Master's thesis

NTNU
Norwegian University of Science and Technology
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Department of Biology

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Nutrient (C, N and P) budgets of *Hediste diversicolor* (OF Müller, 1776) fed salmon smolt sludge

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

Norway is the largest producer of Atlantic salmon (*Salmo salar*) in the world, with a yearly production of approximately 1.3 million tons. With aquaculture of this scale, a large amount of nutrient pollution occurs in the form of spilled feed and excreted nutrients. As this can have adverse effects on aquatic systems, there is an environmental and economic incentive for recycling said nutrients into valuable products. One proposed solution is integrated multi-trophic aquaculture (IMTA), where lower trophic level organisms are cultivated using the waste products of the main species as feed. The Nereid polychaete *Hediste diversicolor* has been shown to utilize aquaculture sludge as a feed source and is therefore a novel candidate for this purpose.

This thesis aimed to investigate the bioremediation potential of *H. diversicolor* fed salmon smolt sludge, by establishing an individual nutrient budget containing the three macronutrients carbon (C), nitrogen (N) and phosphorus (P). This was based on a feeding experiment that investigated the feeding activity, nutrient assimilation, feces production and metabolism at two feeding levels, namely 5% and 40% of the polychaetes total N content. The oxygen consumption of the polychaetes were measured to observe the impact of feed concentration and feeding state on the respiratory rate.

The polychaetes ingested a larger amount of feed in the high feeding level. However, the ingestion rate decreased with an increasing feed availability, with 64 ± 20% and 22 ± 12% of the feed added being ingested for the low and high feeding treatment, respectively. A significantly higher assimilation rate was seen in the high feeding treatment (72-85%) than in the low (50-80%) for all three macronutrients, with N being the most efficiently assimilated and P being the least. For the low feeding treatment, significantly different assimilation rates were seen for all three nutrients, but no difference was found in the high. Due to the low ingestion rate, the assimilation of the total amount of nutrients added was found to be much lower. The total assimilation was higher in the low treatment (28-53%) than in the high (16-21%). The largest feces production was seen in the low treatment for P with 40% of the ingested nutrients. Only P showed a significant difference between the low and high treatment when investigating percentage feces production, but a similar trend was also seen in C and N. The respiratory rate of the polychaete did not differ between the two feeding levels, nor the feeding state of the polychaetes. A rate of -0.04 µmol O_2 $mgDW^{-1}$ h^{-1} was established, giving a CO_2-production of 0.84 µg CO_2 $mgDW^{-1}$ h^{-1}.

It is concluded that *Hediste diversicolor* utilizes salmon smolt sludge as a feed source. The polychaetes efficiently assimilates ingested nutrients, with the lowest ability for assimilation found in P and the highest in N. However, the assimilation ability of the polychaete is strongly limited by its low ingestion rate at higher feeding levels. Thus, *H. diversicolor* has the potential of recycling nutrient from aquaculture sludge, making it a novel candidate for IMTA-applications in the future.

Keywords:

Hediste diversicolor, polychaete, bioremediation, aquaculture sludge, IMTA, nutrient budgets

Sammendrag

Norge er verdens største produsent av atlantisk laks (*Salmo salar)*, med en årlig produksjon på omtrent 1.3 millioner tonn. Med matproduksjon på denne skalaen oppstår det store mengder med næringsforurensning i form av overforing og avføring. Da dette kan ha skadelige innvirkninger på akvatiske miljøer, er det et miljømessig og økonomisk insentiv for resirkulering av disse næringsstoffene til verdifulle produkter. En foreslått løsning er integrert flertrofisk akvakultur (IMTA), hvor organismer fra lavere trofiske nivåer kultiveres med avfall fra hovedproduksjonen som fôrkilde. Den nereide børstemarken *Hediste diversicolor* utnytter slam fra lakseoppdrett som en fôrkilde, og er derfor en lovende kandidat for dette formålet.

Denne oppgaven hadde som mål å undersøke bioremedieringspotensialet til *H. diversicolor* fôret på slam fra laksesmolt, ved å etablere individuelle næringsbudsjetter for de tre næringsstoffene karbon (C), nitrogen (N) og fosfor (P). Dette var basert på et foringsforsøk med søkelys på spiseaktivitet, næringsopptak, avføring og metabolisme på to ulike fôrnivåer, 5 og 40% av markens totale nitrogeninnhold. Oksygenkonsumet til børstemarken ble målt for å undersøke innvirkningen av fôrkonsentrasjon og foringsstatus på respirasjonsraten.

Børstemarkene inntok en større mengde næring ved det høye fôringsnivået. Inntaksraten sank derimot med økende fôrtilgjengelighet, med 64 ± 20 og 22 ± 12% inntak av fôret i henholdsvis det lave og høye fôrnivået. En signifikant høyere opptaksrate ble observert i det høye fôrnivået (72-85%) enn i det lave (50-78%) for alle tre næringsstoffene, hvor opptaket var mest effektivt av N, og lavest av P. For det lave fôrnivået ble det funnet en signifikant forskjell mellom alle tre næringsstoffene, men ingen forskjell ble observert i det høye. Grunnet den lave inntaksraten var opptaket av den totale mengden næring tilsatt observert å være mye lavere. Det totale opptaket var høyere i det lave fôrnivået (28-53%) enn i det høye (16-21%). Den største produksjonen av avføringen ble observert i det lave nivået for P med 40% av den inntatte næringen. For prosent avføring produsert var det høyere produksjon i det lave fôrnivået enn det høye for alle tre næringsstoffene, hvor kun P hadde en signifikant forskjell. Respirasjonsraten til børstemarken var ikke forskjellig mellom de to fôrnivåene eller fôringsstatusene. En rate på -0.04 μmol O_2 $mgTV^{-1}$ t^{-1} ble funnet, som ble omregnet til en CO_2-produksjon på 0.84 μg CO_2 $mgTV^{-1}$ t^{-1}.

Det konkluderes med at *Hediste diversicolor* kan benytte seg av slam fra laksesmolt som sin eneste fôrkilde. Børstemarken tar effektivt opp næringsstoffer den har spist, med den laveste opptaksraten observert i P og den høyeste i N. Opptaksevnen til børstemarken er derimot sterkt begrenset av inntaksraten, spesielt ved høyere fôrnivåer. Dermed har *H. diversicolor* potensialet til å resirkulere akvakulturslam, og er en lovende kandidat for IMTA-applikasjoner i fremtiden.

Nøkkelord:

Hediste diversicolor, børstemark, bioremediering, akvakulturslam, IMTA, næringsbudsjett

Abbreviations

C	Carbon
DIN	Dissolved inorganic nitrogen
DM	Dry matter content
DW	Dry weight
IMTA	Integrated multi-trophic aquaculture
N	Nitrogen
P	Phosphorus
RAS	Recirculating aquaculture system
SD	Standard deviation
WW	Wet weight

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1 Introduction

1.1 Challenges in Norwegian aquaculture

A rapidly increasing world population and an estimated doubled demand for food by 2050, shows that marine food production and aquaculture will play a major role in supplying the necessary protein in the years to come (FAO, 2020). While the need for an increase in marine food production is prominent, the aquaculture industry has multiple environmental challenges that needs to be overcome for it to grow to the required scale in a sustainable fashion (Costello et al., 2020; Grefsrud et al., 2021), the most prominent being sea lice infestations, escaped farmed salmon, and nutrient pollution (Olaussen, 2018).

With a yearly production of approximately 1.3 million tons of Atlantic salmon, the Norwegian salmon farming industry produces a large amount of nutrient discharge (Olaussen, 2018), mainly in the form of excretory products and feed spills (Carroll et al., 2003). In a study done by Wang et al. (2012), annual discharge of 404.000, 50.400 and 9400 tons of carbon (C), nitrogen (N) and phosphorus (P), respectively was estimated. Carbon and nitrogen is mainly lost as dissolved inorganic pollutants, while phosphorus mostly ends up as particulate waste (Wang et al., 2012). This makes aquaculture the biggest contributor to nutrient pollution in Norway (Grefsrud et al., 2021). The open net cage is still the most used cultivation structure in norwegian aquaculture, as these utilize the suited water quality in the fjords. However, open cages do not easily allow for the collection of feces and feed spill, leading to particulate waste settling on the benthic environment below and around the cage, and dissolved nutrients leaking out into the water (Brooks & Mahnken, 2003).

An increasing interest in producing salmon in closed facilities on land has been seen for many years, mainly to avoid salmon lice and escaped salmon (Lekang et al., 2016), the lack of suitable farming locations along the Norwegian coast (Lekang et al., 2016; Olaussen, 2018), limited number of sea-production concessions (Bjørndal & Tusvik, 2019), and the higher degree of control one can achieve in these facilities (Attramadal et al., 2012; Liu et al., 2016), Within a closed, land-based system, such as recirculating aquaculture systems (RAS), efficient solid waste removal is crucial to prevent deterioration of the water quality (Van Rijn, 2013). Intensive collection and treatments of these solid wastes results in sludge, mainly consisting of excrements and feed spillage, and thus containing a high amount of nutrients (Del Campo et al., 2010). The nutritional composition and physical properties of salmon sludge varies, especially in relation to the feed-to-feces ratio and the dry matter content, which is usually between 10-15% (Aas & Åsgård, 2017). The current areas of use for smolt sludge is biogas, fertilizer, and more recently as feed for secondary biomass production (Del Campo et al., 2010; Wang et al., 2019a).

Feed makes up a large fraction of the carbon footprint in Norwegian salmon aquaculture, resulting in an increased focus on creating more sustainable and efficient feed formulas (Olsen, 2011). To reduce the harvesting pressure on feed ingredients, the marine components of salmon feed has been decreased, mainly substituted by land-farmed plant proteins, such as rapeseed, soy and wheat (Salin et al., 2018). While the environmental footprint of plant proteins is smaller than that of marine oil and fish meal, the transport of especially soy represents a large problem in CO_2 emissions (Hognes et al., 2011). An increased fraction of plant proteins in feed has also been shown to present adverse health effects for the fish (Krogdahl et al., 2003), and a lower digestibility than that of marine

proteins, further resulting in increased nutrient discharge (Lazzari & Baldisserotto, 2008) and a lower growth (Egerton et al., 2020; Pratoomyot et al., 2010). New sources of sustainable protein is therefore being investigated, such as insects (Iaconisi et al., 2018), fishery byproducts (Luthada-Raswiswi et al., 2021), and lower trophic level marine organisms (Olsen, 2011).

1.2 Integrated Multi-Trophic Aquaculture (IMTA) and blue circular economy

For a largescale industry to be sustainable, it is necessary with green solutions in the entire production chain (Govindan & Soleimani, 2017). Achieving a closed-loop system where all material- and energy flows, including waste, is utilized at a high level both improves the financial value of said waste products, while lowering the environmental impacts significantly (Campanati et al., 2022; Saavedra et al., 2018). This concept builds on circular economy, where reusing and recycling all materials in the production is a main principle (Kirchherr et al., 2017). For the salmon farming industry, some of the main resources for recycling are the sludge and wastewater from land-based facilities (Grefsrud et al., 2021), and residual raw materials from fish (Šližytė et al., 2017).

With large amounts of uncollected and unutilized nutrients, such as nitrogen and phosphorus, being dissolved into the water or settling on the sea floor, Norwegian salmon farming is losing large amounts of potential value that can be converted into valuable biomass for future economic value (Chávez-Crooker & Obreque-Contreras, 2010; Fossberg et al., 2018; Knowler et al., 2020). Due to these nutrient's role in plant growth, an excessive discharge could also result in harmful algal blooms and eutrophication, which could damage the environment (Folke et al., 1994; Quinones et al., 2019), as well as the farms themselves (Brown et al., 2020). The biogeochemical flow of N and P is considered one of the nine planetary boundaries (Steffen et al., 2015), with both already being disturbed due to the ever increasing food production where these are important in agricultural fertilizer (Slootweg, 2020). With phosphorus being a scarce resource that is currently non-sustainably exploited, there are concerns on the effects this has on future food security (Cordell et al., 2009). Therefore, more efficient use of both N and P, including nutrient recycling, can help with mitigating the imbalance in these nutrient pathways (Brownlie et al., 2021; Peñuelas et al., 2013).

Integrated multi-trophic aquaculture (IMTA) is one potential method of reducing the nutrient pollution seen in Norwegian salmon farming (Ellis & Tiller, 2019). IMTA is the on-site cultivation of lower trophic level organisms using waste-resources of a higher trophic level organism as the feed resource, and thus recycling said waste products (Troell et al., 2009). Some suggested IMTA-candidates for salmon farming are mussels (MacDonald et al., 2011; Sarà et al., 2009), algae (Fossberg et al., 2018; Wang et al., 2014), and polychaetes (Bischoff et al., 2009; Hu et al., 2021; Jerónimo et al., 2020; Marques et al., 2018). These organisms have shown improved growth and development through the ingestion and absorption of the dissolved and particulate waste molecules produced by the salmon from excrements and feed spills, further creating valuable biomass (Buck et al., 2018; Knowler et al., 2020).

1.3 *Hediste diversicolor* (OF Müller, 1776)

Hediste diversicolor is an Annelid polychaete in the family Nereidae. It inhabits the intertidal zones on both sides of the North Atlantic, ranging on the European coast from Northern Europe to the Mediterranean in the south (Scaps, 2002), due to its large tolerance of changes in salinity, temperature (Neuhoff, 1979) and oxygen content (Fritzsche & Von Oertzen, 1995; Vismann, 1990). Through creating and ventilating burrows, it plays an important role in the bioturbation of the sediment (Banta et al., 1999; Hedman et al., 2011), as well as increasing the seabed-water interface (Davey, 1994).

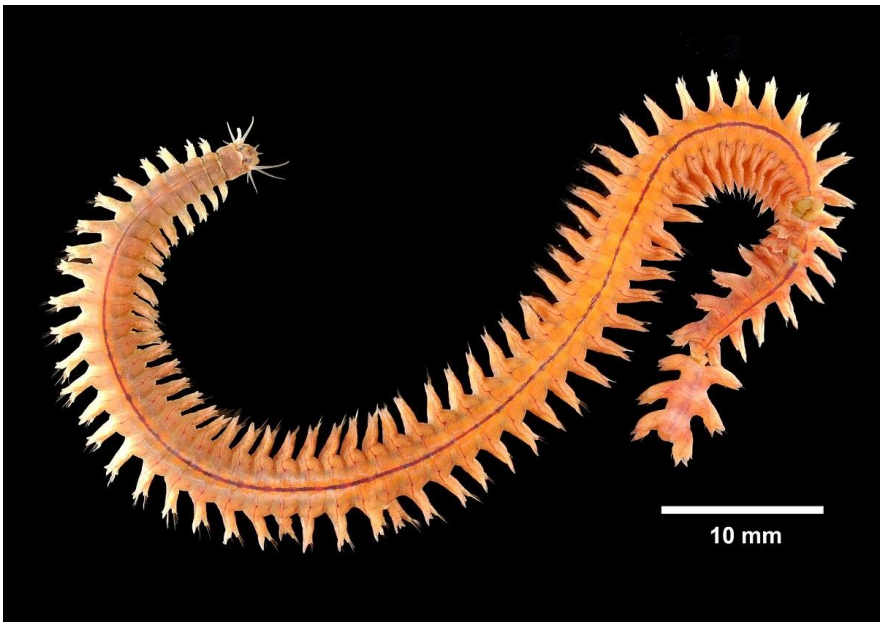

Figure 1.1: *Hediste diversicolor*. Image: Lazo-Wasem (2019)

H. diversicolor is a highly opportunistic feeder, exhibiting a variety of different feeding strategies (Scaps, 2002), such as filter-feeding (Riisgard, 1991), deposit feeding (Aberson et al., 2016), active predation (Rönn et al., 1988), and bacteriolytic activity (Lucas & Bertru, 1997). Thus, this species plays an important part in the removal and conversion of dead organic matter in the estuaries. The preferred feeding strategy has been shown to differ in relation to the environmental conditions and types of available feed resources (Costa et al., 2006). When the food availability is partially suspended *H. diversicolor* exhibits suspension-feeding behavior, trapping small and suspended particles in a net of mucus for easier ingestion (Costa et al., 2006; Vedel & Riisgård, 1993).

H. diversicolor has been used as bait for sports fishing for a long time, mainly being harvested through digging in intertidal zones around the world (Brown, 1993). Concerns about the intensity of said digging has led to cultivation studies being conducted, to relieve pressure of the natural populations of Nereid polychaetes and co-living organisms (Olive, 1999; Watson et al., 2007). Polychaetes can commonly be found as part of the benthic fauna under aquaculture cages, using the particulate waste settling on the seafloor as feed (Tomassetti & Porrello, 2005). Through various feeding experiments, they have proven to grow well on salmon sludge as the sole feed source, making them a potential candidate for the recycling of sludge, with the future use as a marine protein constituent of fish feed

in mind (Seekamp, 2017; Wang et al., 2019b). Studies have also shown that this species has a favorable nutrient and amino acid composition for use in aquaculture feed (Dahl, 2021; Santos et al., 2016). Their bacteriolytic abilities allow for the utilization of bacterial bound nutrients in the sludge, further increasing their value and potential (Wang et al., 2019a). All these factors, combined with long experience in lab rearing of *H. diversicolor* (Scaps, 2002), makes for a promising candidate for use in bioremediation of aquaculture waste.

1.4 Aims

The goal behind this thesis was to further investigate the bioremediation potential of *Hediste diversicolor* fed on smolt sludge from land-based salmon farming, building on previous studies conducted in WP 2 of the NFR project POLYCHAETE (#280836) (Dahl, 2021; Kristensen, 2021; Seekamp, 2017; Wang et al., 2019b). The main aim was to establish a nutrient budget for individuals of *H. diversicolor* fed salmon smolt sludge. As studies conducted on this topic up until now have been performed on groups of polychaetes, this thesis will give an insight into the variation in feeding activity and amount on an individual level. The polychaetes were fed two different feeding levels, one low and one high, to further investigate how nutrient assimilation and ingestion differs with an increasing feed level.

The main aim was reached through two specific sub-aims as following:

1. Establish a nutrient budget for individual polychaetes fed smolt sludge. The ingestion and assimilation rates of the three elemental macronutrients (C, N and P), at two different feeding levels, as well as production of feces and dissolved nutrients were investigated.

2. Investigate the respiration rate of individual polychaetes under two feeding treatments, as well as the impact of resting- and feeding state on the respiration.

The hypotheses tested were as follows:

1. *H. diversicolor* readily accepts smolt sludge as a feed source.

2. The amount of assimilated nutrients and feces production increases with increasing feed level.

3. The respiratory rate of *H. diversicolor* increases with feed level.

This thesis was part of the 'POLYCHAETE' project, short for 'Cultivation of Polychaeta as raw material for feed' (Project number: 280836). POLYCHAETE is a collaborative research project financed by the Research Council of Norway, assessing the potential of cultivating Nereid polychaetes for possible use as fish feed, and for bioremediation of nutrient waste from salmon farming. The experiments were carried out within the framework of the research infrastructure Norwegian Center for Plankton Technology (245937/F50) hosted by SINTEF Ocean and NTNU. The thesis falls under WP 2, investigating the cultivation biology of *Hediste diversicolor*, and more specifically T2.1 looking at feed uptake.

2 Materials and methods

2.1 Preparation work prior to the main experiments

The methods used in this experiment were based on Honda and Kikuchi (2002), Wang et al. (2019b), and experiences made with polychaete handling and feeding behavior during the testing period running up to the feeding experiment. Honda and Kikuchi (2002) which ran a similar experiment looking at nitrogen budgets in individuals of the Nereid *Perinereis nuntia* fed flounder feces*,* served as the main inspiration for the setup of the systems used. Wang et al. (2019b) gave valuable insight to the methods used working with smolt sludge and *Hediste diversicolor*. Scaps (2002) and Kristensen (1983a) were used to understand the behavior of *H. diversicolor*, and the use of plastic tubes compared to sediment.

The polychaetes were obtained by digging in Leangbukta (63°26'23.2"N 10°28'27.7"E), in Trondheim municipality, Norway. Only polychaetes of an appropriate size range were selected, and other species of polychaetes were discarded. After digging, the polychaetes were kept in a flow-through system (Figure 2.1a) with sediment (Robust, Sand box-sand, 0-4mm grain size) at 12°C and an 18:6 dark-light cycle. They were fed biweekly with commercial fish feed (Skretting Gemma Diamond) during the testing period and smolt sludge (FW smolt sludge, Belsvik RAS facility) during the experimental periods. Dead polychaetes were removed from the tanks on observation, and the tank was checked regularly in regard to environmental variables and buildup of excess feed.

Figure 2.1: a) Flow through tank used before the experiment; b) Flow-through tank used during the experiment. Image: Håkon Sæther

The smolt sludge used in the experiment was obtained from Lerøy's juvenile fish facility in Belsvik, Norway. After obtaining, the smolt sludge cannisters were kept at 4°C before being treated. The smolt sludge was thoroughly mixed and centrifuged twice for 5 minutes on 5000rpm, at 12°C (Thermo Scientific, Heraeus, Multifuge X3R), before being frozen in zip lock bags until use in the experiment. A subsample of the treated smolt sludge was freeze-dried overnight and analyzed for carbon, nitrogen, and phosphorus content. Since this analysis could not be executed before the experiments were finished, data from previous smolt sludge analysis from Belsvik was used to determine the feeding amount for the polychaetes (Dahl, 2021; Kristensen, 2021). Three subsamples of the smolt sludge

were freeze-dried (Labconco, Freezone) to determine the dry-matter content for feed calculations.

During a polychaete reproduction and pilot feeding experiment attempted in spring 2021, several methods were developed. Among these were knowledge about the Termaks climate cabinet (Series KB8000L), which was later used during the testing period, as well as familiarity handling adult polychaetes.

2.2 Pilot-testing period

To establish a working method and experimental design for the experiment, preliminary tests were conducted from mid-August through October. Several factors were taken into consideration such as temperature, light regime, environment and how the polychaetes were handled. The main goal was to ensure a stress-free environment for the polychaetas, and thus also consistent feeding as this was crucial for the main part of the experiment. The polychaetes were kept in 150 mL seawater in a climate cabinet (Termaks, Series KB8000L) at a constant temperature of 12°C and a 16:8h light-dark rhythm.

2.2.1 Feeding

At the start of the testing period, it was ensured that the polychaetes had good ingestion activity. Fish feed pellets were used as the feed resource as this was proven to be readily accepted as feed by the polychaetes. To begin with, the feeding amount, frequency and handling were investigated. Different amounts of feed were given to the polychaetes, to see how much feed was ingested before they stopped eating. Towards the middle of the testing period, fish pellets were exchanged with smolt sludge, as this was the food-source that was to be used. Again, the effect of differing feeding levels and handling was observed.

2.2.2 Environment

No sediment was used in the experiment since collection of all feces and leftover feed was crucial for analysis. Using sediments would have allowed the polychaetes to bring the smolt sludge down into their burrows, making it hard to collect uneaten sludge (Scaps, 2002). Thus, several prototypes were tested, with one attempting to mimic the burrow, as shown in Figure 2.2. This consisted of a black plate, with a plastic tube bent in a U-shape, glued to the beaker. The part of the beaker under the plate was then covered in aluminum foil to create a dark environment. This prototype was however discarded quickly, as the polychaetes were not easily willing to inhabit the tube.

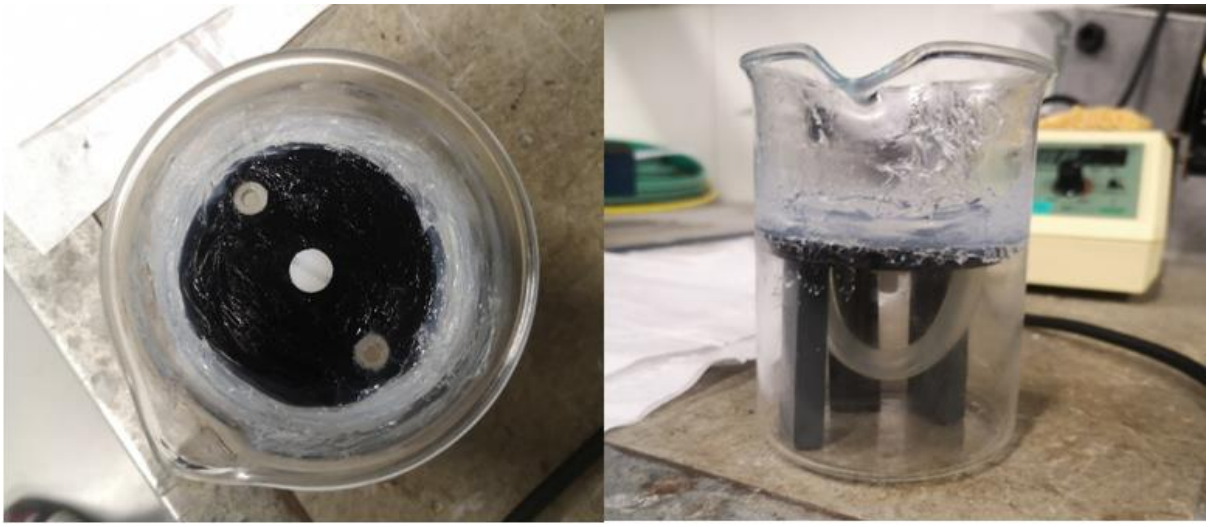

Figure 2.2: The prototype for an artificial burrow shown from the top and the side, consisting of a lightproof plate as 'sediment' and a silicone tube as an artificial burrow. Image: Håkon Sæther

It was decided to use normal 250mL beakers filled with 150mL of seawater for the polychaetes' housing. As completely empty beakers led to excessive mucus production, irregular feeding behavior and mucus entanglement, a variety of different tubes were tested in the beakers as artificial burrows. Parameters such as material (plastic, glass, silicone), inner diameter, and transparency were tested. In the end, a lightproof polyurethane plastic tube with an inner diameter of 3mm, cut to a length of approximately 5cm was chosen as the most suited based on polychaete preference.

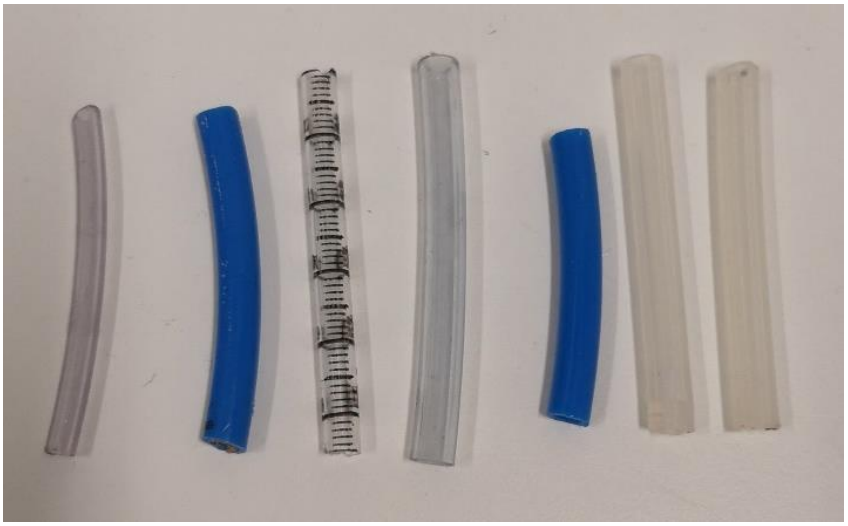

Figure 2.3: A selection of tubes that were tested for burrow preference during the testing period, in varying size, material and transparency. Image: Håkon Sæther

2.2.3 Handling

Ensuring suited behavior and ingestion activity, as well as avoiding stress levels of the polychaetes, was the main focus in order to obtain consistent results in the feeding experiments. Thus, their behavior was observed during the testing period, to establish the procedures to be used. The feeding activity of the polychaetes were observed when disturbed with activity, and when they were left alone in the climate cabinet for the duration of their feeding. The method for exchanging water and supplying feed was also developed to be as non-disturbing as possible for the polychaetes, so that they would continue normal behavior quickly after these routines were conducted.

2.3 Feeding experiment

2.3.1 Experiment

The main experiment was conducted between 13th and 22nd of November 2022. The environmental conditions used were a constant temperature of 12°C, with a light-dark rhythm of 16:8. The setup can be seen in Figure 2.4.
One week before the experiment, polychaetes were moved to a separate holding tank (Figure 2.1b) where they were fed smolt sludge to be accustomed to the diet used in the experiment. They were fed every second day, as well as right before the main experiment would begin.
All polychaetes were weighed prior to the experiment (Sartorius Handy H120). They were dabbed with a paper towel to remove adherent water and added to a petri dish on the scale. The twenty individuals that were closest in weight were selected to be used in the experiment and moved to beakers of filtered seawater at 12°C to defecate overnight, before being weighed again with an empty gut. The plastic tubes used as artificial burrows were added to the beakers, so that the polychaetes could find the tubes during the three-day acclimatization period, where they were not fed. All beakers were covered in plastic wrap to prevent evaporation.

Two feeding treatments were used in this experiment, based on the master theses of Kristensen (2021) and Dahl (2021), making up the highest and the lowest of their feeding concentrations, namely 5% and 40% of the polychaetes' total nitrogen content. The distribution of feeding treatments between the polychaetes were randomly assigned using Excel. The amount of feed given to each polychaete was calculated using Equation 1 (Kristensen, 2021).

$$Amount\ fed = \frac{(WW * DM_p * N_p) * FL}{N_s * DM_s} \qquad \text{(Equation 1)}$$

With,

- WW: Wet weight of the given polychaete [mg]
- DM_p: Mean dry matter content of the polychaetes [%]
- N_p: Nitrogen content of the polychaetes [%]
- FL: The assigned feeding level for the given polychaete [% N]
- N_s: Nitrogen content of the sludge [%]
- DM_s: Dry matter content of the sludge [%]

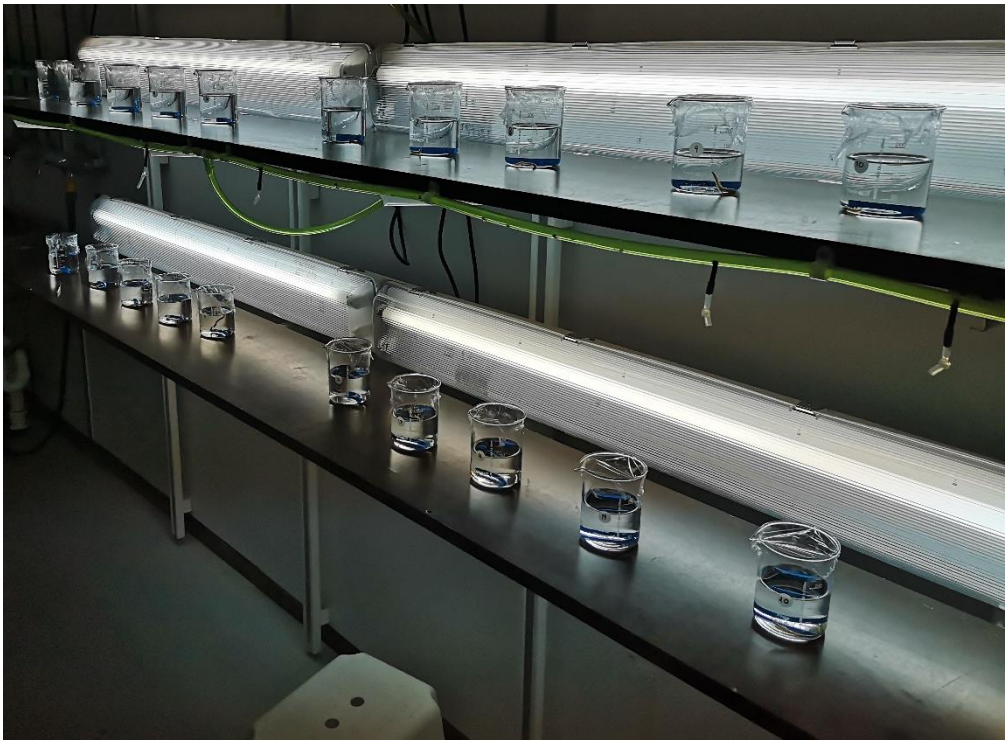

Figure 2.4: The main feeding experiment setup, with the 20 beakers evenly distributed along the top and bottom shelf. Image: Håkon Sæther

A simplified sketch of the experiment is shown in Figure 2.5. At the start of the feeding experiment, twenty new 250mL beakers were filled with 150mL of filtered seawater (0.22µm). The calculated amounts of frozen smolt sludge, from Equation 1, were then measured using a microscale (Mettler Toledo XA204) and added to the beakers. It was noted whether polychaetes were in their tubes before the feeding began. Together with their tubes, polychaetes were transferred from their original beaker to the beakers containing smolt sludge and left undisturbed for 3 h to feed. Adherent feces on the tubes were removed.

Subsequently, the polychaetes were transferred to clean beakers containing seawater. The seawater from the feed beakers containing uneaten smolt sludge was homogenized using a stick blender (Bosch, MSM6B300) and frozen at -20°C. The polychaetes were then left in the new beakers for 36 hours to allow for defecation, after which they were transferred to clean beakers containing sludge, thus starting a new feeding period. In total, the polychaetes were fed three times. The content of the beakers containing seawater and feces were then homogenized and frozen until sampling.

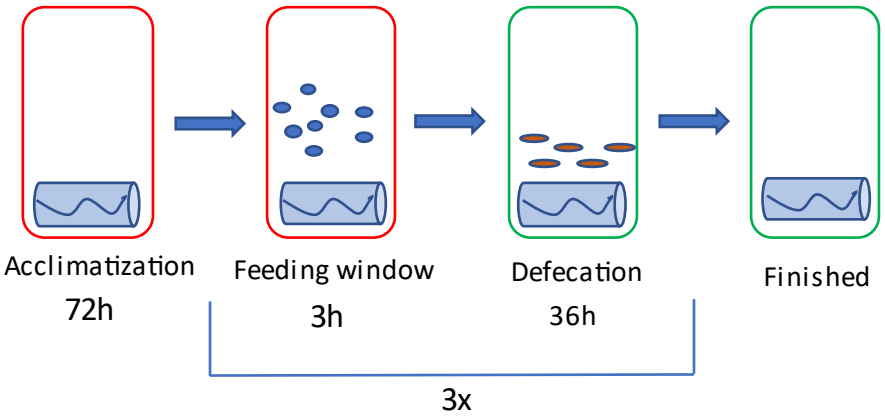

Figure 2.5: Simplified illustration of the feeding experiment. The polychaetes were acclimated for 72 hours, before being fed for three hours. 36 hours of defecation occurs in a clean beaker before they are fed again, for a total of three times. Sampling occurs in the feeding- and defecation beaker after the time period shown in the figure.

2.3.1.1 Controls

Controls were conducted to measure the instant leakage from the smolt sludge, the leakage over three hours and the metabolic compounds produced by the polychaetes. In this way, the amount of dissolved nutrients due to natural leakage could be subtracted from the amount of dissolved nutrients resulting from the activity of the polychaetes. The feeding concentrations were calculated using the mean wet weight of the polychaetes, being 230 mg.

For controls, there were three parameters to cover. The first was leakage over time, the second was precondition and instant leakage right after the smolt sludge was added as a T_0-sample, and the last was the metabolic compounds produced by the polychaetes during the three-hour feeding window. For the first two, four replicates were conducted for both feeding levels, meaning a total of 16 beakers, and the metabolic control consisted of four replicates, giving a total of 20 control samples. The leakage-controls were conducted by adding smolt sludge into 150mL of filtrated seawater with no polychaetes and taking a sample after 3h. For the metabolic compounds, the polychaetes were added to beakers with 150mL of filtrated seawater and left for 3h with no feed.
The samples from the controls were homogenized and frozen at -20°C until sampling.

2.3.2 Sampling

For the budget, both particulate and dissolved carbon (C), nitrogen (N) and phosphorus (P) were investigated. Sampling was done to get an overview of the particulate and dissolved nutrient content and ratio. To separate the particulate- from the dissolved nutrients, filtration of all samples was conducted. Precombusted GF/C 47mm diameter glass fiber filters (Whatman, GF/C, 0.45µm mesh size, CAT No-1822-025) were used.

For the filtration of the water samples from the main feeding experiment, a vacuum pump system that retains the filtrate was used (Figure 2.6). Two pumps were used (Scanvac

VacSafe 15) which could filter four samples at the time. The samples were moved to the refrigerator the day before filtration for thawing.
Four samples were sampled at the time and homogenized to ensure even nutrient distribution on the filters. The homogenized samples were then filtrated through the system, before the filter was placed in sterile petri dishes and the filtrate was poured into sterile centrifuge tubes. Both filters and filtrate were frozen until chemical analysis.

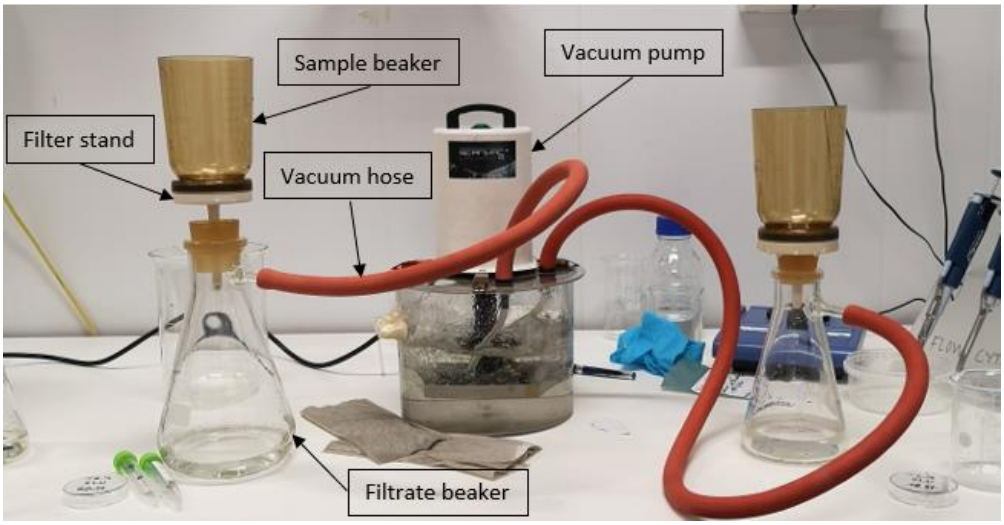

Figure 2.6: One of two identical filtration setups used for the particle sampling and filtrate collection from the main feeding experiment. Image: Håkon Sæther

2.3.3 Chemical analysis

All chemical analyses were conducted at Trondheim Biological Station (TBS) in collaboration with lab technician Siv Anina Etter. In total, three different analyses were conducted, which were (A) particulate C and N, (B) particulate P and (C) dissolved inorganic N and P.

A. Particulate C and N

As the filter from the main feeding experiment was to be used for both particulate C & N, and particulate P, two 13mm diameter subsamples of the filter were punched out. All punching was conducted on a copper plate to ensure no contamination would take place. One subsample was placed in a labeled polyethylene bottle for particulate phosphorus (Section B) analysis, while the other was packed tightly in a tin capsule for C and N analysis.

The well-plates were then placed in a heating cabinet at 60°C overnight for drying. After all samples were packed in tin capsules and dried, the analysis was conducted in an element analyzer (Vario EL cube, ELEMTAR).

The total amount of particulate C and N was calculated using equation 2.

$$Particulate\ nutrients = Nutrients_{Measured} * \frac{Filter_{Total}}{Filter_{Subsample}} \quad \text{(Equation 2)}$$

With,

Measured nutrients: Raw measured value from the nutrient analysis [µg]
$Filter_{Total}$: Measured area of nutrient coverage on the full GF/C-filter
$Filter_{Subsample}$: Area of the subsample, 13mm diameter

B. Particulate phosphorus

To convert the particulate phosphorus from the filter-subsample into dissolved phosphate (PO_4^{3-}), a chemical process was used. 10mL of distilled water, 0.1ml of 4M H_2SO_4, and 2mL of potassium persulfate was added to the polyethylene bottle. The bottles were closed and subsequently autoclaved for 30mins at 120°C (CertoClav EL Autoclav).

After autoclaving, the samples were filtered (VWR syringe filter, 25mm, 0.45µm mesh size) into 4,5mL plastic tubes and placed in an O.I Analytical Autosampler (Model 3360) for analyzing the amount of dissolved PO_4^{3-}, as shown in Figure 2.8.

After the first run of the samples, most measurements were far outside the standard curve (0-250µg L^{-1}), and the samples were therefore diluted in three different amounts. All samples inside the standard curve where left undiluted, samples between 250-1000µg L^{-1} were diluted 1:4, and samples above 1000µg L^{-1} were diluted 1:10. After another run, it became clear that the machine had underestimated the highest samples, which were then diluted again with a ratio of 1:3.

The total amount of particulate phosphorus was calculated using Equation 3.

$$Particulate\ P\ [\mu g] = P_{Measured} * V_{Solution} * D * F_{Proportion} \quad \text{(Equation 3)}$$

With,

$P_{Measured}$: Output from the nutrient analyzer [µg L^{-1}]
$V_{Solution}$: Volume of the chemical solution; 12.1mL.
D: The respective dilution coefficient for each sample.
$F_{Proportion}$: The filter proportion as shown in Equation 2.

Figure 2.7: a) Optifix dispenser used for chemical dosing of distilled water and oxidizing agent; b) row of all polyethylene bottles containing filters for particulate phosphorus. Image: Håkon Sæther

C. Dissolved nitrogen and phosphorus

The filtrate water from the sampling were taken out of the freezer and thawed in the refrigerator overnight. To ensure homogenous distribution of nutrients, the tubes were shaken and 3-4mL were added to tubes. The samples were then analyzed in an O.I Analytical autosampler (Model 3360) (Figure 2.8) where the water was analyzed for dissolved nitrate (NO_3^-) and dissolved phosphate (PO_4^{3-}).

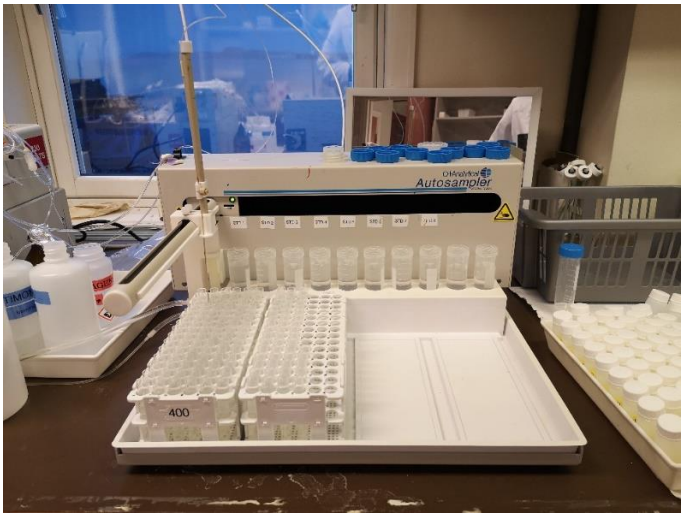

Figure 2.8: O.I. Analytical autosampler (Model 3360) used for the analysis of dissolved nitrogen and phosphorus, as well as particulate phosphorus. Image: Håkon Sæther

After the first run, most of the samples were too high for the standard curve (0-50µg L^{-1}) and had to be diluted and analyzed again to be able to make an accurate estimation of the amount. Four different dilution regimes were used. Samples between 0-60µg L^{-1} were not diluted, between 60-200µg L^{-1} were diluted 1:4, between 200-400µg L^{-1} were diluted 1:10

and all samples above 400µg L^{-1} were diluted 1:20. The dilutant was artificial seawater with 30% NaCl.

The total amount of dissolved N and P was calculated using Equation 4.

$$Dissolved\ Nutrient\ [\mu g] = \frac{C\ [\mu g\ L^{-1}] * D}{V} \qquad \text{(Equation 4)}$$

With,

C: Measured output from the nutrient analysis [µg L^{-1}]
D: The respective dilution coefficient for each sample
V: Total volume of the sample (150mL)

2.4 Respiration experiment

To get information about dissolved carbon, needed for the budget equation, a respiration experiment on the polychaetes was conducted. The impact of feed level and resting state was also investigated.
Twenty polychaetes were acclimatized in clean beakers filled with 150mL of filtered seawater for three days, during which weighing occurred for feed calculations using Equation 1. The acclimatization was done using a climate cabinet (Termaks, Series KB8000L) with the environmental conditions used in the main feeding experiment.

Respiration measurements were done using a respirometer (Unisense, MicroRespiration System) with two 40mL respiration chambers equipped with a magnet stirrer to ensure circulation of the water. The chambers and the probes were submerged in water at 12 degrees, cooled using a lab cooler (Julabo, 300F). The setup is shown in Figure 2.9.

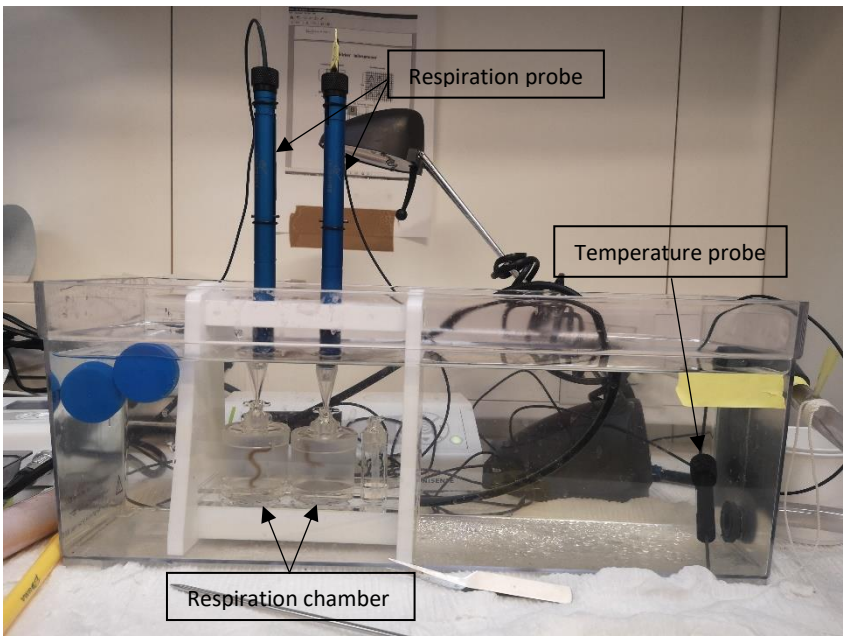

Figure 2.9: Unisense MicroRespiration System used for measuring respiration of the polychaetes. Labels showing the different parts making up the system. Image: Håkon Sæther

Two polychaetes were fed smolt sludge at a time, with a 30-minute interval and were allowed to feed for three hours, after which the oxygen consumption was measured for 30 minutes. Due to varying swimming behavior and occasional air bubbles, the most suited timeframe of at least 5 minutes was chosen as the final measurement to exclude unrepresentative values.
From the oxygen decrease, the amount of dissolved C produced through respiration was calculated using Equation 5.

$$Dissolved\ C\ [\mu g\ mgDW^{-1}\ ] = \frac{R*\ M_C*t*V}{DW} \qquad \text{(Equation 5)}$$

With,

R: Respirometer output showing decrease in oxygen [µmol O_2 L^{-1} h^{-1}]
M_C: Molar mass of C [g mol^{-1} → µg μmol^{-1}]; 12.01
t: Number of hours spent respiring
V: Volume of the respiration chambers [L]
DW: Dry weight of the polychaete [mg]

2.5 Calculations

2.5.1 Nutrient budget

For measuring the pathways of the nutrients added to the system, a budget approach was used. The budget was separated into two parts, where the first found the fraction of ingested nutrients, while the other investigated the dissolved, fecal, and assimilated fraction of the ingested nutrients. Thus, the budget was calculated using Equation 6 and Equation 7.

$$\mathrm{N_{Ingested}} = \mathrm{N_{Added}} - \mathrm{N_{Uneaten}} - \mathrm{N_{Dissolved}} \qquad \text{(Equation 6)}$$

With,

N_{Added}: The total amount added of the respective nutrient [µg], based on the nutrient content of the sludge.
$N_{Uneaten}$: The particulate nutrient amount measured after the three-hour feeding window.
$N_{Dissolved}$: The dissolved fraction of the nutrients measured in the water of the feeding beaker.

$$\mathrm{N_{Assimilated}} = \mathrm{N_{Ingested}} - \mathrm{N_{Feces}} - \mathrm{N_{Dissolved}} \quad \text{(Equation 7)}$$

With,

$N_{Ingested}$: The total amount of nutrients ingested [µg $mgDW^{-1}$]
N_{Feces}: The particulate amount of nutrients in the defecation beaker [µg $mgDW^{-1}$].
$N_{Dissolved}$: The dissolved fraction of the nutrients measured in the water of the defecation beaker [µg $mgDW^{-1}$].

2.5.2 Dry matter content

The dry matter content of the sludge and polychaetes were measured to get an accurate representation of the nitrogen fed to each of the polychaetes. The dry weight of the polychaetes allowed for an accurate biomass to standardize the raw data, making them more comparable. The dry matter content of the polychaetes and sludge was calculated in the same way using Equation 8.

$$DM\ [\%] = \frac{WW}{DW} * 100 \quad \text{(Equation 8)}$$

With,

WW: Wet weight of the sludge or polychaete measured before freeze drying [g]
DW: Dry weight of the sludge or polychaete as measured after freeze drying [g]

2.5.3 Actual amount fed

As the nutrient analysis of the sludge was delayed until after the feeding experiment was conducted, previous sludge data from the same juvenile fish facility was used. As there are large fluctuations in the nutrient composition of sludge (Aas & Åsgård, 2017), a calculation was done to get an accurate representation of the actual amount fed to each polychaete. This calculation was done using Equation 9.

$$Actual\ amount\ fed\ [\%] = \frac{N_p}{N_S * (F_S * DM_S)} * 100 \quad \text{(Equation 9)}$$

With,

N_P: Nitrogen content of the polychaete [µg]
N_S: Nitrogen content of the sludge [µg N $mgDW_S^{-1}$]
F_S: Amount of sludge added to the respective feeding beaker [mgWW]
DM_S: The dry matter content of the sludge [%], calculated in Equation 8.

2.6 Method testing and quality assurance

After observing non-coherent results that indicated loss of nutrients throughout the main feeding experiment, testing was done using the control samples. As the controls containing sludge had no contact with polychaetes there should be no nutrient uptake, thus no loss of nutrients. Comparing the nutrients added with the total amounts of dissolved and particulate nutrients, an estimation of the loss percentage was done using Equation 10.

$$\%\ loss\ of\ nutrients = \frac{N_{Added} - (N_{Particulate} + N_{Dissolved})}{N_{Added}} \times 100 \qquad \text{(Equation 10)}$$

With,

N_{added}: The total amount of nutrients added.
$N_{Particulate}$: The measured value of particulate sludge in the sample.
$N_{Dissolved}$: The measure value of dissolved nutrients in the sample.

As the result from Equation 10 indicated loss in the process, several methods were tested to predict the loss of nutrients from the raw data.
Several linear regression models were investigated to accurately predict the loss seen, with the most accurate (R^2 = 0,94) being modeled on the amounts of nutrients added and the amount of nutrients measured.
In the end the mean percentage loss found using Equation 10 was used as a fixed loss rate for each feeding treatment and macronutrient, respectively. For adjusting the data, Equation 11 was used.

$$Nutrients_{Adj} = \frac{Nutrients_{Initial}}{1 - \%\ loss} \qquad \text{(Equation 11)}$$

With,

$Nutrients_{Initial}$: The measured amount of particulate nutrients.
% Loss: The loss percentage found using Equation 10

2.7 Statistics

For the processing of all raw data collected in this thesis, Microsoft Excel v16.0 was used. The processed data was analyzed and visually presented using the statistical software SigmaPlot v14.0.

For the budget calculation, removal of incoherent data samples was done if the row of data satisfied one of the three criteria below:

1. The percentage assimilation was negative.
2. The percentage assimilation was above 100%.
3. The amount of uneaten nutrients was higher than the amount of nutrients added, resulting in a negative ingestion amount.

When calculating means and standard deviations for the polychaete samples, the sample consisted of all polychaetes in the respective treatment per day (n=10). For the calculations of feces production, the samples consisted of the ten polychaetes across all three feeding days (n=30). When the data was not normally distributed, a median was used instead of the mean. All statistical analysis were conducted with a significance level of $p < 0.05$, and confidence intervals of $\alpha=0.95$.

All tests for normal distribution of the data were done using Shapiro-Wilk's test of normality, while the equal variance testing was done using a Brown-Forsythe test. For all testing for significant differences between treatments, nutrients and feeding days, one-way ANOVAs were conducted if the assumptions of normality and equal variance was met. If the assumptions for normality of the data set and equal variation were not met, a Kruskal-Wallis H test was conducted in its place. For the comparison between different treatment groups within the same test, a post-hoc Holm Sidak's test was conducted if the assumption of equal sample size was met. If the sample sizes differed between the treatments assessed, a Dunn's post-hoc test was conducted instead.
To test for correlations between the nutrient fractions of the budget, polychaete biomass, and tube inhabitance, a Pearson Correlation test was used if the assumption of normally distributed data was met. If the data compared did not meet the assumption of normality, a Spearman correlations test was conducted in its place.

3 Results

3.1 Sludge and actual feeding levels

The dry matter (DM) content of the sludge was 29.8±0.9% measured by weight before and after freeze drying. Through an elemental analysis of the smolt sludge, the macronutrient composition and elemental ratio of the sludge was found and shown in Table 3.1.

As mentioned in section 2.5.3, the data used for calculating the feeding amount was based on old data from the same facility (Dahl, 2021; Kristensen, 2021), and the actual feeding in the experiment was therefore calculated using Equation 9. For the low feeding treatment, a feed level of 7.0 ± 1.6% was found, where the target was 5%. For the high feeding treatment with a target level of 40% N, 53.5 ± 7.8% was found. Data for polychaete biomass and calculation of actual feeding levels are found in Appendix A, Table 6.1 and Table 6.2 respectively.

3.2 Ingestion rate of nutrients

When considering the bioremediation potential of the polychaetes, a principal factor is the feeding activity. How much of the added feed is ingested by the polychaetes is important for determining the most suited feed concentration, to again reduce build-up of leftover feed over time.

The same trends in ingestion could be seen for C, N and P, and no significant difference (Kruskal-Wallis H test) was seen between the three. Similar ingestion rate [%] and amount [µg gDW^{-1}] were found (Kruskal-Wallis H test) for the three feeding days. However, a difference (Kruskal-Wallis H test) was found between the low and high feeding treatment, with the low treatment having the highest ingestion rate (Table 3.2). When considering the amount of nutrients ingested, the high feeding treatment had a larger ingestion (Kruskal-Wallis H test) than the low treatment (Figure 3.1-Figure 3.3).

Testing for correlation (Spearman Correlation test) between the ingestion rate and weight of the polychaetes, a positive relationship was found for all three macronutrients, suggesting an increasing ingestion rate with increasing polychaete biomass. No correlation (Spearman Correlation test) was found between tube inhabitance (Appendix A, Table 6.3) and ingestion rate.

Table 3.1: The nutritional composition (n=6) of the salmon smolt sludge used during the two experiments. Here shown in content [µg $mgDM^{-1}$], and the elemental ratios between the three macro nutrients in question, C, N and P.

Content	C	N	P
	459.8±15.7	62.3±6.5	24.7±2.8
Elemental ratio (%)	**C:N**	**C:P**	**N:P**
	7.5±0.7	18.6±2.0	2.6±0.5

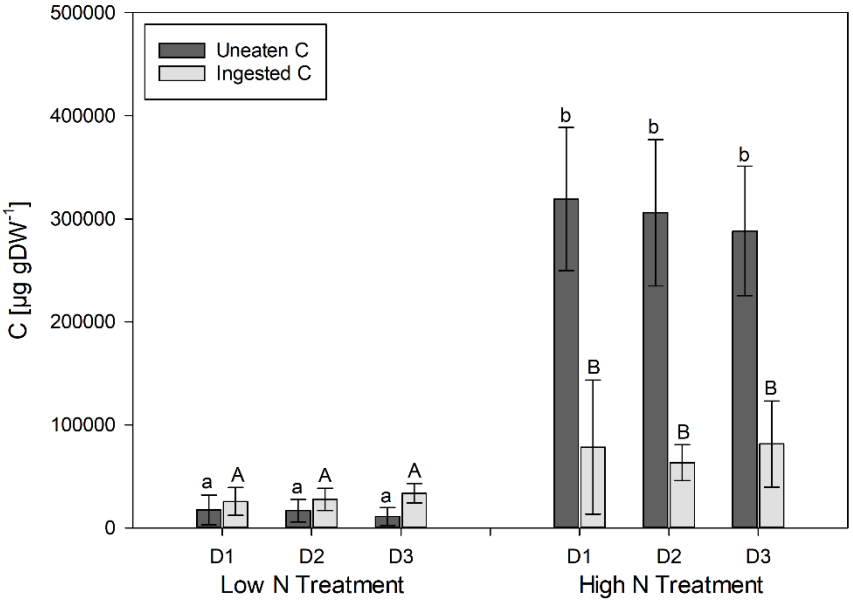

Figure 3.1: Mean±SD (n=10) uneaten and ingested fraction of the C added [µg gDW^{-1}], for the three feeding days (D1-3) and the two feeding treatments. Unequal superscripts within and between the two treatments indicates significant difference.

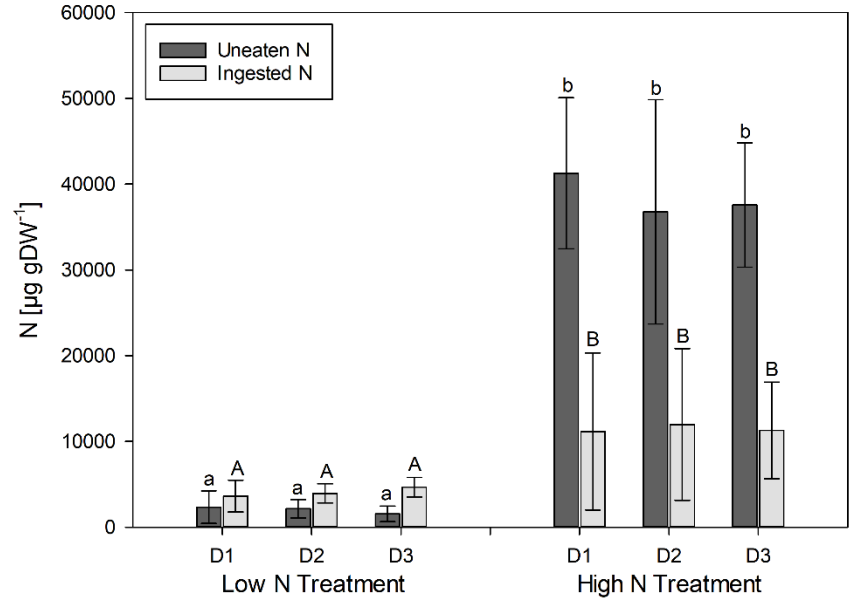

Figure 3.2: Mean±SD (n=10) uneaten and ingested fraction of the N added [µg gDW^{-1}], for the three feeding days (D1-3) and the two feeding treatments. Unequal superscripts within and between the two treatments indicates significant difference.

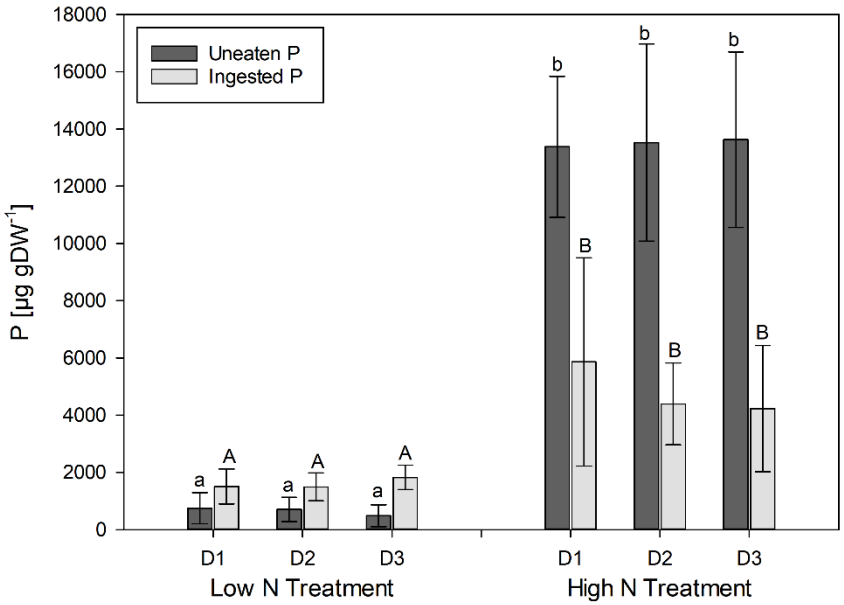

Figure 3.3: Mean±SD (n=10) uneaten and ingested fraction of the P added [µg gDW^{-1}], for the three feeding days (D1-3) and the two feeding treatments. Unequal superscripts within and between the two treatments indicates significant difference.

3.3 Dissolved nutrients and respiration

3.3.1 Dissolved Nutrients

The leakage of N and P from the smolt sludge to the water in the beaker instantly (T_0-controls) and over three hours (Leakage controls) was investigated. As weight measurements of the polychaetes were done after the Leakage controls were conducted, the amount of sludge added to the beakers differed between the two controls. The sludge added and concentrations of dissolved N and P, is shown in Appendix B, Table 7.1.

No correlation (Pearson Correlation test) was found between the amount of smolt sludge added and the dissolved N (µg NO_3^- L^{-1}) in the water, suggesting no leakage occurred. No significant difference (one-way ANOVA) was found between the high and low feeding treatment for the complete data set. Based on the measurements of dissolved N, and the

Table 3.2: The rate of ingestion [Mean±SD] for the three macronutrients, where the ingestion rate is the percentage ingested of the total added of the respective macronutrient (n=10). No significant difference was found between the three feeding days. Significant difference between the Low and High feeding treatment shown through unequal superscripts of the mean value.

Ingestion [%]	% C Ingested [Mean±SD]		% N Ingested [Mean±SD]		% P Ingested [Mean±SD]	
Treatment	Low	High	Low	High	Low	High
Feeding day 1	57.5±27.1	18.8±14.1	61.1±29.1	20.3±15.0	59.0±23.5	26.4±12.7
Feeding day 2	58.9±21.4	17.7±6.2	64.8±17.4	25.4±19.5	59.5±18.4	22.6±8.3
Feeding day 3	70.9±17.7	22.0±11.3	74.8±14.8	22.9±10.9	71.3±8.3	21.7±11.2
Mean	62.4±22.1[a]	19.5±10.5[b]	66.9±20.4[a]	22.9±15.2[b]	63.3±16.7[a]	23.6±10.7[b]

lack of significant difference and correlation, an assumption was made that the measured dissolved NO_3^- stems from the natural background concentration of the intake water. Thus, the dissolved N was not accounted for in the nutrient budget.

A strong correlation was found between the amount of sludge added and the concentration of dissolved P in the water (Pearson Correlation test). There was also a significant difference (one-way ANOVA) between the low and high feeding treatments. As no significant difference was found between the Leakage and T_0-controls, both were included in calculating the average dissolved P for the low and high feeding treatment, being 22.6 ± 5.2 µg and 72.8 ± 10.7 µg respectively.

3.3.2 Respiration

Three aims were investigated for respiration. The first being the effect of the feeding level on respiratory rate, the second being the effect of the resting state, and lastly finding a baseline respiration for calculating the amount of CO_2 in the budget calculation.

On the first day of the respiration experiment, the polychaetes were fed and was therefore considered non-resting. No significant difference (one-way ANOVA) in respiratory rate were found between the two treatments. Nor was a significant difference (one-way ANOVA) found between the two treatments the day after, when the polychaetes were in the resting state. A Spearman correlation test was done to look at the relationship between the dry weight of the polychaetes and the respiration amount [µmol $mgDW^{-1}$], but no correlation was found.

Thus, no significant relationships were found for the resting state, nor the feeding level, suggesting that neither of these had any impact on the oxygen consumption of the polychaetes. The baseline respiratory rate for the polychaetes were calculated to be -0.040 µmol O_2 $mgDW^{-1}$ h^{-1}. The respiration for the two treatments and states can be seen in Figure 3.4.

For calculating the CO_2-production, and therefore the amount of dissolved C in the water, Equation 5 was used. A production of 0.84µg CO_2 $mgDW^{-1}$ h^{-1} was calculated. As no significant difference was found between the two feeding levels, the same dissolved carbon production was used for both treatments in the budget calculations.

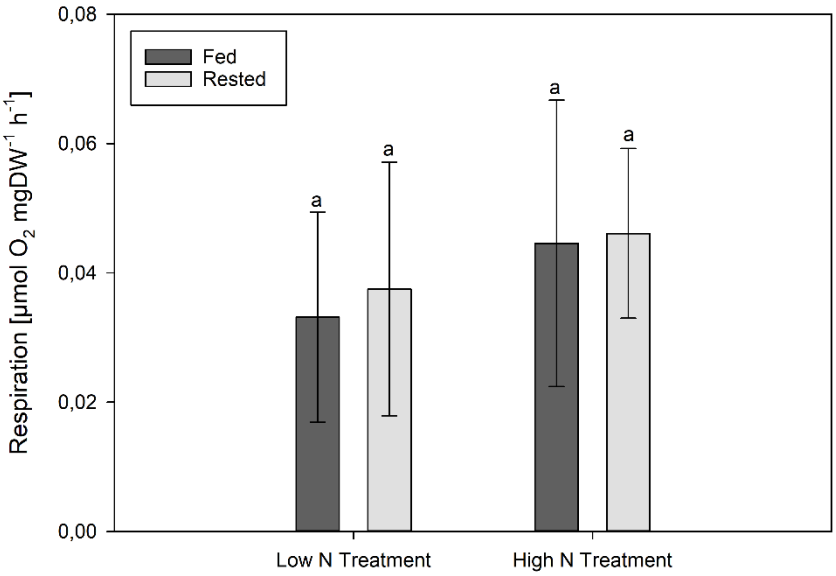

Figure 3.4: Respiration rate [µmol O_2 $mgDW^{-1}$ h^{-1}] for the two feeding levels, and the two states (n=10). No significant difference was found between the feeding levels nor the two states, as indicated by the equal superscripts.

3.4 Feces production and composition

Similar feces production was seen within each treatment between the three feeding days, suggesting that the amount of feces produced did not change during the experimental period. The nutrient concentration of the feces samples can be seen in Figure 3.5-Figure 3.7. When comparing the mean feces production of all polychaetes for each treatment, a trend of a higher amount of nutrients in the high treatment can be seen. However, significant difference (Kruskal-Wallis H test) between the high and low treatment is only found in N and C, but not for P.

Investigating the percentage feces produced compared to the total amount of nutrients ingested, a significant difference (N and P; Kruskall-Wallis H test, C; one-way ANOVA) between the two treatments was found for P, but not for C and N. For P, the median % feces produced was 40.0 for the low treatment and 16.7 for the high, compared to 24.8 and 19.8 for C, and 22.3 and 15.1 for N. Thus, suggesting a lower rate of assimilation of P in the low treatment compared to the two other nutrients. Comparing the percentage feces production for the three nutrients, only N and P showed a significant difference (Kruskal-Wallis H test) in feces production to eachother, with C and P being near significantly different (p=0.09). The percentage feces production can be seen in Figure 3.8.

A negative correlation (Spearman Correlation test) was found between feces production and the biomass of the polychaete, with the only significant being for N. No correlation (Pearson Correlation test) was found for the percentage feces produced of total ingested amount. A positive relationship (Pearson Correlation test) was found for percentage feces produced for C and P, but not for N. When investigating tube inhabitance and amount of feces produced, a significant positive correlation was found for C and N, but not for P.

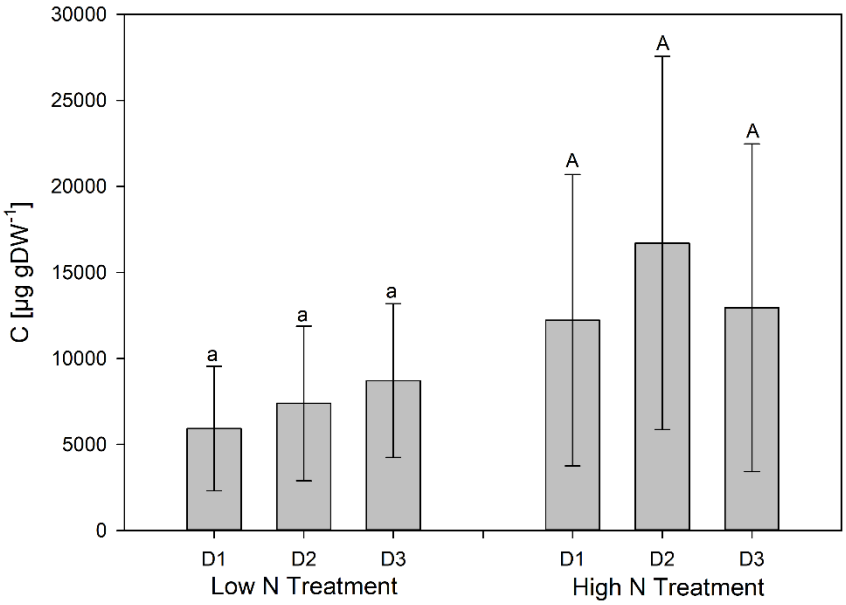

Figure 3.5: Mean±SD (n=10) amount [µg gDW^{-1}] of C measured in the feces between the three feeding days and the two feeding treatments. Significant difference is indicated by unequal superscripts within and between treatments.

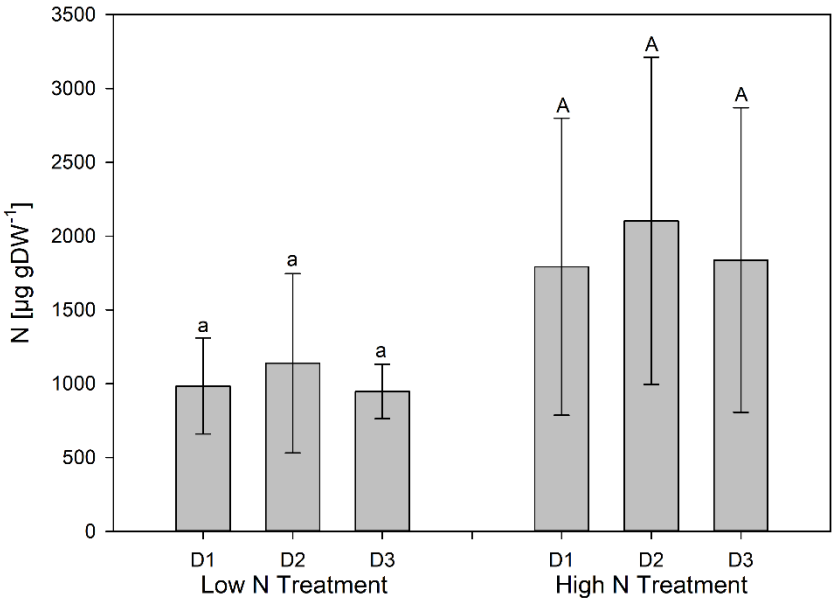

Figure 3.6: Mean±SD (n=10) amount [µg gDW^{-1}] of N measured in the feces between the three feeding days and the two feeding treatments. Significant difference is indicated by unequal superscripts within and between treatments.

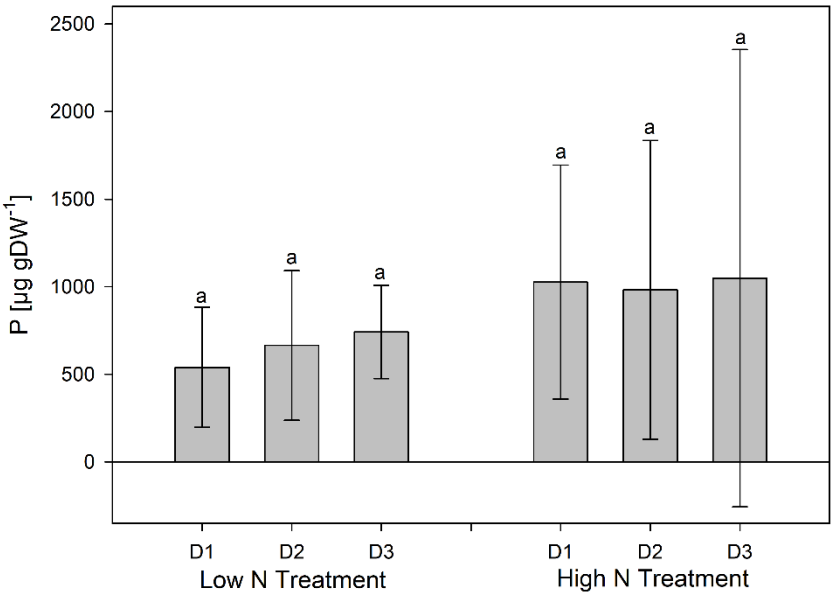

Figure 3.7: Mean±SD (n=10) amount [µg gDW^{-1}] of P measured in the feces between the three feeding days and the two feeding treatments. Significant difference is indicated by unequal superscripts within and between treatments.

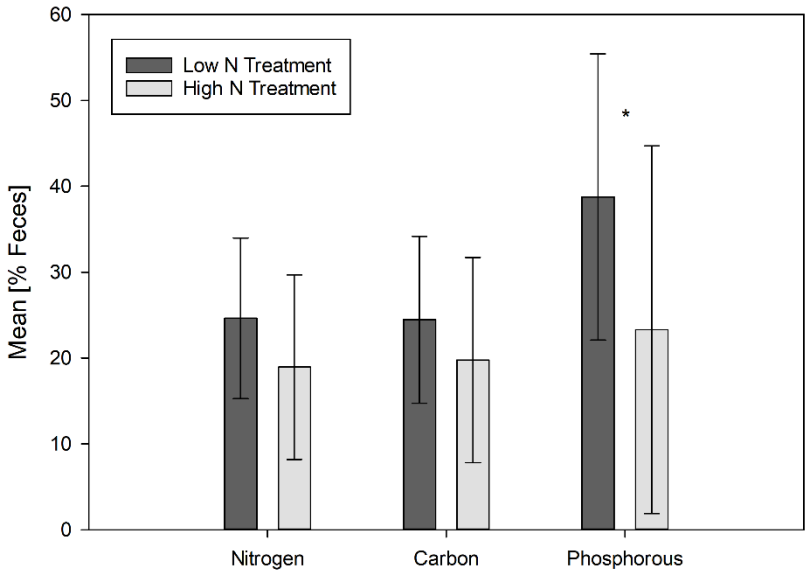

Figure 3.8: The percentage [Mean±SD] feces production (n=30) related to the total amount of ingested nutrients shown for all three macronutrients. Significate difference between feeding treatments indicated through superscript (*) between the respective bars.

The elemental ratios of the feces for the three macronutrients are shown in Table 3.3. Similar feces composition was found for both feeding levels (one-way ANOVA).

Table 3.3: The elemental ratio of the feces (n=30) in the Low and High feeding treatment. High feeding treatment shown as median, due to non-normality of the data, marked with (*).

Elemental ratio	C:N	C:P	N:P
Low	7.5±3.7	11.1±2.4	2.5±3.1
High*	5.5	12.0	1.7

3.5 Assimilation and Nutrient Budget

The nutrient budgets of the polychaetes over the three feeding days and between the low and high feeding treatments, is shown in Figure 3.9-Figure 3.11 and Table 3.4.

The ingested amount of nutrients was divided into three fractions of dissolved nutrients, particulate nutrients in the feces and assimilated nutrients. As explained by the assumption for the dissolved fraction of N in section 3.2.1, this was not taken into consideration for its respective budget.

No significant difference (Kruskal-Wallis H test) was seen between the three feeding days in assimilation numbers. For the assimilation fraction, a significant difference (Kruskal-Wallis H test) was found between the treatments for all three nutrients when looking at the amount ingested. When investigating the percentage assimilated between the two treatments, a significant difference (Kruskal-Wallis H test) was found for C and P, but barely not for N ($p = 0.056$), where the highest median assimilation rate was found in the high feeding treatment for all three nutrients.

Comparing the assimilation rates of the ingested nutrients between the three macronutrients, a difference was found between the two treatments. For the low feeding treatment, a significant difference (Kruskal-Wallis H test) was found between the assimilation rate of all three nutrients, with the median assimilation of C, N and P being 64.3, 77.7 and 49.5% respectively. However, when testing for differences (Kruskal Wallis H test) for the high feeding treatment, no differences were found, with the median assimilation for C, N and P being 74.9, 85.0 and 72.2% respectively.

The size of the polychaetes showed no correlation (Pearson Correlation test) with the assimilation rate of the polychaetes, suggesting that the polychaetes assimilate a similar fraction of the ingested nutrients independent of their biomass. When testing for correlation between tube inhabitance and assimilation rate, a slight negative relationship could be observed for all three nutrients, with P being the only significant ($p = 0.040$). This suggests that the assimilation of phosphorus decreases with an increasing tube inhabitance.

Table 3.4: The complete nutrient budgets (n=30) of the ingested feed for all three macronutrients, C, N and P, showing the respective fractions of assimilated, fecal, and dissolved nutrients. All fractions shown in medians, due to frequent non-normality of the data.

Nutrient	Treatment	% Assimilated	% Feces	% Dissolved
C	Low	64.3	24.8	10.9
	High	74.9	19.8	5.3
N	Low	77.7	22.3	-
	High	85.0	15.0	-
P	Low	49.5	40.0	10.5
	High	72.2	16.7	11.1

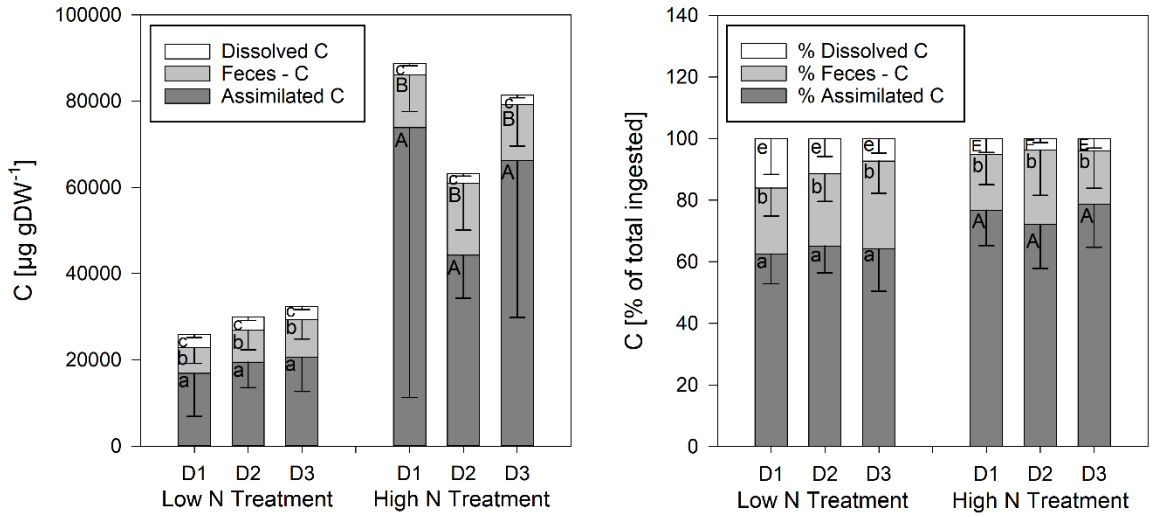

Figure 3.9: Stacked bar charts showing the pathway of the C ingested by the polychaetes. The complete stacked bar (n=10) constitutes the total amount of ingested C [µg gDW^{-1}]. The white fraction represents dissolved C, the light grey fraction represents particulate C in the feces and the dark grey represents the assimilated C. a) Amount of C in the three fractions; b) % of the ingested C in the three fractions. Significant differences are indicated with unequal superscripts, both between and within treatments.

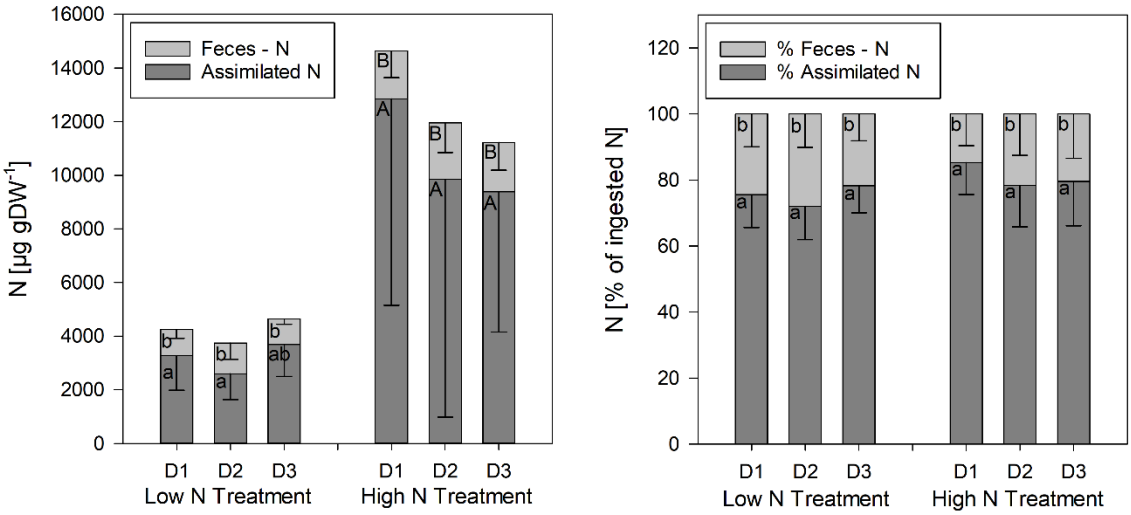

Figure 3.10: Stacked bar charts showing the pathway of the N ingested by the polychaetes. The complete stacked bar (n=10) constitutes the total amount of ingested N [µg gDW^{-1}]. The light grey fraction representing particulate N in the feces and the dark grey represents the assimilated N. a) Amount of N in the three fractions; b) % of the ingested N in the three fractions. Significant differences are indicated with unequal superscripts, both between and within treatments.

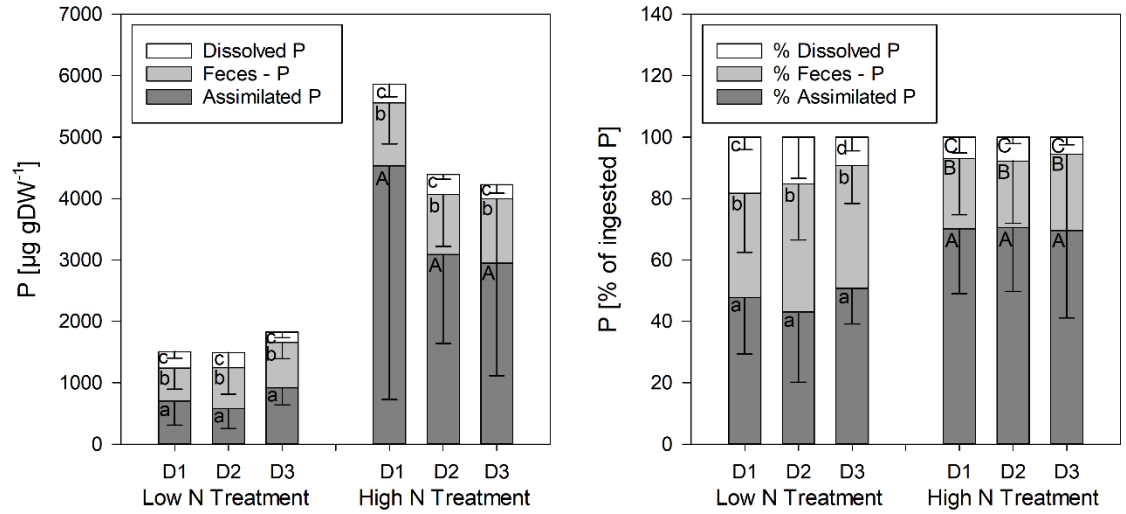

Figure 3.11: Stacked bar charts showing the pathway of the P ingested by the polychaetes. The complete stacked bar (n=10) constitutes the total amount of ingested P [µg gDW^{-1}]. The white fraction represents dissolved P, the light grey fraction represents particulate P in the feces and the dark grey represents the assimilated P. a) Amount of P in the three fractions; b) % of the ingested P in the three fractions. Significant differences are indicated with unequal superscripts, both between and within treatments.

From a cultivation standpoint, another interesting factor is the percentage assimilated of the total amount of nutrients added. When looking at the three feeding days, no significant difference (one-way ANOVA, High N: Kruskal Wallis H test) was found in either of the two feeding treatments, thus the mean assimilation rate of the three days was calculated and shown in Table 3.4 for its respective treatment and macronutrient.

Within the low feeding treatment, N was observed to have the highest assimilation rate of the total amount of nutrients added with 52.9 ± 16.7%, while P had the lowest with 28.2 ± 12.1%. Between the three macronutrients, a significant difference (one-way ANOVA) was found for P compared to the two other nutrients, but no significant difference was found between the assimilation rates of C and N. No correlation (Pearson Correlation test) was found between the biomass of the polychaetes and the total assimilation rate.

For the high feeding treatment, no significant difference (one-way ANOVA) was found between any of the three macronutrients, with assimilation rates spanning from 15.7±9.8 (C) to 21.0±13.5 (N). No correlation (Spearman Correlation test) was found between the biomass of the polychaetes and the total assimilation rate within this treatment.

Comparing the two feeding levels, a significant difference was found for all three macronutrients, were the lowest assimilation occurred in the high feeding treatment for C, N and P.

Table 3.5: The Mean±SD (n= 30) assimilation rate of the total amount of nutrients added to the feeding beakers for the three macronutrients and two feeding treatments. Significant difference between treatments and nutrients is indicated with unequal superscripts.

Treatment	C [%]	N [%]	P [%]
Low[a]	42.0±15.3[A]	52.9±16.7[A]	28.2±12.1[AB]
High[b]	15.7±9.8[B]	21.0±13.5[B]	17.2±10.8[B]

4 Discussion

4.1 Feeding and Nutrient Ingestion

Similar ingestion of each macronutrient within the high and low feeding regimes was found for all three feeding days. A significantly higher amount of feed was ingested in the high treatment than in the low for all three macronutrients, meaning the feeding activity increased with a larger addition of sludge. As feed availability is assumed to be a main limiting factor for growth for an IMTA-application of *H. diversicolor*, a higher addition of feed will result in a higher growth rate, as shown in previous experiments (Galasso et al., 2020; Nielsen et al., 1995). In Kristensen (2021), a positive relationship was found between the specific growth rate and the amount of sludge administered to the polychaetes. This indicates the same trend of a higher nutrient ingestion with increasing feed availability, but the aforementioned study did not quantify the amount ingested. In the present study, the increasing amount of ingested nutrients was not proportional to the increase in nutrients added. The percentage ingested of the total amount added was much higher in the low treatment, ranging from 62-67%, compared to 19.5-23.6% for the high, suggesting a much lower degree of utilization in the high treatment. When regarding this from a production point-of-view, a feed utilization of ~20% is far from ideal, both regarding water quality and feeding efficiency. However, the ingestion rates found in the present study could be influenced by the lack of sediment and other stressors (Bhuiyan et al., 2021; Kristensen, 1983a), and might therefore be lower than in reality.

Comparing different experiments that use sludge as a feed source can be challenging as the composition and physical properties of the sludge can vary greatly between different facilities (Aas & Åsgård, 2017), but also between different periods in the same facility, seen by comparing this study with Kristensen (2021). As the nutrient composition is highly dependent on the degree of spilled feed (Cripps & Bergheim, 2000), it is hard to standardize this feed source. Since only two feeding levels were to be investigated in this experiment, the lowest (5% N) and the highest (40% N) feeding regimes from Kristensen (2021) and Dahl (2021) were chosen to observe both ends of the scale. Thus, there is a large gap between the two feeding levels, where the mid-concentrations are not represented. With older sludge data being used in this experiment, the actual feeding amount differed a lot from the intended feeding levels, making the amount of nutrients added in the higher feeding level even more excessive.

4.2 Nutrient budgets

To the best of the author's knowledge, there are only a few previous studies done on feeding experiments of nereid polychaetes (Fang et al., 2016; Honda & Kikuchi, 2002) with a nutrient budget approach. Earlier feeding experiments have mostly been conducted on groups of polychaetes with a focus on growth and conversion of the total feed amount added, with no quantification of feed ingested. Thus, it is hard to compare literature values with the findings in the present study. It is important to keep the nutrient loss rates in mind, further explained in Section 4.3, as this might have led to higher assimilation values.

The main aim of the respiration experiment was to establish CO_2-production for the C nutrient budget. As similar production of CO_2 was found for both feeding levels and states, all polychaetes were used in the calculation, namely 0.84 µg CO_2 $mgDW^{-1}$ h^{-1}. For the nutrient budget, the percentage dissolved C made up 10.9% and 5.8% of the ingested

nutrients for the low and high feeding treatments, respectively. Fang et al. (2016) found the metabolic fraction of the ingested C to be 56.4 ± 3.1% for *Perenereis aibuhitentis* fed fish feces at 15°C, much higher than what was recorded in this experiment.

Literature on respiration rates of *H. diversicolor* shows a large variation in recorded values, which could be explained by differences in experimental conditions (Galasso et al., 2018). Galasso et al. (2018) found a respiratory rate of 0.45 µmol O_2 h^{-1} (0.25 gWW, 11°C), while Kristensen (1983b) have found a rate of up to 3.5 µmol O_2 h^{-1} (0.5 gWW, 12°C). In the present study, all polychaetes were included in the calculation of the respiratory rate, finding a mean of 1.87 µmol O_2 h^{-1} (0.225 gWW, 0.047 gDW, 12°C), coinciding well with the respiration found by Nielsen et al. (1995) (~0.047 gDW, 15°C). Thus, the respiratory rate found in the present study is well within the range of existing literature.

When conducting the respiration experiments, it was decided to not use tubes as artificial burrows, so not to add tube inhabitance as another variable, since varying tube inhabitance was observed in the main feeding experiment (Appendix A, Table 6.3). However, when doing the respiration measurements, much of the feed seemed untouched, and the observed polychaete activity was quite low which could indicate exhaustion due to stress. As mentioned in Section 2.2.2, polychaetes inhabiting completely empty beakers showed low appetite and excessive mucus production, further pointing towards stress. When comparing the two feeding levels and resting state of the polychaetes, no significant differences were found. As the polychaetes were not fed for the three day acclimatization period, non-eating individuals would have been starved for up to 4 days. Nielsen et al. (1995) saw a sharp decline in respiratory activity after starvation of both *H. diversicolor* and *A. virens*, starting already after 11 hours, suggesting a relationship between polychaete respiration and starvation. There was also no relationship found between the weight of the individuals and their respiratory rate, despite this being well documented in numerous studies (Galasso et al., 2018; Nielsen et al., 1995; Nithart et al., 1999; Shumway, 1979). Doing a linear regression analysis on the respiratory rate and dry weight of the polychaetes, an R^2-value of 0.05 was calculated. As neither starvation nor biomass of the polychaetes showed any relationship with the measured respiration, there is reason to doubt the accuracy of the respiration data acquired through this experiment, which could also explain the much lower fraction of CO_2 in this budget compared to Fang et al. (2016).

The amount of fecal matter produced was seen to be higher in the high treatment than in the low for all three macronutrients, with N and C being significantly different between the two treatments. The lack of significant difference in P could in part be due to large standard deviations because of varying appetite in the polychaetes, also impacting the feces production. When looking at percentage feces produced of the ingested nutrients, it is only P that is significantly different between the two feeding levels. From visual inspection of Figure 3.8 the mean percentage feces produced in the high treatment is lower than in the low treatment, indicating that a smaller amount of the ingested nutrients is excreted as fecal matter. The feces production was between 15-25% for all treatments, except for the low treatment P being 40.0%. This correlates well with the lower assimilation rate seen in the low P treatment, further suggesting this species' limited need to assimilate phosphorus. The feces production is well within the same area as existing literature, where Honda and Kikuchi (2002) and Fang et al. (2016) found a feces production of 21.0 and 24.34% respectively for ingested N. Fang et al. (2016) also found a feces production of 21.6% of the ingested C.

By comparing the elemental ratio of the sludge (Table 3.1) with the elemental ratio of the feces (Table 3.3), differences can be seen. Comparing the composition of the sludge to the feces from the low treatment, only the C:P ratio showed a decrease, with C:N and N:P remaining roughly the same. This suggests that the amount of retained P is lower than that of C. When considering the high feeding treatment, ratios in the feces are in general much lower than those seen in the sludge. With a lower ratio, the two nutrients are closer in concentration, indicating a higher removal of the more abundant nutrient. A clear reduction can be seen in both the C:P and N:P-ratios, once again indicating a lower utilization of P. In the high feeding treatment, the C:N ratio is also lower indicating a higher removal of C than N. As the assimilation rate of N was found to be higher than that of C, this could be explained by the large CO_2-production seen in Fang et al. (2016), even though this rate of respiration was not found in the present study.

Assessing the bioremediation potential of *H. diversicolor*, its ability to assimilate nutrients from the feed ingested is a principal factor. For assimilation of the ingested nutrients, the highest rate (% Assimilated) was found for N in both the high and the low treatment, followed by C and then P. The percentage assimilated N was 77.7 and 85.0% for the low and high feeding treatment, respectively. These assimilation rates are high compared to Honda & Kikuchi (2002) who found an assimilation rate of 62.8% of the ingested N and Fang et al. (2016) with 60.0%, both investigating Nereid polychaetes fed fish feces. However, it should be noted that no dissolved fraction for N was included in this experiment, explained further in Section 4.3, which would then increase the rates of both assimilation and feces production. Thus, if this had been recorded, the assimilation fraction might have been more comparable to Honda and Kikuchi (2002), and Fang et al. (2016), who both found an ammonia excretion of approximately 16%.

The assimilation of ingested C is hard to compare to previous literature, as the author have only found one paper assessing this (Fang et al., 2016). As shown by Nederlof et al. (2020) on two different species of polychaetes, the requirement of C is much higher than that for N. This requirement is reflected in the assimilated amount of C, being much higher than N and P. In the present study, an assimilation rate of 66.5 and 76.1% was found for the low and high treatment, respectively. This high assimilation rate is in line with the findings of Nederlof et al. (2020), where the majority of C ingested was assimilated for growth. However, comparing this to the assimilation rate of 26.0% found in Fang et al. (2016), there is a large difference. In the mentioned study, 52.4% of the ingested C was excreted as CO_2 through metabolism, explaining the significantly lower assimilation rate found. Thus, with potentially non-coherent respiration data, there is a possibility that the assimilation percentage for C found in this experiment is higher than in reality.

For P, no studies have been found to focus on assimilation of ingested nutrients, making comparisons of other than trends difficult towards previous literature. In the present study, the lowest assimilation of the three macronutrients was seen in P, further strengthened by Kristensen (2021), who found the lowest conversion rate of all nutrients added being P. However, the total conversion rate of P found in Kristensen (2021) of ~1% is much lower than the 17.17-28.19% total assimilation rate in the present study. It is important to note that the sludge composition from the mentioned study was used for calculating feeding amount in this experiment as well. When analyzing the smolt sludge used in the present study, it was seen that the concentration of P was much lower than the smolt sludge used in Dahl (2021) and Kristensen (2021), almost by a factor of two. Thus, approximately 50% less P was added in this experiment, meaning an adjusted assimilation rate is closer to 8.6-14.1% assimilation assuming similar ingestion rates. The P content of

H. diversicolor was found to be 8.5 µg $mgDW^{-1}$ (Kristensen, 2021), substantially lower than the sludge which was 24.72 µg $mgDW^{-1}$. Therefore, the low assimilation of P could be explained by the excessive availability of this nutrient. As noted by Frost et al. (2006) in their study conducted on *Daphnia*, low food availability results in a higher fraction of the ingested C being allocated for basal metabolic processes, reducing growth and thus limiting the need for P. This could also be an explanation to the low assimilation of P in the low feeding treatment.

Considering the complete nutrient budgets (Table 3.4), the highest rate of utilization between the three nutrients are seen in N, with both the highest assimilation and the lowest production of feces, corresponding well with previous studies (Fang et al., 2016; Kristensen, 2021). In contrast, P shows the lowest assimilation and the highest feces production, both indicating *H. diversicolor*'s limited ability to utilize this nutrient from the feed source, also shown in previous literature (Kristensen, 2021). C was seen to be efficiently assimilated, almost to the same rate as N, both in the present study and previous literature (Kristensen, 2021; Nederlof et al., 2020). However, these findings contradict Fang et al. (2016), where the majority of ingested C was allocated to metabolic processes, resulting in a much lower assimilation.

4.3 Methodical challenges

No background nutrient analysis was conducted for the seawater used in the experiments, making it difficult to deduct the natural nutrient content of the water from the raw data. For the dissolved nitrogen (NO_3^-), no significant difference was found when considering the leakage controls, as well as no correlation being found between the amount of sludge added and the dissolved N in the water. Forbord et al. (2012) conducted an experiment using the same intake point in the Trondheim fjord at the same time of year. The nitrogen concentration in the water was found to be 148 µg NO_3^- L^{-1}, far higher than the 97.9 µg NO_3^- L^{-1} measured in the present study, suggesting that the dissolved nitrate found in the present work stems from the background concentrations in the water. Unfortunately, the water samples in this thesis were only analyzed for nitrate content, while *H. diversicolor* mainly excretes nitrogen as dissolved ammonia (NH_3) (Christensen et al., 2000; Nithart et al., 1999). Thus, it is feasible that the DIN-fraction went unnoticed as ammonia.

It is important to compare the experimental setup to the natural environment of the polychaetes. Plastic tubes was used instead of sediment in these experiments, as it was crucial to collect all uneaten sludge and fecal particles. Kristensen (1983a) did a study on ventilation behavior in the Nereid polychaetes *Alitta virens* and *A. succinea* in sediment and artificial plastic tubes. The ventilation activity of polychaetes in tubes were up to three times longer than those in natural sediment, suggesting stress. Stress has been seen to impact the feeding behavior of the polychaetes, both in previous studies (Bhuiyan et al., 2021; Clark, 1960), and from personal observations during the testing period. Therefore, it is reasonable to believe that the added stress from not having natural sediment could impact the feeding. The worms were also moved from the defecation beaker to the feeding beaker right before feeding, which could influence their behavior as well.

As mentioned in section 2.6, a substantial amount of loss occurred during the main feeding experiment, further impacting the raw data. As particles can stick to surfaces, a small amount of loss was expected throughout the experiment. However, most likely due to the filtration methods used, a higher degree of loss was obtained than previously anticipated.

To make sure no contamination of the filtrate occurred, it was decided not to flush the walls of the sample beaker shown in Figure 2.6. Thus, some particulate matter could have stuck to the wall of this beaker and not be collected on the filter.

The final calculation of assimilation rates was based on the nutrients that were not present in any of the measurements. Therefore, a loss of nutrients will lead to higher assimilation and ingestion, as well as lower particulate samples of feces and uneaten feed, shifting the entire balance of the nutrient budgets. Using the control samples, a fixed loss percentage was calculated (Table 6.4) for the two feeding levels in their respective nutrients, which was used to account for loss in the budget calculations. As the sludge added to the controls were based on the mean biomass of the polychaetes (229.5 mgWW), the loss percentage most accurately predicts for polychaetes of this size. However, the range of polychaete biomass spans from 150-340 mgWW, meaning that over and underestimation could occur.

4.4 Bioremediation potential of *H. diversicolor* and future research

With the current state of Norwegian salmon farming, seen from a sustainability perspective, there is a need for improvement (Bailey & Eggereide, 2020). The increasing focus on utilizing waste ingredients for a lower environmental footprint and higher financial gain (Campanati et al., 2022; Slootweg, 2020), as well as the increase in sludge production through land-based salmon farming (Bjørndal & Tusvik, 2019) presents the need for new solutions such as IMTA (Ellis & Tiller, 2019). The findings in the present study further strengthens the existing evidence of *H. diversicolor*'s ability to utilize the undigested nutrients found in aquaculture waste (Kristensen, 2021; Wang et al., 2019a; Yousefi-Garakouei et al., 2019), further creating valuable secondary biomass as a potential new aquaculture feed resource (Dahl, 2021; Marques et al., 2018). The IMTA-application of *H. diversicolor* can be conducted in both coupled and de-coupled systems. For coupled systems, the integration of polychaetes into sand filters (Jerónimo et al., 2020) and benthic polychaete cultivation under sea-based cages (Jansen et al., 2019) have been suggested, with both showing potential. Considering de-coupled IMTA, Nederlof et al. (2020) showed a decrease in bioremediation potential when the polychaetes were fed preserved feces in comparison to fresh.

The quantified ingestion rate found in this thesis could provide new and complimentary information to previous studies done on nutrient conversion in *H. diversicolor*, as this data might add valuable insight in regards to the relatively low conversion rates found in Kristensen (2021). The feeding activity of Nereid polychaetes have been shown to be influenced by parameters such as feed quantity (present study), temperature (Fang et al., 2016), density (Yousefi-Garakouei et al., 2019), negative stimuli (Clark, 1960), and polychaete size (Honda & Kikuchi, 2002). With the ingestion rate in the low feeding treatment being ~60%, and the high feeding treatment only being ~20%, there is an incentive for future research on this topic for an optimized feeding regime.

When considering the percentage assimilation of the ingested macronutrients, all three showed a high percentage nutrient uptake. In the low feeding treatment, the percentage assimilation showed the polychaete assimilated between 50-70% of the ingested nutrients, with the highest rate found for N, and the lowest found for P. For the high feeding treatment, the assimilation was even higher, ranging from 72-85%. This suggests that the polychaetes are highly efficient in assimilating the nutrients obtained from the smolt sludge. It must be noted that the high assimilation numbers could in part be due to the

particulate loss discussed (Section 4.3), as all loss directly leads to a higher assimilation percentage. In assimilation of the total amount of nutrients added, the low ingestion rate is visible, with much lower assimilation rates than the ingested nutrients. The assimilation of P is seen to be significantly lower than the two other macronutrients. Considering the current unsustainable exploitation of P, this is an unfortunate finding as efficient recycling of this nutrient will be important for future food security (Brownlie et al., 2021).

Considering some of the nutrients were not assimilated and the relatively low ingestion rate recorded in this study, it could be interesting to combine the polychaete production with other alternatives of nutrient recycling, such as biogas production (Del Campo et al., 2010) and other extractive species, e.g. mussels and algae (Fossberg et al., 2018; MacDonald et al., 2011). With a more integrated system for nutrient recycling, the range of valuable products created from this waste source could become substantial.

For future research, there is still a lot to investigate. As ingestion rate have been shown in the present study to strongly limit the overall nutrient assimilation of *H. diversicolor*, there is a need to investigate decisive factors for polychaete feeding behavior. Examples of this is feed quantity and quality, abiotic parameters such as light and temperature, and rearing density. By determining optimal parameters, a higher degree of ingestion could be expected, both resulting in better bioremediation and polychaete growth. As the assimilation of P is much lower in Kristensen (2021) than in the present study, more research needs to be done on this subject to investigate the actual degree of utilization occurring.

Lastly, it would be interesting to conduct this experiment once more, taking the experiences made during this thesis into account. The trends seen in this thesis, like feces production and 'nutrient preference', fits in well with what is found in previous studies conducted on this topic. However, the incoherent data from the respiration, the lack of dissolved N, and the uncertainty added by the particulate loss means that the exact values found in the present study should be regarded with caution.

5 Conclusion

The feeding activity of the polychaetes differed between the two treatments, where the amount ingested increased with a higher feeding level. However, the ingestion rate decreased with an increasing feed availability, with 64 ± 20% and 22 ± 12% of the feed added being ingested for the low and high treatment, respectively. Thus, the fraction of added feed that was ingested decreased with an increasing feed quantity.

A significantly higher assimilation rate of the ingested nutrients was seen in the high feeding treatment (72-85%) than in the low (50-78%) for all three macronutrients. For both treatments, the highest rate of assimilation was seen in N, followed by C, and the lowest assimilation was found in P. While a significant difference in assimilation rate was seen between all three nutrients in the low feeding treatment, no difference was found in the high. As not all the feed was ingested, the assimilation rate of the total amount of nutrients added were much lower, with the highest amount of assimilated nutrients being observed in the high treatment. However, the total assimilation rate was higher in the low treatment (28-53%) than the high (16-21%). The largest feces production was seen in the low treatment for P, with 40% of the ingested nutrients. While only P showed a significant difference between the low and high treatment when investigating percentage feces produced, a similar trend of increasing feces production with increased feeding activity could also be seen in C and N.

The respiratory rate of the polychaetes did not differ between the two feeding levels, nor the two feeding states. No correlation was found between the weight of the polychaetes and the respiration. An oxygen consumption rate of -0.04 µmol O_2 $mgDW^{-1}$ h^{-1} was established, and from this a production of 0.84 µg CO_2 $mgDW^{-1}$ h^{-1} was calculated.

Through the present study, *Hediste diversicolor* has been shown to readily accept smolt sludge as a feed source, and to efficiently assimilate ingested nutrients from this waste product. This species is therefore a novel candidate for use in IMTA-applications for the salmon farming industry and could play a role in nutrient recycling from land-based facilities in the future. However, the low ingestion rate is a limiting factor in overall bioremediation potential, and use of polychaetes can therefore only play a part in the utilization of aquaculture sludge and should be coupled with other treatments.

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Appendix A – Main feeding experiment

Biomass and nutrient composition of polychaetes

Table 6.1: Data for the polychaetes used in the main feeding experiment, showing biomass and dry weight ratio, as well as CN concentration and ratio for the two feeding treatments, Low (T1.x) and High (T2.x)

Polychaete ID	Wet weight [mg]	Dry weight [mg]	% Dry Weight	C [µg mgDW^{-1}]	N [µg mgDW^{-1}]	C:N
T1.1	290	59.5	20.5	508.2	112.7	4.5
T1.2	245	49.4	20.2	479.9	105.5	4.6
T1.3	338	70.3	20.8	358.7	80.4	4.5
T1.4	204	45.6	22.4	269.6	51.2	5.3
T1.5	297	56.5	19.0	455.3	95.8	4.8
T1.6	214	43.3	20.2	576.1	121.0	4.8
T1.7	279	44.2	15.8	542.1	104.6	5.2
T1.8	212	42.3	20.0	435.7	95.0	4.6
T1.9	176	28.5	16.2	670.1	141.8	4.7
T1.10	296	60.3	20.4	274.1	68.6	4.0
T2.1	205	51	24.9	510.3	94.9	5.4
T2.2	163	32.6	20.0	574.8	114.2	5.0
T2.3	193	30.3	15.7	620.0	83.0	7.5
T2.4	298	52.5	17.6	446.3	90.8	4.9
T2.5	225	43	19.1	526.7	93.9	5.6
T2.6	212	37.9	17.9	518.8	100.6	5.2
T2.7	135	28.7	21.3	545.4	95.1	5.7
T2.8	147	29.6	20.1	521.9	109.2	4.8
T2.9	183	37.7	20.6	513.8	89.7	5.7
T2.10	279	48.5	17.4	474.2	92.1	5.2
Mean±SD	229.6±57.5	44.6±11.7	19.5±2.2	491.0±100.7	97.0±19.1	5.1±0.7

Actual feeding amount

Table 6.2: The actual amount fed in percentage for the low (T1.x) and high (T2.x) feeding treatments. Standard deviation calculated from the mean amount fed per polychaete across the three feeding days. Polychaetes marked with * not included in calculation of mean feeding level and standard deviation, due to error in measuring the dry weight.

Polychaete ID	Target Feeding Level (%)	Actual Amount Fed (%)	Standard Deviation (%)
T1.1	5 %	5.5	0.2
T1.2	5 %	5.9	0.2
T1.3	5 %	7.6	0.2
T1.4	5 %	11.0	0.1
T1.5	5 %	7.0	0.0
T1.6	5 %	5.4	0.4
T1.7	5 %	7.7	0.2
T1.8	5 %	6.8	0.1
T1.9	5 %	5.4	0.0
T1.10	5 %	8.0	2.1
Average - Low	5 %	7.0±1.6	0.4±0.6
T2.1	40 %	43.2	0.2
T2.2	40 %	44.5	0.2
T2.3*	40 %	~~93.3~~	~~1.1~~
T2.4	40 %	63.6	0.4
T2.5	40 %	57.1	0.1
T2.6	40 %	56.9	0.2
T2.7	40 %	51.0	0.4
T2.8	40 %	46.5	0.3
T2.9	40 %	55.3	0.2
T2.10	40 %	63.7	0.2
Average - T2	40 %	53.3±7.7	0.2±0.1

Tube inhabitance

Table 6.3: Tube inhabitance for each polychaete used in the main feeding experiment, with number of observations in and out of the tubes, as well as the percentage inhabitance.

Polychaete ID	Observed in Tube	Observed Out of Tube	Inhabitance [%]
T1.1	2	6	25
T1.2	8	0	100
T1.3	5	3	62.5
T1.4	8	0	100
T1.5	4	4	50
T1.6	5	3	62.5
T1.7	8	0	100
T1.8	4	4	50
T1.9	4	4	50
T1.10	4	4	50
T2.1	8	0	100
T2.2	4	4	50
T2.3	6	2	75
T2.4	0	8	0
T2.5	6	2	75
T2.6	3	5	37.5
T2.7	6	2	75
T2.8	6	2	75
T2.9	8	0	100
T2.10	0	8	0

Loss rates from leakage controls

Table 6.4: The calculated loss [Mean±SD] from the leakage controls for the three macronutrients and both treatments. These loss rates were used for adjusting the data to account for the loss in the budget calculations.

Treatment	Nitrogen [% loss]	Carbon [% loss]	Phosphorus [% loss]
Low	6.09±14.13	12.40±20.29	-
High	39.39±6.77	33.52±8.71	25.94±7.73

Appendix B – Dissolved nutrients and respiration

Dissolved Nutrients

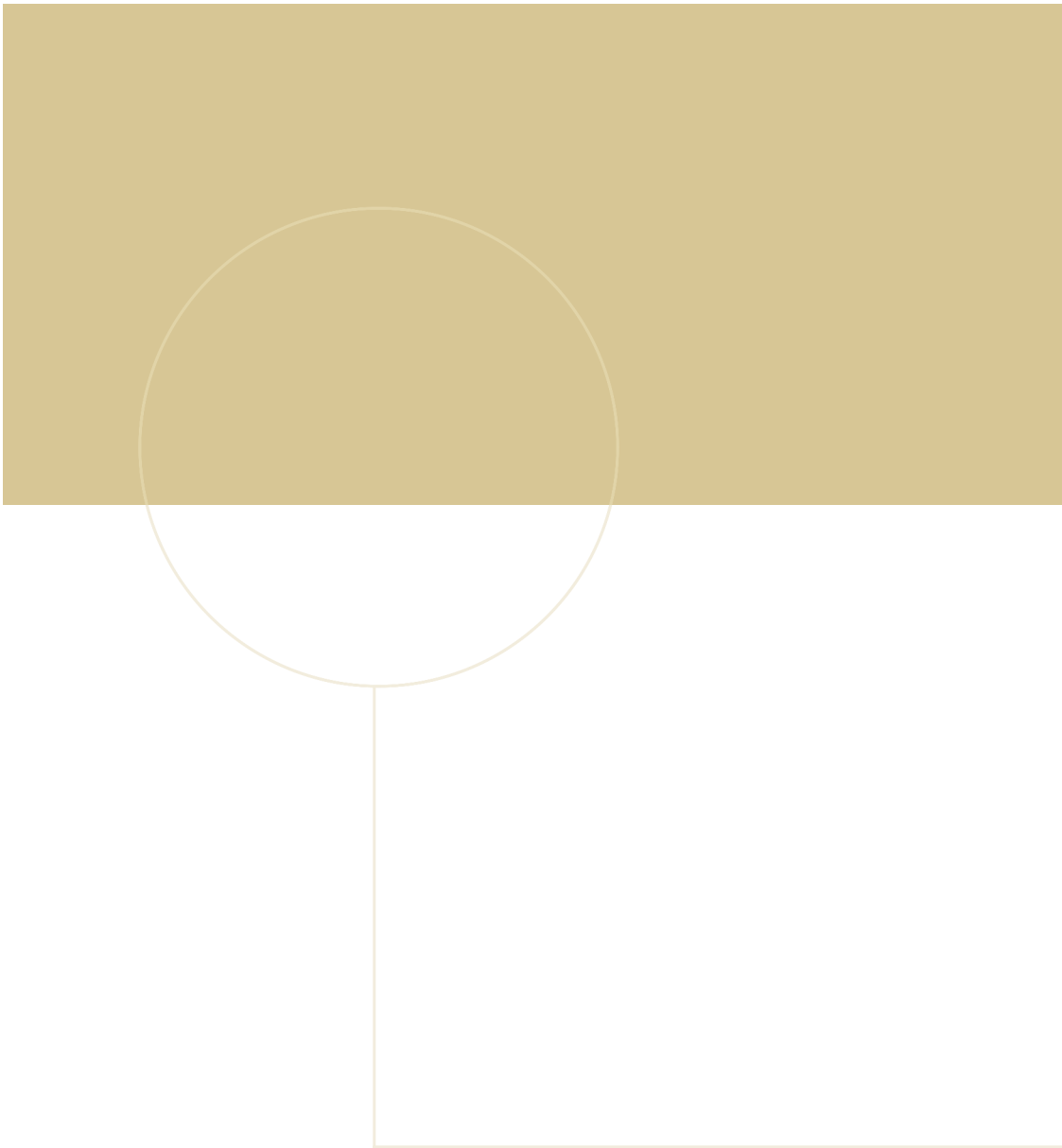
Table 7.1: Amount of dissolved N and P for the two controls investigating leakage of nutrients from the sludge to the water. Here shown in amount of sludge added [mgWW], dissolved N [µg] and Dissolved P [µg] for the two treatments in their respective controls.

Control	Treatment	Sludge added [mgWW]	Dissolved N [µg]	Dissolved P [µg]
Leakage	Low	11.9	15.7	19.4
Leakage	Low	12.1	15.0	18.3
Leakage	Low	11.7	15.3	26.2
Leakage	Low	12.0	15.2	23.6
Leakage	High	95.4	14.9	59.7
Leakage	High	96.3	12.7	68.8
Leakage	High	95.7	14.5	67.0
Leakage	High	96.0	15.2	64.5
T_0	Low	17.6	14.6	33.3
T_0	Low	17.1	15.9	21.1
T_0	Low	17.3	15.1	17.5
T_0	Low	17.6	14.3	21.1
T_0	High	109.0	12.5	68.3
T_0	High	108.1	14.9	78.8
T_0	High	108.6	14.4	88.4
T_0	High	108.3	14.8	87.4

Polychaete biomass

Table 7.2: The weight of the polychaetes used in the respiration experiment, showed in wet weight, dry weight, and the DM ratio. Mean±SD shown for all weight factors in the bottom row. The polychaetes were assigned into the low and high feeding treatment using the random function in Excel.

Treatment	Polychaete ID	Wet weight [mg]	Dry Weight [mg]	% DW
Low	1	190	44.6	23.5
Low	5	320	64.0	20.0
Low	7	170	36.6	21.5
Low	8	150	34.5	23.0
Low	9	220	47.8	21.7
Low	11	160	31.8	19.9
Low	12	180	37.6	20.9
Low	15	200	39.3	19.7
Low	17	330	73.9	22.4
Low	18	160	32.8	20.5
High	2	330	68.0	20.6
High	3	160	39.5	24.7
High	4	200	37.6	18.8
High	6	220	47.4	21.6
High	10	210	45.2	21.5
High	13	190	-	-
High	14	210	41.0	19.5
High	16	250	58.1	23.2
High	19	290	55.8	19.2
High	20	340	58.0	17.1
Average	-	224±63.5	47.0±12.5	21.0±1.9



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