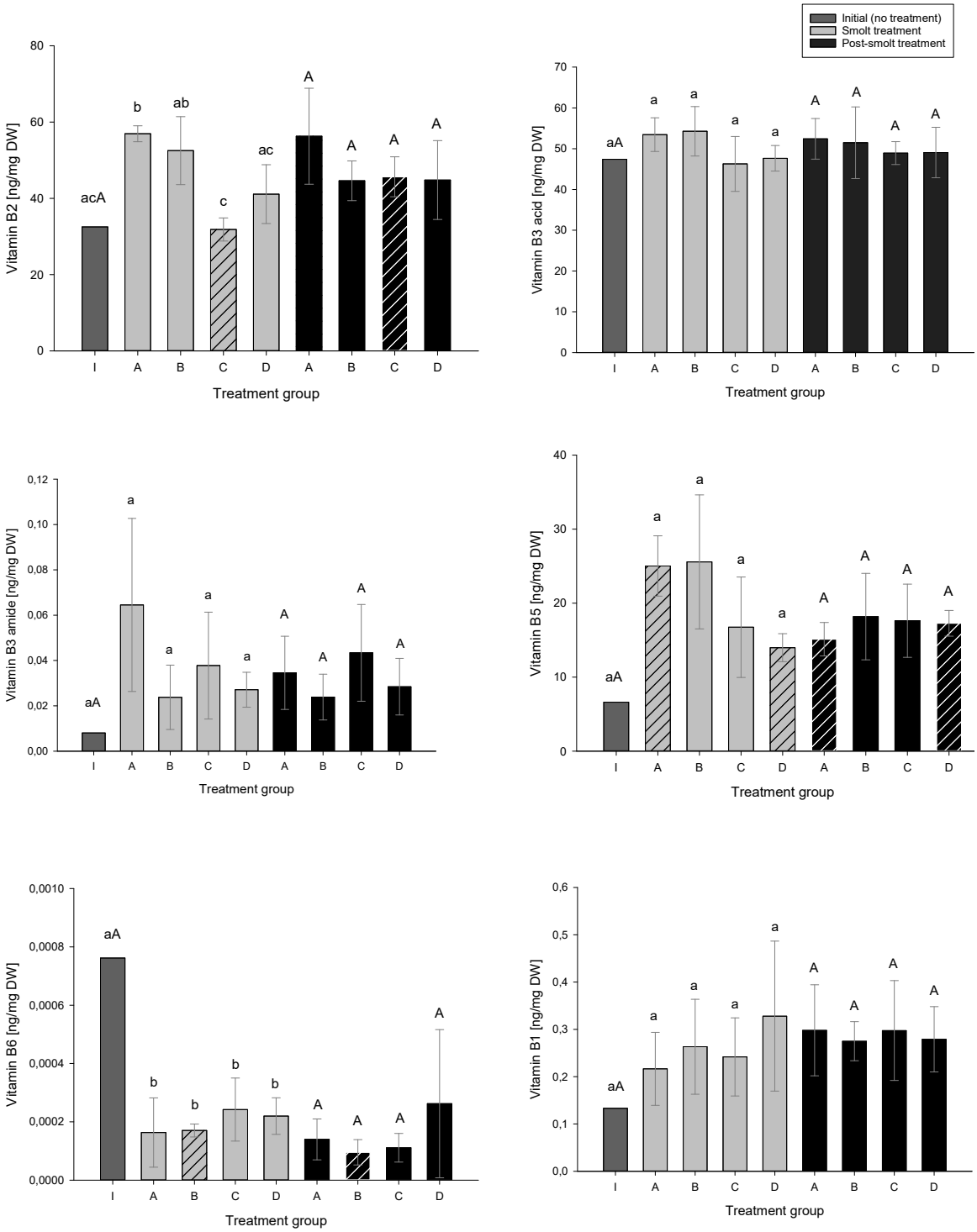

Figure 3.15: Content [ng/mg DW] of the water-soluble vitamins B2, B3 acid, B3 amide, B5, B6 and B1 in the initial polychaetes (I), compared to polychaetes from the smolt and post-smolt treatment groups (A, B, C and D) [ng/mg DW]. (Mean±SD). Significant differences within the smolt treatment groups are marked with lowercase superscripts (P<0.05). Similarly, significantly differences within the post-smolt treatment groups are marked with uppercase superscripts (P<0.05). Non-significant differences between the treatment groups are marked by similar letters (P≥0.05). Significant differences between corresponding treatment groups across the treatments are marked with shaded bars (P<0.05).

The content of water-soluble vitamins in the polychaetes is presented in figure 3.15. The content of vitamin B2 increased across all treatment groups. There was a significant difference between smolt treatment group B compared to the initial as well as treatment group C and D ($P<0.05$). There were no significant differences within the post-smolt treatment groups ($P\geq0.05$). However, there was a significant difference between smolt- and post-smolt treatment group C across the treatments ($P<0.05$). The content of vitamin B3 acid was similar across both treatments. There were no significant differences between any of the treatment groups or across treatments ($P\geq0.05$).

Despite an increase in the mean vitamin B3 amide content in most of the groups, there were no significant differences for neither smolt, nor post-smolt treatment groups compared to each other and the initial polychaetes ($P\geq0.05$). Accordingly, there were no significant differences between the initial polychaetes and any of the smolt- or post-smolt treatment groups ($P\geq0.05$). There was a significant difference between smolt and post-smolt treatment group A, and smolt and post-smolt treatment group D ($P<0.05$).

Vitamin B6 content decreased for all the different treatment groups. There was a significant difference between the initial polychaetes and every smolt treatment group ($P<0.05$); however, no significant differences were found between the post-smolt treatment groups and the initial polychaetes ($P\geq0.05$). There was a significant difference between smolt and post-smolt treatment group B ($P<0.05$). Lastly, despite increases in vitamin B1 from the initial polychaetes to all the smolt and post-smolt treatment groups, there was no significant difference within any of the treatments or between any of the corresponding treatment groups ($P\geq0.05$).

3.4.7 Fat-Soluble Vitamins

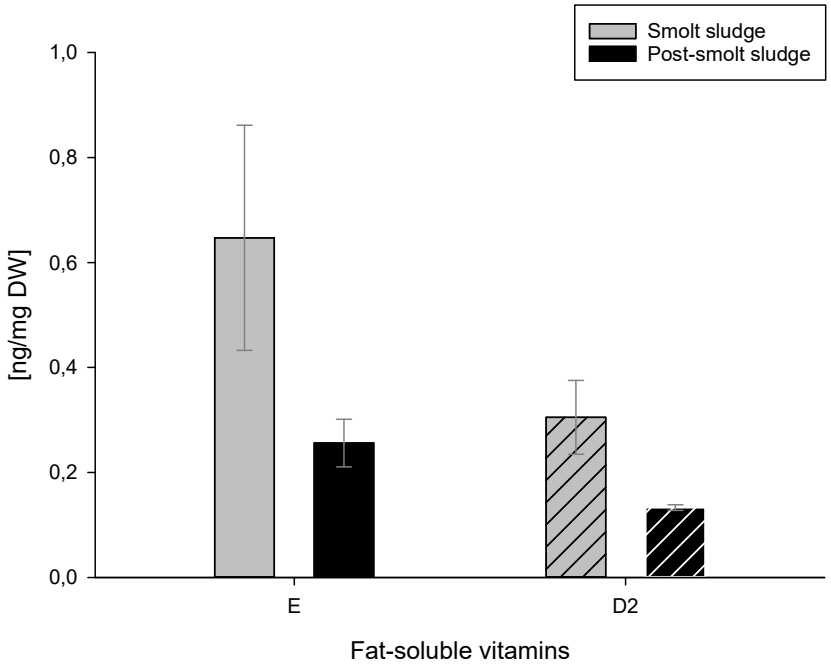

Figure 3.16: Total content [ng/mg DW] of fat-soluble vitamins in in both diets – smolt and post-smolt sludge. Statistically significant differences between the two diets are marked with shaded bars.

Fat-soluble vitamins in the sludge are presented in figure 3.16. Two fat-soluble vitamins were detected in the sludge diets - vitamin E and vitamin D2. The abundance of fat-soluble vitamins was higher in the smolt sludge compared to the post-smolt sludge. There was no significant difference between the two diets in respect to vitamin E ($P \geq 0.05$); there was a significant difference in respect to vitamin D2 between the two diets ($P < 0.05$).

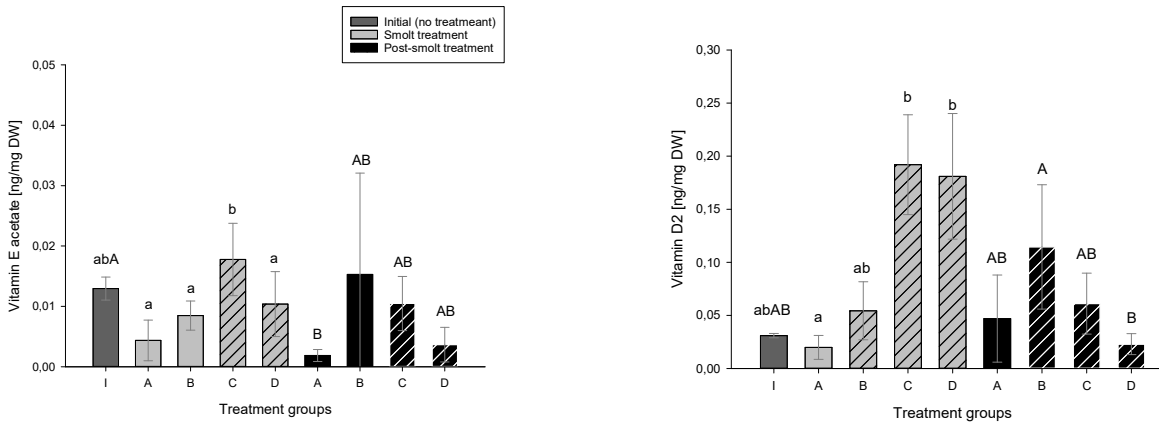

Figure 3.17: Fat-soluble vitamin content [ng/mg DW] of vitamin E acetate and D2 [ng/mg DW] in the initial polychaetes (I) compared to the polychaetes from the smolt and post-smolt treatment groups (A, B, C and D). Significant differences within the smolt treatment are marked with different lowercase letters. Statistically significant differences within the post-smolt treatment are marked with different uppercase letters. Significant differences between corresponding treatment groups across treatments are marked with shaded bars.

The content of fat-soluble vitamins in the polychaetes are presented in figure 3.17. There was a significant difference in the content of vitamin E acetate in smolt treatment group C compared to the other smolt treatment groups ($P<0.05$). Within the post-smolt treatment, there were a significant difference between treatment group A and the initial polychaetes ($P<0.05$). There were also significant differences between smolt and post-smolt treatment group C, and smolt and post-smolt treatment group D across the treatments ($P<0.05$). Considering vitamin D2, there were significant differences between smolt treatment group A and smolt treatment groups C and D ($P<0.05$). There was a significant difference between post-smolt treatment groups B and D ($P<0.05$). No treatment groups were significantly different from the initial polychaetes ($P\geq0.05$). Across the smolt and the post-smolt treatments, there were significant differences between corresponding treatment groups B, C and D ($P<0.05$).

3.4.8 Ash

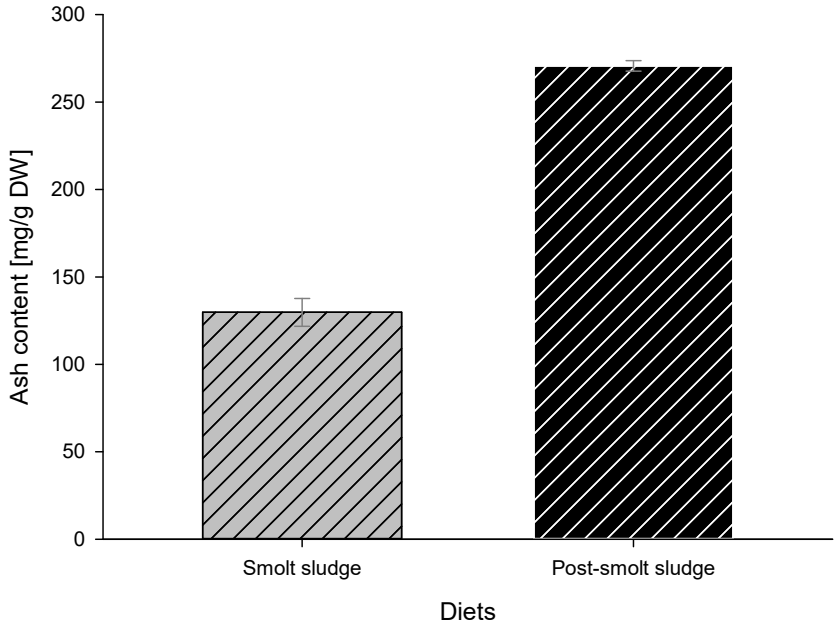

Figure 3.18: Ash content [mg DW] in the smolt- and the post-smolt sludge diets (Mean±SD). Statistically significant difference between the two diets is marked with shades ($P<0.05$).

The ash content in both diets, smolt- and post-smolt sludge respectively, is presented in figure 3.18 The ash content in the smolt sludge is 129.7 mg/g DW; the ash content in the post-smolt sludge has a value of 270.7 mg/mg DW. There was a significant difference between the two sludges ($P<0.05$).

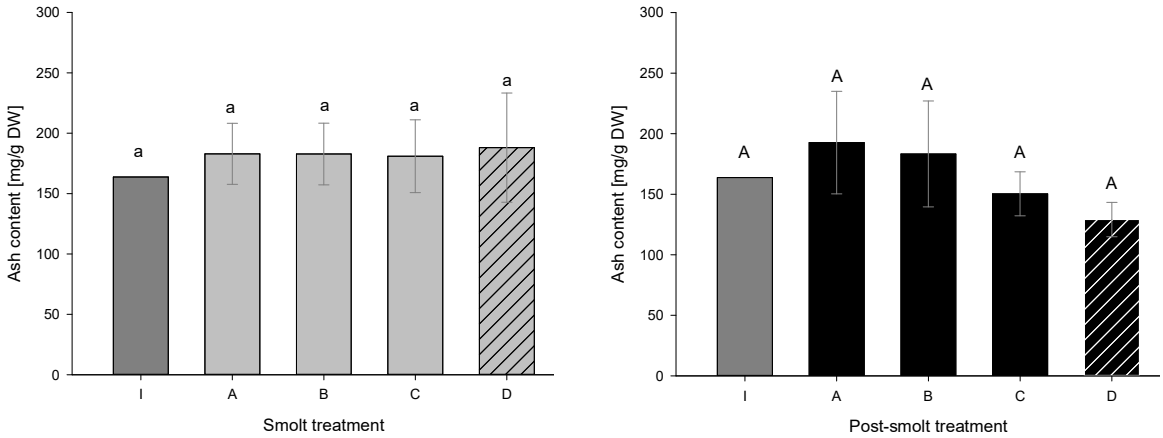

Figure 3.19: Ash content [mg/g DW] in the initial polychaetes as well as all smolt and post-smolt treatment groups (Mean±SD) Similar upper- and lowercase letters indicate no significant differences. Bars which are shaded on indicate significant differences between specific treatment groups across treatments.

The ash content in the polychaetes is presented in figure 3.19. The ash content in the initial polychaetes is 163.8 mg/g DW. There was a no significant difference in ash content within the different smolt treatment groups ($P<0.05$). The average content in the smolt treatment groups ranged between 181-188.1 mg/g DW.

The ash content in the post-smolt treatment groups is declining from treatment group A to treatment group D. The ash content in the post-smolt treatment groups ranged between 129.1-192.7 mg/g DW. Despite these differences, there were no significant differences between the initial polychaetes and any of the post-smolt treatment groups ($P\geq0.05$); there were a significant difference between smolt and post-smolt treatment group D ($P<0.05$).

3.4.9 Unidentified Material

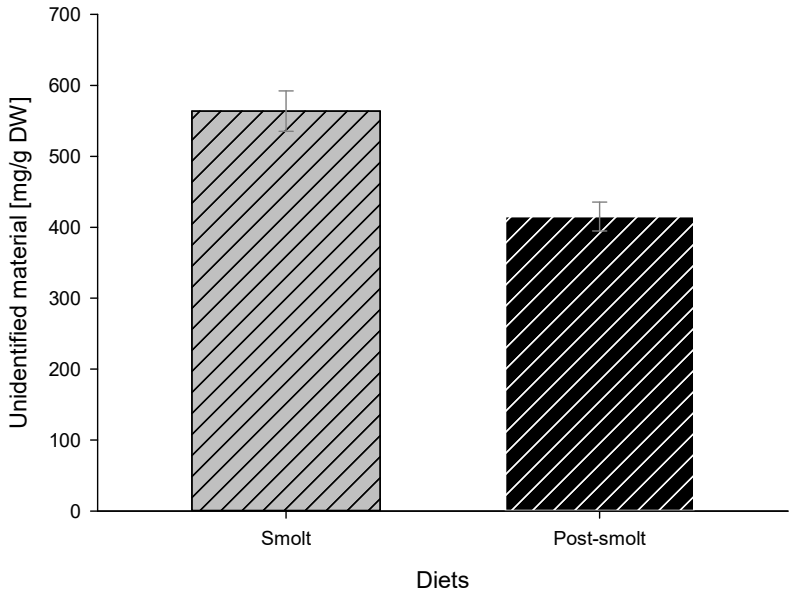

Figure 3.20: Unidentified material [mg/g DW] in the smolt and post-smolt sludge (Mean±SD). Significant differences are marked with shade (P<0.05).

The remaining, unidentified material in the diets is presented in figure 3.20. The amount of unidentified material in the smolt sludge was 564 mg/g DW and the amount of unidentified material in the post-smolt sludge was equal to 415 mg/g DW. There was a significant difference between the smolt- and post-smolt sludge (P<0.05).

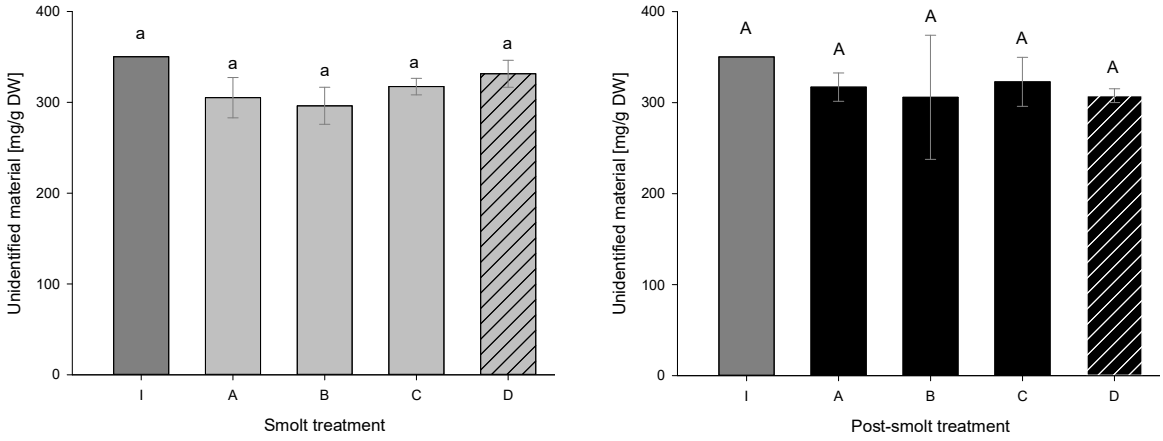

Figure 3.21: Unidentified material [mg/g DW] in all treatment groups including the initial polychaetes (Mean±SD). Non-significant differences are marked with one or more similar lowercase letters within the smolt treatment groups and uppercase letters within the post-smolt treatment groups (P≥0.05). Shaded bars indicate significant differences between corresponding treatment groups across treatments (P<0.05).

Figure 3.21 reveals the amount of unidentified material in the initial polychaetes as well as the smolt- and post-smolt treatment groups. Regarding the smolt treatment groups, there were no significant differences between the initial polychaetes and any of the different treatment groups ($P \geq 0.05$). Similarly, for the post-smolt treatment groups there were no significant differences compared to the initial polychaetes ($P \geq 0.05$). There was a significant difference between the corresponding smolt and post-smolt treatment group D ($P < 0.05$).

3.4.10 Total Composition

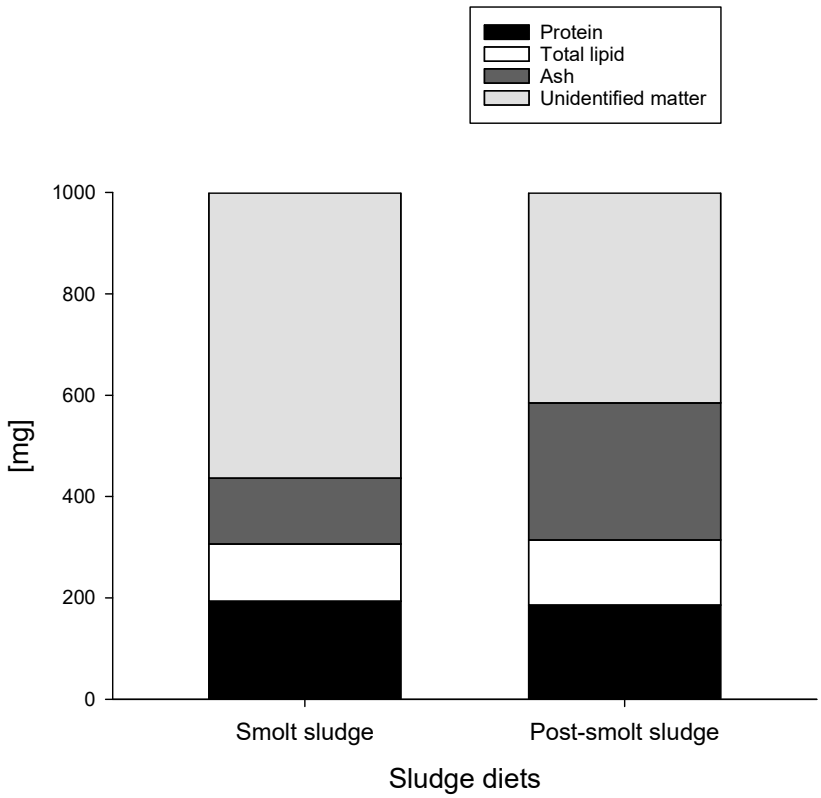

Figure 3.22: Total composition of the smolt and post-smolt sludge diets in 1 g of sample [DW]. The black part of the columns represents the amount of protein; the white parts represent the total lipid content; the dark grey represents the ash content; the remaining unidentified matter is coloured with a brighter grey.

The biochemical composition of the diets is presented in figure 3.22. The contribution of protein to the biochemical composition was 193 mg for the smolt sludge and 186 for the post-smolt sludge. The total lipid content in the smolt sludge was 113 mg and 128 mg in the post-smolt sludge. In the smolt sludge, the ash content was 130 mg, and it was 270 mg in the post-smolt sludge. Lastly, the remaining unidentified material in the smolt sludge was 564 mg and 415 mg in the post-smolt sludge.

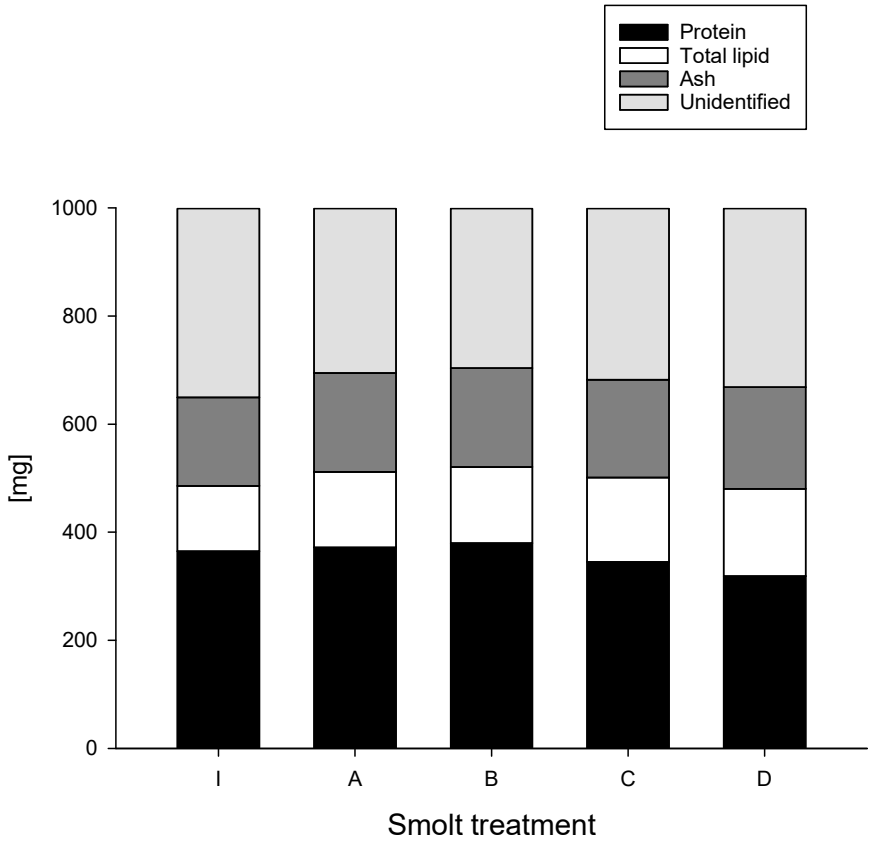

Figure 3.23: Total composition [mg] of the initial polychaetes (I) and the polychaetes from the different smolt treatment groups in 1 g of sample [DW]. The content of proteins forms the black parts of the columns; the white parts consist of the total lipid content; the dark grey covers the ash, and the brighter grey parts of the columns presents the remaining, unidentified parts of the polychaete composition.

The biochemical composition of the polychaetes from the initial group and the smolt treatment groups is presented in figure 3.23. The protein content in the smolt treatment groups ranged from 319-372 mg; the protein content in the initial polychaetes was 365 mg. The total lipid ranged from 140-162 mg across the treatment groups; the content in the initial polychaetes was 121 mg. The ash content in the smolt treatment groups ranged from 181-188 mg; the ash content in the initial polychaetes was 164 mg. Lastly, the unidentified material in the smolt treatment groups accounted for 296-331 mg of the total matter; the amount of unidentified material in the initial polychaetes was 350 mg.

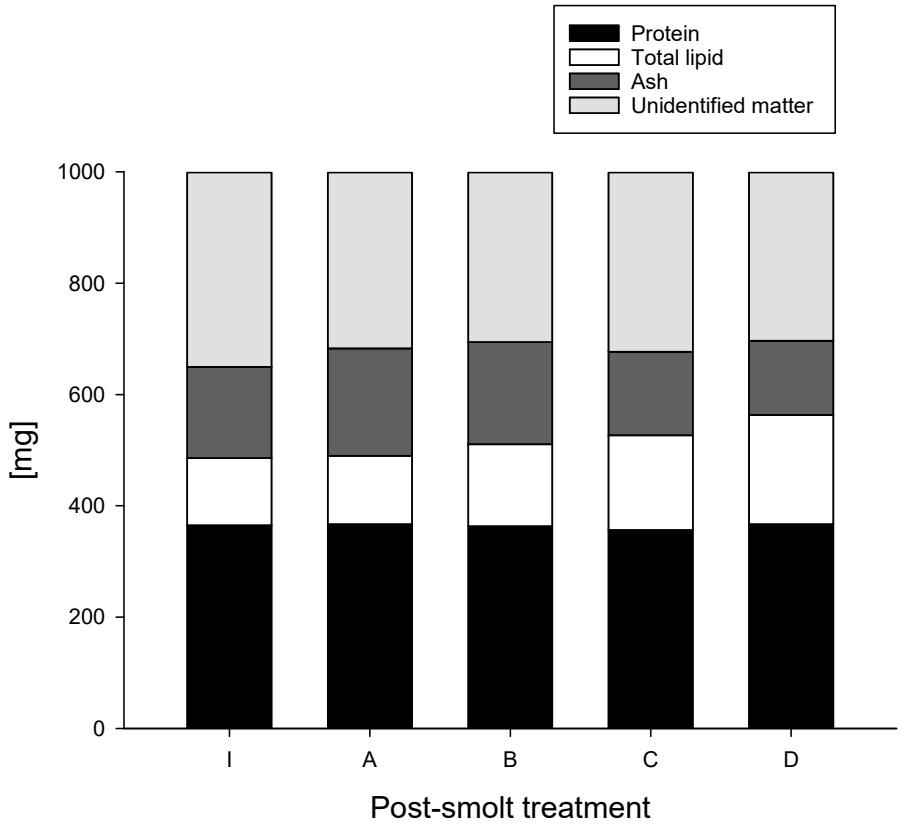

Figure 3.24: Total composition of the initial polychaetes (I) and the polychaetes from the different post-smolt treatment groups in 1 g of sample [DW]. The content of proteins forms the black parts of the columns; the white parts consist of the total lipid content; the dark grey covers the ash, and the brighter grey parts of the columns presents the remaining, unidentified parts of the polychaete composition.

The biochemical composition of the polychaetes from the post-smolt treatment groups is presented in figure 3.24. The protein content in post-smolt treatment groups ranged between 356-367 mg; the protein content of the initial polychaetes was 365 mg. The total lipid content in the different post-smolt treatment groups ranged between 123-196 mg; the total lipid content in the initial polychaetes was 121 mg. The ash content in the treatment groups ranged between 129-193 mg and the ash content of the initial polychaetes was 164 mg. Lastly, the unidentified material in the post-smolt treatment groups was varying between 306-323 mg; the content of unidentified material in the initial polychaetes was 350 mg.

4 Discussion

4.1 Survival Rate

The survival rate within the smolt treatment was between 78-90%. In comparison, the survival rate within the post-smolt treatment ranged between 80-91%. There were no significant differences between any of the treatment groups. The survival rates in this study were relatively high compared to some previous studies (Bischoff, 2007, Seekamp, 2017), but similar to others (Narciso and da Fonseca, 2000, Batista et al., 2003, Pajand et al., 2017, Nesto et al., 2012, Gómez et al., 2019).

Possible explanations for this could be the length of the experiment and the stocking density. The stocking density in this experiment was noticeably lower than similar experiments with higher mortality rates (Bischoff, 2007, Seekamp, 2017). Lower stocking densities leads to less competition and more available feed for the individual polychaete. The experiment was also relatively short (30 days) in comparison to other studies (Bischoff, 2007) (45 days).

Further, one of the tanks within the smolt treatment group D was ended early (11 days) because of accumulation of an unknown red biofilm in fear of it spreading to the other tanks. Despite the biofilm, all 15 individuals were found alive. The actual effect of the biofilm was not conclusive as the respective tank was taken out of the experiment immediately after the biofilm was spotted. It can be argued that early ending of the applicable tank may have a false effect on the survival rate as it was contributing to a higher survival rate than what could have been the result if the tank was left untouched throughout the experiment.

Even though the different treatment groups were fed considerable different amounts of sludge (feeding levels), it did not seem to have any effect on the survival rate of the polychaetes. Similar conclusions were drawn in previous studies (Bischoff, 2007, Seekamp, 2017). There was no indication of starvation due to the high survival rate of the polychaetes.

In some previous studies, low survival rates were linked to either high concentrations of total ammonia nitrogen (TAN) (Bischoff, 2007) or cannibalism (Batista et al., 2003). High concentrations of TAN were related to increased feeding, while cannibalism was related to low feed supply. TAN was not measured in this experiment; the high survival rates could indicate that the TAN levels were well within the threshold values (Bischoff, 2007). Also, there was no indication of cannibalism, which suggests the food supply was sufficient (Batista et al., 2003).

4.2 Growth

The specific growth rate (SGR d^{-1}) for the smolt treatment groups follows a similar pattern like the individual growth rates. The SGR of treatment group A (-0.001 d^{-1}) and treatment group B (0.005 day^{-1}) were significantly different from treatment group D (0.021 d^{-1}). No groups were significantly different from treatment group C (0.015 d^{-1}). In comparison, the SGR of post-smolt treatment group A (0.002 d^{-1}) was significantly different from treatment group D (0.02 d^{-1}). Treatment group B (0.014 d^{-1}) and treatment group C (0.014 d^{-1}) were not significantly different from other treatment groups. In this regard, it seems to be a strong positive correlation between the amount of sludge given to the polychaetes and the corresponding growth rates.

On one hand, all treatment groups had lower SGR than some similar experiments where polychaetes were fed on different aquaculture waste diets (Brown et al., 2011, Pajand et al., 2017, Gómez et al., 2019). On the other hand, smolt treatment groups C and D, and post-smolt treatment groups B, C and D exceeded the SGR of other aquaculture waste feeding experiments (Bischoff, 2007, Seekamp, 2017). It seems very likely that the SGR is strongly affected by the amount of sludge given to the polychaetes (Brown et al., 2011). Growth rates could also be linked to the compositions of the diets (Brown et al., 2011, Seekamp, 2017). Previous studies that worked with considerably more nutritious sludge reported equivalent higher growth rates (Pajand et al., 2017, Brown et al., 2011).

The relative low growth rates could also be explained by internal development-related reasons (Wang et al., 2019b). In this experiment, all polychaetes were caught during the autumn, when the polychaetes initiates their maturation process (Last and Olive, 1999) and invests less energy into somatic growth. Environmental factors like temperature and day length are also effecting the time of maturation, and polychaetes have been susceptible to manipulation of those factors (Last and Olive, 1999). Thus, in this experiment, summer conditions were simulated in terms of day length (16L/8D) and temperature (16 °C).

Another aspect regarding the growth rates is the initial weight of the polychaetes as the growth rate decreases with size (Heip and Herman, 1979). The initial average wet weight of the polychaetes at the start of the experiment ranged between 0.22-0.3g within the smolt treatment groups and between 0.23-0.25 within the post-smolt treatment groups. Thus, the low growth rates in smolt treatment group A could be explained by the high initial average weight (0.3 g) at the start of the experiment. Other studies also reported higher growth rates for smaller polychaetes and decreasing growth rates as they grew bigger (Nesto et al., 2012).

Another factor that could have had influence on the growth rates was the food accessibility (Scaps, 2002a). Polychaetes from group A in both treatments had considerably less feed available then the other treatment groups. Since the polychaetes often prefer not to leave their burrows entirely when feeding (Wang et al., 2019b), some of the feed given to the less fed treatment groups might become out of range. Arguably, polychaetes given less feed could have spent more energy finding their feed.

4.3 Evaluation of Diets

Previous studies of polychaetes fed on aquaculture sludge indicated that the growth rates of the polychaetes were affected by the compositions of the diets (Brown et al., 2011, Seekamp, 2017). The content of total organic matter (TOM), total lipids and protein in the smolt sludge was 87%, 11% and 19%, respectively. In comparison, the content of TOM, total lipids, and protein in the post-smolt sludge was 73%, 13% and 18%, respectively.

The protein content in both sludge diets was lower than previously reported in other studies where aquaculture waste products were given to polychaetes; smolt sludge (25%) (Seekamp, 2017), halibut faecal waste (50%) (Brown et al., 2011). Still, the protein content was higher than reported for yellowtail amberjack (*Seriola lalandi* sludge) (17%) in a similar experiment with the polychaete *Abarenicola pusilla* (Gómez et al., 2019). There were no significant differences in protein content between the smolt and post-smolt sludge in this experiment.

The total lipid content in the smolt and post-smolt sludge diets (11% and 13%) slightly exceeded the total lipid content in the smolt sludge reported by Seekamp (10%) but were considerably lower than the halibut faecal waste (22%) reported by (Brown et al., 2011, Seekamp, 2017). The values were lower than what was found by Gómez in *Seriola lalandi* sludge (14%) (Gómez et al., 2019). The total lipid content was significantly higher in the post-smolt sludge compared to the smolt sludge in this experiment.

Regarding fatty acids, MUFA was the most abundant group with values of 50% and 39% for the smolt and post-smolt sludge, respectively. Followed by the SAFA with values of 28% and 35%, and the PUFA with values of 22% and 26% for the smolt and post-smolt sludge, respectively. In similar studies of smolt sludge, SAFA was the most abundant group followed by MUFA and PUFA (Wang et al., 2019b). The relative content of DHA+EPA in smolt and post-smolt sludge was 6% and 5%, respectively, which was lower than previously reported (10%) (Wang et al., 2019b).

There was also a significant difference in the ash content between the two sludges. The content of ash in the smolt sludge was 13%, while the ash content in the post-smolt sludge was more than doubled at 27%. The differences in ash content between the two sludges could be explained by the content of salts in the marine post-smolt sludge. On one hand, the content of ash in the freshwater smolt sludge was rather low compared to previous similar analysis (43%) (Seekamp, 2017). On the other hand, the content of ash in the marine post-smolt sludge was very similar to some marine sludges (27%) (Brown et al., 2011), but notably higher than in the yellowtail amberjack sludge (18%) (Gómez et al., 2019). Despite the similar ash content, Brown reported considerably higher contents of protein and total lipids in the halibut faecal waste compared to the post-smolt sludge.

The carbohydrate content was not reported in this thesis as there were some difficulties with the method (section 2.6.5). Previous assumptions of carbohydrate content in freshwater smolt sludge reported values of 24% (Seekamp, 2017); studies of the content of carbohydrates in marine yellowtail sludge reported values of 14% (Gómez et al., 2019). The content of carbohydrates in those sludges is reflected in the digestible carbohydrate limit (%) of the respective species; yellowtail (≤10%) and Atlantic salmon (≤20%) (Shimeno, 1991, Helland et al., 1991).

One could assume that most of the unidentified material in the smolt sludge (56%) and the post-smolt sludge (41%) consists of carbohydrates, as Atlantic salmon are poor utilizers of carbohydrates (Hemre et al., 1995, Wilson, 1994).

The overall content of the different minerals was higher in the post-smolt sludge compared to the smolt sludge, but with a few exceptions like manganese, cobalt, arsenic, and copper. As there were no replicates, no statistical analysis was performed, and no present studies were found to compare.

The overall content of the different water-soluble vitamins was higher in the post-smolt sludge compared to the smolt sludge, except B3 amide and B6. Yet, there were no significant differences within any of the vitamins between the two sludge diets. An opposite pattern was discovered regarding the fat-soluble vitamins; the content of fat-soluble vitamins was higher in the smolt sludge compared to the post-smolt sludge. There was a significant difference in the content of vitamins D2. No similar studies were found in comparison for any of the vitamins.

4.4 Composition of *H. diversicolor*

The protein content accounted for most of the biochemical composition in all treatment groups across both treatments. There were no significant differences within either the smolt, or post-smolt treatment groups, and there were no significant differences compared with the initial polychaetes, indicating that the diets did not influence the protein content of the polychaetes.

The protein content in all treatment groups were low in comparison to previous published studies where polychaetes were fed on different aquaculture waste diets; 57% (Brown et al., 2011), 49% (Pajand et al., 2017), 55% (Seekamp, 2017). Another study on wild polychaetes described changes in protein content with the different seasons, with values ranging from 47% during winter and summer, to 60% during early spring (Luis and Passos, 1995). The rather big difference in protein content between this study and previous published results, might be a result of the method used to quantify proteins (Mæhre et al., 2018). On one hand, determining proteins based amino acids content as done in this thesis might result in an underestimated result, as some amino acids (tryptophan) and parts of other amino acids might get destroyed during hydrolysis. On the other hand, protein determination using the traditional Jones factor (6.25), based on the total nitrogen content, will likely result in an overestimation (Mæhre et al., 2018).

The total lipid content in the smolt treatment groups ranged between 14%-16%. In comparison, the total lipid content in the post-smolt treatment groups ranged between 12-20%, and the total lipid content in the initial polychaetes was 12%. Values found by Brown et al (2011) resembles the total lipid content in the treatment groups fed with the most amounts of post-smolt sludge (treatments groups D). Additionally, the values were similar to the natural range of the polychaetes during early spring and autumn (Luis and Passos, 1995, García-Alonso et al., 2008). In other studies, the lipid content in polychaetes fed on eel sludge were even higher (García-Alonso et al., 2008). However, the treatment groups fed with the least amounts of sludge (treatment groups A) across both treatments, resembles values from similar studies where polychaetes were fed on aquaculture sludge diets (Pajand et al., 2017, Seekamp, 2017, Wang et al., 2019b).

Significant differences between the initial polychaetes and the different treatment groups show increasing values of total lipid throughout the experiment. All treatment groups fed on aquaculture sludge increased their total lipid content during the feeding experiment, resembling results from previous similar studies (Seekamp, 2017, Wang et al., 2019b). There seems to be a strong correlation between the total lipid content in the polychaetes and the amount of sludge

fed to the respective treatment groups (available total lipid) (Santos et al., 2016). Further evidence is reflected in the linear relationship of the total lipid content of the polychaetes, and the amount of sludge fed to the different treatment groups. The relationship between the total lipid [mg] and sludge [g] were described through slopes of 0.29 for the smolt treatment and 0.82 for the post-smolt treatment. The difference in slope values indicates a stronger effect of the post-smolt sludge on the total lipid content compared to the smolt sludge, emphasised by the significant difference between the two slopes ($P<0.05$).

The total fatty acid content increased significantly throughout the experiment in all treatment groups except the A treatment groups, resembling the results from earlier studies (Seekamp, 2017, Bischoff et al., 2009). The fatty acid content increased correspondingly with increasing amounts of feed given, resembling the pattern found in the total lipid content of the treatment groups. As in earlier studies, the content of palmitic acid (C16:0), oleic acid (C18:1 n-9) and linoleic acid (C18:2 n-6) were among the most abundant fatty acids in terms of saturated, monounsaturated, and polyunsaturated fatty acids, respectively (Luis and Passos, 1995, Pajand et al., 2017, Santos et al., 2016, Wang et al., 2019b).

The relative content of SAFA decreased within all treatment groups in both treatments compared to the initial polychaetes. The smolt treatment groups showed significantly increased relative content of MUFA. On the contrary, the relative content of PUFA in the post-smolt treatment groups increased significantly. The increase of fatty acids in the smolt and post-smolt treatment groups followed the increasing available fatty acids in their respective diets, resembling findings from similar studies (Bischoff et al., 2009, Seekamp, 2017).

Previous consensus is that microbes almost exclusively account for all the production of omega-3 fatty acids in marine environments. To the contrary, studies shown invertebrates containing elongase and desaturase, and inhabits the ability to de novo synthesise omega-3 polyunsaturated fatty acids (Kabeya et al., 2018). Further studies described the ability of *H. diversicolor* to biosynthesise essential PUFA from shorter saturated FAs (Kabeya et al., 2020). Thus, the decrease of SAFA and increase of PUFA could be explained by the ability of *H. diversicolor* to biosynthesise deficient essential fatty acids. The essential marine fatty acid DHA was completely absent in the initial polychaetes but was found in increasing levels in all treatment groups (0.3-2%), thus providing further proof the abilities of *H. diversicolor* to biosynthesise essential fatty acids. A similar behaviour has also been described by (Wang et al., 2019a).

Similarly, the relative abundance of EPA in most treatment groups (4-8%) exceeded the values reported in previous studies (Luis and Passos, 1995, Pajand et al., 2017), but were similar

to others (Santos et al., 2016). Wang et al (2019) reported an even higher relative content of EPA compared to all treatment groups. The difference in relative content could be explained by differences in the number of fatty acids detected.

On a different note, DHA:EPA ratio increased in both treatments with increased feeding. EPA and DHA are essential for proper development and healthy aging in several organisms (Dunstan et al., 2007). Further, EPA play an important role in immune response an inflammatory processes (Lall and Dumas, 2015). Moreover, fin rot and reproductive performance of male and female fish are related to low EPA concentrations in the diet (Hamre et al., 2013). The relative DHA:EPA ratio within the smolt treatment groups ranged between 0.15-0.34; the relative ratio in the post-smolt treatment groups ranged between 0.10-0.26. Treatment groups C and D within both treatments resembles values reported by (Wang et al., 2019b). Slightly higher values were reported in other studies with more nutrient-rich diets (Santos et al., 2016, Pajand et al., 2017). The differences in DHA:EPA ratios is likely affected by the quality of the feed products, as similar polychaete studies with higher DHA: EPA-values often utilizes feed extra rich in nutrients; particularly fish feed and commercial diets (Santos et al., 2016, Pajand et al., 2017, Seekamp, 2017).

As mentioned in the description of the methods, the carbohydrate analysis did not give any meaningful results. Previous studies assumed that the carbohydrate content in polychaetes were equal to the parts of the TOM not covered by proteins and total lipid (Seekamp, 2017). Presumably, the carbohydrates cover major parts of the unidentified or remaining matter in the polychaetes, but this was not conclusive. The amount of unidentified material in the smolt treatment groups was 30-33%. In comparison, the amount of unidentified matter in the post-smolt treatment was 30-32%, and 35% in the initial polychaetes. A previous study of the polychaete *Perinereis helleri* reported carbohydrate values of 11-16% of wet weight (Palmer et al., 2014).

There were no significant differences in total content of amino acids between the initial polychaetes and either of the smolt nor post-smolt treatment groups. Previous studies have recorded a decrease in the total content of amino acids (Seekamp, 2017, Wang et al., 2019b). The share of essential amino acids of the total content of amino acids in the smolt treatment groups ranged between 41-44%, and 43-44% within the post-smolt treatment groups; both resembling the values from earlier records (Seekamp, 2017, Wang et al., 2019b). All values were lower than the initial 45%, but there were no significant differences. The most abundant essential amino acids in all treatment groups were leucine (6-8%) and lysine (7%), resembling the results of Seekamp (2017) and Wang et al (2019). Due to destruction during hydrolysis

(Fountoulakis and Lahm, 1998, Laboratories, 2008), tryptophan was not included in the results. The lack of tryptophan data therefore affected the total composition of essential amino acids.

The most abundant non-essential amino acids across the treatments were glutamine + glutamic acid (12-13%) and proline (8-11%), resembling previous studies (Seekamp, 2017, Wang et al., 2019b). There was a significant increase in proline from the initial polychaetes (4%) to all treatment groups in both treatments. The alanine content in the initial polychaetes (11%) was notably higher than every treatment group (6-8%), but no significant differences was found. There was no evidence of any effect of the different feeding levels on the amino acids composition in any of the treatment groups, strengthened by the fact that the amino acid composition of organisms largely is affected by the genes, and excess amino acids are catabolized for energy (Owen et al., 1979).

The ash content was 18-19% in the smolt treatment groups and 13-19% in the post-smolt treatment groups with no significant differences within any of the treatment groups or to the initial polychaetes (16%). Seekamp (2017) reported lower ash content in the polychaetes fed with aquaculture sludge, while other studies reported even higher values (Brown et al., 2011, Luis and Passos, 1995).

There were no considerable differences in the content of minerals between the initial polychaetes and any of the treatment groups. No statistical analysis was performed as there were no replicates. A previous study reported a similar pattern in the relative amounts of minerals in the polychaete *Perinereis helleri* (wet weight) (Palmer et al., 2014).

4.5 Potential of *H. diversicolor* as an Alternative Feed Resource

Table 4.1: Essential amino acids requirements of salmon (percentage of the protein) (Jobling, 2012).

Essential amino acids [%]	Requirement of salmon
Arginine	6
Histidine	1.8
Isoleucine	2.2
Leucine	3.9
Lysine	5.0
Methionine	4.0
Phenylalanine	5.1
Threonine	2.2
Tryptophan	0.5
Valine	3.2
Total content [%]	33.9

Essential amino acid requirements of salmon are presented in table 4.1. Salmon require the same 10 amino acids as most monogastric animals (Jobling, 2012). Additionally, cystine and tyrosine is in some cases characterized as essential as they are synthesized from methionine and phenylalanine (Lall and Dumas, 2015). The amino acid composition of different feed resources is usually presented on a total (not available) content basis. Thus, in formulating fish feeds to meet amino acid requirements, the total amino acid content of the feed ingredients must be corrected for availability to allow the optimum amounts of amino acids in the diet (Jobling, 2012). In general, essential amino acids are important for marine fish in several regards like prevention of fin erosion, scoliosis (curved spine) and lordosis (curved spine) and cataract (Lall and Tibbetts, 2009). Essential amino acids also have a strong effect on normal growth, reproduction and immune and metabolic functions (FAO, 2021).

All treatment groups in both treatments showed exceeding amounts of the essential amino acids arginine, histidine, isoleucine, leucine, lysine, threonine and valine in regard of the recommended requirements (Jobling, 2012). The values are presented in appendix III. The exceptions were from smolt treatment group D and post-smolt treatment groups C and D (arginine) and smolt treatment group B (histidine) which showed contents below recommendation. Additionally, all treatment groups in both treatments showed contents of methionine and phenylalanine slightly below the recommended levels. Tryptophan content was not successfully measured in this experiment due to destruction during hydrolysis, but was

detected in previous studies suggesting it should be present in the polychaetes (Palmer et al., 2014, Seekamp, 2017). There is a few suggested values for recommended protein levels in feed for Atlantic salmon (36-45%) (NRC, 2011, FAO, 2021). However, the quality of the protein in fish feed is largely dependent on the content of amino acids as some, particularly vegetable sources, may be entirely deficient in one more essential amino acids for marine fish (Lall and Dumas, 2015). Smolt treatment groups A and B were within the recommended levels, while treatment groups C and D were below. In comparison, post-smolt treatment groups A, B and D were well within the recommended levels, but treatment group C was just below. The findings suggests that the polychaetes cultivated on aquaculture sludge can serve as a source of essential amino acids and protein in future fish feed.

The total lipid content in the treatment groups given the most feed (treatment groups C and D) were well within the recommended levels for marine fish (NRC, 2011). The relative content [%] of DHA+EPA of the total fatty acids in the polychaetes ranged between 4-10% among all treatment groups within both treatments; all values were well within the recommended threshold value (>2.7%) for DHA+EPA of the total fatty acids in fish feed with respect to normal growth (Rosenlund et al., 2016). Despite of other studies also confirming normal growth rates at only 5% DHA+EPA, such low levels of DHA+EPA also resulted in higher occurrences of gill infections and other physiological disorders (Sissener et al., 2016). Anyhow, most of the treatment groups were very similar to the commercial "standard" of 8% DHA+EPA per 2016 (Sissener et al., 2016). Thus, indicating that polychaetes fed on aquaculture sludge are well suited as an alternative feed resource with respect to LC-PUFA in Atlantic salmon feed.

Vitamins are essential micronutrients required in the diets in relatively small amounts. Generally, vitamins are important for health, growth and functioning in animals (Jobling, 2012). Recommendations for content of vitamins [mg/kg] in feed for Atlantic salmon is well documented (Jobling, 2012, Hemre et al., 2016). Several of the essential vitamins for salmon were found in all treatment groups within both treatments. Two different fat-soluble vitamins were found in the polychaetes; vitamin D_2 (0.019-0.19 mg/kg) and vitamin E acetate (0.004-0.017 mg/kg). No specific recommendations were found for those fat-soluble vitamins in regard of content in fish feed. Furthermore, it is reported that fish generally utilize D_2 very poorly or not all at (Jobling, 2012). For the water-soluble vitamins, thamin, riboflavin, niacin, pantothenic acid and pyridoxine were found in all polychaetes. They are all essential for Atlantic salmon (Jobling, 2012). Absence of one or more of these essential amino acids can lead to loss of equilibrium, lens cataract, skin and fin lesions, anaemia and rapid breathing, respectively (Jobling, 2012, Lall and Tibbetts, 2009).

The content of thamin in the polychaetes (0.2-0.3 mg/kg) was considerably lower than the recommended 10 mg/kg in Atlantic salmon feed (Jobling, 2012). The content of niacin (45-54 mg/kg in the polychaetes), was just below the recommended minimum of 65 mg/kg (Hemre et al., 2016). The content of pyridoxine was very low and ranged between $5*10^{-5}$-$2.6*10^{-3}$ mg/kg, whereas the recommended content is 10 mg/kg for salmonids (Jobling, 2012, Hemre et al., 2016). Still, the riboflavin content in the polychaetes exceeded the minimal requirement of 10-12 mg/kg with values ranging from 32-56 mg/kg in all treatment groups (Hemre et al., 2016). Further, the content of pantothenic acid ranged between 13-25 ng/mg in the polychaetes and were somewhat within the range of the 22 mg/kg recommended lower limit (Hemre et al., 2016).

Polychaetes from all treatment groups were abundant in several essential microelements. In terms of microelements, the polychaetes were richest in iron (≈600 mg/kg), which is an essential element for Atlantic salmon, and heavily exceed the recommended dietary content of 60 mg/kg in feed (Lall and Milley, 2008). In addition, zinc (≈175 mg/kg) was another abundant essential mineral in the polychaetes that exceeded the recommended content (37-67 mg/kg) in Atlantic salmon feed (Lall and Milley, 2008). The manganese content in the polychaetes was doubled (≈20 mg/kg) in comparison to the recommended content (10 mg/kg) in feed (Lall and Milley, 2008). The content of copper in the polychaetes (≈20 mg/kg) was also relatively high in comparison to the recommended 5 mg/kg in feed for Atlantic salmon (Lall and Milley, 2008).

Additionally, the polychaetes were also abundant in terms of macroelements. The relative phosphorous content in the polychaetes (≈0.7%) equals the recommended minimal requirement of Atlantic salmon feed (Jobling, 2012). Furthermore, the relative magnesium content (≈0.4%) was higher than the lower recommended limit of 0.05% (Jobling, 2012). Potassium was the most abundant mineral in the polychaetes (≈1%), which is slightly over the recommended content of (0.7%) in the feed (Council, 1993).

On the contrary, the content of the toxic element arsenic in the polychaetes (≈7 mg/kg) was well below the upper threshold value of 25 mg/kg in fish feed (Lovdata, 2002). Furthermore, the content of cadmium in the polychaetes (≈0.1 mg/kg) was also below the threshold value of 2 mg/kg in fish feed (Lovdata, 2002). In addition, the threshold value for lead (≈10 mg/kg) in fish feed was not breached as the content in the polychaetes only reached 0.6 mg/kg. Lastly, the exact content of mercury was not determined as it was below the detection limit of the analysis (<0.7 mg/kg). Therefore, although it is reason to believe so, it is not conclusive if the content of mercury is below the threshold value (0.2 mg/kg) set for fish feed (Lovdata, 2002). Overall,

H. diversicolor appears to be a viable resource for feed in terms of high contents of essential elements and low content of toxic elements.

5 Conclusion

The result from this study demonstrates that the polychaete *H. diversicolor* can successfully be cultivated on different types and levels of aquaculture sludge. Looking at growth and survival, there were no significant differences between the corresponding treatment groups within the two sludges, proving that both sludges can work equally well. Additionally, increased levels of feed given to the polychaetes resulted in increased growth between the respective treatment groups. High survival rates and relatively high growth rates proves that the nutritional value in smolt and post-smolt sludge alone are sufficient to cover the basic needs of the polychaetes in those terms.

Feeding different levels of sludge (treatment groups) did not have a strong influence on the biochemical composition of the polychaetes regarding protein, amino acids, minerals, and vitamins. However, it was notable that the total lipid content in the polychaetes increased with increasing amounts of feed given to the different treatment groups, resulting in significant differences between several treatment groups and the initial polychaetes. Furthermore, the effect of the post-smolt sludge was even greater compared to the smolt sludge, which likely correlates with the significantly higher content of total lipid in the post-smolt sludge.

The polychaetes fed on aquaculture sludge contained high levels of LC-PUFA which include DHA+EPA, with adequate levels in terms of feed for Atlantic salmon. The content of omega-3 and omega-6 fatty acids increased throughout the trial, providing further indication of the polychaetes' abilities to incorporate and recycle valuable nutrients from its diets that otherwise would have been lost to the environment.

The polychaetes were proven to be rich in minerals which are essential for Atlantic salmon and well above the recommended lower levels for feed. *H. diversicolor* demonstrated low levels of toxic minerals which also were well below the given threshold values for fish feed resources. In this study, *H. diversicolor* was shown to contain several of the vitamins essential for healthy development, growth, and survival of Atlantic salmon. The polychaetes also contained all the essential amino acids for Atlantic salmon (tryptophan not measured) in sufficient quantities. Thus, the overall biochemical composition of the polychaetes reared on aquaculture sludge can be a well-rounded and sustainable feed resource for future fish feed, possibly replacing parts of other marine ingredients like fishmeal and fish oil.

6 Future Prospects

The polychaetes showed increased growth with increased feeding and the growth rates did not flatten out, indicating that the polychaetes might be susceptible to even higher feeding levels. Further studies in terms of determining the optimal feeding levels could be of interest to the project.

With increased feeding the amount of biofilm accumulating on top of the sediment, increased correspondingly. One tank even developed a red bacterial biofilm, leading to an early ending to that respective experimental tank because of lack of knowledge concerning possible consequences. Even so, all polychaetes were found alive. Thus, possible zoological challenges and effects of bacteria- and biofilm on water quality and the survival of polychaetes need to be examined further.

A large-scale production of polychaetes for feed will probably demand farming in higher densities than what was applied in this trial (≈137 individuals m^{-2}). However, earlier studies with doubled densities (275 individuals m^{-2}) resulted in considerable higher mortalities (Seekamp, 2017). On the contrary, survival rates of 93% with 250 individuals m^{-2} during a 60-day experiment has been reported, but with increasing mortality with correspondingly increasing density (Yousefi-Garakouei et al., 2018). Further studies of cultivation-densities should be of interest to the project.

The polychaetes in this experiment were all collected from wild populations. Development of cultivation methods for reproduction and broodstock would be essential for a sustainable production. Collection of polychaetes from wild populations could be considerably less sustainable in several regards, such as effects on the environment and populations dynamics (Cole et al., 2018). Additionally, considerable manpower and economy, as well as predictability regarding availability for the producers and the customers are other factors that need to be taken under consideration (Nernberg, 2019).

The experimental trial in this study lasted only 30 days and started with fully developed polychaetes. Further studies in terms of polychaete development, growth and survival from earlier life stages are crucial for the development of reliable large-scale cultivation systems, as one of the biggest challenges of polychaete mass production is the supply of offspring (Mandario, 2018).

Looking at the biochemical composition of the polychaetes, carbohydrates were not successfully determined. Thus, further analysis of carbohydrates is necessary to obtain a

complete and accurate description of the biochemical composition of the polychaete *H. diversicolor*. There were also some uncertainties in regard of the protein and amino acid content. Limitations to the method led to failure of measuring the tryptophan content due to hydrolysis (Mæhre et al., 2018). It is also not unlikely that parts of the other amino acids got destroyed during hydrolysis, leading to a slight underestimation of the total protein content of the polychaetes. With that in mind, further investigation of the protein content of the polychaetes should be of interest in future studies.

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Appendices

Appendix I

Table I: The table shows an overview of the amount of sludge [g] fed to the polychaetes in the smolt treatment every other day during the 30-day feeding experiment. The standard deviation for each group is included in the table. The amount of feed is based on the total nitrogen content of polychaetes in each tank, respectively. The amount of feed in the A treatment groups corresponds to 5% of the total nitrogen content in polychaetes. B corresponds to 10%, C =20% and D = 40%.

Smolt treatment		
	Feeding	
Tank #	**Aim [g]**	**Actual feed [g]: mean ± STD**
A1	1.24	1.23 ± 0.0295
A2	1.15	1.15 ± 0.0013
A3	1.05	1.05 ± 0.0022
A4	1.72	1.71 ± 0.0018
B1	2.11	2.11 ± 0.0024
B2	2.46	2.46 ± 0.0015
B3	1.96	1.96 ± 0.0019
B4	2.38	2.38 ± 0.0011
C1	4.22	4.22 ± 0.0018
C2	3.41	3.41 ± 0.0012
C3	4.87	4.87 ± 0.0024
C4	5.26	5.26 ± 0.0014
D1	6.94	6.94 ± 0.0021
D2	8.37	8.37 ± 0.0019
D3	7.98	7.98 ± 0.0025
D4	7.66	7.66 ± 0.0024

Table II: The table shows an overview of the amount of sludge [g] fed to the polychaetes in the post-smolt treatment every other day during the 30-day feeding experiment. The standard deviation for each group is included in the table. The amount of feed is based on the total nitrogen content of polychaetes in each tank, respectively. The amount of feed in the A treatment groups corresponds to 5% of the total nitrogen content in polychaetes. B corresponds to 10%, C =20% and D = 40%.

Post-smolt treatment		
	Feeding	
Tank #	**Aim [g]**	**Actual feed [g]: mean ± STD**
A1	0.57	0.57 ± 0.0015
A2	0.76	0.76 ± 0.0019
A3	0.92	0.92 ± 0.0026
A4	0.63	0.63 ± 0.0034
B1	1.44	1.44 ± 0.0025
B2	1.67	1.67 ± 0.0025
B3	1.45	1.45 ± 0.0022
B4	0.97	0.97 ± 0.0019
C1	4.07	4.07 ± 0.0020
C2	2.89	2.89 ± 0.0028
C3	2.34	2.34 ± 0.0018
C4	2.72	2.72 ± 0.0024
D1	4.51	4.51 ± 0.0012
D2	5.55	5.55 ± 0.0021
D3	5.27	5.27 ± 0.0024
D4	6.70	6.70 ± 0.0026

Appendix II

Table III: Estimated nitrogen content in the polychaetes. The numbers come from a previous similar experiment from Inka Seekamp (Seekamp, 2017). The nitrogen content in the DM (mg) of polychaetes is based on the estimated DW of polychaetes [mg] and the estimated nitrogen content of the DM (%).

	Polychaete	**Smolt sludge**	**PS-sludge**
Estimated WW (mg)	195		
Estimated DW content (%)	20	16	16
Estimated DW pr. polychaete (mg)	39		
Estimated % N content of DM	9.50	4	4
Estimated N content of DM (mg)	3.7		

Appendix III

The parts of the protein [%] covered by essential amino acids in the smolt and post-smolt treatment groups are presented in tables 8.4 and 8.5, respectively. The total content of essential amino acids in the protein decreases non-significantly in all treatment groups compared to the initial polychaetes ($P \geq 0.05$).

Table IV: Essential amino acids as percentage of the total protein content (Mean) in the initial polychaetes (I) and the different smolt treatment groups (A, B, C and D).

EAA	**Smolt treatment groups**				
[% of protein]	**I**	**A**	**B**	**C**	**D**
Arginine	6.5	6.3	6.2	6.4	5.6
Histidine	3.3	1.8	1.3	2.1	2.3
Isoleucine	4.6	4.6	5.2	4.6	4.9
Leucine	8.0	7.9	6.5	7.7	8.0
Lysine	8.0	6.0	5.8	6.0	6.0
Methionine	3.4	2.9	3.3	3.0	3.0
Phenylalanine	4.8	4.7	3.9	4.7	4.6
Threonine	3.5	4.5	4.2	3.4	3.4
Tryptophan	-	-	-	-	-
Valine	4.5	5.8	4.8	5.9	6.0
Total EAA in protein [%]	46.6	44.4	41.2	43.7	43.8

Table V: Essential amino acids as percentage of the total protein content (Mean) in the initial polychaetes (I) and the different post-smolt treatment groups (A, B, C and D).

EAA	**Post-smolt treatment groups**				
[% of protein]	**I**	**A**	**B**	**C**	**D**
Arginine	6.5	6.1	6.3	5.3	5.8
Histidine	3.3	1.8	2.2	2.2	2.1
Isoleucine	4.6	4.5	4.4	4.7	4.7
Leucine	8.0	7.8	7.7	7.9	7.8
Lysine	8.0	6.1	6.1	6.1	7.7
Methionine	3.4	2.8	2.9	3.0	2.9
Phenylalanine	4.8	4.5	4.7	4.8	4.5
Threonine	3.5	4.4	3.7	3.7	4.2
Tryptophan	-	-	-	-	-
Valine	4.5	5.6	5.6	5.7	5.8
Total EAA in protein [%]	46.6	43.6	43.3	43.3	45.6

Appendix IV

Table VI: The mass spectrometer was operated in positive ionization mode for both water- and fat-soluble vitamins, and the mass transitions and their respective collision energies and fragmentor voltages.

Compound Name	Precursor Ion	Product Ion	Fragmentor	Collision Energy
Vitamin B1 (Thiamine)	266.1	122.1	50	17
Vitamin B2 (Riboflavin)	377.2	243.1	170	25
Vitamin B2 (Riboflavin)	377.2	172.1	100	22
Vitamin B3 Acid (Nicotinic acid)	124	106	110	17
Vitamin B3 Acid (Nicotinic acid)	124	80.1	110	25
Vitamin B3 Acid (Nicotinic acid)	124	78.1	110	25
Vitamin B3 Amide (Nicotinamide)	123.1	96.1	110	17
Vitamin B3 Amide (Nicotinamide)	123.1	80.1	110	25
Vitamin B3 Amide (Nicotinamide)	123.1	78.1	110	29
Vitamin B5 (D-Pantothenic acid)	220.1	202.1	50	9
Vitamin B5 (D-Pantothenic acid)	220.1	98	50	25
Vitamin B5 (D-Pantothenic acid)	220.1	90.1	50	13
Vitamin B6 (Pyridoxine)	170.1	152.1	50	13
Vitamin B6 (Pyridoxine)	170.1	134.1	50	25
Vitamin B6 (Pyridoxine)	170.1	77.1	50	35
Vitamin B7 (Biotin)	245.1	227.1	110	13
Vitamin B7 (Biotin)	245.1	123	110	33
Vitamin B7 (Biotin)	245.1	97	110	35

Vitamin B9 (Folic acid)	442.2	295.1	50	17
Vitamin B9 (Folic acid)	442.2	193.2	50	13
Vitamin B9 (Folic acid)	442.2	120.1	50	35
Vitamin B12 (Cyanocobalamin)	1355.6	1209.5	215	46
Vitamin B12 (Cyanocobalamin)	678.6	359.1	150	20
Vitamin B12 (Cyanocobalamin)	678.6	147.1	150	40
Vitamin C (Ascorbic acid)	177	141	70	5
Vitamin C (ascorbic acid)	177	95	95	10
Vitamin C (ascorbic acid)	177	85	95	14
Vitamin A (Retinol)	269.2	81.1	110	17
Vitamin A (Retinol)	269.2	93.1	110	25
Vitamin A (Retinol)	269.2	95.1	110	9
Vitamin D2 (Ergocalciferol)	397.4	83.1	50	25
Vitamin D2 (Ergocalciferol)	397.4	107.1	50	33
Vitamin D2 (Ergocalciferol)	397.4	159.2	50	29
Vitamin D3 (Cholecalciferol)	385.4	159.1	50	29
Vitamin D3 (Cholecalciferol)	385.4	259.2	50	13
Vitamin D3 (Cholecalciferol)	385.4	367.4	50	9
Vitamin E (a-Tocopherol)	431.4	97.1	50	25
Vitamin E (a-Tocopherol)	431.4	111.1	50	21
Vitamin E (a-Tocopherol)	431.4	165.1	50	21

Vitamin E Acetate (Tocopheryl acetate)	490.4	165.1	50	35
Vitamin E Acetate (Tocopheryl acetate)	490.4	207.1	50	25
Vitamin E Acetate (Tocopheryl acetate)	490.4	473.4	50	9
Vitamin E Succinate (Tocopherol-succinate)	548.4	165.1	50	35
Vitamin E Succinate (Tocopherol-succinate)	548.4	265.1	50	25
Vitamin E Succinate (Tocopherol-succinate)	548.4	531.4	50	13
Vitamin K1 (Phylloquinone)	451.4	185.15	100	30
Vitamin K1 (Phylloquinone)	451.4	187.1	170	35
Vitamin K2 (Menaquinone)	445.3	81.1	110	35
Vitamin K2 (Menaquinone)	445.3	95.1	110	35
Vitamin K2 (Menaquinone)	445.3	187.1	110	25
Vitamin K3 (Menadione)	173.1	77.1	120	42
Vitamin K3 (Menadione)	173.1	105	120	22
Vitamin K3 (Menadione)	173.1	51.1	120	50

Appendix V

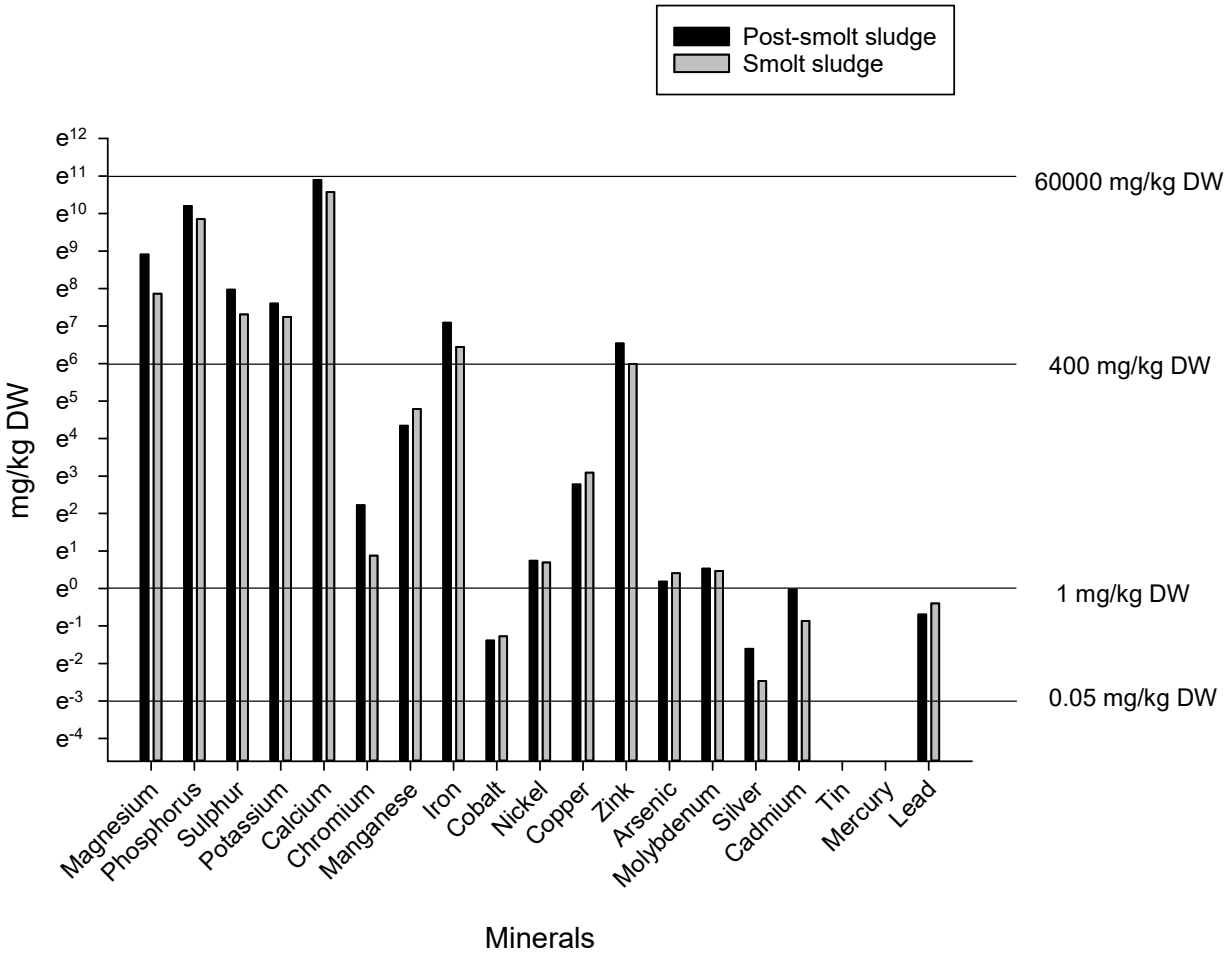


Figure I: Overview of the content of different minerals in the smolt and post-smolt sludge diets. Because of the huge variation in content, the Y-axis is made of a logarithmic scale.

Appendix VI

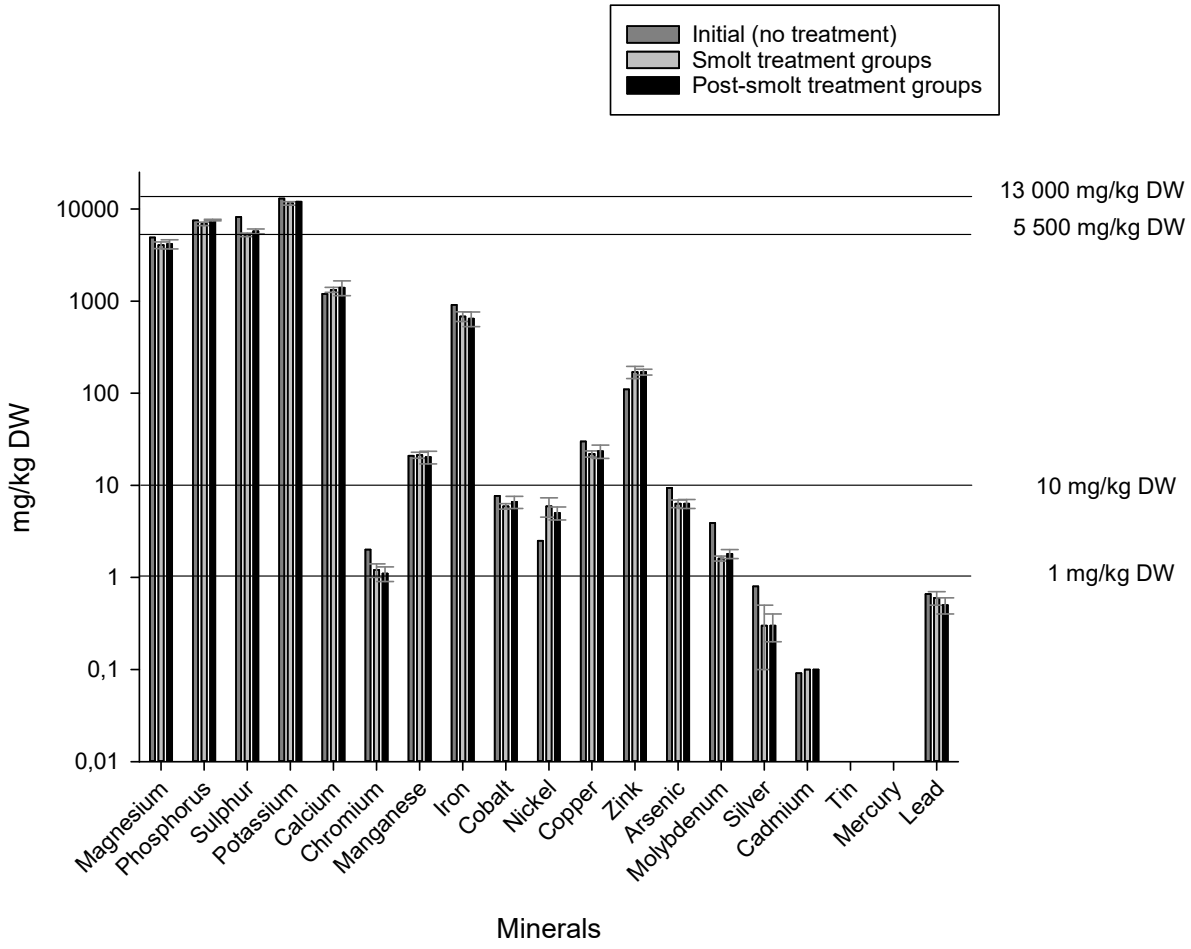
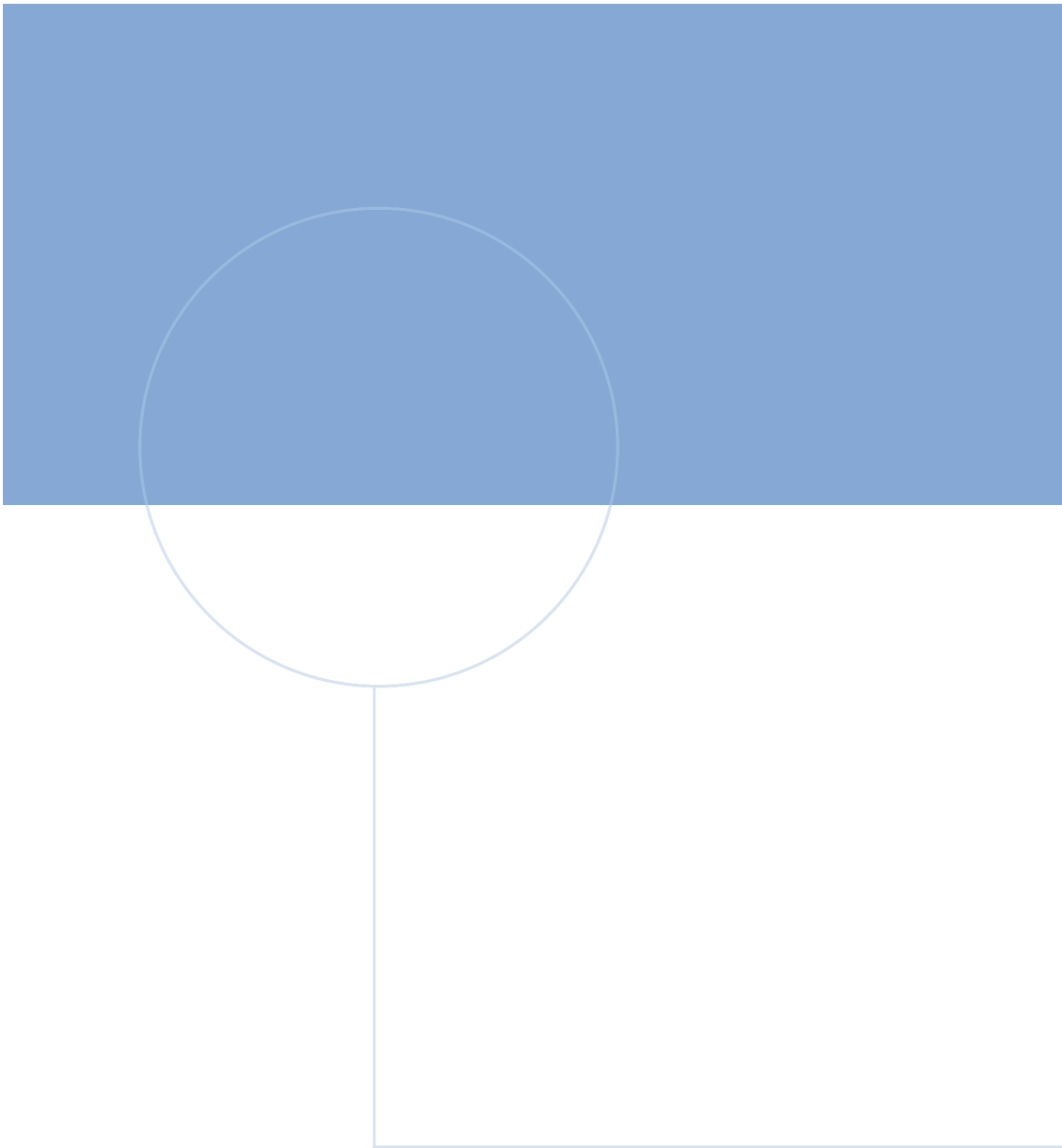


Figure II: Mineral content in initial polychaetes as well as pooled samples from all smolt treatment groups (Mean±SD) (A, B, C and D) and post-smolt treatment groups (A, B, C and D). The mineral content is presented in mg/kg DW. Because of large differences between in amounts between the minerals, the Y-axis is based on a logarithmic scale.



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