Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Trygve Moflag Krogli

Sludge from Atlantic salmon sea cages: composition, quantification and potential applications

Master's thesis in Ocean Resources
Supervisor: Kjell Inge Reitan
Co-supervisor: Inka Anglade
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Acknowledgements

Two years have flown by, and all of a sudden I have finished my master here at NTNU Trondheim. This is not something I could have done without the help from my superb supervisors. A big thanks to Kjell Inge Reitan with his broad knowledge, always having the answer to any question you might ask him and being the anchor behind this thesis. And Inka Anglade, thank you for helping me conduct the field work, hours of assistance in the lab, and your fast and precise feedback whenever I needed help or guidance.

I would also like to thank Siv Anina Etter and Zdenka Bartosova for your help with the chemical analyses of the samples. Further on, a big thank you to Måsøval for letting me collect my samples at your location “Lamøya”. To all the fantastic employees at the location which helped me during the collection period, whether it be by being my personal boat captain or bringing me to the ER after cutting my thumb, thank you.

Finally, a big appreciation to both my family and my girlfriend Camilla for helping me keep my spirit up when everything did not go to plan or when my motivation was lacking. I could never have done this without you.

Abstract

For the Atlantic salmon industry to be more sustainable, several challenges need to be addressed. One of the areas is resource management, where large amounts of waste product in the form of sludge are released from open-cage farms, while still containing valuable resources. Most of the sludge collected and treated today comes from land-based production, but collection of sludge from sea-based production should also be a priority. For this to be implemented, the contents and amounts of sludge produced need to be investigated, ensuring that the resources in the sludge are effectively utilised.

The aims of this thesis were to investigate the contents of sludge released from open-cage salmon farms and to quantify the amount of sludge sedimented directly beneath the cages. This was performed by collecting sludge released from two different cages at an open-cage salmon farm, at two different depths, beneath the cages. The contents of carbon, nitrogen, phosphorous, amino acids, protein, lipids, fatty acids and ash were all analysed and a quantification of the sludge sedimented directly beneath the cages was performed based on the amounts of sludge collected. With this information, an evaluation of using the sludge in IMTA was performed, while additionally suggesting other possible areas of use.

It was found that sea-based sludge from open-cage salmon farms has a lower nutritional content compared to sludge from land-based smolt production, where only the lipid content was found to be of similar values. Further on, it was found that using the sea-based sludge as a resource in IMTA is likely to be less efficient compared to land-based sludge due to the lower nutritional content, in particular the low content of nitrogen. The sea-based sludge also had a relatively high content of phosphorous which will be poorly utilised by most marine species. The amount of sludge sedimented directly beneath the investigated cages were found to range between 19-40 kg d^{-1}, while the theoretical amount produced ranged between 170-332 kg d^{-1}. Based on this, an average of 13% of the sludge produced sedimented directly beneath the cages. When comparing the different cages and depths the sludge was collected at, no significant differences were found for either nutritional contents or amounts of sludge collected. Due to the variations between different salmon farms, further evaluations of both contents and amounts of sludge sedimented beneath open cage salmon farms should be assessed to get a broader knowledge, providing information to find areas of use where the resources in sludge are utilised in an efficient and sustainable way.

Sammendrag

For at lakseindustrien skal bli mer bærekraftig må flere utfordringer adresseres. En av disse områdene er ressurshåndtering, hvor store mengder avfall i form av slam blir sluppet ut fra oppdrett i åpne merder, mens slammet enda inneholder verdifulle ressurser. Mesteparten av slammet som samles og behandles i dag kommer fra landbasert produksjon, men det å samle opp slam fra sjøbasert produksjon burde også bli en prioritet. For at dette skal bli implementert må innholdet i og mengdene slam undersøkes, slik at man kan forsikre seg om at ressursene i slammet blir utnyttet på en effektiv måte.

Målene i denne avhandlingen var å undersøke innholdet av slam sluppet ut ved produksjon av laks i åpne merder og kvantifisere mengden slam som sedimenteres rett under merdene. Dette ble gjort ved å samle opp slam fra to forskjellige åpne merder ved samme lokalitet, ved to ulike dybder. Innholdet av karbon, nitrogen, fosfor, aminosyrer, protein, lipider, fettsyrer og aske ble analysert, og en kvantifisering av slammet som ble sedimentert rett under merdene ble gjort, basert på mengdene slam som ble samlet opp. Med denne informasjonen ble det gjort en evaluering rundt bruk av slammet i IMTA, og andre potensielle bruksområder ble foreslått.

Det ble funnet at sjøbasert slam fra produksjon i åpne merder har et lavere næringsinnhold sammenlignet med slam fra landbasert smoltproduksjon, hvor kun innholdet av lipider hadde et likt innhold i de ulike slamtypene. Videre ble det funnet at det sjøbaserte slammet mest sannsynlig er mindre effektivt som en ressurs i IMTA sammenlignet med landbasert slam på grunn av det lavere næringsinnholdet, spesielt nitrogeninnholdet. Det sjøbaserte slammet inneholdt også en relativt høy mengde fosfor, noe som vil bli dårlig utnyttet av de fleste marine arter. Mengden slam som ble sedimentert rett under de undersøkte merdene varierte mellom 19-40 kg d^{-1}, mens den teoretiske mengden som ble produsert varierte mellom 170-332 kg d^{-1}. Basert på dette ble bare 13% av det produserte slammet sedimentert rett under merdene. Sammenligninger mellom de forskjellige merdene og dypene slammet ble samlet opp på viste at det ikke var noen signifikante forskjeller mellom hverken næringsinnhold eller mengde slam som ble samlet opp. På grunn av variasjoner mellom ulike oppdrettsanlegg burde videre evalueringer rundt både innhold og mengde slam som sedimenteres under oppdrettsanlegg med åpne merder gjøres for å få et bredere kunnskapsgrunnlag som kan gi informasjon som gjør at man finner bruksområder hvor ressursene i slam utnyttes på en effektiv og bærekraftig måte.

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Abbreviations

AA	Amino acids
C	Carbon
C1B	Cage 1 bottom traps
C1T	Cage 1 top traps
C2B	Cage 2 bottom traps
C2T	Cage 2 top traps
DHA	Docosahexaenoic acid, C22:6 n-3
DW	Dry weight
EAA	Essential amino acids
EPA	Eicosapentaenoic acid, C20:5 n-3
FA	Fatty acids
IMTA	Integrated multi-trophic aquaculture
MUFA	Monounsaturated fatty acids
N	Nitrogen
Non-EAA	Non-essential amino acids
P	Phosphorous
PD	Pancreas disease
PUFA	Polyunsaturated fatty acids
SAFA	Saturated fatty acids
SD	Standard deviation
WW	Wet weight

1 Introduction

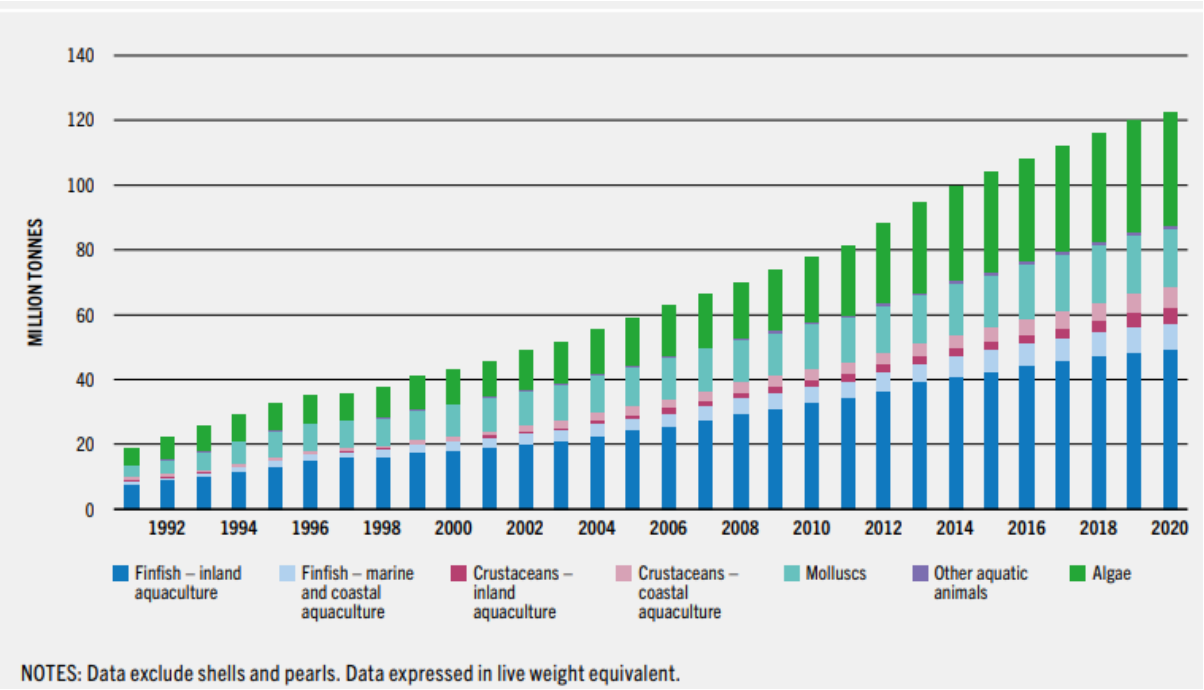

Figure 1 World aquaculture production from 1991 to 2020 (FAO, 2022).

With an ever-growing population, aquaculture has the potential to be one of the main answers to the rising demand of food in the coming years. On a global level, aquaculture and consumption of aquatic foods have grown rapidly in the last decades (FAO, 2022). The consumption of aquatic food per capita has more than doubled from the 1960s to 2020, going from 9.9 kg to 20.2 kg. The aquaculture production has grown even faster with an increase of 600% since 1991 (Figure 1). Furthermore, the production is expected to increase by another 15% until 2030 (FAO, 2022). With such rapid growth, sustainability can be a challenge.

The first Atlantic salmon (*Salmo salar*) produced in Norway was slaughtered in 1971 (Misund, 2022). Since then, the production has only increased, reaching an all-time high in 2021 with a production of over 1.5 million tonnes (Fiskeridirektoratet, 2022a). On a global basis, this is more than half of the total global salmon production (Misund, 2022), which is enough to make Norway the top producer of aquaculture in Europe and one of the biggest in the world (FAO, 2022). This gives a great responsibility of how we utilise the resources used in the production.

1.1 Sustainability in Norwegian salmon production

Sustainability is defined as "meeting the needs of the present without compromising the ability of future generations to meet their own needs." (Brundtland, 1987), and can be divided into three pillars: economic, social and environmental sustainability, where the environmental pillar is the one of highest relevance to this thesis. In an industry where a shared resource such as the ocean is used, it is important to show consideration and take care of the used resources. This should be done in a way that is not harming or causing imbalance in the existing ecosystems it contains. By not leaving a harmful footprint from the production and not over-exploiting resources, future generations can further utilise the resources, meeting their own needs.

The production of Atlantic salmon in Norway has proved to be an efficient production when looking at carbon footprint and area used for production (Hognes et al., 2011). It also has one of the lowest feed conversion ratios (FCR), referring to the necessary feed input to achieve a certain amount of growth being low (Fry et al., 2017). Additionally, it has one of the highest protein and calorie retentions when compared to both terrestrial animal and other aquatic animal productions, meaning it has a high amount of protein/calories left in the edible product in relation to the amount in the feed it is given (Fry et al., 2017).

Even with the many positive sides of Norwegian salmon production, it is facing several challenges (Taranger et al., 2014). Escapes is one of the commonly mentioned problems of the production, mostly because of the risk of genetic introgression between wild and farmed salmon. Between 2014 and 2022, the number of escapees per year has ranged from 17 000 to almost 300 000 (Fiskeridirektoratet, 2022b). Salmon lice (*Lepeophtheirus salmonis*) have also been a substantial problem for the industry. The large lice infection pressure created by the farms could have a negative impact on wild salmonids while also leaving the farmed fish with severe and sometimes deadly wounds, causing poor welfare (Serra-Llinares et al., 2014; Torrissen et al., 2013). Due to the high intensity and heavy feeding activities at salmon farms, large amounts of sludge consisting of feed waste and faeces are released, causing environmental impacts on the benthic areas underneath and in proximity to the farms. (Sæther et al., 2013). At greater volumes, this discharge of nutrients can cause eutrophication and oxygen depletion caused by severe organic enrichment (Wildish & Pohle, 2005).

1.2 Salmon waste sludge

In this context, sludge is the collective term for leftover feed not eaten by the fish, fish faeces and biofouling. When looking at the nutrients within the feed Atlantic salmon is given, up to 70% is digested, leading to 30% or more of the nutrients being left in the faeces and thereby not utilised by the salmon (Ytrestøyl et al., 2016). Sludge is a poorly utilised resource which is considered to be a problem today, because of the impacts it can have on the environment when not collected and treated correctly. An improvement in this area is needed to keep salmon production sustainable.

The feed given to Atlantic salmon is very energy dense, with an energy content of around 25 MJ/kg (Aas & Åsgård, 2017). Salmon is able to digest approximately 80% of the energy in the feed, resulting in a theoretical amount of 5 MJ kg^{-1} energy left in the faeces. By looking at the theoretical amount of energy in sludge from salmon production in Norway, the total amount of energy potentially going to waste each year is 11 785 235 GJ from sea-based production and 242 880 GJ from land-based production (Aas & Åsgård, 2017).

The amount of carbon (C), nitrogen (N) and phosphorous (P) released by salmon farms are rather high, whereas from the total feed input, as much as 70% C, 62% N and 70% P is released to the environment through excretion, respiration, faeces and feed waste (Wang et al., 2012). Numbers from 2019 indicate that this results in 224 000 tonnes C, 66 000 tonnes N and 14 000 tonnes P being released on a yearly basis (Hilmarsen et al., 2021).

Table 1 Overview of waste categories coming from salmon farms.

	Particulate organic matter (POM)	**Dissolved organic matter (DOM)**	**Dissolved inorganic matter (DIM)**
Carbon	POC	DOC	DIC
Nitrogen	PON	DON	DIN
Phosphorous	POP	DOP	DIP

The types of waste coming from salmon farms can be divided into three categories: particulate organic matter (POM), dissolved organic matter (DOM) and dissolved inorganic matter (DIM), as shown in the overview in Table 1 (Sæther et al., 2013). POM containing C, N and P has its origin from feed waste and faeces. This can be consumed by organisms in the water masses as well as benthic organisms. DOM, in the form of DOC, DON and DOP, are small molecules and particles (< 2µm) containing C, N and P, leaking from uneaten feed and faeces (Fredriksen et al., 2011). The dissolved organic waste is a small fraction of the total waste while also being stable substances with a long turnover time, meaning that they have a low impact on the environmental conditions of the water masses. The DIM are nutrients released by the fish through excretion and respiration, and are consumed by phytoplankton in the euphotic zone as well as by macroalgae in the littoral zone (Sæther et al., 2013). The effect of the addition of nutrients to the water masses and benthic areas from these different types of waste could cause a change in biodiversity, where fast growing species such as macroalgae with large surface areas and opportunistic species such as polychaetes often dominate the areas close to salmon farms.

Collecting sludge is a requirement for all new land-based facilities and most of the sludge collected today is collected from this source (Aas, 2021). Sludge collected from land-based facilities is mostly used as a fertilizer, in biogas production or it is sent to a waste treatment facility (Kraugerud, 2022). As most of this collected sludge comes from land-based production of smolt and post smolt in fresh or brackish water, it has a relatively low content of salt. If we were to start collecting sludge from sea-based production at a great scale, the salt content of the sludge would become a problem for the currently used utilisation processes. This is because the salt inhibits the effectiveness both in biogas production and when using it as a fertilizer (Vangdal et al., 2014).

If sludge could be utilised by other species that also live in the ocean, the salt content would not be a problem. Integrated multi-trophic aquaculture (IMTA) could be a solution to this. In IMTA, two or more species are cultivated together, and the waste produced from one species is used as a resource for other species (Fiskeridirektoratet, 2018). A fed primary species, such as salmon, creates bi- and waste products which can be used for growth by one or more secondary species. The secondary species can be seaweed assimilating dissolved nutrients (Fossberg et al., 2018), mussels collecting particulate matter (Handå, 2012) or polychaetes consuming sedimented particles (Nederlof et al., 2020). By doing this, we are getting closer to maximizing the use of the resources put into the system.

1.3 Monitoring of benthic conditions

As the high feeding intensity in salmon farms can cause harmful environmental effects on ecosystems, the farmers are obligated to perform B- and C-investigations to monitor the ecological health of the benthic areas underneath the farms (Fiskeridirektoratet, 2020a, 2020b). These investigations monitor the benthic area under and in proximity to the farms, giving them a score between 1 (best) and 4 (worst). The B-investigation has its focus right underneath the farm, performed as a trend analysis, while the C-investigation focuses on impacts further away from the farm and is more comprehensive. The results from these investigations are of high importance to the farmer, as negative results indicate that the farmer is causing harm to the environment while also leading to longer fallowing periods at to allow for the ecosystems to recover. The results of the investigations also decide when the next investigation will be. In cases where the best grade is given, the next investigation will be during next production cycle at maximum feeding loads, but if a lower grade is given, a new investigation will be performed before a new batch of fish is put out at the farm, as well as during its maximum feeding load.

1.3.1 B-investigation of Lamøya – the collection site

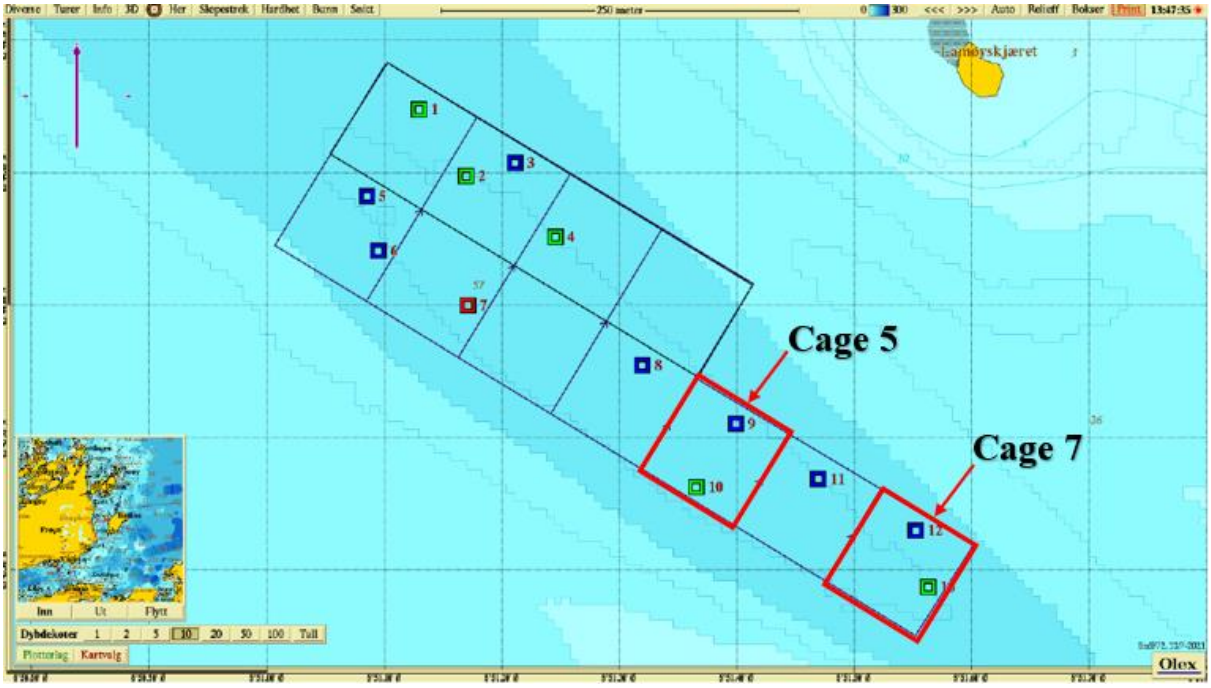

Figure 2 Bathymetric map showing the overall results of the samples. Blue=1, green=2 and red=4. The score ranges from 1-4, where 1 is the best and 4 is the worst (Knutshaug, 2021)). Cage 5 and cage 7 were the cages used during the sludge collections.

The latest B-investigation at the location "Lamøya", from which the samples in the present study were collected, was performed during maximum feeding load at the end of June 2021, performed by Åkerblå (Knutshaug, 2021). 13 samples were collected at different positions at the location using a "Van Veen" grab. The results of these samples showed that the area was mostly dominated by soft mineral sediments, with minor areas covered by hard bottom. The sediments had a light colour with firm or soft texture, and 12 of the 13 samples received the highest grading on the sensory evaluation. In the areas with soft bottom, an average of 30 polychaetes per grab was observed (ranging from 4 to 70). The chemical analysis of the samples showed natural values at six of the positions, lower values at four and very low values at one of the positions. There were no observations of sludge or formation of gas in the samples taken (Knutshaug, 2021).

The results of the investigation were mostly positive, and the highest grade was given. The fauna in the samples showed that the sediments received a great supply of organic matter, but it was able to utilise it at such a rate that a harmful overload was avoided. But even with the highest grade given and mostly good results, some of the areas underneath the farm showed signs of overload. During an earlier B-investigation, the same position (7 – red square in Figure 2) had similar results, meaning that this area most likely acts as an accumulation point,

collecting higher amounts of organic matter than the rest of the area underneath the farm (Knutshaug, 2021).

1.4 Aims of the Study

The aim of this study was to assess the composition of sludge released from open cage Atlantic salmon aquaculture facilities. The nutrient composition was assessed by taking measurements of the contents of carbon, nitrogen and phosphorous, as well as amino acids, protein, lipids, fatty acids and ash. The composition of the sludge was used to evaluate the sludge as a resource in IMTA. Further, a quantification of the sludge sedimented beneath the cages was performed, based on the collected sludge. The specific objectives were as followed:

1. Assessing the composition of sludge sedimented to the seafloor beneath open cage salmon farms.
2. Evaluating the quality of the sludge released from open cage salmon farms to be used as a resource in IMTA.
3. Quantifying the amount of sludge sedimented and collected directly beneath open salmon cages and compare this to the theoretical amount of sludge produced in the cages.

1.5 Projects related to the thesis

This thesis was connected to the RCN funded projects “Cultivation of Polychaeta as raw material for feed (POLYCHAETE)” (Project number: 280836) and “Nutrients in a Circular Bioeconomy: Barriers and Opportunities for Mineral Phosphorous Independence in Norway” (Project number: 268338).

2 Materials and Methods

2.1 Locations of the registrations

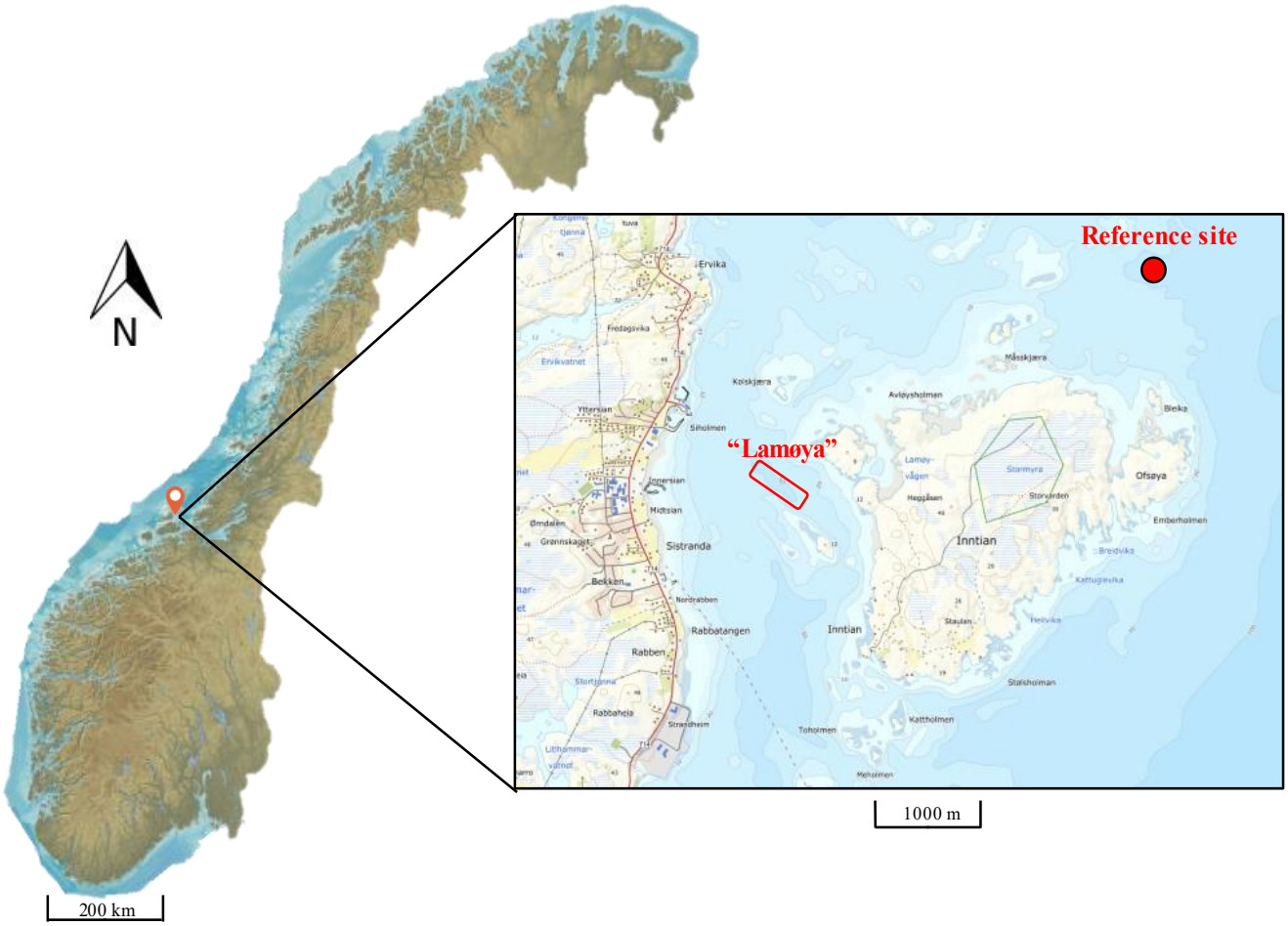

Figure 3 Map showing Lamøya, the location of the sludge collection, as well as the location of the reference site. Map is modified after ©norgeskart.no.

The collection of sludge was performed at Måsøval's location "Lamøya" (63°43'56.5"N 8°51'23.8"E) just outside Sistranda in Trøndelag county. The cages used for the collection were cage 5 and 7 (shown in Figure 2). During the rest of this thesis, cage 7 will be referred to as Cage 1 and cage 5 as Cage 2. At the time of sampling, Cage 1 held 105 000 fish with an average weight of 900 g while Cage 2 held 75 000 fish with an average weight of 850 g. For the reference site, a location about 3.5 km away from "Lamøya" and outside of the impact zone of other aquaculture sites was used (63°45'6.3"N 8°55'15.9"E). "Lamøya" and the reference site are marked on the map in Figure 3.

2.2 Overview of the sludge collection

Table 2 Overview of the time frame for the collections performed during this study.

Collection	From	To
I	9/8/22	11/8/22
II	15/8/22	17/8/22
III	22/8/22	24/8/22
Reference	24/8/22	31/8/22

Table 3 Overview of the replicates used for the collections. The replicates used were the different dates of which the samples were collected. C1/C2 refers to Cage 1/Cage 2, T/B refers to top traps/bottom traps and T1-T3/B1-B3 refers to the three traps placed at either the top or bottom position under the cage (placements are illustrated in Figure 5).

Replicates	Collection points			
	C1		C2	
11/8/22	C1T (T1-T3)	C1B (B1-B3)	C2T (T1-T3)	C2B (B1-B3)
18/8/22	C1T (T1-T3)	C1B (B1-B3)	C2T (T1-T3)	C2B (B1-B3)
24/8/22	C1T (T1-T3)	C1B (B1-B3)	C2T (T1-T3)	C2B (B1-B3)

Three collections of sludge were performed, where each collection period lasted 48 hours. For the reference site samples, the collection period was set to one week. The collection period lasted from 9/8/22 to 31/8/22, shown in Table 2. A total of three replicates were used for the sludge collections, where the three different dates were the different replicates, shown in Table 3. The different chemical analyses of the sludge used different sample sizes, where analyses of the contents of carbon, nitrogen, phosphorous and dry matter were performed for n=9, meaning that all the three samples from each collection point were used individually. For the other analyses the three traps placed at either the top or bottom position from the same cage were pooled, resulting in n=3.

2.3 Feeding of the salmon during the registration period

During the collection period, the salmon at the location was fed using a feed produced by EWOS. The feed used was called RAPID HP 500 50A, a feed with a 7 mm diameter. A total of 10.1 tonnes of feed was supplied to Cage 1 and 10.4 tonnes to Cage 2 during the sludge collection period. The specific growth rate (SGR), which refers to the amount of daily growth in percentage of the body weight, was low during the period, especially in Cage 1. Cage 1 had an average SGR of 0.64% d^{-1} and Cage 2 of 0.97% d^{-1}, reported by Måsøval. This could have been caused by a reduced appetite due to the fish being infected by pancreas disease (PD) (Veterinærinstituttet, n.d.). The disease was first diagnosed in early June, before the fish was confirmed infested in the end of July. A full overview of the average weight, number of fish, total biomass, feed supplied and SGR on a per day basis is presented in Table 4.

Table 4 Overview of average weight (g), number of fish, total biomass (kg), feed supplied (kg) and specific growth rate (SGR) during the registration period. All data was provided by Måsøval.

	Average weight (g)		**Number of fish**		**Total biomass (kg)**		**Feed supplied (kg)**		**SGR (% d^{-1})**	
Date	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
9/8/22	859	797	105 845	75 641	90 928	60 321	474	522	0.50	0.83
10/8/22	863	804	105 779	75 460	91 312	60 679	463	527	0.49	0.83
11/8/22	865	809	105 683	75 350	91 425	60 976	205	405	0.21	0.64
12/8/22	869	814	105 662	75 275	91 791	61 299	404	403	0.42	0.63
13/8/22	873	821	105 561	75 181	92 168	61 704	488	506	0.51	0.79
14/8/22	878	826	105 558	75 127	92 672	62 063	532	423	0.55	0.65
15/8/22	884	835	105 554	75 083	93 308	62 724	671	732	0.69	1.12
16/8/22	891	845	105 438	75 055	93 895	63 428	724	764	0.74	1.16
17/8/22	899	853	105 255	74 965	94 596	63 973	907	652	0.92	0.98
18/8/22	902	863	105 255	74 965	94 906	64 672	326	734	0.33	1.09
19/8/22	908	872	105 170	74 875	95 466	65 315	669	757	0.67	1.12
20/8/22	914	882	105 170	74 875	96 153	66 031	722	752	0.72	1.10
21/8/22	921	892	105 109	74 732	96 839	66 664	781	797	0.77	1.15
22/8/22	929	901	105 039	74 617	97 546	67 193	814	663	0.80	0.95
23/8/22	938	913	105 010	74 538	98 481	68 061	1 015	987	0.99	1.40
24/8/22	946	923	104 870	74 454	99 221	68 701	922	756	0.89	1.06

2.4 Collection design

Sediment traps were used to collect the sludge. Each trap consisted of four tubes with a removable cup at the bottom (shown in Figure 4). The tubes were held together by a plate shaped as a cross, with holes for the tubes on each arm of the cross. In the middle of the cross, there was a detachable knotting point going through the cross, giving knotting points on the upper side and on the lower side of the trap. The tubes were 47 cm long with an inner diameter of 6.5 cm, giving a collection area of 33.2 cm^2 per tube. 12 traps were used during this experiment, distributed under the two cages.

Figure 4 One of the sediment traps used during the collections.

The traps were placed in pairs of two with 5 meters of depth apart from each other. The first trap was placed at a depth of 39 meters and the second one at 44 meters below surface. To keep the traps steady in the water masses, a 10 kg weight was placed 1.8 meters underneath the bottom trap. The cages used in this experiment had a diameter of 120 meters. Therefore, the three trap pairs placed under each cage were placed 40 meters (1/3 of the cage diameter) apart from each other to evenly distribute them under the cage.

The traps were marked with a cage number (C1 and C2) as well as a marking to tell the top and bottom trap apart (T1-T3 and B1-B3). The numbers represent the pair the trap belongs to, meaning that T1 and B1 is one pair. C1 and C2 were the two different cages. Figure 5 illustrates how the traps were placed under the cages.

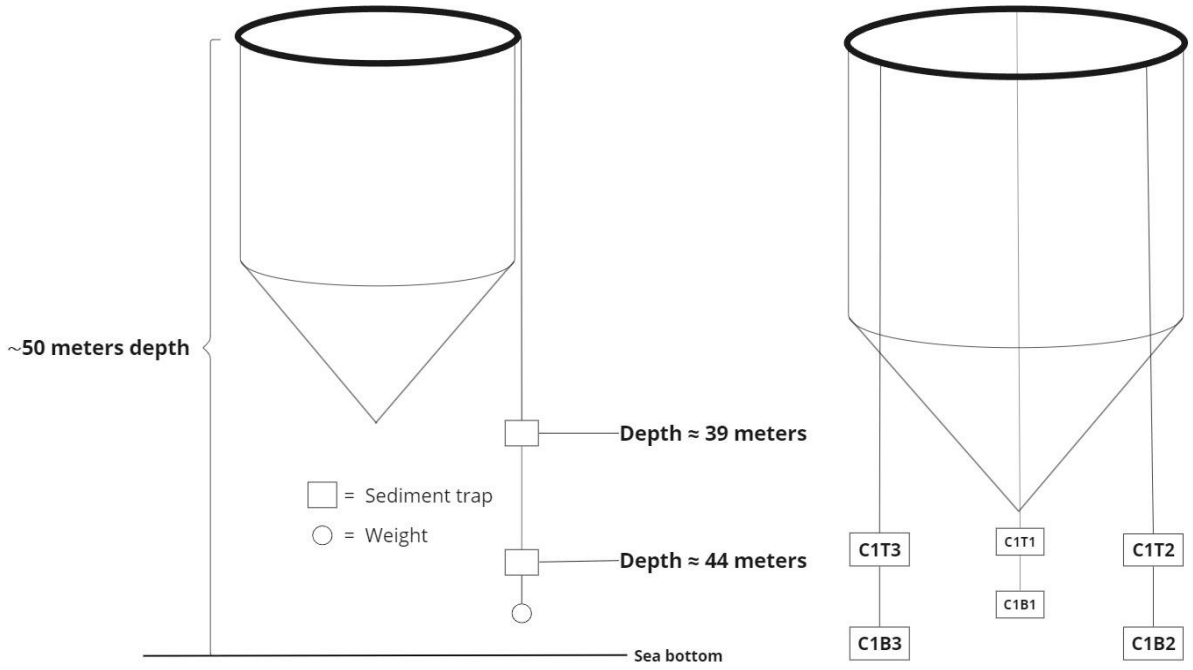

Figure 5 Illustration showing the trap setup (left side) and how the traps were placed and marked (right side).

2.5 Collection process

To ensure that the traps were collecting sludge for 48 hours, the time of when the traps were put out was noted each time. When putting out the traps, the rope was tied to the railing of the cage before the traps were immersed. This was done to prevent losing the traps in case the rope was lost during the immersion. The weight and the first, lower trap was put out and let down slowly before the higher, second trap also was put out. They were both slowly let down until all the rope was out. The same proscess was repeated for each trap every time.

After 48 hours, the traps were collected. When pulling the traps up, they were slowly pulled at a steady pace to prevent losing particles due to turbulence within the traps. When they reached the surface, they were carefully lifted while keeping enough distance from the cage to avoid any scraping along the bottom side of the floating pipe. This was done to prevent unwanted particles attached to the floating collar of the cages, such as barnacles and seaweed, from entering the tubes.

The sediment traps were transported to the feed barge and the sediments were allowed to settle for 10 minutes. After this, excess water above the level of the collection cups was pumped out, using a handpump with a 300 µm filter on the inlet and 200 µm on the outlet. The 200 µm filter

was added to make it possible to see if any particles were accidentally sucked in by the pump, as we would then see it on the inside of the filter on the outlet. This was never observed.

Larger objects of seaweed and shells were removed from the cups. The cups were now yet again left to sediement before more excess water was removed from the cups. Finally, the samples were transferred into sampling containers. Each trap was treated as one sample, meaning that all four cups from one trap were pooled into the same sampling container. All samples were frozen after being transferred into sampling containers. After all the samples were collected, they were brought back to Trondheim in a frozen state and kept in a freezer at -20°C.

For the reference site, the sediment traps were set out for 7 days instead of 48 hours and the samples were treated the same way as the sludge samples. Three pairs of traps were used for the reference collections. The location used was a location unaffected by aquaculture, but with approximately the same depth as the collection site. The location we used had a depth of 55 meters and was located about 3.5 km away from Lamøya (shown in Figure 3).

The setup used for the reference site was modified, as there were no cages to hold the traps in place. To compensate for this, an extention of 10 meters of the rope from the bottom trap to the bottom weight was added. This led to the weight being located on the sea floor as an anchor. A floating element was attached about 1 meter above the top trap to keep the traps standing vertically in the water masses and maintain the traps in the same position throughout the collection period. Lastly, a bouy was attached at the surface to be able to locate the traps. The setup is illustrated in Figure 6.

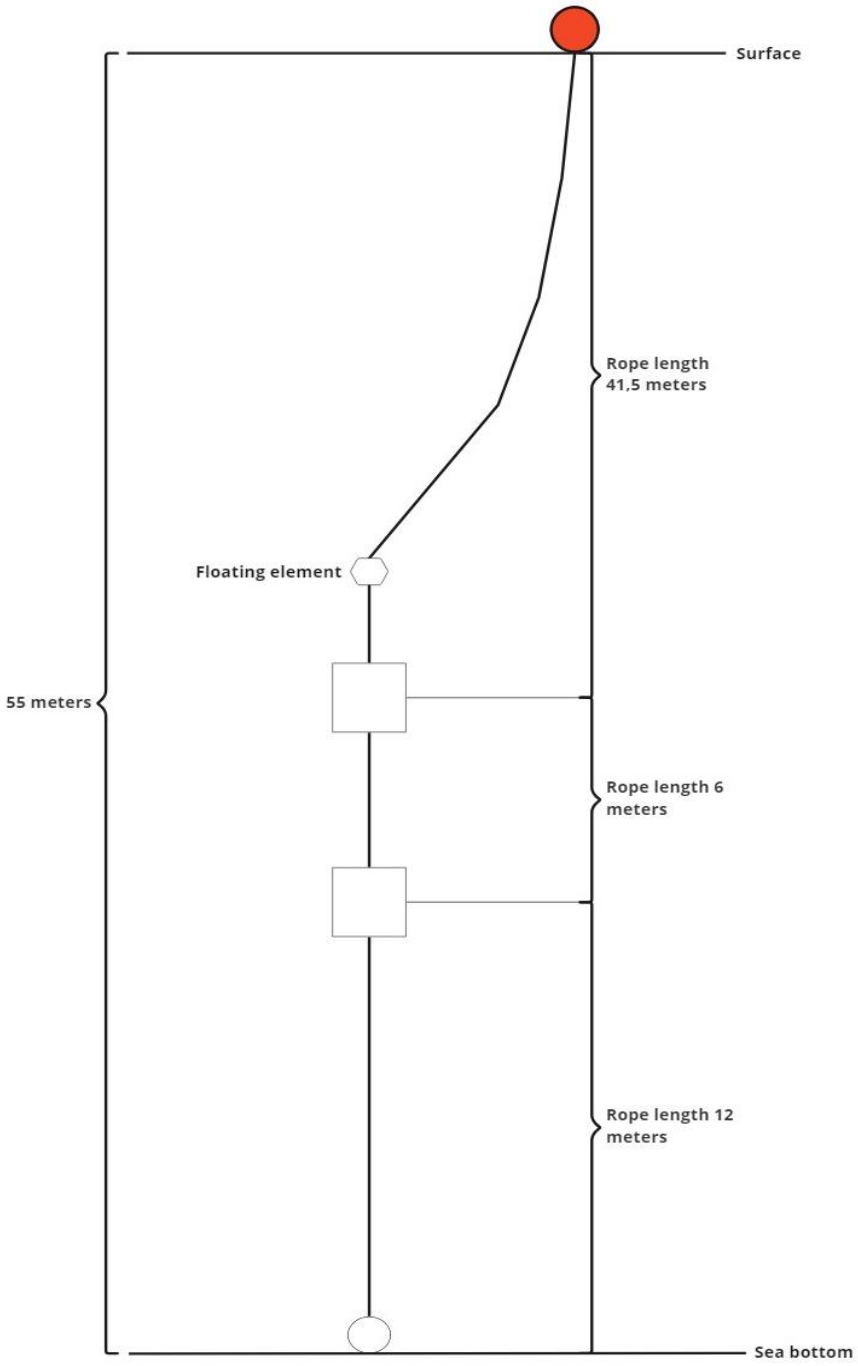

Figure 6 Illustration showing the reference setup.

2.6 Chemical analyses

2.6.1 Dry matter

All the sludge samples were centrifuged using a Sorwall RC5C for 5 minutes at 5000 rpm. Half of the water volume was poured out before each sample was split into two 50 ml sample containers. The sludge samples were then centrifuged two times using a Heraeus Labofuge 400R for 5 minutes at 5000 rpm, removing water between each round. The final 15-20 ml of water was removed using a pipette. Finally, the sludge and feed samples were freeze-dried in a Beta 1-8 LSCbasic freeze dryer for 48 hours. The feed samples had to be dried in a heating cabinet at 110°C for an additional 24 hours before they were fully dried and weighed.

2.6.2 Carbon and Nitrogen

500-1000 µg of dry matter were weighed into tin capsules, using a Mettler Toledo UMT2 scale. The capsules were then sealed by folding them several times and stored in a heating cabinet at 60°C overnight. The tin capsules containing samples were then analysed using a Vario El cube elemental analyser (Elementar). The software compared the elemental peak to a known standard of Acetanilide, and the contents of C and N were converted to and expressed in mg gDM^{-1}. The analysis was performed by Siv Anina Etter at NTNU TBS.

2.6.3 Phosphorous

500 µg of dry matter was added to separate polyethylene scintillation vials. 15 ml distilled water, 0.15 ml 4 M H_2SO_4 and 3 ml potassium persulfate was added to the vials to convert particulate phosphorous into dissolved phosphate. The samples were then autoclaved at 120°C for 30 minutes and cooled down subsequently. 4 mL of the samples were filtered using a VWR syringe filter, 25 mm, 0.45 µm mesh into 4.5 mL plastic tubes. An O.I Analytical Autosampler (Model 3360) was then used to analyse the samples, where the phosphate content of the samples was measured. The analysis was performed by Siv Anina Etter at NTNU TBS.

2.6.4 Amino acids

50-100 mg of dry matter were analysed using a method developed by Agilent and Pickering laboratories. The samples were first hydrolysed in 6 M HCl containing 0.4% mercaptoethanol for 24 hours at 110°C. During the HCl hydrolysis, glutamine and aspargine were converted to glutamic and aspartic acid. Following this, the pH was adjusted before the samples were filtered and diluted using a citrate buffer. A HPLC system (Agilent Infinity 1260, Agilent Technologies) was then used to analyse the samples. The amino acid analysis was performed by SINTEF Ocean.

2.6.5 Protein

The protein content was calculated using the recommended method to calculate protein content, according to FAO (2003). The formula calculates the sum of individual amino acid residues by removing the molecular weight of the water and calculating the dehydrated weight of each amino acid. This was then multiplied by the content of the measured amino acid to get the protein content for each amino acid, giving the following formula:

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

The total protein content was then determined by summarizing the protein content of each individual amino acid.

2.6.6 Total lipids

The total lipid extraction of sludge and feed samples were done according to Folch et al. (1957). For the sludge, 200 mg of the grounded sample was used and for the feed 100 mg was used. The samples were homogenized with 6 mL of chloroform:methanol (2:1), using an Ultra Turrax T8. 1.5 mL of 0.88% KCl was then added to the solution. A vortex mixer was used to mix the samples before they were centrifuged at 4000 rpm for 4 minutes at 4°C. By using a glass pipette, the organic phase in the lower part of the tube was collected and transferred into a 15 mL kimax tube. Another 2 mL of chloroform was added to reextract the samples to assure that as much as possible of the organic phase was extracted. The samples from the two extractions were then pooled and filtered using 0.2 µm PTFE mesh filters. The solution was

then dried using gaseous nitrogen and put in a desiccator for 1 hour for further drying. The total lipid content was finally measured by weighing.

2.6.7 Fatty acids

A stock solution of 5 mg/ml was made by resolving the total lipids (from 2.6.6) in chloroform:methanol (2:1). 40 µl of this stock solution was added to a new kimax-tube. 1 mL of $CHCl_3$ x/ISTD 23:0 and 2 mL 1% H_2SO_4:MeOH was then added, and the tubes were flushed with N_2-gas. After this, the samples were incubated on a 50°C heating block for 16 hours. The next day, the samples were cooled down to room temperature and 5 mL of saturated NaCl and 2 mL isooctane was added. The samples were mixed using a vortex mixer, before they were centrifuged at 4000 rpm for 3 minutes at 4°C. The fatty acid methyl esters (FAME), which were now in the upper layer, were transferred into new kimax-tubes using glass pipettes. This process of adding isooctane, centrifuging and transferring FAME was performed two more times, first adding 2 mL more of isoocatane and the last time 1 mL. The FAME was then dried using gaseus nitrogen before being resolved in 200 µl isooctane. Finally, the FAME were analysed using gas chromatography, performed by Zdenka Bartosova at NTNU Department of Biology.

2.6.8 Ash

To measure the ash content of the samples, a pre-weighed amount of the grounded sludge, feed and reference site samples were put in different crucibles and placed in a muffle furnace for 5 hours at 450°C. After the samples had been cooled down over night, the samples were weighed again using the same scale. The ash content was determined using the following formula:

$$Ash = \frac{Weight\ before\ combustion\ (mg) - Weight\ after\ compustion(mg)}{Sample\ weight\ before\ combustion\ (g)}$$

The weight before and after combustion includes the weight of both the crucible and the sample.

2.7 Quantification of sediments

The calculated amount of sludge sedimented directly beneath the cages were calculated by the following formula:

$$Calculated\ amount\ of\ sedimented\ sludge\ = \frac{Cage\ area\ (\mathrm{m}^2)}{Area\ of\ three\ traps\ (m^2)} * Collected\ sludge\ (kg)$$

The values represent the calculated amount of sludge sedimented directly beneath the cages, based on the amount of sludge captured at each depth. The only factors considered for these calculations are the area of the cage, the area of the traps and the amount of collected sludge.

By using the assumptions for sludge production in Norwegian salmon farms as described in Aas and Åsgård (2017), the theoretical amounts of sludge produced by the salmon in the present cages were calculated. The calculations were based on the average amount of DW of feed supplied to the salmon during each of the two-day collection periods.

The following assumptions were used:

1. Salmon eats 87% of the feed it is given, meaning that 13% of the feed ends up as feed waste.
2. Salmon is able digest 70% of the DW of the feed that is given, meaning that 30% of the content is left in the faeces.

This gave the following formula:

$$\begin{aligned} Sludge\ produced &= Feed\ waste\ (kg) + Faeces\ (kg) \\ &= (DW\ Feed\ (kg) * 13\%) + (DW\ Feed\ (kg) * 87\% * 30\%) \end{aligned}$$

2.8 Data treatment and statistical analyses

Calculations of all data presented in this study were performed using Microsoft® Excel. Tables were made using Microsoft® Word and Microsoft® Excel. Creating of graphs and statistical analyses were performed using SigmaPlot® for Windows version 14.0.

Normal distribution of data was tested using the Shapiro-Wilk test, and for variance of data the Brown-Forsythe test was used. To determine statistical differences between the data, t-tests were performed. Contents of dry matter, carbon, nitrogen, phosphorous, amino acids, protein, total lipids, fatty acids and ash, as well as elemental ratios, the quantified amounts of sedimented sludge and theoretically produced sludge were all tested for significant differences. The values were cross tested between top and bottom traps and between Cage 1 and Cage 2. When the assumptions for normality of data was not met, log transformation or a Mann-Whitney Rank Sum test was used. Statistical analysis was performed at the 95% confidence level ($P<0.05$). When analysing the correlation between feeding and sludge collected, a linear regression test was performed.

When analysing the contents of dry matter, carbon, nitrogen and phosphorous, all the collected samples were analysed individually, giving a sample size of n=9 for each group (three top/bottom samples at each collection point for each of the three replicates). For amino acids, protein, total lipids, fatty acids and ash, the three samples from each depth at each cage were pooled, giving a sample size of n=3 for each group (one pooled top/bottom sample at each collection point for each of the three replicates).

The results from the chemical analyses conducted on the sample from C1B2 17/8 was removed due to the sample container breaking during centrifuging, giving bad results. During the collection period, no data was gathered from trap C1B1 11/8 due to the trap being lost. The values for this trap used during the quantifications of sludge were an estimate based on the average collected WW and DW of trap C1T1 multiplied by 0.97 since the C1B1 trap collected an average of 97% of what the C1T1 trap collected during the other collection periods.

3 Results

3.1 Chemical composition

3.1.1 Dry matter

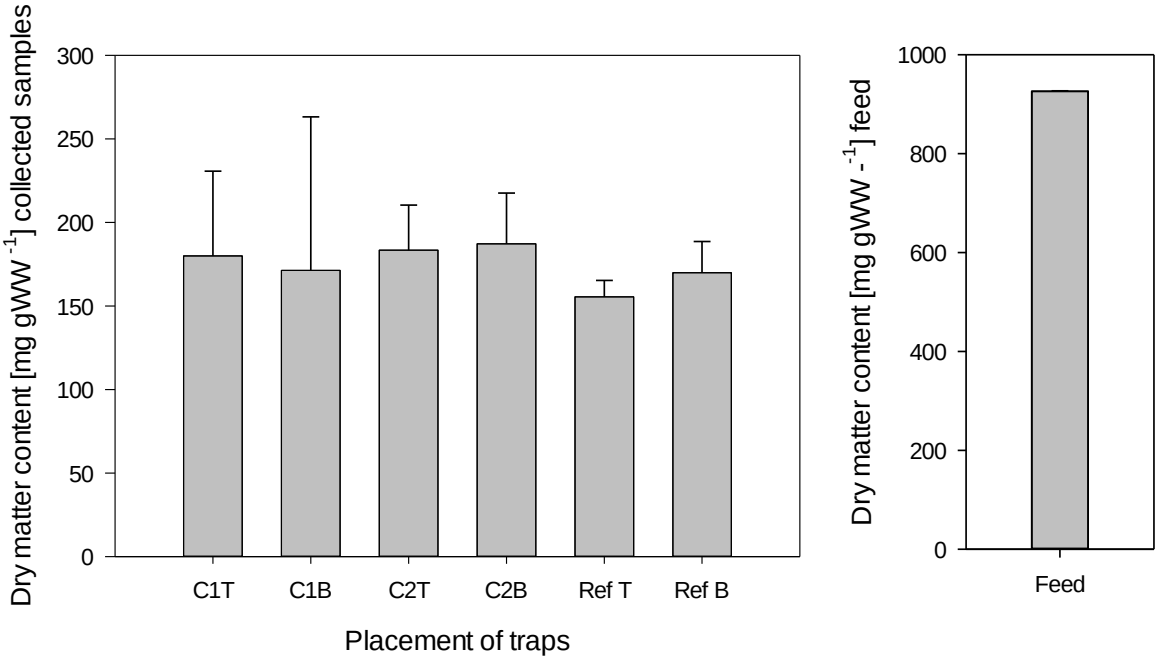

Figure 7 Mean±SD (n=9 for sludge samples, n=3 for reference samples) dry matter (DM) content (mg gWW^{-1}) (left). The displayed samples are from cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B), as well as from the reference site top traps (Ref T) and reference site bottom traps (Ref B). Mean±SD (n=3) dry matter content (mg gDM^{-1}) of the feed samples (right).

Mean DM contents (mg gWW^{-1}) of samples collected at each cage and depth are shown in Figure 7. The mean DM content of the sludge ranged between 171-187 mg gWW^{-1}, while the samples from the reference site ranged between 156-170 mg gWW^{-1}. The feed samples had a significantly higher DM content of 926 mg gWW^{-1}. No significant differences were found between the dry matter contents captured at any depth or cage ($P \geq 0.05$).

3.1.2 Carbon

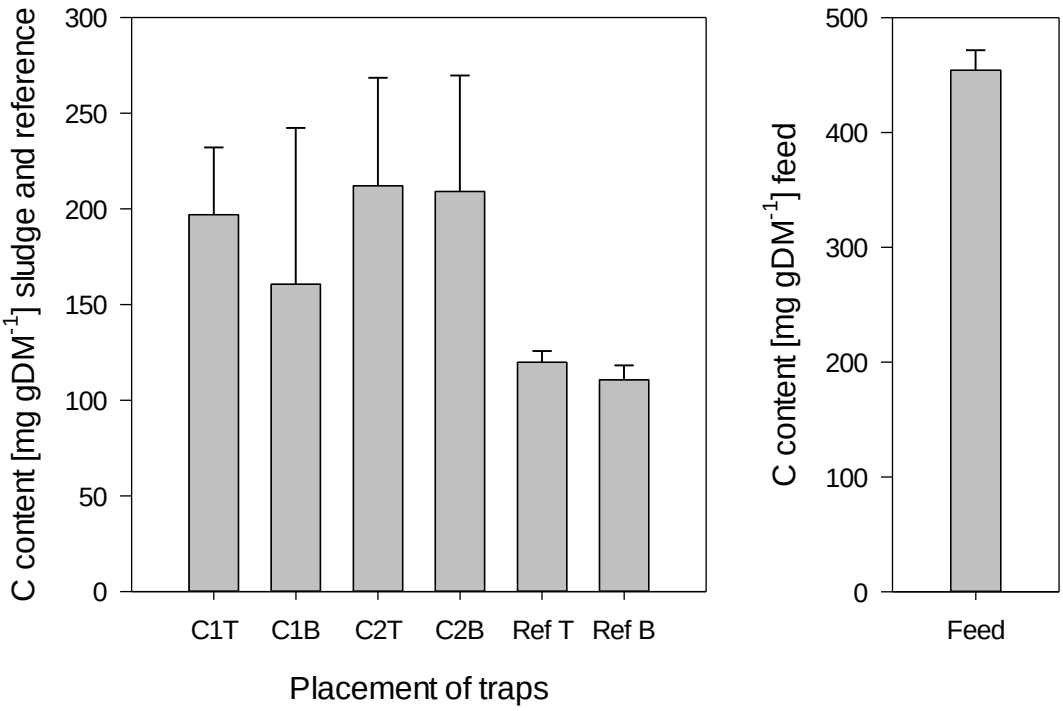

Figure 8 Mean±SD (n=9 for sludge samples, n=3 for reference samples) carbon (C) content (mg gDM^{-1}) of the collected sludge from the traps in cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B) (left). The samples from the reference site top traps (Ref T) and reference site bottom traps (Ref B) are also displayed. Mean±SD (n=3) C content (mg gDM^{-1}) of the feed samples (right).

Figure 8 shows the mean content of carbon (mg gDM^{-1}) in the collected samples. The mean C content ranged between 161-212 mg gDM^{-1} for the sludge samples. At the reference site, the samples had a mean C content around half of the amount of the sludge samples, ranging between 111-120 mg gDM^{-1}. The feed samples had a mean C content of 454 mg gDM^{-1}, more than double the amount of the sludge samples. No significant differences were observed between the measured C contents at any depth or cage ($P \geq 0.05$).

3.1.3 Nitrogen

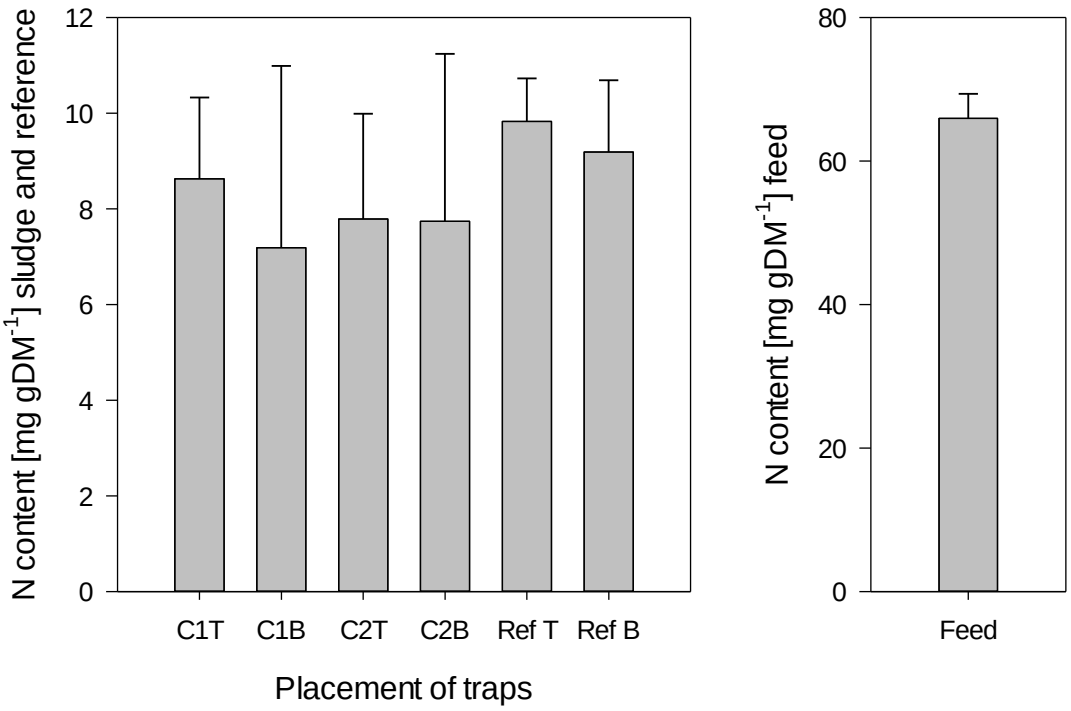

Figure 9 Mean±SD (n=9 for sludge samples, n=3 for reference samples) nitrogen (N) content (mg gDM^{-1}) of the collected sludge from the traps in cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B) (left). The samples from the reference site top traps (Ref T) and reference site bottom traps (Ref B) are also displayed. Mean±SD (n=3) N content (mg gDM^{-1}) of the feed samples (right).

The mean contents of nitrogen (mg gDM^{-1}) are shown in Figure 9. For the sludge samples, the N content ranged between 7.2-8.6 mg gDM^{-1}. The samples from the reference site were somewhat higher, ranging from 9.2-9.8 mg gDM^{-1}. The feed samples had the highest N content of 66 mg gDM^{-1}. No significant differences were found between the measured N contents at any depth or cage ($P \geq 0.05$).

3.1.4 Phosphorus

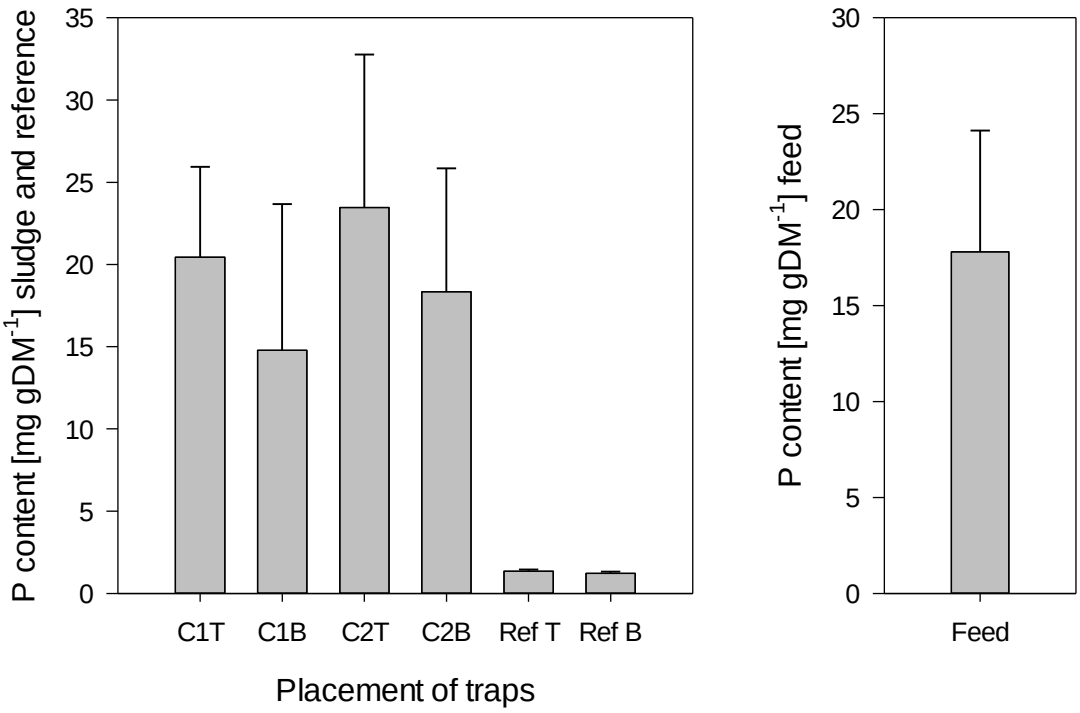

Figure 10 Mean±SD (n=9 for sludge samples, n=3 for reference samples) phosphorous (P) content (mg gDM^{-1}) of the collected sludge from the traps in cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B) (left). The samples from the reference site top traps (Ref T) and reference site bottom traps (Ref B) are also displayed. Mean±SD (n=3) P content (mg gDM^{-1}) of the feed samples (right).

Mean phosphorous contents (mg gDM^{-1}) of the collected sludge, reference samples and feed samples are presented in Figure 10. The P content of the sludge samples ranged between 15-24 mg gDM^{-1}. The P content of the reference site samples was only a fraction of this, ranging between 1.2-1.4 mg gDM^{-1}. In the feed samples, the P content was similar to the sludge samples, having a mean of 18 mg gDM^{-1}. No significant differences were observed between the measured P contents at any depth or cage ($P \geq 0.05$).

3.1.5 Elemental ratios

Table 5 Median C:N, C:P, N:P and C:N:P ratios for the sludge samples, reference site samples and feed samples. Median values were used due to non-normality of data.

	C:N	**C:P**	**N:P**	**C:N:P**
Sludge	25.4	10.4	0.4	10:0.4:1
Reference site	12.3	87.4	7.1	97:7:1
Feed	6.6	31.6	4.5	32:5:1

The median elemental ratios for the sludge samples, reference site samples and feed samples are shown in Table 5. The sludge had the highest C:N ratio of 25.4, compared to 12.3 in the reference site samples and 6.6 in the feed. For the C:P ratio, the sludge had the lowest with 10.4, where the reference site samples had a ratio of 87.4 and the feed samples 31.6. The sludge samples also had the lowest N:P ratio of 0.4, where the reference site samples had a ratio of 7.1 and the feed samples 4.5. Finally, the C:N:P ratios were 11:0.4:1, 97:7:1 and 32:5:1 for the sludge, reference site and feed samples, respectively. No significant differences were observed within any of the elemental ratios ($P \geq 0.05$), but non-normality of data occurred, which is why median values were used.

3.1.6 Amino acids

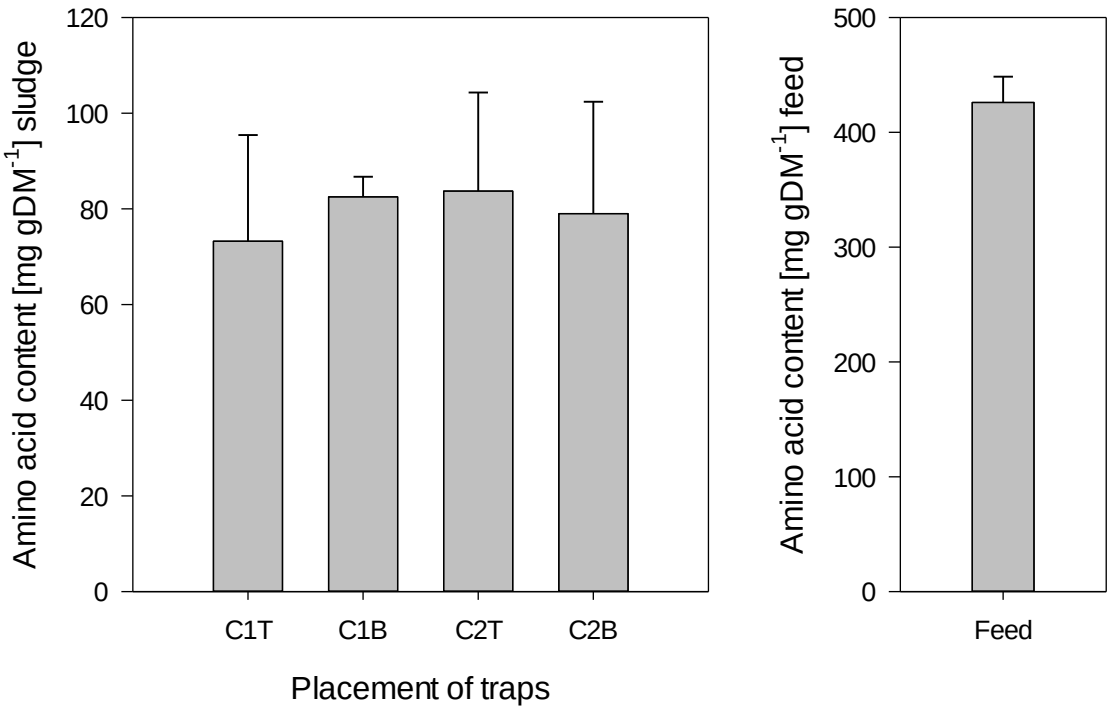

Figure 11 Mean±SD (n=3) total amino acid content (mg gDM^{-1}) of the collected sludge from the traps in cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B) (left). Mean±SD (n=3) total amino acid content (mg gDM^{-1}) of the feed samples (right).

Figure 11 shows the mean total amino acid content (mg gDM^{-1}) of the samples. The sludge samples all had a similar mean total amino acid content, ranging between 73-84 mg gDM^{-1}. The feed samples had a mean total amino acid content five times higher of 426 mg gDM^{-1}. No significant differences were observed between the measured amino acid contents at any depth or cage ($P \geq 0.05$).

Table 6 Mean±SD (n=3) amino acid content (% of total amino acid content) in cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B), as well as feed.

Essential amino acids (EAA)					
(%)	**C1T**	**C1B**	**C2T**	**C2B**	**Feed**
Arginine	3.19±0.61	3.07±0.29	3.87±0.56	3.11±0.32	6.54±0.28
Histidine	2.36±0.18	2.35±0.16	2.74±0.52	2.20±0.24	2.16±0.02
Isoleucine	6.50±0.68	6.77±0.82	6.34±0.28	6.18±0.18	4.41±0.03
Leucine	9.19±0.78	9.02±0.67	9.49±0.48	8.83±0.52	7.62±0.03
Lysine	4.60±0.43	4.61±0.5	4.20±0.22	4.54±0.15	6.96±0.19
Methionine	2.52±0.31	2.35±0.48	2.40±0.08	2.15±0.19	2.08±0.06
Phenylalanine	8.00±0.51	7.74±0.79	7.76±0.26	7.41±0.43	4.66±0.07
Threonine	4.55±0.29	4.63±0.04	4.58±0.47	4.50±0.09	3.16±0.07
Tryptophan	-	-	-	-	-
Valine	6.64±0.17	6.19±0.1	6.5±0.06	6.26±0.16	5.09±0.09
Total EAA (%)	47.55±0.47	46.73±1.16	47.88±0.74	45.18±1.18	46.04±0.57
Total EAA (mg gDW^{-1})	34.84±8.89	38.56±0.87	40.10±8.24	35.70±9.30	196.19±8.68
Non-essential amino acids (non-EAA)					
Alanine	7.29±0.31	7.37±0.44	7.53±0.67	7.95±0.57	5.68±0.02
Aspartic acid + Asparagine	5.76±0.25	6.58±0.71	6.58±0.51	6.55±0.29	6.53±0.17
Cystine	2.44±0.27	2.00±0.50	2.13±0.25	1.95±0.25	1.56±0.10
Glutamic acid + Glutamine	9.12±0.76	9.93±1.74	8.54±0.36	8.53±0.41	21.27±0.52
Glycine	5.70±0.36	6.12±0.50	5.63±0.57	5.58±0.61	4.12±0.05
Proline	6.28±1.33	6.73±0.94	7.25±1.25	8.97±1.45	8.26±0.44
Serine	7.51±1.01	7.31±0.69	7.48±0.14	7.83±0.16	6.41±0.21
Taurine	0.16±0.16	0.12±0.09	0.40±0.11	0.24±0.10	0.08±0.02
Tyrosine	5.65±0.43	4.97±0.58	5.39±0.26	4.98±0.19	3.18±0.08
Methionine sulfoxide	0.22±0.11	0.20±0.13	0.17±0.09	0.31±0.14	0.04±0.02
Hydroxyproline	1.36±0.66	1.83±1.11	0.87±0.57	1.35±0.6	0.09±0.02
Hydroxylysine	0.96±0.58	0.13±0.02	0.13±0.10	0.59±0.32	0.10±0.09
Total non-EAA (%)	52.45±0.47	53.27±1.16	52.12±0.74	54.82±1.18	53.96±0.89
Total non-EAA (mg gDW^{-1})	38.43±9.23	43.96±2.73	43.64±8.62	43.32±9.83	229.97±10.28
Total AA (mg gDW^{-1})	73.26±18.12	82.52±3.46	83.74±16.82	79.02±19.11	426.16±18.32

Amino acid (AA) composition (% of total AA content) of sludge and feed samples are displayed in Table 6. EAA made up for 45-48% of the total AA content in the sludge samples and 46% of the AA content in the feed samples. Leucine, phenylalanine and lysine were the most abundant EAA with contents of 8.8-9.5%, 7.4%-8.0 and 4.2-4.6% of total AA, respectively, in the sludge samples. The contents of the same EAA's in the feed samples were 7.6%, 4.7% and 7.0% of total AA content. The mean content of non-EAA ranged between 52-55% of total AA content in the sludge samples and 54% in the feed samples. The most abundant non-EAA in the sludge samples were glutamic acid + glutamine, alanine and serine with contents of 8.5-9.9%, 7.3-8.0% and 7.3-7.8% of total AA content, respectively. For the feed, the contents of the same non-EAA were 21%, 5.7% and 6.4% of total AA content. No significant differences were observed between the contents of EAA, non-EAA or total AA ($P \geq 0.05$).

3.1.7 Protein

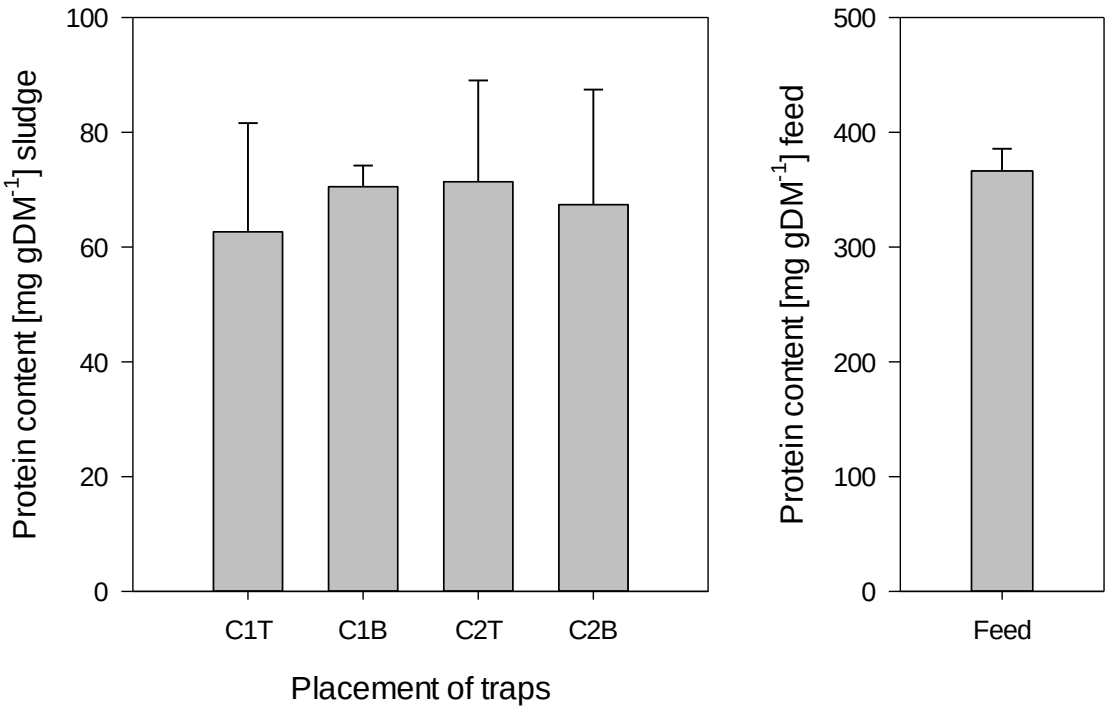

Figure 12 Mean±SD (n=3) protein content (mg gDM^{-1}) of the collected sludge from the traps in cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B) (left). Mean±SD (n=3) protein content (mg gDM^{-1}) of the feed samples (right).

Mean protein contents (mg gDM^{-1}) are presented in Figure 12. The mean protein content of the sludge samples ranged from 63-71 mg gDM^{-1}. The protein content of the feed samples was more than five times higher than the sludge samples with a content of 366 mg gDM^{-1}. No significant differences were observed between the measured protein contents at any depth or cage ($P \geq 0.05$).

3.1.8 Total lipids

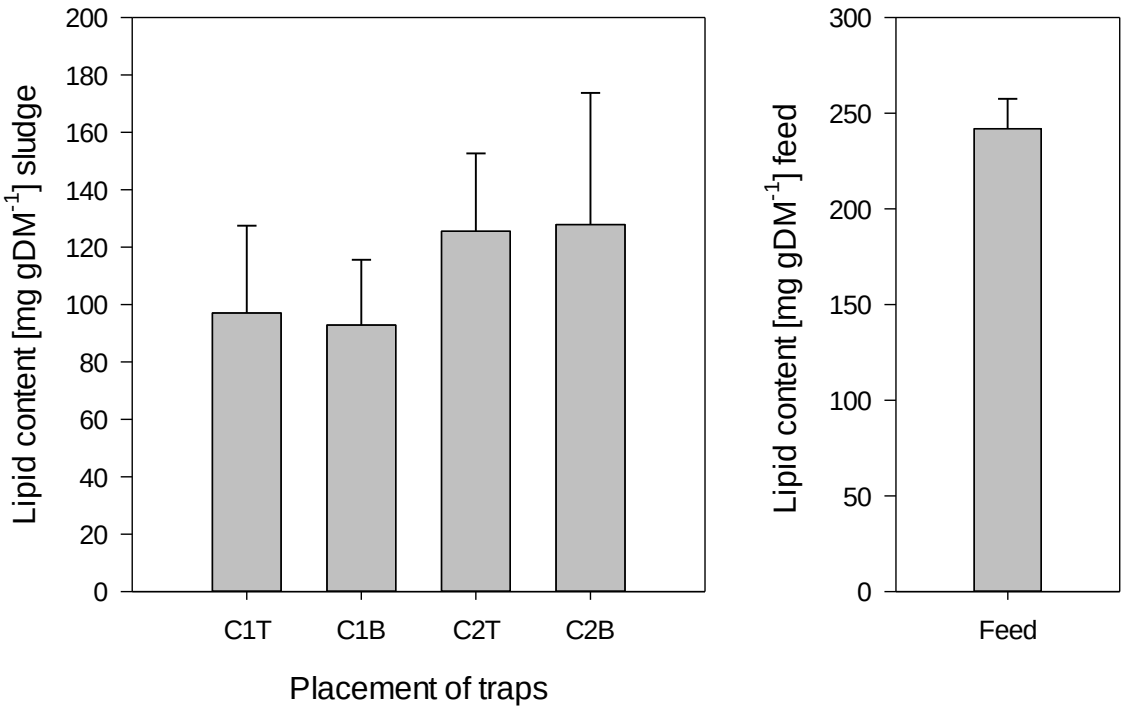

Figure 13 Mean±SD (n=3) total lipid content (mg gDM^{-1}) of the collected sludge from the traps in cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B) (left). Mean±SD (n=2) total lipid content (mg gDM^{-1}) of the feed samples (right).

Figure 13 presents the mean content (mg gDM^{-1}) of total lipids in the samples. For the sludge samples, the mean total lipid content ranged between 93-128 mg gDM^{-1}. The mean total lipid content of the feed samples was found to be 242 mg gDM^{-1}, approximately double the content of the sludge samples. No significant differences were observed between the measured total lipid contents at any depth or cage ($P \geq 0.05$).

3.1.9 Fatty acids

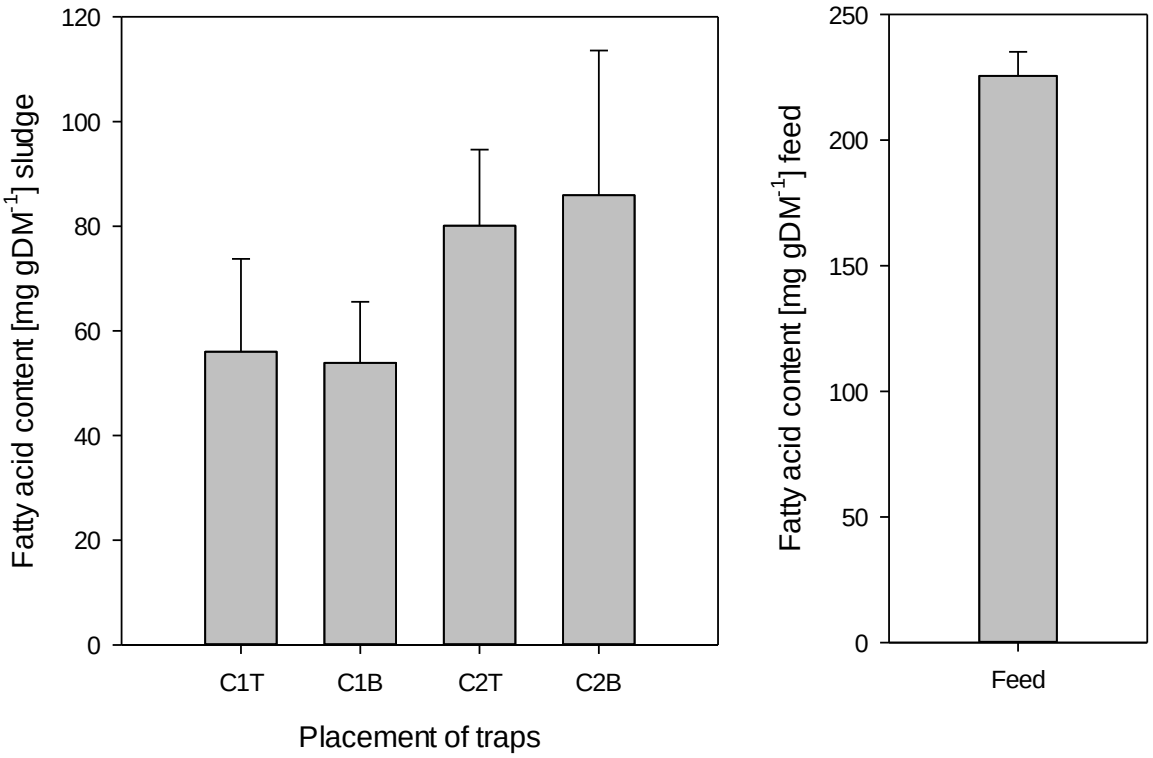

Figure 14 Mean±SD (n=3) fatty acid content (mg gDM^{-1}) of the collected sludge from the traps in cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B) (left). Mean±SD (n=2) fatty acid content (mg gDM^{-1}) of the feed samples (right).

Mean fatty acid contents (mg gDW^{-1}) ranged between 55-86 mg gDW^{-1} for the sludge samples (Figure 14). The mean content in the feed samples was found to be significantly higher at 223 mg gDW^{-1}. No significant differences were observed between the measured fatty acid contents at any depth or cage ($P \geq 0.05$).

Table 7 Mean±SD (n=3 for sludge samples, n=2 for feed samples) fatty acid content (mg gDW^{-1}) in the collected sludge from cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B), as well as feed. Contents are presented in mg gDW^{-1} instead of percentages due to some unidentified peaks occurring during the gas chromatography, resulting in the possibility of some missing fatty acids.

	C1T	**C1B**	**C2T**	**C2B**	**Feed**
Total FA (mg gDW^{-1})	56.04±17.73	53.91±11.65	80.11±14.52	85.92±27.66	225.64±9.51
C14:0	1.62±0.42	1.53±0.29	2.16±0.32	2.28±0.64	4.57±0.23
C15:0	0.18±0.04	0.17±0.03	0.21±0.10	0.22±0.05	0.41±0.02
C16:0	10.27±2.26	9.81±1.73	12.22±5.97	13.04±3.37	22.78±0.91
C17:0	0.29±0.08	0.27±0.07	0.27±0.10	0.25±0.06	0.45±0.02
C18:0	13.24±3.04	11.36±2.89	13.46±6.8	13.51±3.5	9.24±0.42
C20:0	0.84±0.21	0.78±0.19	1.05±0.53	1.15±0.32	1.03±0.05
C22:0	0.48±0.07	0.49±0.06	0.57±0.28	0.66±0.09	0.66±0.03
ΣSAFA	26.92±5.98	24.41±5.11	29.94±14.93	31.11±80	39.14±1.67
C14:1 n-5	0.03±0.00	0.03±0.01	0.04±0.03	0.05±0.02	0.15±0.02
C16:1 n-9	0.07±0.02	0.07±0.01	0.10±0.05	0.11±0.03	0.26±0.01
C16:1 n-7	1.06±0.36	1.05±0.20	1.77±0.95	1.86±0.62	5.56±0.26
C16:1 n-5	0.11±0.02	0.11±0.03	0.13±0.05	0.15±0.03	0.33±0.01
C18:1 n-9	14.42±6.36	14.54±3.76	25.8±11.66	29.76±11.33	85.81±3.94
C18:1 n-7	1.34±0.43	1.35±0.26	2.37±1.32	2.54±0.78	5.45±0.28
C20:1 n-9	1.87±0.79	1.82±0.52	3.45±1.91	3.69±1.28	6.73±0.28
C22:1 n-11	0.29±0.10	0.29±0.09	0.51±0.30	0.54±0.18	0.70±0.03
C22:1 n-9	1.80±0.76	1.70±0.49	3.06±1.65	3.22±1.04	5.83±0.22
C24:1	0.49±0.13	0.45±0.13	0.62±0.32	0.63±0.17	0.66±0.03
ΣMUFA	21.47±8.93	21.41±5.41	37.86±18.26	42.55±15.43	111.47±5.09
C16:2	0.03±0.02	0.04±0.01	0.08±0.05	0.09±0.05	0.41±0.02
C18:2 n-6	4.38±1.61	4.53±0.50	7.11±3.89	7.19±2.62	35.16±1.06
C18:3 n-3	1.12±0.58	1.23±0.24	2.14±1.13	2.12±0.84	18.77±0.81
C18:4 n-3	0.16±0.07	0.18±0.07	0.24±0.15	0.27±0.11	1.88±0.14
C20:2 n-6	0.13±0.03	0.12±0.04	0.19±0.12	0.20±0.06	0.26±0.01
C20:4 n-6	0.13±0.02	0.19±0.08	0.09±0.01	0.15±0.04	0.63±0.03
C20:3 n-3	0.03±0.01	0.03±0.01	0.05±0.03	0.05±0.02	0.11±0.01
C20:5 n-3 (EPA)	0.32±0.10	0.41±0.08	0.53±0.30	0.50±0.20	7.95±0.34
C22:5 n-3	0.40±0.12	0.39±0.09	0.52±0.25	0.67±0.27	1.73±0.04
C22:6 n-3 (DHA)	0.95±0.32	0.96±0.21	1.35±0.77	1.28±0.48	8.12±0.27
ΣPUFA	7.65±2.84	8.09±1.24	12.31±6.72	12.53±4.65	75.03±2.74
Σn-3	2.98±1.17	3.20±0.63	4.84±2.64	4.89±1.89	38.57±1.62
Σn-6	4.64±1.65	4.85±0.61	7.39±4.02	7.55±2.72	36.05±1.10
DHA:EPA	2.99±0.03	2.43±0.64	2.51±1.31	2.58±0.11	1.02±0.01

Table 7 displays the fatty acid content (mg gDW^{-1}) of the sludge samples as well as the feed samples. Oleic acid (C18:1 n-9) was found to be the most abundant fatty acid for all sludge samples and for the feed, with a content of 14-30 mg gDW^{-1} for the sludge samples and 86 mg gDW^{-1} for the feed samples. Other fatty acids with a high content were stearic acid (C18:0) with a content of 11-14 mg gDW^{-1} in the sludge samples and 9 mg gDW^{-1} in the feed, palmitic acid (C16:0) with a content of 10-13 mg gDW^{-1} in the sludge sample and 23 mg gDW^{-1} in the feed, and linolelaidic acid (C18:2 n-6) with a content of 4.4-7.2 mg gDW^{-1} in the sludge and 35 mg gDW^{-1} in the feed. The content of saturated fatty acids (SAFA) ranged between 24-31 mg gDW^{-1} for the sludge samples and 39 mg gDW^{-1} for the feed. The content of monosaturated fatty acids (MUFA) was found to range between 21-43 mg gDW^{-1} for the sludge and 111 mg gDW^{-1} for the feed. For the polyunsaturated fatty acids (PUFA), the sludge had a content between 8-13 mg gDW^{-1} while the feed had a content of 75 mg gDW^{-1}. The total content of omega-3 fatty acids (n-3) was found to range from 3.0-4.9 mg gDW^{-1} for the sludge while the feed had a content of 39 mg gDW^{-1}. Looking at omeage-6 fatty acids (n-6), the sludge had a total content ranging between 4.6-7.6 mg gDW^{-1} and the feed had a content of 36 mg gDW^{-1}. The DHA:EPA ratio was found to range from 2.4-3.0 for the sludge samples while the feed had a ratio of 1.0. No significant differences were observed for the contents of SAFA, MUFA, PUFA, EPA, DHA or total fatty acids in the sludge samples ($P \geq 0.05$).

3.1.10 Ash

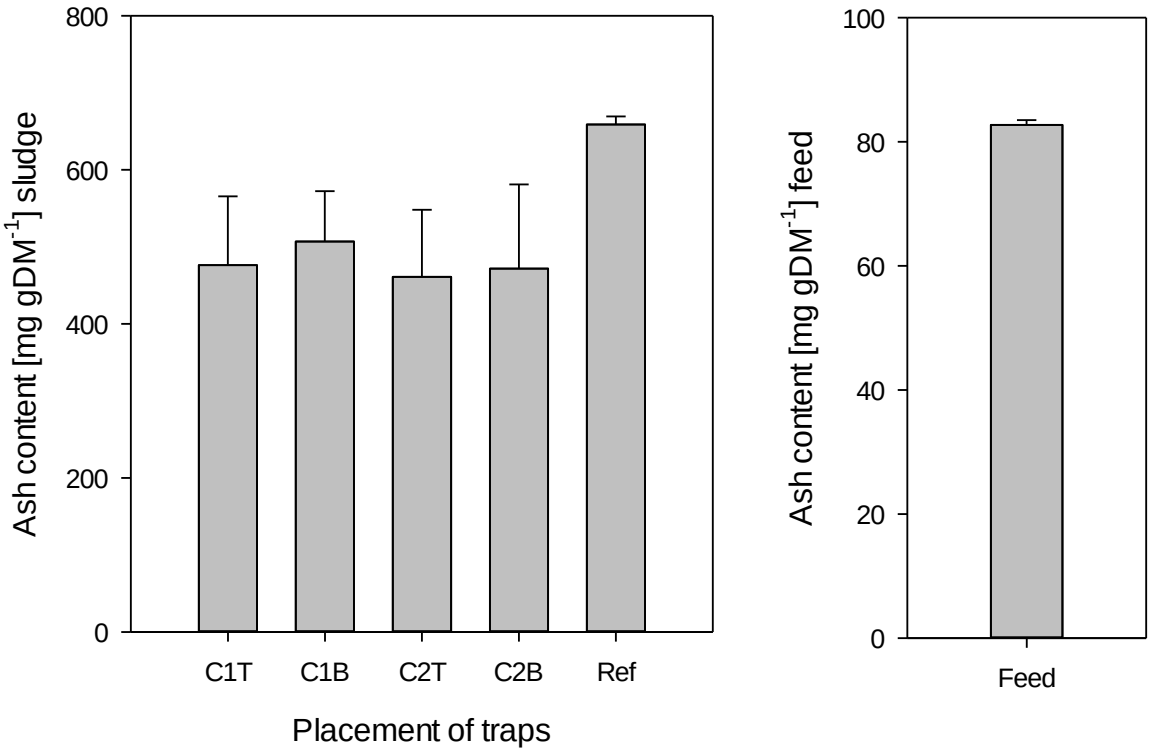

Figure 15 Mean±SD (n=3) ash content (mg gDM^{-1}) of the collected sludge and from the traps in cage 1 top (C1T), cage 1 bottom (C1B), cage 2 top (C2T) and cage 2 bottom (C2B) (left). The mean±SD (n=3) ash content of the reference site samples (Ref) is also displayed. Mean±SD (n=3) ash content (mg gDM^{-1}) of the feed samples (right).

Mean ash contents (mg gDM^{-1}) are displayed in Figure 15. The mean ash content of the sludge samples amounted between 461-507 mg gDM^{-1}. For the reference site, the mean ash content amounted to 659 mg gDM^{-1} and for the feed samples the mean content was found to be 83 mg gDM^{-1}, almost six times lower than in the sludge samples. No significant differences were observed between the measured ash contents at any depth or cage ($P \geq 0.05$).

3.2 Quantification of sediments

3.2.1 Dry weight of the collected samples

Table 8 Total dry weight (mg DW d^{-1}) collected at each depth per day from cage 1 (C1), cage 2, (C2) and from the reference site traps (reference).

	Top traps	Bottom traps
C1 11/8	811	675
C2 11/8	717	797
C1 17/8	1 373	1 374
C2 17/8	1 263	1 137
C1 24/8	1 274	1 185
C2 24/8	1 326	1 273
Reference	29	38

Table 8 shows the total dry weight (mg DW d^{-1}) of the sludge samples and reference site samples at each depth. The total amount of sludge collected ranged between 675-1374 mg DW d^{-1}. The top traps collected more or equally as much sludge as the bottom traps at all days/cages, except C2 11/8. In this instance, the bottom traps collected 10% more than the top traps. When looking at the trend between the amount of sludge captured by the top and bottom traps, the top traps collect an average of 5% more sludge than the bottom traps. For the reference site, the top traps captured an average of 29 mg DW d^{-1} and the bottom traps 38 mg DW d^{-1}. No significant significances were observed for the DW of sludge collected from any of the depths or cages ($P \geq 0.05$), but non-normality of data was observed.

3.2.2 Calculated DW of sludge sedimented directly beneath the cages

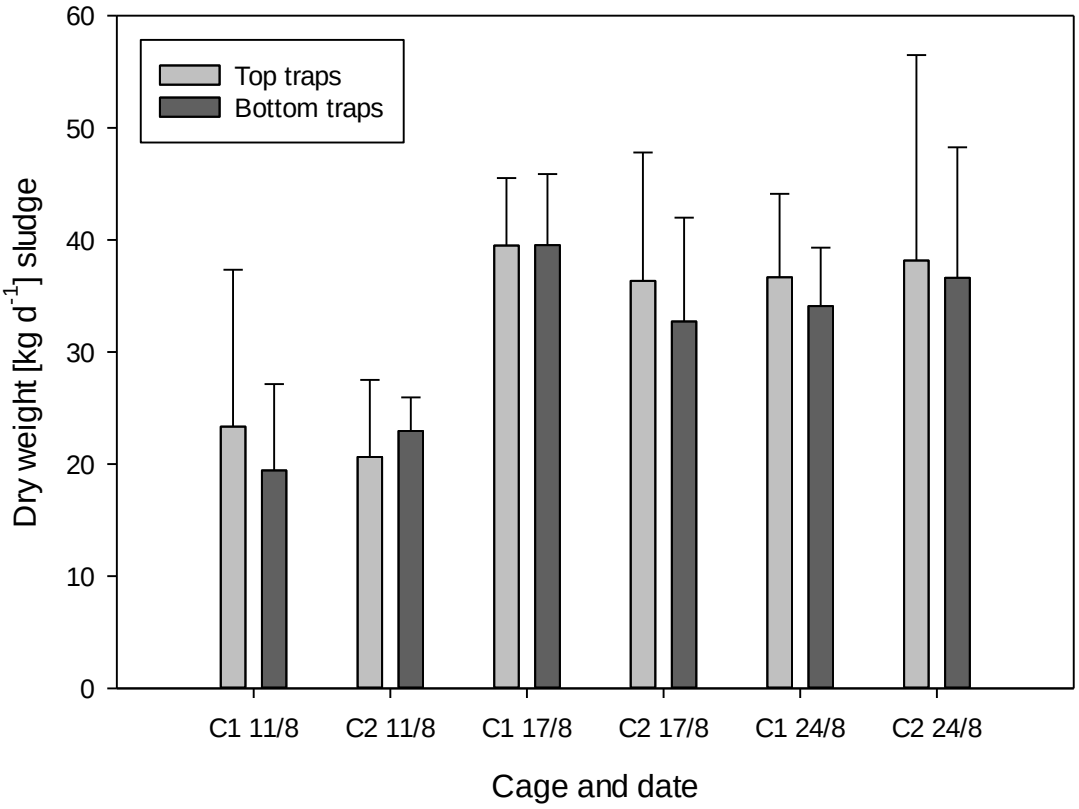

Figure 16 Calculated dry weight of sludge sedimented (kg d^{-1}) (n=3) directly beneath cage 1 (C1) and cage 2 (C2), at the given depths. The calculations are based on the amounts of sludge collected, the area of the cages and the area of the traps.

Figure 16 illustrates the calculated amount of sludge sedimented directly beneath each cage, based on the amounts of sludge captured during the collection periods, the area of the traps and the area of the cage. The amount varies between 19-40 kg d^{-1}. There were no significant differences between the DW of sludge sedimented at any of the depths or cages ($P \geq 0.05$), but non-normality of data was observed.

3.2.3 Theoretical amount of sludge produced in the cages

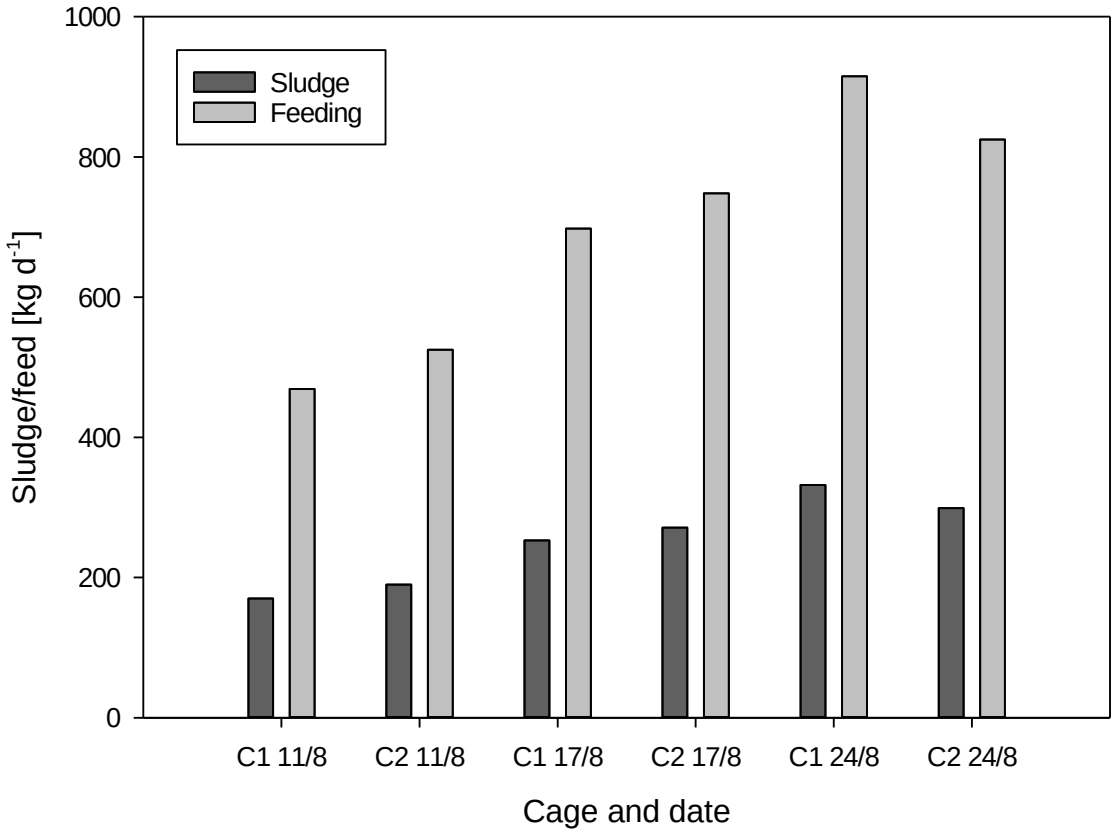

Figure 17 Theoretical amount of sludge produced (kg d^{-1}) from cage 1 (C1) and cage 2 (C2) during each collection period, calculated by using the assumptions described in Aas and Åsgård (2017). Amount of feeding (kg d^{-1}) for the same period is also displayed in the graph.

The theoretical amount of sludge produced (kg d^{-1}) in the cages during each collection period is presented in Figure 17. The amounts of sludge produced varied from 170-332 kg d^{-1}, where the increase in sludge produced was due to the increased feeding during the period. The amount of feeding varied from 469-915 kg d^{-1}. There were no significant differences between the theoretical amounts of sludge produced ($P \geq 0.05$).

3.2.4 Correlation between collected sludge and feeding

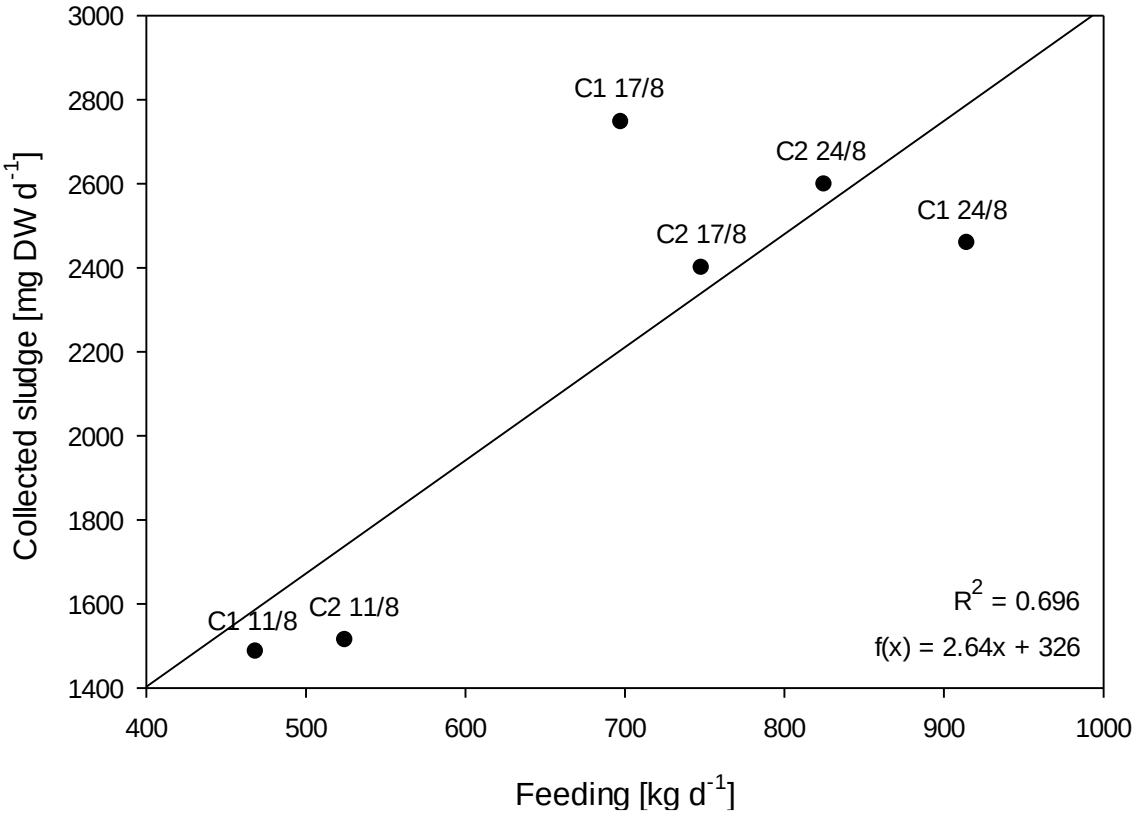

Figure 18 Correlation between the total amount of collected sludge (mg DW d^{-1}) and the feeding (kg d^{-1}) during the different collection periods. The collected amounts of sludge are the total amount of sludge collected from both top and bottom traps combined per day.

Figure 18 displays the correlation between the total amount of collected sludge (mg DW d^{-1}) and the feeding (kg d^{-1}) during the same period. The correlation coefficient for this linear regression plot is R^2= 0.696, showing a correlation between the amount of collected sludge and the feeding. This shows that an increase in feeding also results in an increase of collected sludge. C1 17/8 was the biggest outlier due to the high amount of sludge collected this day.

4 Discussion

4.1 Composition of the sludge

The mean contents of the sludge in the present study was found to be 195 mg gDW^{-1} for the content of C, 8 mg gDW^{-1} for the content of N and 19 mg gDW^{-1} for the content of P. The total amino acid content was found to be 80 mg gDW^{-1}, giving a protein content of 68 mg gDW^{-1}. These values were all lower than values reported by previous studies investigating the composition of land-based Atlantic salmon sludge (Anglade et al., 2023; Dahl, 2021; Kristensen, 2021; Seekamp, 2017). The ash content of the sludge was found to amount to 479 mg gDW^{-1}, proving the sludge to contain a large amount of inorganic matter. The lipid content of 111 mg gDW^{-1} stood out, as this was found to have a similarly high content as the sludges from the previous studies. When comparing the analysed contents from the different cages and depths, no significant differences were found. A comparison of the chemical composition of sea-based sludge from the present study, faecal samples from Atlantic salmon (Wang et al., 2013), land-based sludge from smolt production (Dahl, 2021; Kristensen, 2021; Seekamp, 2017) and post smolt production (Dahl, 2021; Kristensen, 2021) is displayed in Table 9.

Wang et al. (2013) investigated the chemical composition of Atlantic salmon faeces by taking samples directly from the latter half of the hindgut of the fish. When looking at the C content, Wang et al. found that the faeces had a mean C content of 366 mg gDM^{-1}, almost twice the content of the sludge in the present study. For the N, the faeces samples were found to have a mean content of 27 mg gDM^{-1}, more than three times the content of the sludge. The P content was more similar, with the faeces containing a mean P content of 23 mg gDM^{-1} only 1.2 times higher than what was found in the sludge. The faeces had a lipid content of 74 mg gDM^{-1} , measuring 1.5 times lower than in the sludge. The lipid content of the sludge compared to the faeces stood out, as this was the only nutritional content where the sludge had a higher content than the faeces. The DM content of the faeces and sludge was found to be similar, where the mean content of the faeces was 150 mg gWW^{-1} and 180 mg gWW^{-1} for the sludge.

Table 9 Comparison of chemical composition (mg gDW^{-1}) of sludge and faeces from different studies. "Sea-based sludge" is the results from the present study, "Faecal samples" is faeces from Atlantic salmon in open sea cages (Wang et al., 2013), "Smolt sludge A" is smolt sludge from land-based production (Seekamp, 2017), "Smolt sludge B" is also smolt sludge from land-based production (Dahl, 2021; Kristensen, 2021) and "Post smolt sludge" is post smolt sludge from land-based production (Dahl, 2021; Kristensen, 2021).

	Sea-based sludge	**Faecal samples**	**Smolt sludge A**	**Smolt sludge B**	**Post smolt sludge**
Carbon	195	366	287	424	363
Nitrogen	8	27	40	49	46
Phosphorous	19	23	-	45	49
Amino acids	80	-	117	236	223
Protein	68	-	249*	193	186
Total lipids	111	74	86	113	128
Fatty acids	70	-	47	96	117
Ash	479	-	427	130	271

*Calculation based on nitrogen content while the other protein calculations are based on amino acid content.

When comparing the sea-based sludge in the present study to sludge coming from land-based production of smolt and post smolt, it is shown that the sea-based sludge also has a lower nutritional content than land-based sludge. The C content of the sea-based sludge was found to be 1.5-2.2 times lower than in the other sludge types, N content was 5.0-6.1 times lower, and P was content 2.4-2.6 times lower. The amino acid content was found to be 1.5-3.0 times lower, and the protein content was 2.7-3.7 times lower. The lipid and fatty acid contents were the most similar among the sludges, where the sea-based sludge had a higher content than Smolt sludge A, but lower than both Smolt sludge B and Post smolt sludge. The ash content was only 1.1 times higher than Smolt sludge A, but 3.7 times higher than Smolt sludge B and 1.8 times higher than Post smolt sludge.

These differences in composition may be due to different factors. The lipid content having a similar value compared to the other samples in Table 9 stands out, as the content of C, N, P, amino acids and protein are all substantially lower in the sea-based sludge compared to the other samples. A possible explanation to this could be the reduced digestion of lipids caused by PD (Røsæg et al., 2019), as a reduced digestion of lipids would cause a higher content of lipids in the faeces. The apparent digestibility coefficients (ADCs) for protein and lipids have been found to be 86% and 94%, respectively, for healthy Atlantic salmon (Einen & Roem, 2003). During a PD outbreak, this percentage will be reduced, as found by Røsæg et al. (2019).

During their sampling period, the lowest ADCs for protein and lipid was found to be 57% and 75%, respectively. A reduced digestion would cause the faeces coming from infected fish to have a higher content of nutrients than the faeces coming from healthy fish. Therefore, it is possible that the disease outbreak could have affected the results in this study, causing higher nutritional contents in the sludge. As the nutritional contents were found to be generally low except for the lipid content, the lipid digestion seems to have been affected the most by the disease.

For the other contents, a possible explanation to why the sea-based sludge samples have a lower nutritional content than the land-based sludge samples could be affected by the likeliness of the land-based sludge to have a higher content of pure sludge compared to the sea-based sludge. This is because the sea-based sludge samples contain an unknown and varying amount of particles such as biofouling, shells and sand, lowering the concentration of the sludge, which can be seen by the high inorganic content (ash) in the sea-based sludge. As the land-based sludge is collected directly from the tanks the fish is reared in, only faeces and feed wastes are collected.

Furthermore, the ratio between faeces and feed waste could be different for the different sludge types. This difference in the amount of feed waste the sludge contains will affect the nutritional composition of the sludge, as a lesser amount of nutrient rich feed in the sludge gives a lower nutrient content (Aas & Åsgård, 2017). If this is the case, it could indicate that the sea-based sludge in the present study contains a lower amount of feed waste compared to the other sludges presented in Table 9.

The sludge from the present study containing a low amount of feed waste can be supported by comparing the ash and DM content of the sludge to the contents of the feed. The sludge was found to have an ash content of 479 mg gDW^{-1} compared to 83 mg gDM^{-1} in the feed, while also having a DM content of 180 mg gWW^{-1} compared to 926 mg gWW^{-1} in the feed. Furthermore, as salmon faeces has been found to have a DM content of 150 mg gWW^{-1} (Wang et al., 2013; Aas et al., 2016), it is very similar to the contents of the sludge found in the present study. Feed waste being consumed by wild fish could also impact the ratio between feed waste and faeces in the sludge, as it is found that salmon farms attract a large number of wild fish feeding on the feed wastes coming from the cages (Dempster et al., 2010; Dempster et al., 2009).

4.2 Feasibility of using sea-based sludge in IMTA

The feasibility of using sludge in IMTA is highly dependent on the nutritional composition of the sludge. Seekamp (2017), Kristensen (2021) and Dahl (2021) all fed polychaetes (*Hediste diversicolor*) on sludge collected from land-based smolt production (Smolt sludge A, Smolt sludge B and Post smolt sludge in Table 9), with the intention of investigating the feasibility of using polychaetes fed sludge as a salmon feed resource. For an area of use like this, it is of high importance that polychaetes utilise and incorporate the nutrients in the sludge at such a rate that polychaetes can be considered a feed resource of high quality.

Seekamp (2017) found that polychaetes received an increase in C:N ratio as the C content increased while the N content decreased. As the sea-based sludge in the present study was found to have a C:N ratio of 25.4 compared to 7.2 in Smolt sludge A, it is likely that an increase in C:N ratio would also be observed if the sea-based sludge was used to feed polychaetes. Kristensen (2021) also saw an increase in C:N ratio, where an increase in feeding levels gave an increased C:N ratio. It was found that polychaetes gained more C than N, and even less P. There was found no significant increase in P levels for the polychaetes, even though a high content of P was available in the sludge. This indicated a low P demand in polychaetes in comparison to the high availability. The P content of the sea-based sludge was lower than the land-based sludge, but as the C content also was lower, the C:P ratio was somewhat similar at 10.4 for the sea-based sludge, 9.4 for Smolt sludge B and 7.4 for Post smolt sludge.

For amino acids, Dahl (2021) found no significant differences between polychaetes fed different feed levels. On the other hand, Seekamp (2017) observed a significant decrease in amino acid content. It is worth mentioning that the amino acid content of the sludge used by Seekamp (2017) contained around half the content of the sludge used by Dahl (2021). The sea-based sludge from the present study had an even lower content of amino acids, possibly causing a reduced protein content and a reduced growth if used to feed polychaetes (Anglade et al., 2023).

Protein was found to be the largest share of biochemical composition in polychaetes by both Seekamp (2017) and Dahl (2021). While Seekamp (2017) saw a decrease in protein content, no significant differences in protein content was observed by Dahl (2021), even with increasing amount of feeding. This indicated that the diets given to the polychaetes did not affect the

protein content of the polychaetes in the study performed by Dahl (2021). Seekamp (2017) used the N content of the sludge to calculate the protein content, which could have led to an overestimation compared to calculations based on amino acid contents (Mæhre et al., 2018). This could possibly explaining why the decrease in protein content was observed while reporting a higher protein content than Dahl (2021). As the sea-based sludge had a protein content 2.7-2.8 times lower than the land-based sludge used by Dahl (2021), it is possible that the lower content could affect the protein content of polychaetes.

Both Seekamp (2017) and Dahl (2021) found that with an increase in lipid content in the fed diet, the lipid content of polychaetes also increased. As the lipid content of the sea-based sludge is similar or higher compared to the sludge in the mentioned studies, similar results for polychaetes fed on the sea-based sludge can be expected.

The nutritional composition of an organism can give an indication of the dietary need of the organism (Hillebrand et al., 2009; Wagner et al., 2013). For blue mussels, their tissue contain 41.7% C (Van der Schatte Olivier et al., 2021), 8.7-9.4% N and 1.0-1.1% P (Buer et al., 2020; Van der Schatte Olivier et al., 2021). This gives blue mussels a C:N:P ratio of approximately 40:9:1, where the sludge in the present study was found to have a ratio of 11:0.5:1. This could indicate that only a small part of the P content of the sludge would be utilised by blue mussels fed on the sludge, as their need for P appears to be relatively low.

Handå (2012) found that blue mussels have a better capacity of utilising the nutritional contents in salmon feed compared to salmon faeces, and as the C:N:P ratio of salmon feed was found to be 32:5:1, it is similar to that of blue mussels. Based on the faecal samples presented in Table 9, salmon faeces have a C:N:P ratio of 16:1:1, being somewhat closer to the ratio of blue mussel than the sea-based sludge from the present study. This could indicate that the utilisation would be even lower from sea-based sludge compared to faeces. The same study also suspected that a low lipid content in the faeces could have affected a reduced growth rate that was observed on the mussels fed salmon faeces compared to salmon feed. The lipid content of the sea-based sludge was somewhat higher than the faeces in Table 9, but as the sludge still had less than half of the lipid content of the feed samples in the present study, a reduced growth compared to salmon feed is likely.

Looking at the C:N:P ratio from the sludge from the present study compared to the reference site samples, the reference site samples had a ratio of 97:7:1 while the sludge samples had a ratio of 11:0.5:1. The Redfield ratio, which is the consistent ratio between C, N and P found in marine biomass samples, is known to be 106:16:1 (Redfield, 1934, 1958). The reference site samples somewhat resemble this ratio, but with a lower relative content of C and N. The fact that the sludge has a ratio of 11:0.5:1 is largely affected by the low content of N and the high content of P. It is especially the relatively high P content that makes it difficult to find areas of use for the sludge where all the nutritional contents are utilised efficiently. Newer studies have found the optimal N:P ratio of phytoplankton to be in the range between 8 and 45 (Klausmeier et al., 2004), and the ratio for blue mussels was found to be 9 (Buer et al., 2020; Van der Schatte Olivier et al., 2021). As the sea-based sludge has a N:P ratio of 0.5, it really shows how skewed this ratio is in relation to what the need is for marine species. While the C and N might be utilised efficiently, the high P content will be hard to fully utilise.

The nutritional contents of sludge will vary during the year within the same facility and between different farming facilities due to variation in the ratio between feed waste and faeces (Aas & Åsgård, 2017). As previously discussed, diseases such as PD could also influence the nutritional contents of the sludge. Because of this variation, the usability of the sludge as a source of nutrition in IMTA could fluctuate between sludge from different facilities, seasons and from sick or healthy fish. While the sea-based sludge collected and analysed for the present study might not prove to be optimal for IMTA, other studies have found (land-based) sludge to have higher nutritional contents and be more fitted for IMTA (Dahl, 2021; Kristensen, 2021; Seekamp, 2017).

It should also be mentioned that the salmon industry is striving to lower the amount of feed waste from the production while also making the feed more digestible through several ongoing projects and companies specialising in these fields (Business Norway, n.d.; Forskningsrådet, n.d.; Nofima, u.d.; Svendsen, 2020; Aas, 2021). If the industry successfully manages to reduce the amount of feed waste to a minimum while also increasing the digestibility of the feed, sludge from the salmon industry might not be optimal for IMTA due to low nutritional contents. Or to put it the other way around, IMTA might not be needed to utilise the resources in the production of salmon. As this is still not a reality, it is important to continue the research in finding ways to utilise the waste products from the production to make the salmon industry as sustainable as possible.

4.3 Quantification of sludge from the cages

Based on the captured amounts of sludge, it was found that 19-40 kg d^{-1} of sludge sedimented directly beneath the cages investigated in the present study during the collection periods. This amounted to 4-6% of the feeding during the same periods. There were found no significant differences between the captured amounts of sludge when comparing the different cages and depths the sludge was collected at, but the top traps were found to collect an average of 5% more sludge than the bottom traps.

Comparing the calculated amount of sludge sedimented beneath the cages to the theoretical amount of sludge produced by the cages (based on Aas and Åsgård (2017)), which ranged between 170-332 kg d^{-1}, an average of 13% of the total sludge output was sedimented directly beneath the cages. This indicates that a large amount of the sludge produced by the cages does not sink down directly beneath the cages but seem to be spread out in proximity to the farm over an area depending on environmental conditions such as current speed and direction (Broch et al., 2017; Carvajalino-Fernández et al., 2020; Law & Hill, 2019), diversifying the pressure created from the output of sludge. This can be supported by the B-investigations performed at "Lamøya" (Knutshaug, 2021), where the highest grading was given, proving the benthic ecosystems beneath the cages to be healthy and having a turnover rate high enough to handle the sedimented sludge.

There are some factors that could have influenced the calculated amounts of sludge sedimented directly beneath the cages. The amounts of collected sludge are very likely to be affected by the water currents at the location, which are unknown. A strong current would cause more sludge to drift off, causing lower amounts of sludge to be collected. During some of the registrations, large variations between the amounts of sludge captured between the traps under the same cage at the same depth were observed, illustrated by high standard deviations in Figure 16, likely due to currents causing the sludge to drift off in the direction of the current. The placements of the traps were also not as optimal as they possibly could have been, as additional collection points more towards the middle of the cages could have given results of interest, but executing this was found to be too difficult. Furthermore, the total area of a trap was very small in comparison to the area of a cage (1:86 332), making the calculations very sensitive regarding the amounts captured.

The fish being infected by PD is also very likely to have reduced the amounts of sludge collected, as the disease is known to reduce the appetite of the fish (Veterinærinstituttet, n.d.), leading to less feeding and less output of faeces. The amount of sludge produced and released by salmon cages is almost entirely based on the feed and feeding, causing variations in the amounts of feeding, different feeding regimes and different feed types to heavily influence the amounts of sludge released and sedimented beneath salmon cages (Aas, 2021).

It has been reported that salmon farms attract an average of 10 tonnes of wild fish during periods of the summer, feeding on feed wastes from the cages (Dempster et al., 2010; Dempster et al., 2009). These estimations might even be an underestimations, as others have reported up to 100-200 tonnes of wild fish being observed under some farms (Sæther et al., 2013). Therefore, an unknown amount of feed waste could possibly have been consumed by wild fish that otherwise would have been captured by the traps, causing a lower amount of sludge to be collected.

For the theoretical amount of sludge produced by the cages, 13% feed waste was used in the calculations, which is the estimation Aas and Åsgård (2017) used in their calculations. Other studies have used feed waste estimates significantly lower at 3-5% (Cromey et al., 2002; Otterå et al., 2009; Wang et al., 2012), indicating a possible overestimation of the calculated amounts of sludge produced by the cages.

4.4 Alternative applications for sea-based sludge

If not used in IMTA, other areas of use for the sludge should be considered. As the sludge from the present study had a relatively high content of lipids, an area of use where this energy is utilised could be of relevance. Biogas production could be a possible solution, but there are some obstacles. Firstly, a high salt content of the sludge will reduce the effectiveness of the gas production (Vangdal et al., 2014). For sludge coming from land-based production using brackish water with a salinity of around 12 ppt., effective dewatering through filtration should be able to lower the salt content to a level where it is no longer a problem (Aas & Åsgård, 2017). As sea water has a salt content of over 30 ppt, additional treatment might be needed for sea-based sludge to be a suitable resource in biogas production. Effective dewatering of the sludge is also essential both in regards of lowering the weight of the sludge to reduce the transportation costs, but also to receive a high calorific value (Aas & Åsgård, 2017). For the operation of producing biogas from sea-based sludge to be worth considering, an energy surplus needs to be possible. This means that the total energy used to dewater, transport and potentially further treat the sludge to remove salt would have to have a lower energy cost than the amount of energy produced from the biogas production.

Using the sludge as an energy source in cement production is also a possibility. When producing cement, a lot of heat is required (>2000°C), and combustion of sludge could contribute to creating this heat (Aas, 2021). Heidelberg Materials (2016) states that as much as 400-500 kg of coal can be replaced per tonne sludge used in the production of cement. Transportation and dewatering are also challenges when using the sludge in cement production. As there is only two cement producing facilities in Norway, long transporting distances will reduce the cost benefit, and effective dewatering will be of high importance to reduce the transportation cost while also being important to keep a high calorific value.

When it comes to utilising all the different nutritional contents of the sludge, both the production of biogas and of cement primarily uses the energy content of the sludge as a resource. For biogas production, the rest product after the production can still be used, e.g. as a soil conditioner, further utilising the nutritional contents of the sludge (Aas, 2021). For cement production, where the sludge is incinerated, all the nutrients in the sludge are lost. This is especially critical due to the amounts of P that will be lost, as P is a limited resource (Reijnders, 2014).

4.5 Future perspectives

The results from B- and C-investigations performed at sea-based salmon farms in Norway are mostly positive. In the period between 2016 and 2021, 87-93% of B-investigations performed at salmon farms received the highest or second highest grading (Barentswatch, 2023). This indicates that most ecosystems underneath salmon farms are heathy and have a turnover that is at a rate capable of handling the organic matter that sediments under the farms. This also indicates that using resources to collect, transport and treat sludge from sea-based salmon production is not necessary for most farms, when only taking environmental conditions into consideration. On the other hand, a future increase in production could change this. Figure 19 (appendix) illustrates the consequences an increasing amount of organic matter has on benthic ecosystems, causing adverse effects such as a reduction in biodiversity and the possibility of eutrophication (Tett, 2008). If the conditions of the ecosystems are not within the limits of "optimal" conditions, actions should be taken in the form of protecting the ecosystems by collecting the output of sludge coming from the farms, or by changing the way the production is performed to closed or land-based production where the sludge is collected (Nilsen et al., 2020).

While the benthic ecosystems might be able to handle the output of organic matter from salmon farms, it can be questioned if it is good resource management to let large amounts of sludge go to waste. Especially when considering that the sludge contains a rather high amount of P. As the supplies of P are estimated to run out within the next 80-200 years (Cordell et al., 2009; Reijnders, 2014; Sverdrup & Ragnarsdottir, 2011), recycling of P is of high importance. Finding solutions that allow for effective recycling of the P content in sludge would be a big step in a sustainable direction.

As the present study only investigated the sludge coming from one salmon farm during a relatively short period, involving infected fish, further research is recommended to retrieve more data regarding the nutritional contents of Atlantic salmon sludge and the amounts of sludge sedimented directly beneath their cages. If a broader study where several facilities with fish of different size during different seasons were investigated, the potential of using sea-based sludge as a resource could be further evaluated, contributing to strengthening the sustainability of the salmon industry.

5 Conclusion

The nutritional contents of sludge from Atlantic salmon sea cages were found to be generally lower than sludge coming from land-based production of smolt and post smolt, where only the lipid content of the sea-based sludge was found to have a similar content as land-based sludge. The salmon being infected by PD, causing a reduced lipid digestion, was suggested as a possible cause of this. It was suspected that the low nutritional contents in the sea-based sludge could have been caused by different ratios between feed waste and faeces in the different sludge types, as a higher content of feed waste gives higher nutritional contents. Based on this and the high content of ash and low content of dry matter, it was indicated that the sea-based sludge had a low content of feed waste. The analysed contents of the sludge were found to have no significant differences between the different cages and depths.

Finding efficient areas of use for the sea-based sludge within IMTA was found to be challenging. It was especially due to the low content of N in combination with the high content of P that finding areas of use where all the nutritional contents of the sludge are efficiently utilised was difficult. It was suspected that if polychaetes were to be fed using the investigated sludge, an increase in C:N ratio, poor P utilisation, and a reduced growth and protein content due to low amino acid contents would be observed. The ratio between C, N and P was also suspected to be a problem if the sludge was to be used as a resource in cultivation of other marine species, e.g. blue mussels, as the C:N:P ratio of the sludge had far different values than what is found in marine biomass. Biogas and cement production was suggested as alternative applications for sea-based sludge, where the energy content of the sludge would be utilised. While all nutritional contents of the sludge would be lost during cement production, the rest product after biogas production could still be used as a soil conditioner to maximize the output from the sludge.

A dry weight of 19-40 kg d^{-1} of sludge was found to be sedimented directly beneath the investigated cages, amounting to 4-6% of the dry weight of the feed supplied during the same period. The theoretical amounts of sludge produced by the cages were estimated to 170-332 kg d^{-1}, indicating that an average of only 13% of the sludge produced by the cages is sedimented on the seafloor directly beneath the cages. The top traps were found to collect 5% more sludge than the bottom traps, but no significant differences were found between the different cages and depths.

6 Reference List

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Appendix I

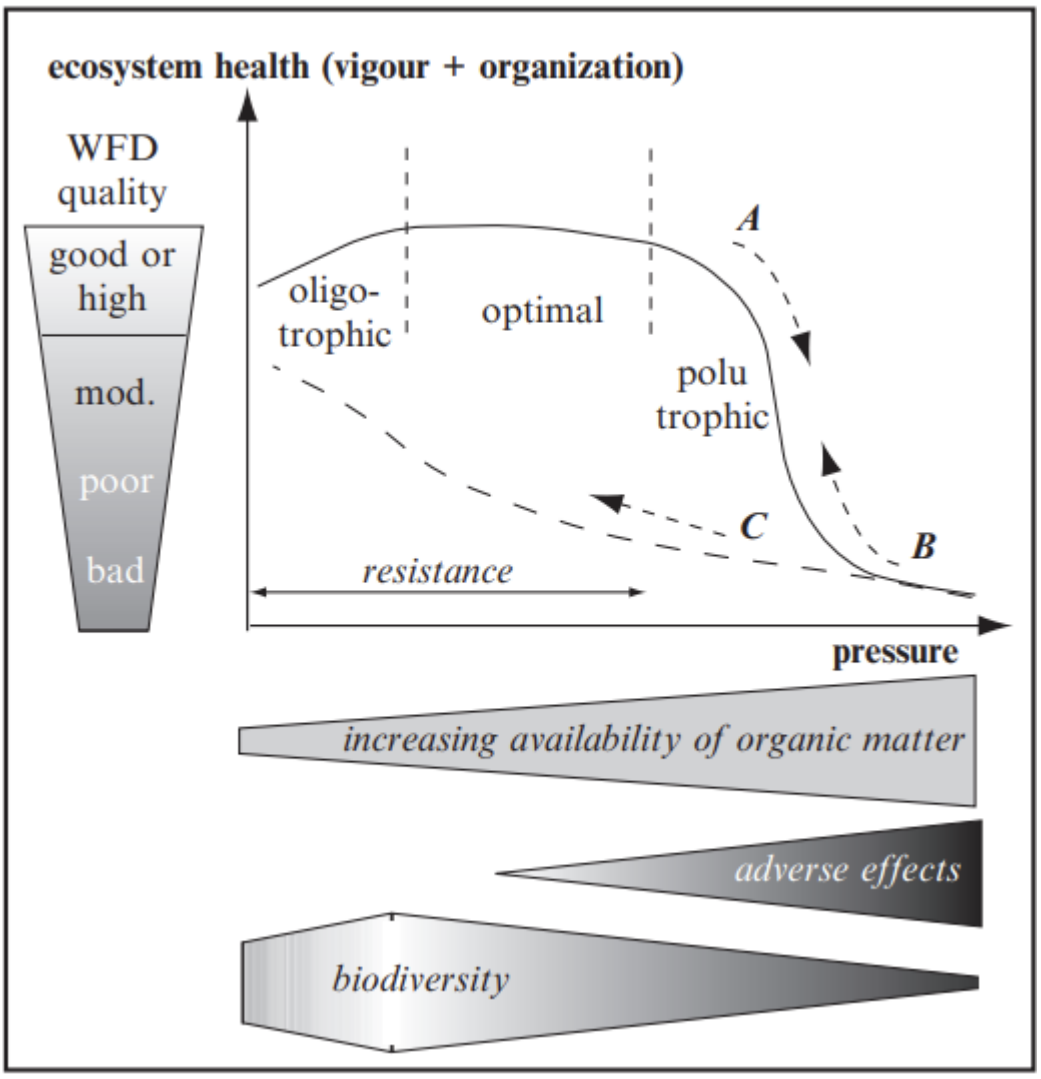
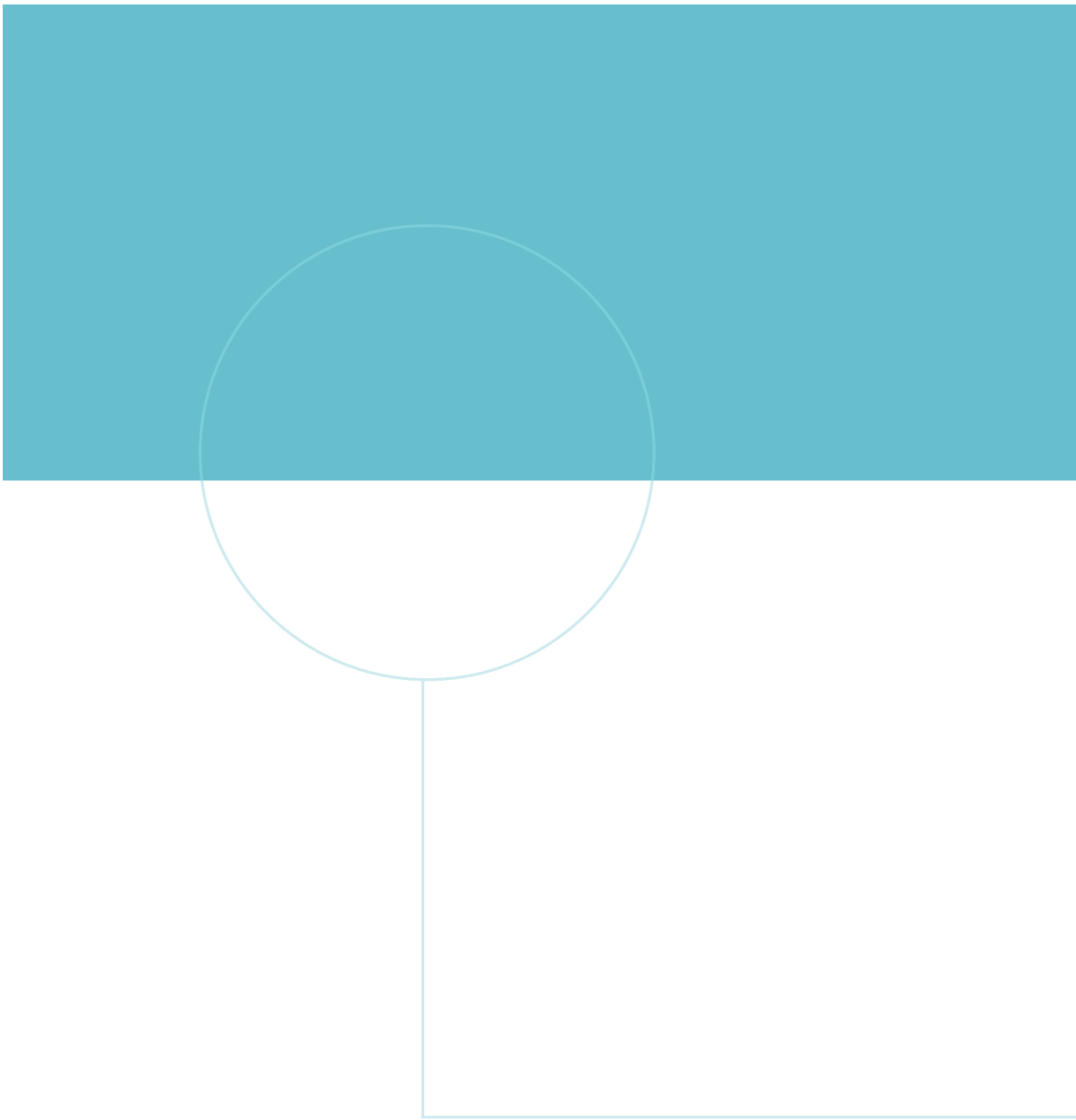


Figure 19 Impacts on ecosystem health when an increasing amount of organic matter put pressure on benthic areas (Tett, 2008).



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