Trace element composition in Norway lobster (*Nephrops norvegicus*) in the proximity of fish farms

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Abstract

The aquaculture industry in Norway is expected to grow with potential impact on the environment. The common seafood delicacy Norway lobster (*Nephrops norvegicus*) was studied to see how the vicinity to aquaculture might affect the trace element concentration. A total of 79 nephrops have been caught in four different locations in Romsdalsfjorden, Norway. Two locations (A and B) were in increasing distance from a fish farm, one location was considered a control group (C) several km from any fish farms, and the last location (D) was near, but upstream fish farms with potential effect. Hepatopancreas and muscle tissue have been analysed for the non-essential trace elements Cd, Hg, Pb, and As, in addition to the essential elements Cu, Zn, Fe, Mn and Se. The main objective of this study was to investigate the element composition of trace elements in this benthic invertebrate species in the vicinity of the fish farms. The trace elements were also investigated regarding distribution of concentration in the sampled organs (tail muscle and hepatopancreas) and differences between sexes.

The hepatopancreas samples showed significantly decreasing concentrations of Cu with increasing distance from aquaculture. The highest mean concentration was found closest to the fish farm (538.7 ± 106.4 mg/kg ww). Additionally, there were trends of the same elevation of concentration near fish farms in Zn concentration in the hepatopancreas, and for As, Cu, Se, and Zn in the muscle tissue. However, these trends were not statistically significant. The analysis of stable carbon and nitrogen isotopes in muscle tissue samples suggested a change in dietary niche of the nephrops close to the fish farm. Indications of a more specialized niche near the fish farm suggested that the nephrops have access to a more similar range of food, meaning they are likely directly or indirectly feeding on organic waste from the fish farm. When looking at all the collected individuals (irrespective of distance to fish farm), all the investigated elements were found to have higher concentrations in the hepatopancreas than in the muscle tissue. Different accumulation in the sexes were found, where females had significantly higher concentrations of Cd, Hg and Cu in the hepatopancreas samples and of Cd, Hg and Mn in the muscle samples. The males were found to have higher concentrations of Fe in the hepatopancreas samples, and higher concentration of As and Se in the muscle samples. In the muscle tissue, which is mainly what is eaten by humans, none of the trace elements were found to have higher values than the maximum legal limit. In the hepatopancreas, there were found elevated levels of Cd.

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1 Introduction

1.1 Background

Aquaculture is an important and still growing industry in Norway, and it is essential to explore how it impacts the environment. One species to consider found in the surrounding fauna of aquaculture in the Norwegian fjords is the Norway Lobster (*Nephrops norvegicus),* hereafter referred to as nephrops. Nephrops is a benthic omnivorous species relevant for studies on potential effects of aquaculture activities, as their feeding behaviour exposes them to potential organic waste effluents originating from fish farms. The nephrops can serve as a food source for other pelagic and benthic species, thereby establishing an inherent connection between the benthic and the pelagic food webs. As Nephrops are a highly sought after seafood option for humans, in addition to other species in both the benthic and pelagic food zone, studying the impact of aquaculture on nephrops is highly relevant for maintain food safety.

Analysis of trace elements is an important part of investigating potential impacts of aquaculture on the environment as well as regarding food safety. Levels of non-essential trace elements like cadmium, lead, mercury, and arsenic are necessary to investigate because of their toxicity and tendency to accumulate. A recent article from a medical perspective speculated that elevated values of cadmium, lead and mercury found in patients after consumption of crab are a result of pollution from fish farm activities (Averina et al., 2022). In their article, Averina et al. (2022) concluded that fish farming activities in the fjords are likely to result in bioaccumulation of toxic elements in the surrounding marine environment. Other essential trace elements like copper, zinc, iron, manganese, selenium are relevant to include in the analysis because of their use in aquaculture, either as antifouling agents (Grefsrud et al., 2024) or supplement to fish feed (Sele et al., 2023). Several studies have previously investigated levels of cadmium and other undesirable elements and their tendency to accumulate in the digestive gland (hepatopancreas) in crabs (Falk, 2014; Pedersen et al., 2014; Rouleau et al., 2001; Wiech et al., 2018). While there have been several studies on crabs, there is a lack of knowledge of impact on other species, which is necessary to develop our understanding of the impact fish farming activities have on the marine environment and food safety.

1.2 Aim of study

To investigate the effect of aquaculture, inductively coupled plasma mass spectrometry (ICP-MS) was used to analyse for trace elements in hepatopancreas and tail muscle of nephrops caught at different locations in different distances to fish farms. The concentrations of undesirable trace elements were also compared to the maximum levels in food, set by the European Commission (EC2023/915). In addition to the analysis of trace elements, stable isotopes of carbon and nitrogen in tail muscle were analysed to investigate dietary changes connected to fish farm effluents. This study was conducted in collaboration with students at Norwegian University of Science and Technology (NTNU) investigating fatty acids in the same specimens.

We tested the following hypotheses:

1) There is difference in the trace element composition between the two sampled organs muscle and hepatopancreas.
2) There is a difference in the trace element composition between males and females.
3) The trace element composition changes with the distance to fish farms.
4) The food composition changes with distance to fish farms.
5) Vicinity to fish farms may lead to food safety issues.

2 Theory

2.1 The Norway lobster

The Norway Lobster (*Nephrops norvegicus,* nephrops) is a pale orange, clawed lobster belonging to the subphylum Crustacea. The name nephrops stems from their kidney-shaped eyes. Nephrops belong to the order Decapoda (also called “ten-footer”), which includes crabs, lobsters, and prawns. It has a life-span of up to 15 years and can grow up to 24-25 cm total length (IMR, 2022b). A nephrops body contains of a cephalothorax covered by the carapace and a tail. The digestive gland, also called hepatopancreas is inside the cephalothorax.

Figure 2.1 Picture of *Nephrops norvegicus* (photo: personal)

The nephrops is found in the Western Mediterranean Sea and Northeast Atlantic from Morocco to Lofoten, Norway (IMR, 2022b). The species lives on seabed of mud sturdy enough to dig burrows in, at depths of 20 – 800 m. Its burrows extend 20 – 30 cm below the seabed and are often occupied by a single specimen (Provenzano & Bliss, 1985, p. 181). A study involving tagging indicates that the adult nephrops tend not to migrate extensively, possibly only some local movements (Farmer, 1975). The nephrops eyes are very sensitive to light, because of the degeneration of its photoreceptors cells in the eyes when exposed to light (Loew, 1976). For this reason, nephrops are nocturnal, emerging only under a certain level of light intensity. Thus depending on the depth, lobsters found at shallower depths tend to spend more time inside its burrows while lobster at greater depths are more active during daytime (Provenzano & Bliss, 1985, p. 181). Regarding feeding pattern, previous studies reported that nephrops are omnivorous, opportunistic feeders with feeding pattern not tentative to their sex or size (Farmer, 1975, p. 36). Studies show that nephrops is a generalist species in terms of food sources, with a broad dietary niche (Cristo & Cartes, 1998). According to Cristo and Cartes (1998) sand, mud or foraminifera can be passively ingested from the sediment.

The commercial fishery of nephrops in Norway have increased from 1977 (15 tons) to 2000 (346 tons) (Zimmermann et al., 2022). According to Zimmermann et al. (2022), 75% of the nephrops are caught by traps, rather than trawlers. There are indications of increase in recreational fishery of nephrops as well. Limited data indicate a doubling of participation in recreational fishery from 2005 to 2010 and a further doubling from 2010 to 2014 (Zimmermann et al., 2022).

2.2 Aquaculture

Aquaculture is an economically important industry in Norway, who is the largest exporter of farmed salmonids in the world. In 2022 Norway exported over 1.23 million metric tons of farmed salmon (*Salmo salar)* and 56 912 tons of farmed trout (*Oncorhynchys mykiss)* (Grefsrud et al., 2024). Globally, the aquaculture sectors contribution to food security and nutrition is increasingly acknowledged. Reporting on 'the State of World Fisheries and Aquaculture', FAO (2022) estimated that aquaculture contributed to 49% of the global production of aquatic animals in 2020. Of the total aquaculture production and fisheries of aquatic animals over 157 million tonnes (89%) were used for human consumption, and the rest to produce fish meals and oil.

Most of the fish farms in Norway operate with open cages (Grefsrud et al., 2024), meaning that organic releases from the fish farms presumably will settle at the seabed. Potential effluents from the fish farms include organic waste like fish feeds and faeces. There are two main release sources of trace elements from aquaculture. The first is the release of organic waste containing partly artificially enriched fish feed and faeces, and the second is the usage of antifouling agents. The Institute of Marine Research, Bergen (IMR) published their yearly report in February 2024. In this most recent report on risk assessment of Norwegian fin fish aquaculture, Grefsrud et al. (2024) evaluate the impact of fin fish aquaculture on the marine environment. They indicate that the waste from fish feed consists of dust and various sizes of pellets. The fish feed waste can vary depending on the individual farms, the type of feed used, and the size and health of the fish. The amount of organic waste was calculated, where Grefsrud et al. (2024) estimated the effluent of faeces to be 29.2% and amount of effluent of feed waste to 5-11% out of the total amount of fish feed. In the report they estimated the total amount of organic waste to up to 817 494 metric tons originating from the Norwegian fish farms. Particles from fish farms normally precipitate within a radius of one kilometre from the facility, and most of it within 500 metres. The amount of effluent accumulating at the bottom depends on local factors including water current, topography, biotic fauna, and sediment characteristics. The organic waste might contain different pollutants. It was reported that fish feed composition in 2020 consisted of 73% plant based material and 24% marine-derived materials (Aas et al., 2022). Especially marine materials may contain heavy metal substances like copper, zinc, mercury, arsenic and cadmium (Grefsrud et al., 2024), and also plant based materials may contribute certain elements including cadmium (Zhang et al., 2021). In addition, fish feed is supplemented with certain trace elements including zinc, selenium, manganese, and iron to increase both fish welfare and growth rate (Kokkali et al., 2023).

The other reason for aquaculture raising concern regarding potential pollutants in the marine environment is the usage of antifouling agents. Fish farming in open cages submerged in seawater makes the surfaces of the fish farm facilities prone to biofouling (Fitridge et al., 2012). Biofouling is an accumulation of algae and sessile organisms, and can affect both the farm operations and fish health (Bannister et al., 2019). Therefore, antifouling agents are used to prevent biofouling, which in turn can affect the surrounding marine environment. One commonly used antifouling agent has been copper oxide (Cu_2O), often in combination with inorganic zinc to increase overall toxicity (Guardiola et al., 2012). The nets were washed with large amounts of copper, toxic for growing organisms, to prevent them from surviving on the

surfaces of the cage (Grefsrud et al., 2024). Over time, up to 80% of the copper used as antifouling agent is estimated to be released into the surrounding environment, ending up in the sediment where it potentially accumulates and becomes available to benthic including nephrops (Grefsrud et al., 2024; Svanevik et al., 2021). Usage of Cu_2O has not been reported since 2022 (Grefsrud et al., 2024). Zinc pyrithione, copper pyrithione and tralopyril are now often used as alternatives. Copper and zinc pyrithione can be very harmful to aquatic life, but does not show tendencies to accumulate in the sediment (Turley et al., 2000). There are concerns being raised regarding tralopyril as well, given the limited information about its toxicity (Hoddevik, 2024).

2.3 Trace elements

According to the International Union of Pure and Applied Chemistry (IUPAC) (2019) the term "trace element" describes an element abundant in average concentrations of less than 100 parts per million atoms (ppma) or less than 100 mg/kg. Trace elements can either be essential or non-essential. Trace elements are natural components of the environment, but only in smaller amounts. For example copper and zinc are heavy metals that are essential for both organisms and humans (Poléo, 1997). There is a range of optimal concentration in which these elements are crucial for living (figure 2.1). There are also levels of concentrations of these essential elements that are too low for survival or too high, making them toxic and even lethal.

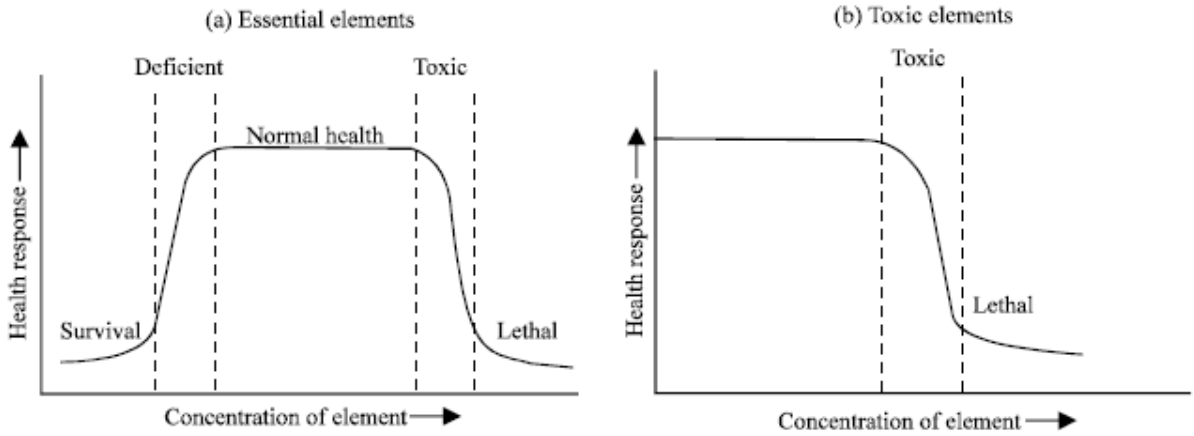

Figure 2.2: Essential and non-essential metals and their relation between concentration and health response (Ansari et al., 2004).

Relevant trace elements for this study are listed in the table below (table 2.1), together with the maximum levels in food for the undesirable elements Pb, Cd and Hg in crustaceans set by the European Commission (EC, 2023). The maximum level of these elements applies to the muscle

from the appendages and abdomen of crustaceans (EC, 2023). The essential elements (table 2.1) have also been found in excess in fish feed (Sele et al., 2023), they are also relevant in terms of potential marine environmental impacts. The essential elements listed below (table 2.1) are relevant in the present study because they are toxic in high concentrations. In the annual monitoring program, Sele et al. (2023) found that selenium, zinc, manganese and iron had higher concentrations in the fish feed than allowed by regulations. For this reason, they are included in this study. These nine elements are hereby expressed as "the relevant elements".

Table 2.1: non-essential and essential elements relevant to this study, the maximum levels (mg/kg ww) of the undesirable elements.

	Element		**Maximum levels (mg/kg ww)**
Non-essential	Cd	Cadmium	0.50
	Pb	Lead	0.50
	Hg	Mercury	0.50
	As	Arsenic	
Essential	Cu	Copper	
	Zn	Zinc	
	Fe	Iron	
	Mn	Manganese	
	Se	Selenium	

2.4 Non-essential elements

2.4.1 Cadmium

Cadmium (Cd, atomic number 48) is a rare element and occurs only in smaller quantities naturally. In aquatic environments with low salinity, cadmium predominantly exists as free Cd^{2+} ions with cadmium (II) hydroxide and in organic complexes (EFSA, 2009). Natural sources include volcanos and decomposition, but these natural levels are generally low, and often found with zinc (Brittanica, 2024a). The concentrations of cadmium in earth's crust is found to be about 0.1 mg/kg on average (Tchounwou et al., 2012). Cadmium is distributed in the water column, meaning it is a mobile element that is consumed at the surface by planktons, sinks with dead planktons and further enriches the deep sea, where organic materials is broken down by bacteria and releases trace elements to the water column (Falk & Nøst, 2013). The main sources of anthropogenic polluted cadmium in the nature are mining, waste and combustion of oil and coal.

2.4.2 Lead

Lead (Pb, atomic number 82) is a non-essential element abundant in many parts of the nature (Brittanica, 2024b). Lead is not frequently found in free form, rather in minerals. PbS (lead sulfide) is the most common source of lead production, but it is also found in $PbSO_4$ and $PbCO_3$. Lead and substances containing lead are toxic and tend to accumulate over time (Brittanica, 2024b). According to Poléo (1997), Pb is a significant environmental problem in Norway since it is toxic in high concentrations. Lead gasoline have since this been phased out, and anthropogenic emission of lead have decreased (Boyle et al., 2014). The release of lead throughout the history still makes it relevant to include as one of the significant elements to be investigated in this study.

2.4.3 Mercury

Mercury (Hg, atomic number 80) occurs naturally in drops or larger fluid masses in proximity to volcanoes or hot springs (Brittanica, 2024c). Anthropogenic sources of mercury are mainly industry and from batteries and thermometer thrown away (Poléo, 1997). Due to governmental decision there has been a substantial decline in anthropogenic emission of mercury into the water and land environments in developed countries (Baird & Cann, 2012, p. 522). Mercury occurs in three chemical forms: elemental mercury, inorganic mercury and organic mercury (methylmercury, MeHg) (VKM et al., 2019). Most of the mercury found in the environment exists as inorganic compounds, primarily as Hg^{2+} ion or complex inorganic ions formed by the ion (Baird & Cann, 2012, p. 524). Atmospheric mercury is subject to long-range transport and can therefore be found on sites far from the release source (ATSDR, 2022b). Mercury can be methylated into methylmercury by anaerobic bacteria in the water (ATSDR, 2022b). Methylmercury was more toxic to nephrops than inorganic mercury (Canli & Furness, 1993), and is readily bioavailable and bioaccumulates within aquatic food webs, resulting in elevated concentrations of mercury in predatory fish (VKM et al., 2019). VKM (2019) assumes in their article that all mercury found in fish is methylmercury.

2.4.4 Arsenic

Arsenic (As, atomic number 33) is one of the elements found in fish feed. Arsenic generally occurs in natural waters at levels below 1-2 µg/l, though concentrations as high as 12 mg/l have been observed in areas containing natural sources (WHO, 2022). Arsenic is naturally occurring in the environment in rocks and sediment, with additional attributions from anthropogenic

sources such as coal burning, copper smelting, and mineral ore processing (Rose et al., 2010). Concentration of arsenic are higher in aquatic environment because it is water soluble, making concentrations in fish and seafood high through absorption (Rose et al., 2010). Studies have revealed that most of the arsenic in seafood exists in a form of no toxicological concern, arsenobetaine (Chain, 2009).

2.5 Essential elements

2.5.1 Copper

Copper (Cu, atomic number 29) is an essential element that occurs naturally, and is a vital trace element for all living organism necessary for cellular functioning (Lall & Kaushik, 2021). In crustaceans copper has the important role of transporting O_2 as respiratory pigment (Poléo, 1997). Copper is mainly used as antimicrobial, antibacterial and antifungal agents (ATSDR, 2022a). Aquaculture is one of the primary sources of copper pollution, as a result of the antifouling of the fish nets, in addition to discharge of copper-enriched fish feed (Bannister et al., 2019; Svanevik et al., 2021). Other anthropogenic contamination sources include paint, industrial melting, mining etc (ATSDR, 2022a).

2.5.2 Zinc

Zinc (Zn, atomic number 30) is a trace element that is essential to life, making up about 65 grams per ton of the earth crust (Brittanica, 2024d). In animals it is more common to be deficient of zinc rather than be poisoned by it, some species are even dependent on a large zinc supply to survive (Poléo, 1997). Zinc have been shown to have a tendency to bioaccumulate in invertebrates (Rainbow & Luoma, 2011). Poléo (1997) establishes some anthropogenic sources of zinc. For one, zinc is used in the making of industrial metal, as it is a component in several alloys. Secondly, zinc is used as galvanic anode for iron and steel in seawater to prevent corrosion. The last source established by Poléo (1997) is drainage of mines.

2.5.3 Iron, manganese and selenium

Iron (Fe, atomic number 26), is involved in biochemical processes such as gene regulation, transport of oxygen and regulation and cell growth (Lall & Kaushik, 2021). Iron is amongst the most abundant metals present in the earth's crust (WHO, 2022). Iron is usually added to fish feed in aquaculture as iron salts, to prevent iron deficiency (Bury & Grosell, 2003).

Manganese (Mn, atomic number 25) is an essential dietary mineral for humans and other animals. It is present both naturally and as a result of anthropogenic activities such as manufacturing of iron and steel alloys, and cleaning, bleaching and disinfection (WHO, 2022). Manganese is involved in several biochemical processes, and deficiency has been shown to reduce growth and increase mortality (Lorentzen et al., 1996). For this reason it was recommended to supply fish feed with manganese to meet the dietary Mn requirement (Lorentzen et al., 1996).

Selenium (Se, atomic number 34) is widely distributed in the Earth's crust. It is an essential element to all living organisms (ATSDR, 2003). Selenium compounds are capable of protecting against harmful effects of toxic heavy metals such as cadmium and mercury (Watanabe et al., 1997). Selenium deficiency can result in growth depression, and can be prevented by supplementation in fish feed diet (Watanabe et al., 1997). The main anthropogenic source of selenium is coal combustion, while natural sources include volcanic eruptions and weathering of rocks and soil (ATSDR, 2003).

2.6 Trace elements in Crustaceans

Several previous studies have shown that cadmium readily accumulates in other large crustaceans (Falk, 2014; Rouleau et al., 2001; Wiech et al., 2018), mainly in the hepatopancreas. The hepatopancreas accumulates primarily cadmium, zinc and copper due to the presence of metallothionein (further explained in 2.7). It has been shown that cooking and thawing significantly increased concentrations of cadmium in claw meat of brown crabs (Wiech et al., 2017). In this study, Wiech et al. (2017) found that the processes of freezing and thawing increased the concentration of cadmium when the claws were attached to the carapace, suggesting a transfer of cadmium from hepatopancreas into the claw during these handling processes. The dietary route is considered a more important uptake of cadmium than the aqueous route in crabs (Davies et al., 1981; Wiech et al., 2018). Therefore, if nephrops exhibit the same uptake pattern as the crabs, the organic waste from fish farms potentially consumed could serve as a potential source of cadmium. Pedersen et al. (2014) investigated the accumulation and distribution among organs when exposed to levels of cadmium aligning with environmentally realistic exposure through food consumption. They found that more than 95% of the assimilated cadmium was bound in the hepatopancreas and increasing with the level of exposure. The levels of cadmium in the gills did also prevail this same pattern. In the muscle,

on the other hand, there was only one case of detection of cadmium of the highly exposed crabs. Regarding the non-essential elements, Canli and Furness (1993) found that inorganic mercury, methylmercury, cadmium and lead were accumulated by nephrops, where methylmercury was mostly accumulated in tail muscle while inorganic mercury, cadmium and lead mostly accumulated in hepatopancreas.

There are several previous studies on the effect of sex on trace metal concentrations. Elahi et al. (2012) found that in the crustaceans *Penaeus semisulcatus,* the level of mercury in females was significantly higher than in males. A study on nephrops in Italy found a higher increase of mercury in females than males and explained it by the female growth rate (Barghigiani et al., 2000). The growth rate of nephrops is dependent on moult frequency and after maturity, moult frequency is more reduced in females than males (Bell et al., 2013), meaning females are older than males of same size. In a previous study on hepatopancreas and gills in nephrops conducted by Canli et al. (1997), they found that the concentration of copper and zinc increased significantly only in the gills (and not in the hepatopancreas) of males when exposed to the metals, but not in females. Canli and Furness (1993) found that hepatopancreas in males had higher concentrations of mercury than females. The higher concentrations were explained by the fact that the males were smaller and more active than the females. Canli and Furness (1993) further suggested that the difference in metabolic rate is the main reason for sex and size differences in the accumulation rate. A study on brown crab found that females had higher concentrations of arsenic in hepatopancreas and cadmium in muscle (Barrento et al., 2009), while Cenov et al. (2018) found higher values of arsenic in males and in spring in nephrops. Marsden and Rainbow (2004) suggests that in reproductively active females the maturing ovarian tissue may be a temporary reservoir for zinc.

Copper, zinc and other trace elements may be found in elevated concentrations near fish farms due to release of fish feed, faeces and antifouling agents. Falk (2014) indicated that the elevated concentrations of cadmium found in brown crab could not be explained by organic waste from fish farms, but rather by upwelling deep sea water. The study by Falk (2014) also suggested that effluents of organic waste and antifouling agents resulted in a trend of increasing concentration of zinc and copper with decreasing vicinity to fish farms. In their study on fish, Signa et al. (2017) found that mercury was more bioavailable to benthic fish than pelagic fish. They also found that with increasing trophic position (increasing $\delta^{15}N$), the cadmium

concentration decreases, indicating a more efficient transfer of cadmium from lower trophic levels to higher trophic levels due to scavenging feeding behaviour.

2.7 Metal handling in Crustaceans

Invertebrates possess different cellular detoxification pathways that help reduce the concentration of harmful metal in their bloodstream (Ahearn et al., 2004). One of these are detoxification by the protein metallothionein. Other pathways for metal ions include transportation into mitochondria, accumulation by lysosomes, transferring into endoplasmic reticulum and efflux across the basolateral cell membrane to the blood (Ahearn et al., 2004). In the context of trace element accumulation, while size might be a significant factor, the age of the organism might be more crucial as it provides a time frame for the accumulation of the trace elements. (Marsden & Rainbow, 2004).

Metallothionein is found in all invertebrate phyla and in all vertebrates and exhibits a strong affinity for metal ions and can bind the ions from low intracellular concentrations (Ahearn et al., 2004). It is a cysteine-rich metal binding protein and are characterized by the presence of metal-thiolate clusters (Calvo et al., 2017). Some of the fundamental roles of metallothionein is maintaining the balance and regulation of essential trace elements like zinc and copper, as well as high affinity for metal ions and protecting organisms from environmental toxic metals such as cadmium, mercury and lead (Calvo et al., 2017). Ahearn et al. (2004) explained these processes of storing and sequestering metals. For the essential trace metals copper and zinc, the metallothionein act as a reservoir, making them available for biochemical processes. In the metal-detoxification process the metal is bound to the metallothionein and carried into the lysosome where the proteins are broken down and the metal is released (Ahearn et al., 2004). The numerous sulfhydryl groups within metallothionein allows it to bind Cd^{2+} ions and the complex is then excreted in the urine (Baird & Cann, 2012). When the amount of cadmium intake exceeds the binding capacity of metallothionein, cadmium is stored in the liver and kidneys (Baird & Cann, 2012). Metallothionein concentrations have been shown to increase in gills and hepatopancreas of nephrops when exposed to cadmium, copper and zinc (Canli et al., 1997). The metallothionein are present in higher concentration in the hepatopancreas, making the concentration of cadmium, mercury, lead, copper and zinc higher in this organ (Canli et al., 1997). Pedersen et al. (2014) suggested that soluble pool of zinc could be regulated by pumps in crustaceans as it is in vertebrates. Another study suggested that manganese in nephrops rarely

enters muscle tissue cells, but binds to sulphates, chlorides or proteins and are excreted via the urine and the faeces (Baden et al., 1995).

2.8 Food safety

Due to its toxic effect for humans (Poléo, 1997) and its tendency to bioaccumulate (Falk, 2014), cadmium needs to be considered for food safety. Worst-case scenario toxic effects of cadmium in humans are kidney failure and damaged bone tissue due to chronic exposure (ATSDR, 2012). Most forms of cadmium compounds are carcinogenic (Tchounwou et al., 2012). For most of us, the greatest proportions of exposure to cadmium comes from our food supply. Some of the cadmium exposure comes from seafood, but the majority of it comes from potatoes, wheat, rice and other grains (Baird & Cann, 2012, p. 554). Humans, like crustaceans, are protected against chronic exposure to low levels of cadmium through the presence of metallothionein. A study in Portugal showed high levels of Cd and methylmercury in edible crab brown meat, where they conclusively suggested moderate consumption of brown meat of crabs (Maulvault et al., 2013). The Panel on Contaminants in the Food Chain have established a tolerable weekly intake (TWI) of 2.5 µg/kg body weight (EFSA, 2009). Most of the mercury present in humans is in the form of methylmercury originating from the fish in our food chain (Baird & Cann, 2012, p. 532). EFSA (2012) have established PTWI for methylmercury (table 2.2), which is highly toxic (more so in pregnant women), and humans are generally exposed to methylmercury through their diet (WHO, 1991). The non-essential elements are the only elements discussed here, because of their toxicity.

Table 2.2: Provisional Tolerable Weekly Intake (PTWI) for the undesirable non-essential elements and the PTWI for an average person weighing 70 kg.

	PTWI (µg/kg body weight)	**PTWI for person at 70 kg (mg)**	**Reference**
Cd	2.5	0.175	(EFSA, 2009)
Pb	25	1.750	(WHO, 2022)
MeHg	1.3	0.091	(EFSA, 2012)

2.9 Analytical techniques

In this section the theoretical principals of wet digesting, inductively coupled plasma mass spectrometry (ICP-MS) and determining trophic relations through stable isotopes of C and N are given. The applied methods will then be further explained in section 3.

2.9.1 Wet digestion

To be able to accurately detect the amount of trace elements by ICP-MS, it was necessary to decompose the sample. The method used in this study is wet digestion by acid and microwaving to speed up the process. The aim of the acid digestion is to transfer the sample to liquid state with low viscosity to be able to introduce the sample to the analytical instrument. Nitric acid (HNO_3) is one of the most widely used digestion reagents, and is the reagent used in the present study. Nitric acid is ideal for organic samples since it does not interfere with the determinations and HNO_3 works as an oxidizing agent to decompose the samples to release the elements within the sample (Matusiewicz, 2003).

2.9.2 Inductively coupled plasma mass spectrometry

ICP-MS is a method to analyse the occurrence and concentration of elements. The main components of a typical ICP-MS instrument are shown in figure 2.3.

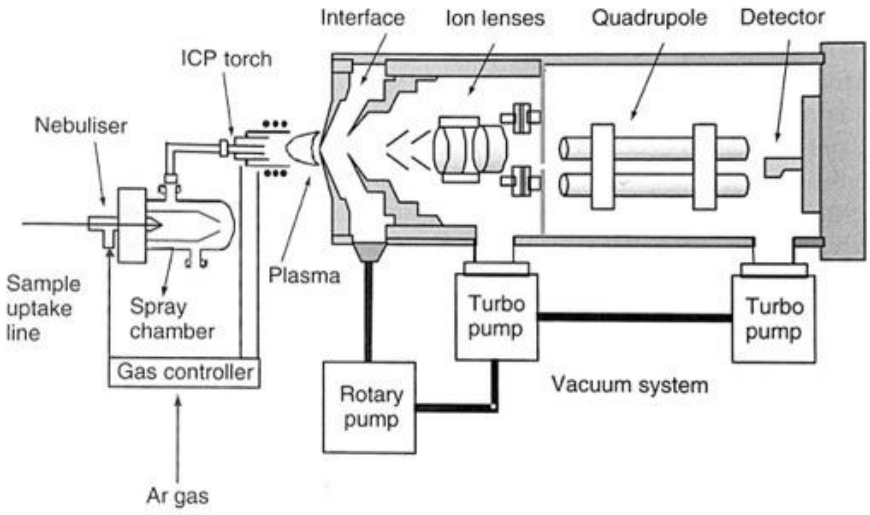

Figure 2.3: Schematic of an Inductively Coupled Plasma Mass Spectrometer ICP-MS (Cullen, 2004).

The samples to be analysed are firstly introduced to a nebuliser, before a stream of argon gas breaks the liquid into a fine aerosol (Cullen, 2004). In the spray chamber the larger droplets are extracted out, while the smaller droplets are delivered to the central channel of argon plasma. This introductory step is the greatest source of noise in an ICP-MS analysis (Skoog & Leary, 1992, p. 235). In the plasma there are argon ions capable of absorbing sufficient power from an external source to maintain temperatures as high as 10 000K (Skoog & Leary, 1992, p. 234). A

load coil surrounding the plasma generates inductive coupling making the electrons in the argon excited (Cullen, 2004, p. 26). The aerosols are exposed to temperatures up to 8000K in the plasma, resulting in atomization and ionization (Cullen, 2004, p. 23). The ions enter the interface containing cones whose main role is to extract a representative sample of the ions and transfer it to higher vacuum regions. The vacuum system maintains the low pressure in the mass analyser, allowing the transfer of ions from plasma to the detector (Cullen, 2004, p. 30).

The next section of the schematic (figure 2.3) is the mass spectrometer. A quadrupole is ideally suited for inorganic mass spectrometry. The quadrupole consists of four parallel metal rods with applied constant voltage and radio-frequency oscillating voltage (Harris et al., 2020, p. 513). As the ions migrate from the ionization chamber, the electric field generated by the quadrupole allow only ions with one selected mass-to-charge ratio reach the detector, while other ions are removed (Harris et al., 2020). The ions allowed to pass through to the detector are then registered as hits per second.

An external standard curve is used to quantify the elements. Internal standards are used for correcting drift in the selected mass range. Potential elements that can be used as internal standards are rhodium (Rh), germanium (Ge), indium (In) and thulium (Tm) (IMR, 2023a). The chosen internal standard cannot occur naturally in the sample material or have low or no interference with the elements you are trying to determine. If possible, the internal standard should be within the mass range of the analytes. Gold is used to stabilize the mercury ions and is added to the internal standard and the external standard curve.

2.9.3 Determining trophic relationships with analysis of stable isotopes

The stable isotope signatures in the biological components of a system can be used as proxies to trace element cycles and how the components are incorporated into food webs (Catry et al., 2016). Carbon and nitrogen are among the elements that can occur in more than one isotope, with change only in the number of neurons in the nucleus (Ben-David & Flaherty, 2012). Isotopic composition is typically expressed as parts per thousand differences from a standard (δ), which is the ratio between the amounts of heavy to light isotopes in a sample (Peterson & Fry, 1987): $\delta^{13}C$ refers to the carbon isotope composition, and $\delta^{15}N$ refers to the nitrogen isotope composition. There is often dissimilarity in $\delta^{13}C$ among primary producers in marine food webs (e.g. phytoplankton and macroalgae; Rau, 1978), making it a good tracer to distinguish the

origins of carbon in the food chain. And because of small trophic enrichment from diet to consumers for $\delta^{13}C$, it is also indicative of diet and possible dietary changes (Tieszen et al., 1983). In contrast, due to big trophic enrichment of $\delta^{15}N$ (3-4‰) (Woodcock et al., 2017), $\delta^{15}N$ can be used to estimate trophic position of consumers in the food web (Post, 2002).

Studies have shown that $\delta^{13}C$ and $\delta^{15}N$ can be used to trace long term exposure of organic waste from fish farms in marine organisms (Woodcock et al., 2017). Woodcock et al. (2018) used ($\delta^{13}C$ and $\delta^{15}N$ to investigate if epibenthic fauna found close to a fish farm (brittle stars, sea urchins, and brown crab) were consuming organic waste originating from the farm. They found distinct lower $\delta^{13}C$ and $\delta^{15}N$ in the specimens collected adjacent to the fish farm than those collected further from the fish farm, and such difference was observed in samples collected from up to one kilometre away. Other studies also found that the sediment near fish farms are more $\delta^{13}C$-depleted and $\delta^{15}N$-enriched, possibly due to a mixing of $\delta^{15}N$-enriched fish oils and vegetable sources (Cutajar et al., 2022; Yokoyama et al., 2006). Analyses of stable isotopes ratios is thereby a valuable method for investigating the trophic relations among food web components.

2.10 Quality assurance and statistics

It is imperative that the chemical analyses are held to a high quality, to generate accurate results. Typically, quality assurance is employed to ensure fitness for purpose, which may entail minimizing error in the sampling and analytical method. The assurance of quality essentially begins with sampling. Collected samples must be representative and preserved properly after collection. For example, samples for trace metal analysis are usually collected in plastic or Teflon containers (and not glass) because metal ions found on glass surfaces leach into the sample over time (Harris et al., 2020, p. 101). Quality assurance of the analytical procedures include maintenance log for the instrument, analysis of certified reference material and blind samples. Certified Reference Materials (CRM) contain certified levels of analytes in realistic matrices, and are used to verify the analytical measurements (Harris et al., 2020, p. 55). Applied analytical methods should produce an answer acceptably close to the certified level (Harris et al., 2020, p. 102).

2.10.1 Precision, accuracy, and trueness

When describing quality of measurement in analytical chemistry, precision, accuracy, and trueness are commonly used. Precision is a measurement of how closely independent test results match each other when they are conducted under stipulated conditions (Menditto et al., 2007). The accuracy is an estimate of the total error, a combination of trueness and precision, and it can be expressed quantitatively as the measurement uncertainty (figure 2.4). Accuracy describes how closely a measured quantity aligns with the true value of what is being measured, and lastly trueness is described as whether the average value obtained from a large number of test results aligns with the CRM (Menditto et al., 2007).

There are two types of errors regarding uncertainty in analytical measurements, systematic errors, and random errors. The figure below (figure 2.4) shows the relationships between these terms. A systematic error occurs when a measurement consistently deviates from the true value in a predictable matter and can be quantified as bias (Menditto et al., 2007; Nabi et al., 2021). On the other hand, random errors occurs when repeated measurements of the same quantity result in varying values (Nabi et al., 2021). Random errors can be quantified as the standard deviation of repeated measurements on the same samples using the same method (Menditto et al., 2007).

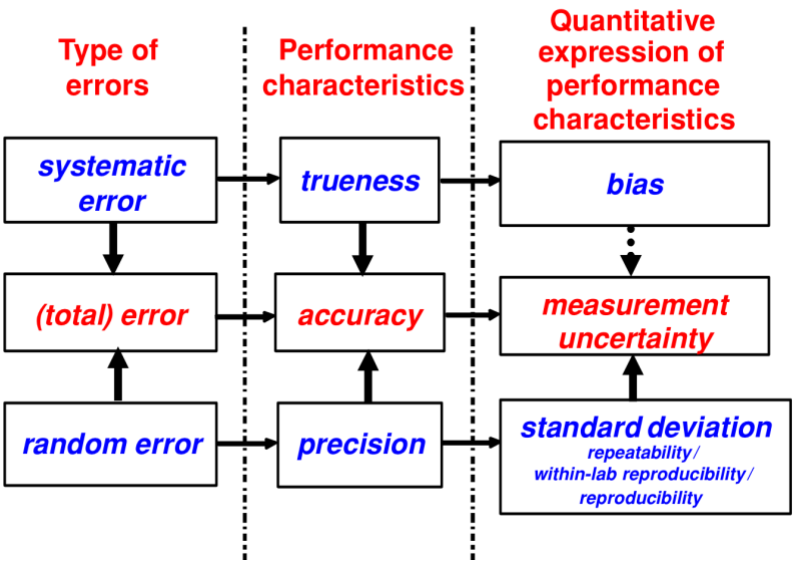

Figure 2.4: Relationships between type of error, qualitative performance characteristics and their quantitative expression (Menditto et al., 2007)

2.10.2 Limits of measurement

The range of measurement is the range of concentration for which the method has been validated and where it provides acceptable trueness and precision (IMR, 2023a). There are two relevant concepts regarding the range of measurement: Limit of detection (LOD) and limit of quantification (LOQ). The LOD is defined as the threshold concentration for detection of the analyte (Cullen, 2004). On the other hand, LOQ relates to the minimum concentration of which the uncertainty of the method becomes acceptable. LOD is commonly determined by 3xSD of at least 20 blind samples, and LOQ is determined by 10xSD of at least 20 blind samples (IMR, 2023a).

2.10.3 Statistical methods

Principial component analysis (PCA) is a multivariate technique used to analyse relationships between multiple variables within a dataset by finding the principal components (PC) that explain the most variance in the data (Abdi & Williams, 2010, p. 433). The goals of PCA are to extract the most important information from the data, compressing and simplifying the important parts and analysing the structure of the observations and the variables (Abdi & Williams, 2010, p. 434).

3 Method

3.1 Sampling

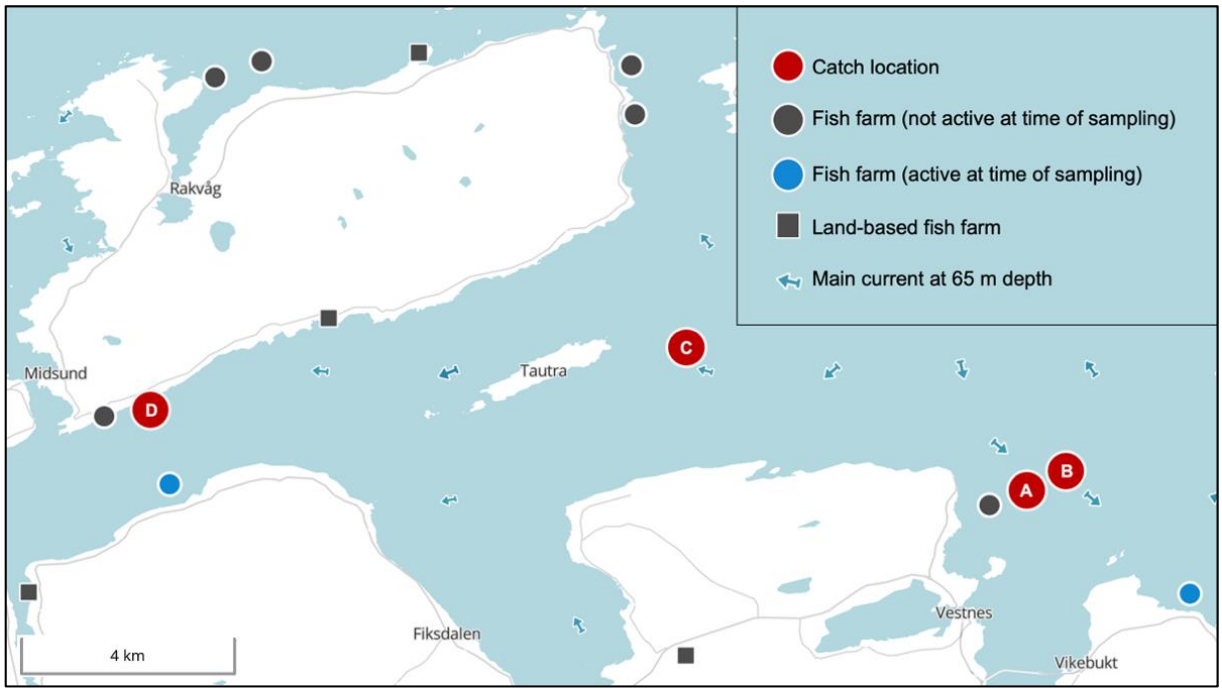

Figure 3.1: Overview map of Romsdalsfjorden, with catch locations and fish farm activities in the area, and the main current along the depth 65 m. Information about fish farm locations was gathered from the BarentsWatch website.

The nephrops (n=103) were caught in Romsdalsfjorden with commercially used nephrops pots by commercial fishers on 25th and 27th of November 2023. The bait was placed into solid perforated plastic containers in the respective pots to prevent the nephrops from consuming the bait. Several aquaculture facilities were in vicinity to the sampling locations (figure 3.1). To find out which fish farms were active, data about salmon lice counting were collected from BarentsWatch. Active fish farm facilities are required to count and report the number of lice weekly. Fish farms that have not been reporting the number of lice for four or more consecutive weeks are said to be inactive and without fish. Four strings of pots were placed at different locations (A, B, C and D) in the fjord (figure 3.1). A and B were in increasing distance from one fish farm, C was furthest from any fish farm and was therefore considered a control group, and D was in between two sea-based fish farms and one land-based. The direction of current at 65 m in the area is shown by arrows (figure 3.1).

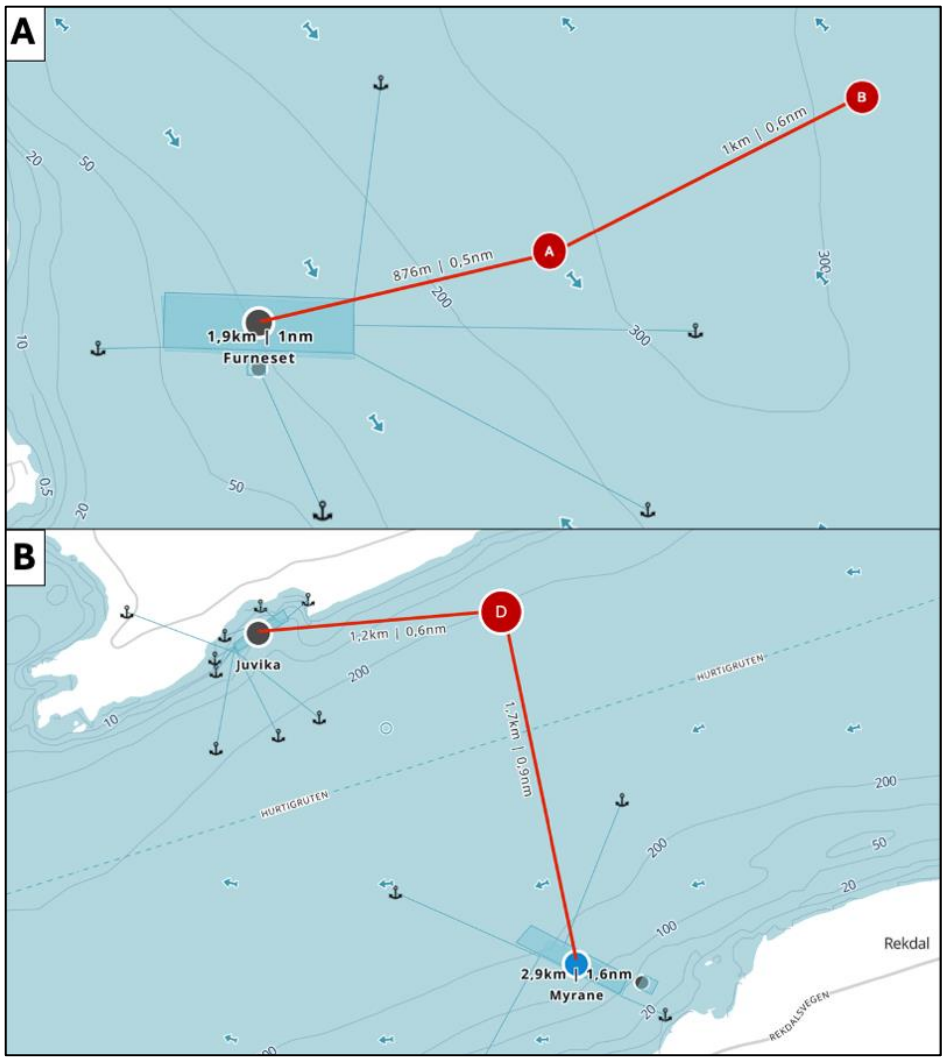

Figure 3.2: Close up map of the three catch locations in vicinity of fish farms, with approximate distances and direction of current (arrows) at 65 m depth (map created on BarentsWatch). **A)** The group A and B in increasing vicinity from the fish farm Fureneset. **B)** The group D in between one in-active (Juvika) and one active fish farm (Myrane).

For sampling location A (figure 3.2A) nephrops were caught in the first 8 pots of two separate strings, while nephrops in group B were caught in the last 8 pots on the same two strings. The fish farm Fureneset, where nephrops from sampling location A and B were caught, was last active in the beginning of October 2023 (6.5 weeks before sampling). A was approximately 880 metres from the centre of fish farm, while B was 1.9 kilo metres from the centre of the fish farm. The direction of current is North-west (figure 3.2A). Station D (figure 3.2B) was in vicinity of two fish farms, Juvika and Myrane. Juvika was last active in July 2022, while Myrane was active at time of sampling. D was 1.2 km from the fish farm Juvika, and 1.7 km from the active fish farm (at sampling time). The main current around D was towards west.

The nephrops were stored alive in a tank without access to food before they were dissected (<24 hours). The dissection of the samples of nephrops took place on the 27th and 28th of November 2023. On the first day nephrops in the groups A, B and C were dissected. On the second day nephrops from D were dissected, and these specimens were caught the previous day. Before dissection the nephrops were euthanized using an electric stunner (Crustastun, Mitchell & Cooper, Uckfield, UK). The carapace length was measured using a calliper, and sex of the specimen was determined before further dissection. Determination of sex was done by visually examining the size of the abdominal appendage of the nephrops, where females are smaller in size. Additionally the first pair of pleopods, meaning the swimming legs, of the males are firm and pointing forwards while the pleopods of females looks hairy and thin (Lauritzen et al., 2010). The tail of the nephrops was cut off just behind the carapace using a knife. The cephalothorax was opened using a scissor. The digestive gland hepatopancreas was then revealed (figure 3.2a) and collected using forceps. The sample of hepatopancreas was roughly homogenised and divided into two separate plastic vials. Further, the tail was cut in half sagittally (figure 3.2b), and the muscle on each side was sampled using a small metal spoon and put into two different plastic vials. The used instruments were wiped clean using paper to avoid cross contamination. This procedure was then repeated for each specimen.

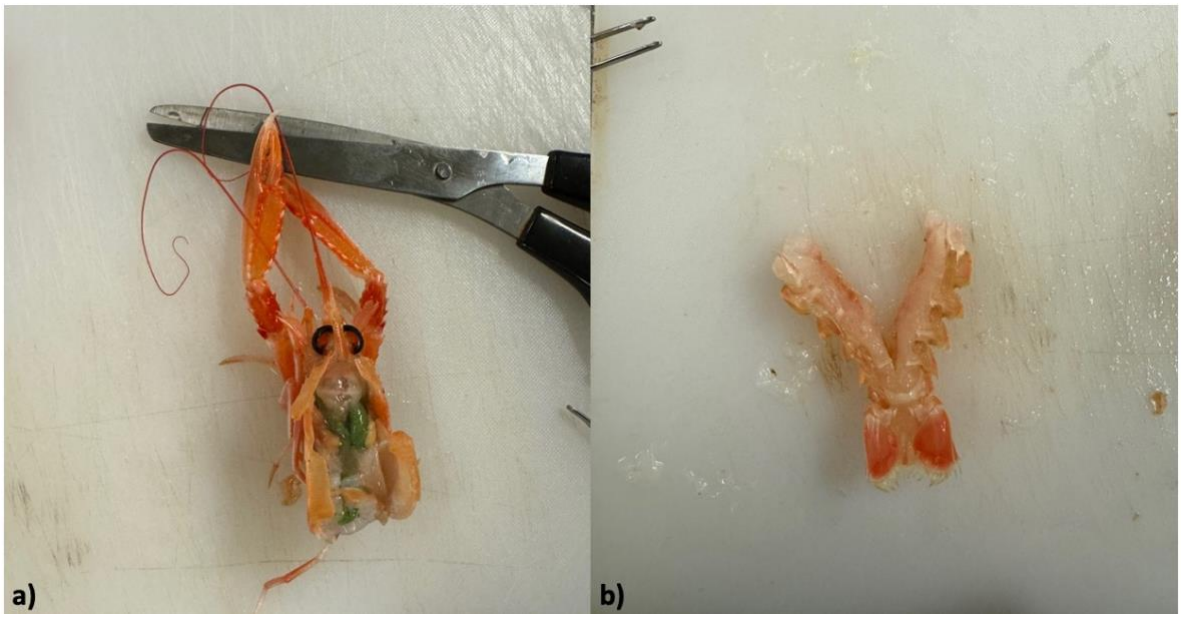

Figure 3.3a) The hepatopancreas is revealed when opening the head of the nephrops.
Figure 3.3b) Nephrops tail cut in half.

Since the materials collected from the nephrops were shared between this study and a study conducted by students at NTNU, both the samples of hepatopancreas and tail muscle was separated into two, given that the sample contained enough material to conduct both our individual analysis. Where hepatopancreas and tail muscle were too small to separate into two separate samples, the total amount of hepatopancreas and/or muscle was given to one of the projects. In cases where the hepatopancreas was not big enough to be separated, but the tail was, all hepatopancreas was given to one of the projects and the tail was still separated. All samples were frozen on site and subsequently transported by plane, stored in a polystyrene box, to the facilities at IMR in Bergen and stored at -20°C until January for further sample preparation.

3.2 Sample preparation

3.2.1 Freeze-drying and homogenization

Both the samples of tail muscles and hepatopancreas were freeze-dried at IMR, following the internal method (IMR, 2022a). Samples are first weighted before introducing it to the freeze-dryer. The frozen sample was put into the freeze dryer at -20°C (Freezone 18 liter, Labanco, Kensas, USA). Inside the freeze dryer the sample was frozen, and the water was removed by the vacuum where the water in the sample directly transforms from solid state to vapour. After 24 hours, the temperature of the plate was increased to +25°C. After 72 hours, the samples were finished and weighted. The weight of the freeze-dried sample was then recorded in LIMS (Laboratory Information Management System). In LIMS the dry matter content was automatically calculated (Eq. 1), where a is the weight of sample and container before freeze-drying, b is the weight of the container and c is the weight of the sample and container after freeze-drying. The percentage of dry matter is later used to calculate the concentration of elements in wet weight.

$$\%\ dry\ matter = \frac{(c-b)\cdot 100}{(a-b)} \qquad \text{(Eq. 1)}$$

Directly after freeze drying, muscle samples were homogenized by manual grinding with mortar and pestle. The hepatopancreas samples were not grounded after freeze-drying due to small amounts in the samples and as homogenization with mortar and pestle might have led to loss of sample material.

3.2.2 Acid digestion

To prepare the samples for the ICP-MS trace elements analysis, sample digestion was required, and performed using the IMR internal method "Oppslutning med Ultrawave og UlltraClave" IMR (2023b). The method is based on EN 15763, AOAC 2013.06 and NKLM 186. First 0.20 – 0.25 g of sample was weighed into a quartz test tube, with 0.5 ml deionized water (MilliQ) and 2 ml concentrated nitric acid (HNO_3). The process of digestion was sped up by heat with UltraWave microwave (Milestone, Sorisole, Italia). The test tubes were placed in a teflon container inside the microwave chamber. The Teflon container contained 130 ml MilliQ water and 5 ml H_2O_2.

Following, the digested samples were diluted to 25 ml and transferred to 50 ml sample tubes. The sample was then ready for further analysing by ICP-MS. Some of the hepatopancreas samples did not contain enough material to reach 0.2 g, and the whole sample was attempted to be transferred to the test tube and weighted. If the weight of the material was above 0.12 g the sample was still diluted to 25 ml, but if the sample weighted below 0.12 g the sample was diluted to 10 ml. The reason for this is that the dilution factor is important for further calculations. A standard curve was used in the analysis to detect the concentration of the elements (standard curve is explained further in section 3.3). If the value of dilution factor is too large, the intensity of the element detected by the instrument will be smaller than the intensity of any data points on the standard curve. With lower intensity, one would have to extrapolate the curve giving a concentration outside the area of measurement. Dilution to 10 ml instead of 25 ml will then rather result in a smaller value of dilution factor in samples ≤ 0.12 g, meaning the intensity will be measured within the area of measurement created by the standard curve. Diluting the sample to 10 ml instead of 25 ml can introduced additional uncertainty during measuring, due to the volumetric flask being small of size. The small size of the flask makes it hard to visually observe when the mark of precise volume has been reached. To reduce this uncertainty in the visual measurement process, the sample was diluted by weighing. The dilution by weighing was done by transferring the digested sample to the tared sample tube and adding deionized water until 10 g of total weight was reached. In this weighing process it was assumed that the density of the sample is similar to that of water.

When diluting the samples, LIMS automatically calculated the dilution factor f (Eq. 2). The weight (m) of the dry sample was automatically transferred into the system when weighing. The rest of the variables are entered via LIMS, V is the volume in ml (25 ml or 10 ml, according

to the dilution) and F is how many dilutions that were conducted. The dilution factor was then used to calculate concentration of elements (Eq. 3). In this equation the C_{pr} is the counts measured in the sample, C_{bl} is the counts measured in the blank sample, k is the coefficient of the calibration curve and f is the dilution factor.

$$f = \frac{V \cdot F}{m \cdot 1000} \quad \text{(Eq. 2)}$$

$$(C_{pr} - C_{bl}) \cdot k \cdot f \quad \text{(Eq. 3)}$$

In the subsequent microwave treatment, 15 test tubes were run in parallel. Between each run, the test tubes were washed with an acid wash to avoid cross contamination. By washing the equipment with acid any traces of the prepared samples left on the glass surface. All the tail muscle samples were digested first, followed by one run of blank samples containing only HNO_3 before the tubes were ready for the last matrix, hepatopancreas. Blank samples were run to make sure there was no trace of previous samples of the former matrix.

3.2.3 Stable isotopes analysis

In addition to preparing the samples for ICP-MS, subsamples of the freeze-dried muscle samples were analysed for $\delta^{13}C$ and $\delta^{15}N$. Briefly, 1.0 ± 0.2 mg was weighed into an 8x5 mm tin capsule (D1009, Elemental Microanalysis, UK) on a semi-microbalance (Ohaus EX225D, USA). The tin capsule was then pressed flat rolled up to ensure no leakage and the formation of a small cube. All capsules were placed in a well plate and sent to Iso-Analytical Ltd (Crewe, UK), where the samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ by Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS). The external standard used in this analysis was muscle tissue of cod ($\delta^{13}C = -18.9 \pm 0.0‰$, $\delta^{15}N = 12.9 \pm 0.1‰$) (Zhu et al., 2019). The reference material used was IA-R042 ($\delta^{13}C = -21.6 \pm 0.1‰$, $\delta^{15}N = 7.6 \pm 0.1‰$) (Zhu et al., 2019).

The isotopic niches of the four groups of nephrops were investigated in RStudio using SIBER (Stable Isotope Bayesian Ellipse in R). We investigated several parameters of standard ellipses, including sample size-corrected standard ellipse area (SEA_C), Bayesian posterior estimated standard ellipses (SEA_B), eccentricity (E), the angle of the major axis with respect to x-axis in degree (θ), and the overlap of standard ellipses between groups. E explains potential variances on the x- and y- axes, where low values of E refer to similar variance in both axes with a more

circular shape and high values of E refer to a stretched isotopic niche along on of the axes. θ values close to 0° show dispersion along the x-axis ($\delta^{13}C$) which indicates potential feeding of multiple production sources. On the other hand, θ values close to 90° show dispersion along the y-axis ($\delta^{15}N$) which indicate feeding across multiple trophic positions (Zhu et al., 2019).

3.3 ICP-MS analysis

After digestion, the analysis by ICP-MS was conducted by the staff following the IMR internal method (IMR, 2023a). The instrument being used for the analysis was ICPMS Thermo iCapQ (Thermo Scientific, Waltham, USA) with FAST SC-4DX autosampler (Elemental Scientific, Omaha, Nebraska). Additionally, the program Qterga was used to set the method and the program Instrument Control were used to control the ICP-MS settings. A calibration curve, also called standard curve, was used to convert the intensities detected to concentration. Standards for creating the calibration curve was made by extracting 10 – 20 – 50 – 100 – 200 and 500µL of stock solution (multi element standard), adding 20µL Au (100mg/L) and diluted with 5% HNO_3 to 10 mL (IMR, 2023a). The standards are made daily before an analysis.

Before starting the analysis, the tubes were visually examined and if approved, the plasma was lit. Tuning of the instrument was then run by placing the probes in tune B containing concentrated HNO_3 and tuning-solution. After 15 minutes the performance report of the instrument was inspected. If passed the probes could then be washed in 5% HNO_3 and placed in its corresponding solutions internal standard, and carrier solution (concentrated HNO_3 and gold). The instrument is then ready to conduct the analysis. Prior to running the samples, 5-6 sample blanks and the standards to make the calibration curve was analysed. Four control samples (CRM) were also analysed, and if they were within the control chart limits the analysis of the samples could begin. Between each matrix two runs of standard blanks were done, to make sure the tubes were properly clean.

Table 3.1 shows the LOQ and LOD for the analysis by ICP-MS in dry weight. LOQ for the trace elements are calculated based on the weighted samples, automatically by LIMS (IMR, 2023a).

Table 3.1: LOD and LOQ for the trace elements in mg/kg dry weight

	LOD (mg/kg dw)	**LOQ (mg/kg dw)**
As	0.003	0.01
Ag	0.003	0.01
Co	0.006	0.02
Cr	0.009	0.03
Cu	0.03	0.1
Fe	0.1	0.5
Hg	0.003	0.007
Mn	0.009	0.03
Mo	0.03	0.1
Ni	0.09	0.3
Pb	0.009	0.03
Se	0.003	0.01
V	0.001	0.005
Zn	0.2	0.5

3.4 Data analysis

3.4.1 Statistical analysis

Statistical analysis and calculations including mean, min, max and standard deviation (SD) were performed in R (version 4.3.2; RStudio, 2023). All tables were created using the function "DescribeBy", and boxplots and scatterplots were used to portray the results. To conduct hypothesis testing (analysis of variance, ANOVA) on the dataset, it had to be tested for normality. This was done by the Shapiro-Wilks test. The Shapiro-Wilk test is widely used to check assumptions of normality in statistical analysis. It is based on correlation within observed data and expected normal scores (Das & Imon, 2016). A p-value below 0.05 indicated that concentration data were not normally distributed, and with p-values above 0.05 the measurements are normally distributed. There were four elements not normally distributed in the subset of hepatopancreas (As, Cd, Se and Pb), and four elements not normally distributed in the subset of muscle (Cd, Fe, Mn and Pb) (appendix I). The QQ-plots of the subsets with and without log-transforming were then checked. The plots were not significantly changed after log-transforming the dataset (appendix I), and the dataset was therefore further used without log-transformation.

3.4.2 Detection of outliers

The method of detecting outliers in this study was the InterQuantile Range (IQR) method (Srinivasan, 2023). The IQR was first calculated by subtracting the first quantile from the third quantile (Eq. 4).

$$IQR = Q3 - Q1 \quad \text{(Eq. 4)}$$

The calculated IQR was then used to identify outliers by limits set to 3 times the IQR. Above the third quantile (Q3) the upper bound was set (Eq. 5), and below the first quantile (Q1) the lower bound was set (Eq. 6). Objects above the upper bound and below the lower bound was considered outliers. The calculation of IQR was done by R (appendix III) and the most extreme outliers were removed from the dataset.

$$Upper\ bound = Q3 + 3 \cdot IQR \quad \text{(Eq. 5)}$$

$$Lower\ bound = Q1 - 3 \cdot IQR \quad \text{(Eq. 6)}$$

3.4.3 Modelling

When using the IQR method detecting outliers, the value of multiplication was increased from 1.5 to 3 to remove only the most extreme outliers and still maintain a representative number of specimens for the study. Before deciding which models and which subsets to use, two different subsets were tested for AIC (Akaike information criteria): all samples and without the extreme outliers detected by IQR. The lower the value of AIC, the better the model. The AIC was notably reduced when the outliers were removed (appendix III), which indicated that the exclusion of the outliers was an appropriate choice. This subset without outliers was used while investigating the effect of organ and sex, while during the assessment of the effect of fish farms the female samples were excluded as well. This was because the males made a better premise for comparing the trace elements due to the lack of female specimens caught (for instance no females in sampling location B).

Data for the nine relevant elements for this study were statistically analysed for the effect of three factors: organ, sex and the effect of sampling location. The exploration of all these effects were done by a linear mixed effect model (LME). All 49 models were evaluated (appendix II). Firstly, models for the carapace length were run for the all the specimens against sex and against the sampling location (M1-M4, appendix II). To examine the effect of the organs on the element

concentrations, models for all relevant elements were run against organ (O1-O9, appendix II). As a clear effect of organ was seen, the effect of the sexes was examined by running all relevant elements against sex in both, samples of hepatopancreas (SH1-SH9, appendix II) and samples of muscle (SM1-SM9, appendix II). Lastly the models for all relevant elements were run against the sampling locations in the hepatopancreas samples (GH1-GH9, appendix II) and the sampling locations in the muscle samples (GM1-GM9, appendix II) to account for the effect of fish farms. All the models were tested by ANOVA (appendix (IV). The models where variables were run against sampling location were additionally tested with post-hoc Tukey HSD for comparing multiple means (appendix V). For correlation analysis Pearson correlation was used.

3.4.4 Principal component analysis (PCA)

The dataset in the present study consists of 79 specimens and the variables of measured concentration of 15 different elements in addition to physical parameters as sex, carapace length as well as collection area. This makes PCA a suitable method of analysing the dataset. The generating of PCA biplots was conducted by Sirius (PRS, Norway). The dataset analysed by PCA was standardised by subtracting the mean and dividing by the standard deviation, automatically conducted by Sirius. This was to make sure that all the variables contribute equally to the analysis, since all the variables had different ranges of concentration. The outliers previously detected by the IQR in R were also removed.

The PCA biplot contains of a loading plot and score plot on two axes: the first principal component and the second principal component. The variables are shown by the loading plot, and the samples are shown by the score plot. The placement of variables in the loading plot can indicate how much they influence the components PC1 and PC2, as well as how the variables correlate to each other. The influence of the variables on the samples will affect how the samples are placed (and clustered) in the score plot and was therefore used to investigate trends among the sampling locations and the correlations and influence of the variables.

4 Results

4.1 Overview

The means, SD (standard deviation), minimum values, and maximum values of the carapace length (mm) of the sampled nephrops, are shown in table 4.1, for each sampling location and sex, as well as overall. Group A and B are in increasing distance from one fish farm, C was the control area and D was between two sea-based fish farms.

Table 4.1: Overview of the nephrops sampled in different distances to salmon aquaculture farms in Romsdalsfjorden, Norway. Catch location (latitude, longitude) and carapace length (mm) of the nephrops, sorted by sampling location and sex, are given. The denotations of significance are explained below the table.

	Area			Carapace length (mm)	
	Lat, Long	Sex	n	Mean ± SD	(Min - Max)
A	62.65, 7.13	M	11	46.01 ± 5.43	(38.41 – 53.92)
		F	1	42.67	42.67
		All	12	45.73 ± 5.27	(38.41 – 53.92)
B	62.65, 7.13	M	20	48.65 ± 5.0 [c]	(36.92 – 57.78)
C	62.68, 6.99	M	15	44.87 ± 5.07	(37.06 – 56.53)
		F	7	38.35 ± 1.63	(36.94 – 40.91)
		All	22	42.79 ± 5.25	(36.94 – 63.33)
D	62.66, 6.73	M	15	41.60 ± 5.94 [d]	(35.24 – 54.72)
		F	10	37.71 ± 3.78	(33.91 – 47.12)
		All	25	40.05 ± 5.45	(33.91 – 54.72)
Overall		M	61	45.51 ± 5.86 [a]	(35.24 – 57.78)
		F	18	38.24 ± 3.13 [b]	(33.91 – 47.12)
		All	79	44.34 ± 5.89	(35.24– 57.78)

a) was significantly different mean from the opposite sex **b)** (ANOVA, $p < 0.05$, appendix IV)
c) was significantly different mean from group denoted by **d)** within samples of males (appendix V)

The nephrops (n = 79) varied in carapace length within each sampling location (table 4.1). On average, nephrops from location D had the lowest mean of carapace length of 40.05 ± 5.45 mm, followed by 42.79 ± 5.25 mm (C), 45.73 ± 5.27 mm (A) and 48.65 ± 5.0 mm (B). However, only nephrops from group B were significantly larger than the rest of the groups (appendix V). Overall, there were more males (n = 61) caught than females (n = 18) (table 4.1). The carapace lengths of the males are generally significantly larger than of the females (appendix V), with respectively 45.51 ± 5.86 mm and 38.24 ± 3.13 mm.

4.2 Effect of organ

Concentrations of the relevant elements in the hepatopancreas and muscle tissue of the sampled nephrops are provided in table 4.2. The mean concentration of Cu was the highest measured in the samples of hepatopancreas at 432.6 ± 177.2 mg/kg ww, followed by the mean concentration of Zn at 85.9 ± 30.7 mg/kg ww and of As at 32.8 ± 9.1 mg/kg ww. The highest mean concentrations found in the muscle tissue samples were Zn at 10.8 ± 1.2 mg/kg ww, As at 7.9 ± 2.5 mg/kg ww and Cu at 5.8 ± 1.7 mg/kg. All the relevant elements showed a significant difference between the concentration measured in the hepatopancreas and the muscle tissue (Table 4.2, appendix IV) and the difference between the organs were visualised by a scatterplot (figure 4.1).

Table 4.2: Mean and SD, minimum and maximum concentration (mg/kg ww) of the different elements sorted by organ. Both females and males are included. Percentage difference from the concentration in the muscle sample to the hepatopancreas sample. All samples of hepatopancreas were significantly higher in concentration than the samples of muscle tissue (appendix IV, O1 – O9).

	Hepatopancreas (mg/kg ww)		**Muscle tissue (mg/kg ww)**		**Percentage difference**
	Mean ± SD	**Min – max**	**Mean ± SD**	**Min - max**	**(%)**
Cd	6.15 ± 2.9	1.7 – 15.7	0.0037 ± 0.0010	0.0012 – 0.0085	166 116
Hg	0.07 ± 0.03	0.02 – 0.16	0.04 ± 0.01	0.02 – 0.07	75
Pb	0.1 ± 0.06	0.02 – 0.31	0.01 ± 0.004	0.01 – 0.02	900
As	32.8 ± 9.1	11.4 – 52.9	7.9 ± 2.5	2.8 – 15.1	315
Cu	432.6 ± 177.2	55.3 – 962.6	5.8 ± 1.7	2.9 – 10.2	7 359
Zn	85.9 ± 30.7	36.5 – 163.8	10.8 ± 1.2	8.3 – 13.1	695
Fe	24.8 ± 7.1	8.6 – 46.4	0.91 ± 0.34	0.42 – 2.08	2 625
Mn	1.9 ± 0.5	1.1 – 3.8	0.18 ± 0.05	0.09 – 0.31	956
Se	6.6 ± 2.9	2.0 – 15.4	0.62 ± 0.11	0.41 – 0.88	965

The percentage difference from the mean concentrations of muscle tissue samples to the hepatopancreas samples were of large values (table 4.2), with Cd being 166 116% higher in the hepatopancreas than the muscle tissue, Cu being 7 359% higher, and Fe 2 625% higher. The smallest percentage difference was seen in Hg, were the mean of hepatopancreas was 75% higher than in the muscle tissue samples.

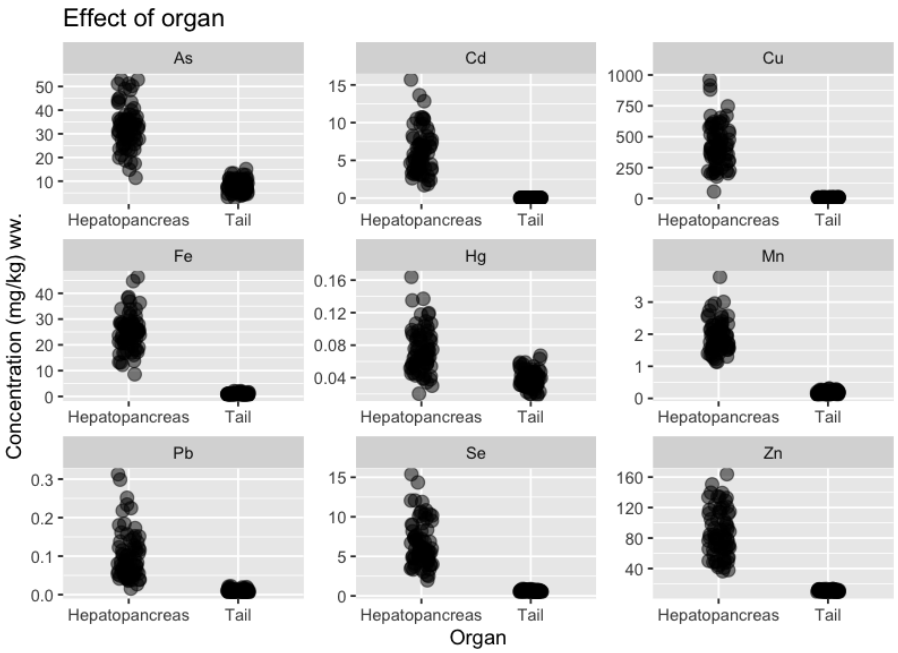

Figure 4.1: Scatterplots of the relevant elements sorted by organ, visualizing the difference in concentration between hepatopancreas ad muscle tissue.

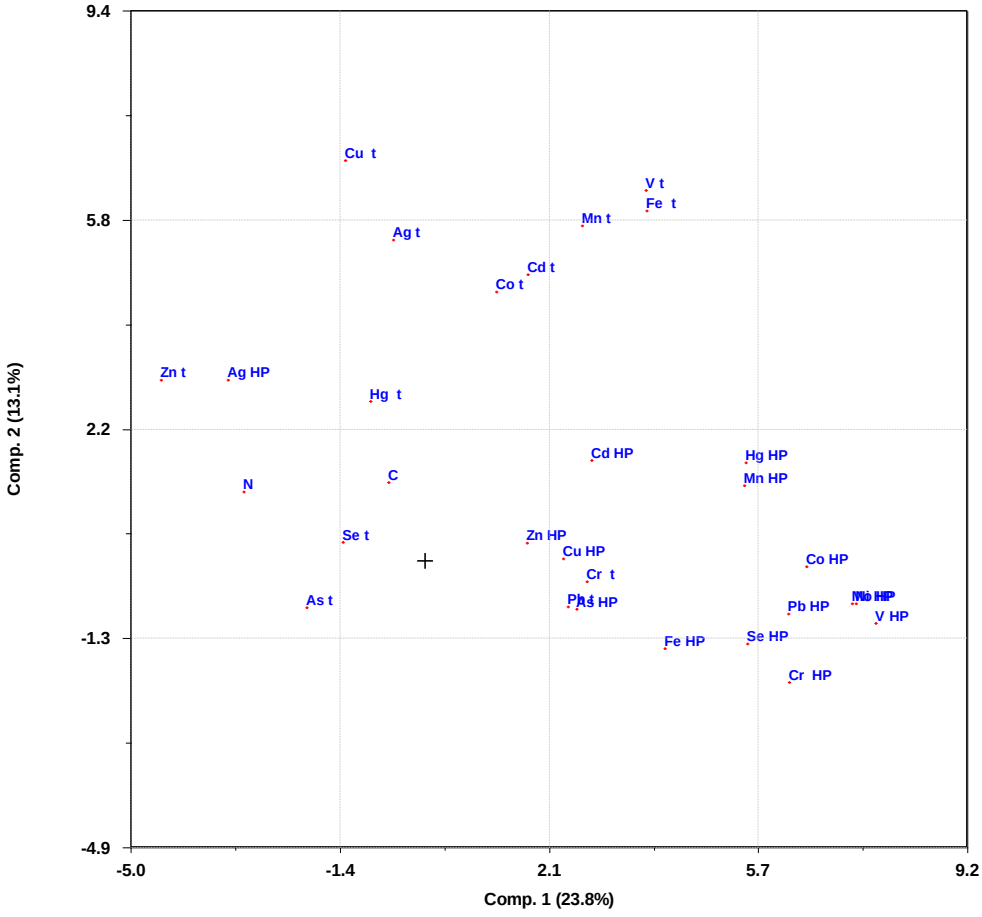

Figure 4.2: PCA loading plot of both the tail samples and hepatopancreas samples. Both males and females are included (t = tail muscle tissue, HP = hepatopancreas tissue).

The PCA loading plot (figure 4.2) indicated the most important contributors to the sample concentration. In total the plot explained 36.4% of the variance, 23.8% in the first component and 13.1% in the second component. Among the muscle tissue variables, all the elements except Cr and Pb are all similarly placed. Out of the elements in the muscle tissue, the most dominating appeared to be Zn, Cu, Fe, V and As. The loading plot indicated that the most correlated elements within the muscle tissue are Cu and Ag, as well as Co, Cd, Mn, Fe and V. All the elements in the hepatopancreas samples are tightly clustered, except for Ag. Most of the elements in hepatopancreas contribute to the first component and the most dominating elements in this component appeared to be Ag, V, Cr, and Mn. In the second component Ag, Cd, Hg, and Cr appeared to contribute the most. Among the hepatopancreas variables the correlations seemed to be more prominent, where one group of correlated elements was Zn, Cu and As, another was Hg and Mn, and lastly a somewhat correlated group of Fe, Se, Cr, Pb, Co, Mo, Ni and V. The stable isotopes (C and N) are placed inside the clustering of muscle tissue, and somewhat correlated to each other.

4.3 Effect of sex

The result of the analyses of the effect of sex on the different trace element concentration in the organs is presented in table 4.3.

Table 4.3: The concentrations (mg/kg ww) of different trace elements hepatopancreas and muscle sorted by sex. Statistically significant differences between the sexes within each tissue and element are indicated by letters explained below the table.

	Hepatopancreas		**Muscle**	
	Female n = 13 (mg/kg ww)	**Male** n = 59 (mg/kg ww)	**Female** n = 18 (mg/kg ww)	**Male** n = 61 (mg/kg ww)
Cd	8.6 ± 3.2 [a]	5.5 ± 2.6 [b]	0.0044 ± 0.0017 [a]	0.0035 ± 0.0016 [b]
Hg	0.096 ± 0.029 [a]	0.071 ± 0.026 [b]	0.047 ± 0.011 [a]	0.035 ± 0.008 [b]
Pb	0.12 ± 0.06	0.096 ± 0.06	0.0099 ± 0.003	0.010 ± 0.004
As	28.5 ± 8.7	33.7 ± 9.0	5.8 ± 2.4 [a]	8.5 ± 2.3 [b]
Cu	610.5 ± 246.3 [a]	393.4 ± 131.2 [b]	5.3 ± 1.7	5.9 ± 1.6
Zn	82.5 ± 26.6	86.7 ± 31.6	9.8 ± 1.0 [a]	11.1 ± 1.1 [b]
Fe	21.2 ± 6.3 [a]	25.6 ± 7.1 [b]	1.02 ± 0.35	0.88 ± 0.34
Mn	2.1 ± 0.5	1.9 ± 0.5	0.21 ± 0.05 [a]	0.18 ± 0.05 [b]
Se	6.7 ± 3.3	6.6 ± 2.8	0.56 ± 0.09 [a]	0.64 ± 0.11 [b]

a) Significantly different mean than corresponding mean (b) of the opposite sex within the organ ($p<0.05$, appendix IV)

In samples of hepatopancreas Cd had significantly higher concentration in the females than the males with 8.6 ± 3.2 mg/kg and 5.5 ± 2.6 mg/kg (table 4.3). The same result was found in Hg for females and males (respectively, 0.096 ± 0.029 mg/kg and 0.071 ± 0.026 mg/kg) and Cu (respectively, 610.5 ± 246.3 mg/kg and 393.4 ± 131.2 mg/kg). Only in Fe did the males have higher concentration than the females with respectively 25.6 ± 7.1 mg/kg and 21.2 ± 6.3 mg/kg.

In the tail muscle samples the relevant elements found to differ significantly between females and males are Cd, Hg, As, Zn, Mn, and Se (table 4.3). The elements with higher values found in the females than the males are Cd (respectively, 0.0044 ± 0.0017 mg/kg and 0.0035 ± 0.0016 mg/kg), Hg (0.047 ± 0.011 mg/kg and 0.035 ± 0.008 mg/kg), and Mn (0.21 ± 0.05 mg/kg and 0.18 ± 0.05 mg/kg). While As with 8.5 ± 2.3 mg/kg and 5.8 ± 2.4 mg/kg were found to have a higher mean value in males than females. This was the case for Zn (11.1 ± 1.1 mg/kg and 9.8 ± 1.0 mg/kg) and Se (0.64 ± 0.11 mg/kg and 0.56 ± 0.09 mg/kg) as well. The non-essential elements with significant differences (As, Cd and Hg) showed different slopes when investigating the relationship between carapace length and the concentration in the muscle (figure 4.3).

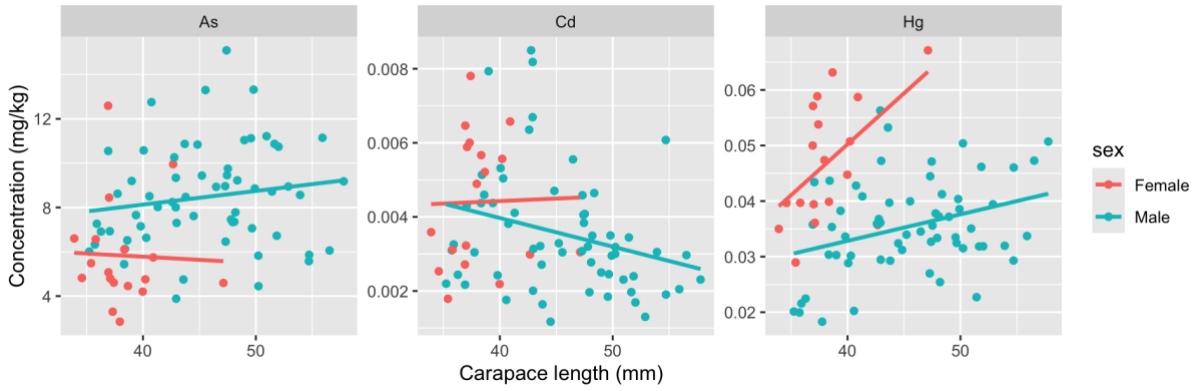

Figure 4.3: Non-essential elements and the relationship between concentration (mg/kg) in muscle and the carapace length (mm) samples coloured by sex.

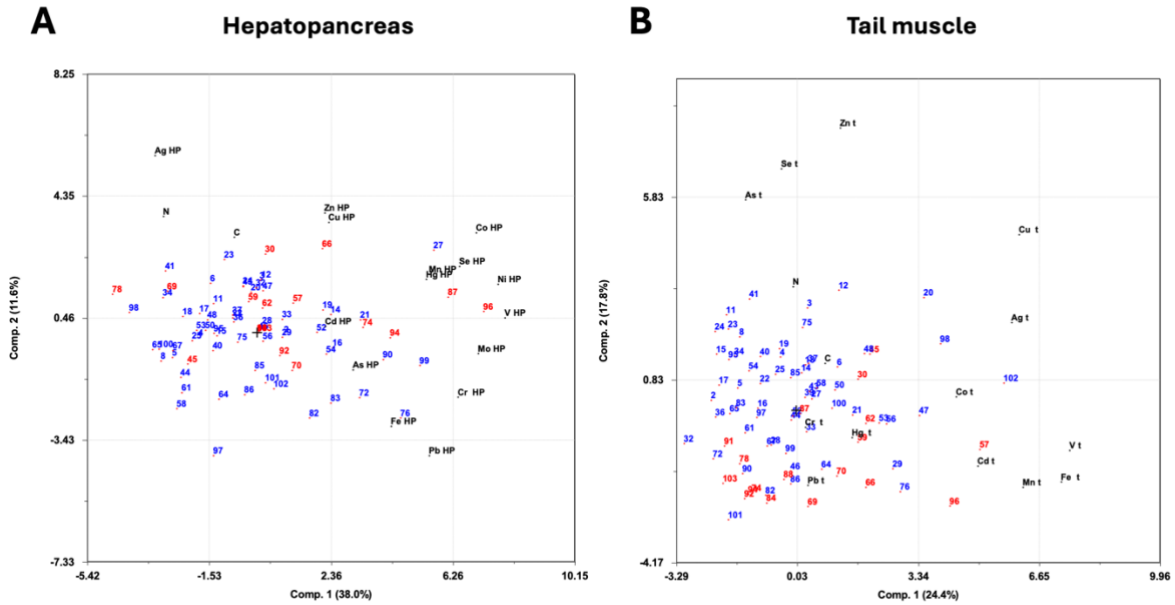

Figure 4.4: PCA plots of the concentrations of trace elements with females (red) and males (blue), **A)** in the hepatopancreas samples and **B)** in the tail muscle samples.

The PCA plot of hepatopancreas (figure 4.4A) explained a total of 49.6% of the variance with 38.0% in the first component and 11.6% in the second component. With including both the females and the males, the PCA plot of the hepatopancreas (figure 4.4A) revealed a correlation between Cu and Zn, as well as Mn, Hg and Se. The females were somewhat clustered and placed further upwards along the second component. The spread of females and males along the first component appeared to be relatively similar. The PCA plot of tail muscle (figure 4.4B) explained a total of 42.2% of the variance with 24.4% explained in the first component and 17.8% explained in the second component. Like in the PCA of hepatopancreas, the sexes were similarly spread along the first component. Most distinct was the clustering of females further down along the second component, most likely explained by Se and Zn being some of the main contributors to the second component having higher values in males than in females (table 4.3).

4.4 Effect of fish farms activities

The females were excluded when examining the difference between the groups A, B, C and D regarding trace element concentrations.

4.4.1 Trace elements

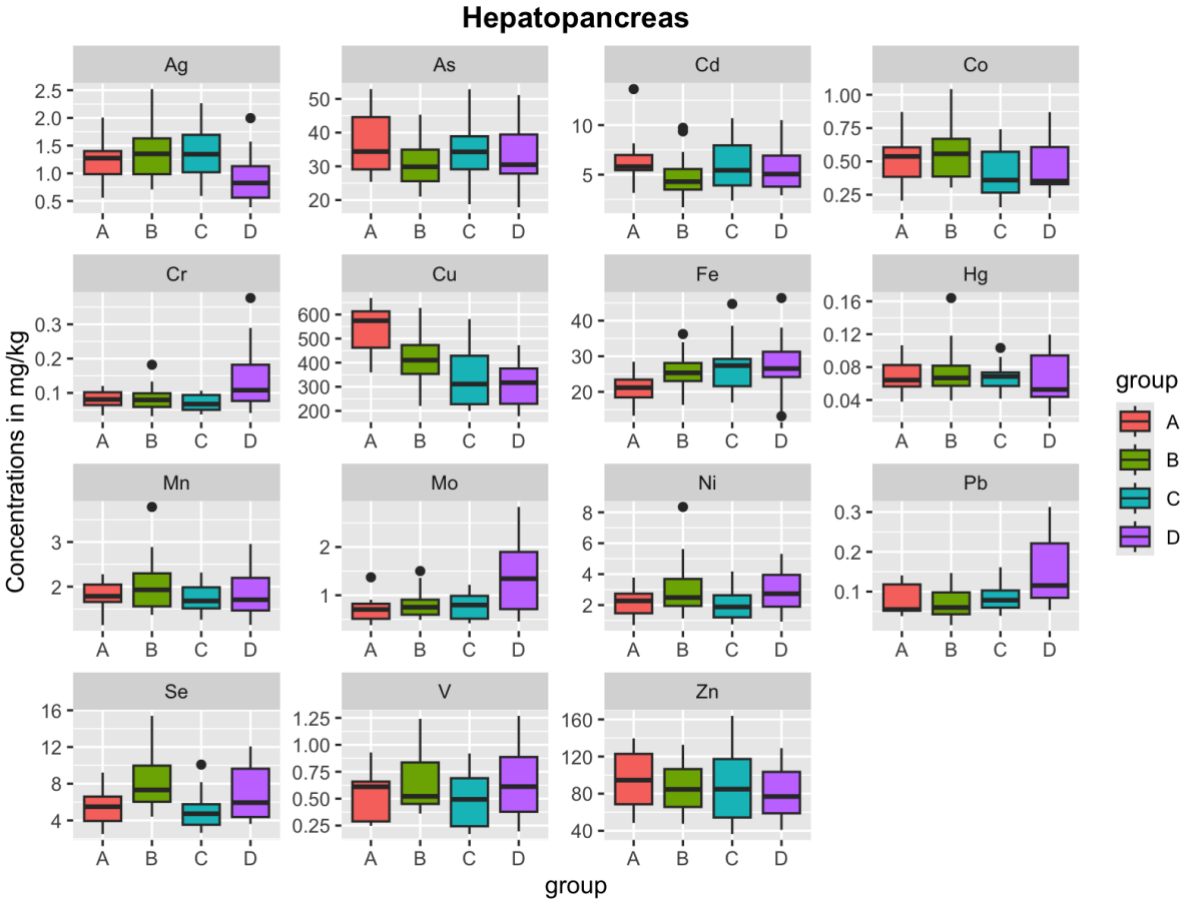

Figure 4.5 Concentration of the trace elements in hepatopancreas in males sampled in different distances to salmon aquaculture farms in Romsdalsfjorden, Norway. Locations A and B were in increasing distance from the same fish farm. Location D was in proximity to three fish farms, but upstream in current. Location C was considered as a control group, being located several km away from the nearest aquaculture facility.

Trace element concentrations measured by ICP-MS in the samples of hepatopancreas are presented by boxplots in figure 4.5. Pb in D (0.15 ± 0.09 mg/kg) and Cu in A (538.7 ± 106.4 mg/kg) were both significantly higher than in all other locations.

Focusing on the groups A, B, and C, with increasing distance to a fish farm, several elements had higher concentrations in A compared to B including As, Cd, Cu, Pb and Zn, however, the differences were small. Group D, which was near two sea-based fish farms, generally have a bigger range and higher means of several elements (Cr, Fe, Mn, Mo, Ni, Pb, Se, V). Elements with highest mean closest to a fish farm (sampling location A) were Cd (6.5 ± 2.8 mg/kg), As (37.4 ± 9.8 mg/kg), and Zn (95.7 ± 32.0 mg/kg), but all of these elements had similar concentrations in the control group C, respectively 6.2 ± 3.0 mg/kg, 34.4 ± 10.1 mg/kg and 89.4 ± 41.3 mg/kg (table 4.4). Statistically, this was not significant. Cu was the only element with a significant pattern of higher concentration with increasing distance from the fish farm with A (538.7 ± 106.4 mg/kg), followed by B (414.9 ± 93.7 mg/kg), and both being higher than the mean concentration in the control group C (341 ± 130.1 mg/kg).

Table 4.4: The most relevant elements and their mean and SD of the concentrations (mg/kg ww) in the samples of hepatopancreas (only males). Mean values of groups with significant difference are denoted as described below the table.

		Hepatopancreas			
		A (n = 11) (mg/kg ww)	**B (n = 19) (mg/kg ww)**	**C (n = 14) (mg/kg ww)**	**D (n = 15) (mg/kg ww)**
Non-essential	**Cd**	6.5 ± 2.8	4.8 ± 2.2	6.2 ± 3.0	5.5 ± 2.3
	Hg	0.07 ± 0.02	0.08 ± 0.03	0.07 ± 0.02	0.07 ± 0.02
	Pb	0.08 ± 0.04 [b]	0.07 ± 0.04 [b]	0.09 ± 0.04 [b]	0.15 ± 0.09 [a]
	As	37.4 ± 9.8	31.0 ± 6.7	34.4 ± 10.1	33.7 ± 9.7
Essential	**Cu**	538.7 ± 106.4 [a]	414.9 ± 93.7 [b, c]	341 ± 130.1 [b]	307.4 ± 91.7 [b, d]
	Zn	95.7 ± 32.0	85.5 ± 26.9	89.4 ± 41.3	79.1 ± 27.8
	Fe	21.3 ± 4.6	25.3 ± 5.5	27.3 ± 7.8	27.6 ± 8.7
	Mn	1.8 ± 0.3	2.1 ± 0.6	1.7 ± 0.3	1.9 ± 0.5
	Se	5.5 ± 2.1 [b]	8.1 ± 2.9 [a]	5.1 ± 2.1 [b]	7.0 ± 3.0

Concentration of element in sampling location denoted with a) were significantly different than sampling locations denoted with b), as well as the denotation of c) and d) being significantly different (appendix V)

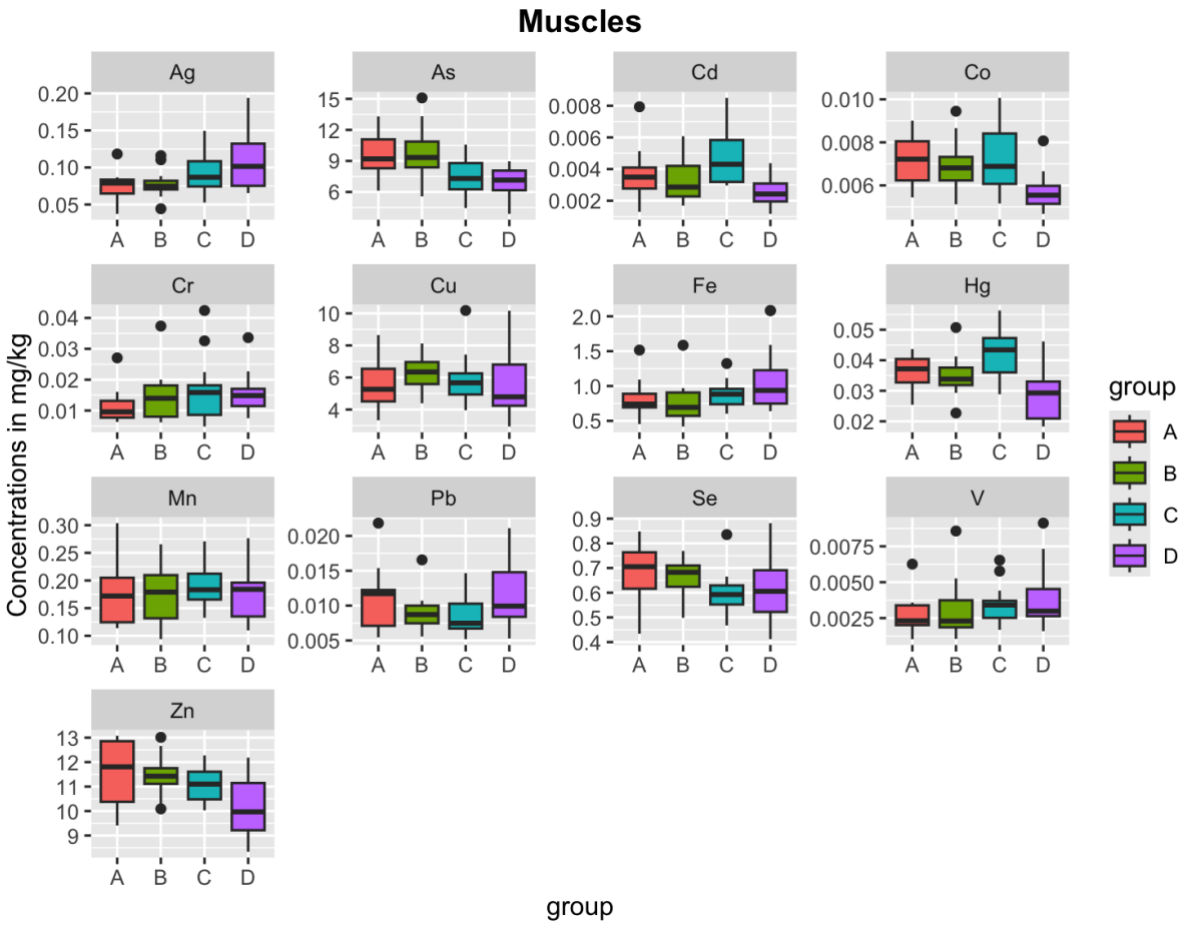

Figure 4.6: Concentration of the trace elements with more than 50% above LOQ in the muscle tissue for the males in the different sampling locations.

Figure 4.6 shows the concentration of different trace elements in the muscle with elements with more than 50% measured concentrations above LOQ. Some of the same pattern observed in the hepatopancreas samples, can also be seen here, where As, Pb, Se and Zn appeared to decrease as the distance to a fish farm increased. There are no significant patterns appearing by visual inspection of the boxplots. None of the non-essential elements As, Cd, Pb and Hg have been found to have levels above the maximum limit of 0.50 mg/kg.

Table 4.5: Mean concentration (mg/kg ww) and SD of the relevant elements in the tail muscle samples (only males). Statistically significant differences are denoted as described below the table.

Tail muscle					
		A (n = 11) (mg/kg ww)	**B (n = 20) (mg/kg ww)**	**C (n = 15) (mg/kg ww)**	**D (n = 15) (mg/kg ww)**
Non-essential	**Cd**	0.0037 ± 0.0017	0.0033 ± 0.0013 [b]	0.0047 ± 0.001 [a]	0.0026 ± 0.0009 [b]
	Hg	0.036 ± 0.005	0.035 ± 0.005 [b]	0.042 ± 0.008 [a]	0.029 ± 0.009 [b]
	Pb	0.011 ± 0.005	0.0086 ± 0.0025	0.0089 ± 0.0031	0.012 ± 0.0048
	As	9.7 ± 2.2 [a]	9.7 ± 2.3 [a]	7.4 ± 1.9 [b]	7.0 ± 1.4 [b]
Essential	**Cu**	5.5 ± 1.6	6.3 ± 1.0	5.8 ± 1.5	5.8 ± 2.4
	Zn	11.6 ± 1.4 [b]	11.4 ± 0.7 [b]	11.1 ± 0.8	10.2 ± 1.2 [a]
	Fe	0.82 ± 0.29	0.75 ± 0.26 [a]	0.88 ± 0.20	1.1 ± 0.5 [b]
	Mn	0.17 ± 0.06	0.17 ± 0.05	0.19 ± 0.04	0.17 ± 0.05
	Se	0.69 ± 0.12	0.66 ± 0.07	0.60 ± 0.09	0.61 ± 0.14

Concentration of element in sampling location denoted with a) were significantly different than sampling locations denoted with b) (appendix V)

The mean concentration of the muscle tissue samples (table 4.5) were generally lower than in the hepatopancreas samples (table 4.4). Cd had the highest mean in group C (0.0047 ± 0.001 mg/kg) and was significantly higher than in B (0.0033 ± 0.0013 mg/kg) and in D (0.0026 ± 0.0009 mg/kg). The mean concentrations of As in A (9.7 ± 2.2 mg/kg) and B (9.7 ± 2.3 mg/kg) were significantly different from the mean concentrations in C (7.4 ± 1.9 mg/kg) and in D (7.0 ± 1.4 mg/kg). In the essential element Zn, the groups A (11.6 ± 1.4 mg/kg) and B (11.4 ± 0.7 mg/kg) were significantly higher than D (10.2 ± 1.2 mg/kg).

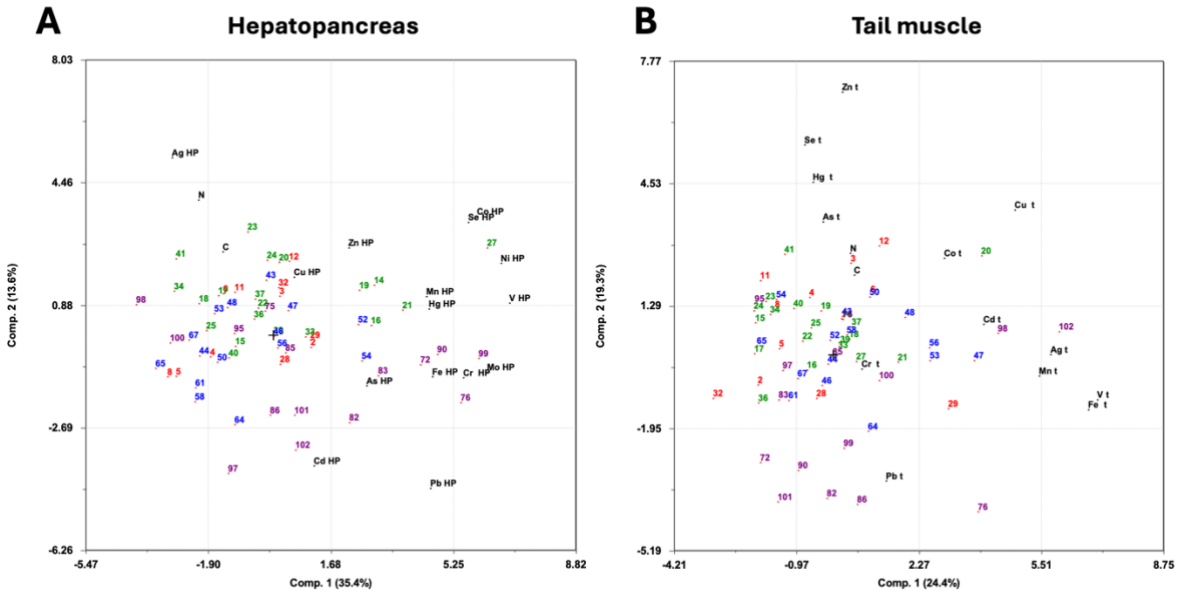

Figure 4.7: PCA biplot of the groups (A = red, B = green, C = blue, D = violet) where **A)** shows the PCA biplot of all the hepatopancreas samples, coloured by group and **B)** shows the PCA biplot of the tail muscle samples.

The PCA biplot of hepatopancreas (figure 4.7A) explained 49% of the variance. In the first component (35.4%) the variables contributing the most to explaining are the elements Mo, V and Ni. The variables Ag, N, Pb and Cd were the most contributing to the explanation of variance in the second component (13.6%). Out of the relevant elements in this study Cu and Zn, Hg and Mn, as well as N and C were correlated. Ag seemed to be correlated to the stable isotopes C and N. The groups A (red) and B (green) were similarly placed in the biplot, but B was more spread in the first component. The control group C (blue) was clustered further down along the second component.

The PCA biplot of the tail muscle samples (figure 4.7B) was found to explain a total of 43.7% of the variance, with 24.4% in the first component and 19.3% in the second component. V, Fe, Mn and Ag were variables with the most contribution to component 1, while Zn, Se and Pb contributed most to component 2. The stable isotopes C and N were correlated here as well but did not contribute to any of the components in the same degree as in the hepatopancreas PCA plot. Zn, Se, Hg and As were somewhat correlated to each other, while they were all negatively correlated to Pb. The groups A (red), B (green) and C (blue) in the tail muscle samples were more clustered together than in the hepatopancreas samples. Group C had higher scores along

the first component. The group D (violet) was more spread across both component in the PCA plot of muscles (figure 4.7B), as well as in the PCA plot of hepatopancreas (figure 4.7A).

4.4.2 Stable isotopes

Table 4.6: Mean and SD of the isotopic ratios $\delta^{13}C$ and $\delta^{15}N$. Significant differences are denoted by letters, where sampling locations denoted with a) is significantly higher than sampling location denoted with b) (appendix V).

Samling location	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
A	-18.76 ± 0.31 ^{b}	11.12 ± 0.36
B	-18.78 ± 0.54 ^{b}	11.24 ± 0.37 ^{a}
C	-18.83 ± 1.02 ^{b}	10.87 ± 0.30 ^{b}
D	-19.54 ± 1.17 ^{a}	10.99 ± 0.54

$\delta^{13}C$ in samples from location D (-19.54 ± 1.17‰) was significantly different than those from the other locations. A, B and C show increasing $\delta^{13}C$ as the distance to a fish farm decreases, but this is not statistically significant. The most substantial difference in the group means of $\delta^{13}C$ was between D and A and was an increase of 0.78‰. $\delta^{15}N$ in samples from location B (11.24 ± 0.37‰) was found to be significantly higher than that from location C (10.87 ± 0.30‰), with a difference in the group means of 0.37‰. There were no other statistically significant differences in $\delta^{15}N$ between other pairs of sampling location.

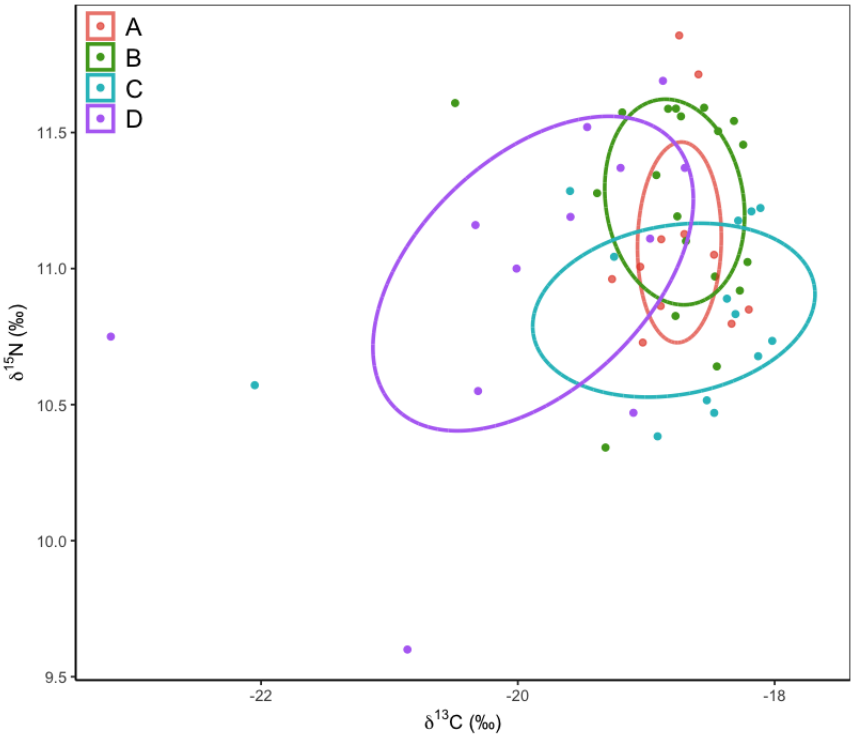

Figure 4.8: Plot of $\delta^{15}N$ against $\delta^{13}C$ and standard ellipse of the sampled nephrops for the four sampling locations.

Samples from location D had the largest isotopic niche (SEA_C and SEA_B, table 4.7), followed by C then B, while those from location A had the smallest isotopic niche. Samples from location D had lower $\delta^{13}C$ than A, B and C, while C was more spread along the axis of $\delta^{13}C$ (figure 4.9). The samples from locations A and B had higher $\delta^{15}N$ than those from C. Samples from location C and D had similar values of E (table 4.7), respectively 0.96 and 0.92, followed by B (0.74), and then A (0.48). The angle θ was found to be highest for A (78.6°), meaning it was more vertically spread (θ > 45°). The samples from location B showed a negative angle (-9.7°), and more spread along the x-axis (θ < 45°). C and D were also spread along the x-axis, at respectively 3.3° and 14.5°. There were certain levels of overlap in standard ellipses of samples between sampling locations. A and B highly overlapped with each other (0.352‰^{2}), and they both overlapped greatly with D (0.237‰^{2} and 0.395‰^{2}). C overlapped partly with A (0.261‰^{2}), B (0.250‰^{2}), and D (0.483‰^{2}). The sampling locations with the most overlap was C and D (table 4.8), but all sampling locations are somewhat overlapped with each other.

Table 4.7: Isotopic niche area (‰^{2}) estimates for each sampling location given by sample size-corrected standard ellipse area (SEAc) and the mode of Bayesian standard ellipse area (SEAb), and credible intervals (CI). As well as eccentricity E, and the angle between the semi-major axis of the SEAc and the x-axis (θ, °) for the different sampling locations of nephrops.

	SEA_C (‰^{2})	**E**	**θ (°)**	**SEA_B (‰^{2})**	**95% CI**
A	0.41	0.48	78.6	0.38	0.18 – 0.66
B	0.64	0.74	-9.7	0.64	0.38 – 0.98
C	1.16	0.96	3.3	1.08	0.57 – 1.84
D	2.15	0.92	14.5	2.05	1.02 – 3.48

Table 4.8: Overlap (‰^{2}) between standard ellipses between sampling locations.

Sampling location	**A**	**B**	**C**
B	0.352		
C	0.261	0.250	
D	0.237	0.395	0.483

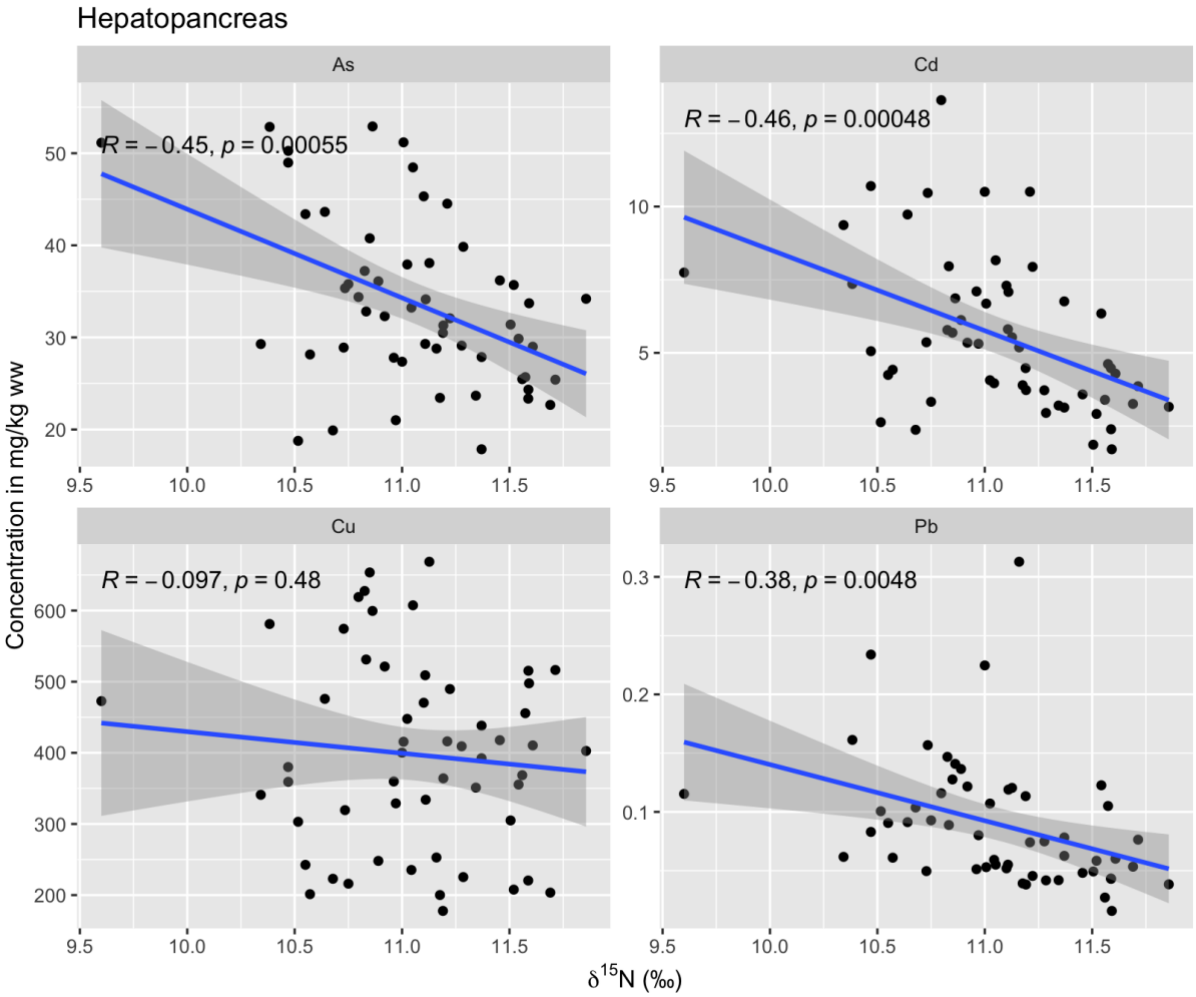

Figure 4.9: Relationship between concentration of trace elements As, Cd, Cu and Pb (mg/kg) and $\delta^{15}N$ in the hepatopancreas samples.

In the hepatopancreas there were four elements As, Cd, Cu and Pb found to significantly decrease as the $\delta^{15}N$ increase (figure 4.9). As (R=-0.45, p=0.00055), Cd (R=-0.46, p=0.0005) and Pb (R=-0.38, p=0.0048) were the only elements where correlation between $\delta^{15}N$ and the elements concentrations were significant. In the samples of muscle tissue Cu and Zn was found to be significantly increasing as the $\delta^{15}N$ increase (figure 4.10). The correlation appeared to be significant in both Cu (R=0.34, p=0.011) and Zn (R=0.44, p=0.00087).

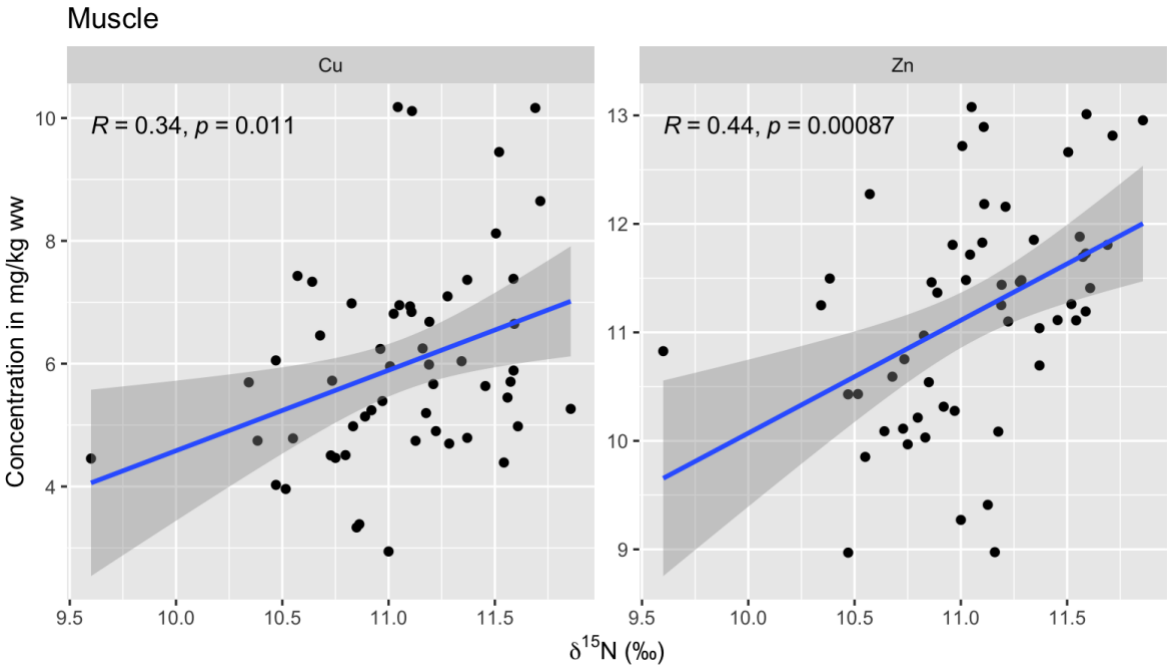

Figure 4.10: Relationship between concentration of trace elements Cu and Zn (mg/kg) and $\delta^{15}N$ in the muscle tissue samples.

4.5 Certified reference material

Quantified element concentrations for certified reference materials, oyster tissue (OT) and lobster hepatopancreas (Tort-3) in the ICP-MS are given in in appendix VII. For the controls of Tort-3, there were three values below 2SD in Mn. For the controls of OT there was one value above 2SD in Cr.

5 Discussion

5.1 Trace element concentrations in the different organs

One objective of this study was to examine the difference in concentration between the two organs that were sampled: hepatopancreas and tail muscle. This study found that all the investigated elements had significantly higher concentrations in the samples of hepatopancreas than the samples of tail muscle (table 4.2). The mean concentration of Cd was found to be significantly higher in the hepatopancreas (6.15 ± 2.9 mg/kg ww) than in the muscle tissue (0.0037 ± 0.001 mg/kg ww). These results are in line with findings of previous studies of Cd concentration in other crustaceans (Davies et al., 1981; Jennings & Rainbow, 1979; Pedersen et al., 2014; Rouleau et al., 2001; Signa et al., 2017; Wiech et al., 2018). Cd was found to have the highest percentage increase from concentration of muscle to that of hepatopancreas (166 116%). One likely explanation for this elevation in the hepatopancreas is the high abundance of the protein metallothionein in the hepatopancreas, which has the role of protecting against the toxic metals Cd, Hg and Pb (Calvo et al., 2017). The ions form complex bindings to the sulfhydryl group of metallothionein to later be excreted (Baird & Cann, 2012), but when the amount of metal exceeds the binding capacity it might accumulate in tissues like hepatopancreas as preventing the organism from metal toxicity. The large percentage difference between the hepatopancreas and the muscle tissue might suggest that nephrops have efficient detoxification processes to prevent a non-essential element like Cd from accumulating in other organs. The other non-essential elements Hg, Pb and As were also found to have increased concentration in the hepatopancreas, though not at the same percentage difference. This might suggest a more efficient uptake of Cd in nephrops than the other non-essential elements, perhaps due to Cd binding more efficiently to metallothionein.

The hepatopancreas samples did also have higher concentrations of all essential elements compared to muscle tissue samples. Cu and Zn have previously been linked to the metallothionein and its function as an regulator of these elements (Calvo et al., 2017). Cu (432.6 ± 177.2 mg/kg) and Zn (85.9 ± 30.7 mg/kg) has the highest concentration in the hepatopancreas out of the essential elements, which might be explained by this regulatory and storing function of the metallothionein in the hepatopancreas. These high concentrations of Cu and Zn are amongst the variables influencing the PCA the most (figure 4.2) and might be explained by higher levels of Cu and Zn compared to the other essential elements in the environment of the sampled area. It was indicated in the loading plot that Zn and Cu in the hepatopancreas are

correlated to each other, as well as them being correlated to Cd. This might be due to these being the trace elements with highest affinity to metallothionein, thus being somewhat similarly accumulated. Further investigations of the distribution of trace elements in organs of nephrops should include analysis of amount of metallothionein and concentration of trace elements in other organs as well to fully understand the distribution pattern.

5.2 Effect of sex

One other objective was to study if there was a possible difference in trace element concentration in the females and males. In the analysis of hepatopancreas samples Cd, Hg, and Cu were found to have significantly higher concentration in the females than in the males, while Fe was shown to have significantly higher concentration in males. The most significant differences were found for Cd and Cu (table 4.3). The higher concentration of Cu in females could perhaps be linked to its function as a respiratory pigment transporting O_2 (Poléo, 1997). Another possible explanation could be connected to the reproductive processes of a female nephrops. However, due to lack of data and time, a comprehensive investigation into the reproductive processes of female nephrops was not feasible. One explanation for the high concentration of both Cd, Hg and Cu could be that the females are older than the males, giving them longer time to accumulate trace elements. This is because growth rate of the nephrops is more reduced in females than males after maturity (Bell et al., 2013), females could be much older than males of same size.

The results of trace element analysis in the tail muscle showed a significantly pronounced increase in Hg concentrations in the females compared to males (figure 4.3). This further corroborates the explanation of the large females being older, having more time to accumulate Hg. Cd does show the same trend (figure 4.3), but not to the same extent as Hg. In contradiction to this finding, Canli and Furness (1993) found that the males had higher Hg concentrations than the females. In their study the males were smaller and more active than the females. The present study found smaller females than males. Therefore, the different results between the present study and Canli and Furness (1993) might suggest that size and age of the nephrops may be contributing more to the accumulation of concentration rather than the sex. The finding of the present study is corroborated by Wiech et al. (2021), who also revealed higher values of Hg in the females. For Zn it is previously suggested that maturing ovarian tissue could be a temporary reservoir in reproductively active females (Marsden & Rainbow, 2004). This could

be the reason for the concentration of Zn in the muscle tissue being significantly lower in the females than the males. Further research on the reproductivity processes of female nephrops is necessary to provide a more thorough understanding of how it may affect the trace elements concentration in the females. Concentration of Zn in hepatopancreas of females were also lower than in males, but not statistically significant. Other elements may also follow a similar pattern of being stored in different organs; however, this study lacks the necessary data to make such conclusions.

5.3 Effect of aquaculture

5.3.1 The sampling location and their trophic relationships

Another objective of this study was to investigate the effect of aquaculture the element composition of nephrops. Grefsrud et al. (2024) established that particles from fish farms normally precipitate within a radius of one kilometer from the facility, and most of it within 500 metres. This was also corroborated by Woodcock et al. (2018) in their study, finding evidence of feeding on organic waste from fish farm up to 1 kilometre from the fish farm. In the present study the sampling locations A, B and C were in increasing distance to one fish farm, Furneset (figure 3.2B). From the centre of the fish farm there was about 876 metres to group A, and 1.9 km to group B. The measurements were calculated from the centre of the fish farms, meaning that sampling location A was about 650 metres from the outer cage of the farm and sampling location B was about 1.7 km from the outer cage of the fish farm. This means that sampling location A falls within the radius of one kilometre where organic effluents likely would precipitate. Samling location B falls just outside the radius of one kilometre but might also experience some precipitation since the radius of one kilometre was a suggested estimate, and local variations may occur. The fish farm Fureneset was active until 6.5 weeks prior to sampling and is assumed to have had some lingering effect on the surrounding fauna from the time it was active. Sampling location C did not have any active or inactive fish farms within a 4-6 km radius and is therefore a control group for this study. Sampling location D was collected in between two farms, one active at sampling and one not active since July 2022. It is relatively close (1.2 km) from one of the fish farms (Juvika, figure 3.2A), but since this fish farm has been without production since more than one year prior to sampling and the main current was in the opposite direction, the effects of from this fish farm are uncertain but were assumed to be moderated. The selected locations for collecting nephrops were deemed suitable for assessing the effect of

fish farm activities on nephrops. Especially if focusing mainly on sampling locations A, B and C, since these are in increasing distance to one fish farm active until recently.

Samples from location A had the lowest standard ellipse area (Table 4.7) compared with other locations suggesting its relatively narrow trophic niche. The standard ellipse area of samples from location B was also low. This similarity is likely caused by the two locations being somewhat close to each other (1 km, figure 3.2A). Compared to sampling locations C and D, A and B are more sheltered from the ocean. This could indicate that samples from sampling location A and B might feed exclusively on the sediments originated from the fish farm waste. However, there are small differences in the parameters of standard ellipse between samples from location A and B. One explanation may be the nephrops in sampling location B being larger than those from A, meaning they might need to scavenge more to maintain their energy levels due to their increased body size.

Samples from locations C and D are less sheltered and closer to the open ocean, and may therefore have been influenced by both the fish farm and natural producers resulting in greater standard ellipse areas than seen in A and B. The standard ellipses of both C and D are flat, suggesting samples from these locations were feeding on a wider range of food items. There are some differences in both $\delta^{13}C$ and $\delta^{15}N$ between C and D. The higher $\delta^{15}N$ in samples from D are potentially a result of the influence of fish farm waste, which is also seen in the $\delta^{15}N$ in A and B. The higher $\delta^{15}N$ suggest that fish farm waste from locations in the sampling area were likely more enriched in $\delta^{15}N$ compared with the natural background. The lower $\delta^{13}C$ in samples from D might be explained by lower $\delta^{13}C$ in oceanic phytoplankton than inner fjord primary producers. This explanation is supported by earlier research indicating that sedimentary organic matter in the inner fjord was more $\delta^{13}C$-enriched compared to the outer fjord, as demonstrated by Kumar et al. (2016). The overlap between the relevant sampling locations A, B and C (table 4.8) showed that A and B were overlapping the most with each other, and none of them overlapped with sampling location C in the same degree. This suggests that A and B are more similar in diet than C. The observed overlap among the dietary niches of sampling locations A, B and C does also suggests potential similarities in access to resources despite indications of a narrower niche closer to the fish farm. This could be due to the nephrops omnivorous feeding pattern, so some consumption resources are still in common between the sampling locations.

There are indications of higher mean of $\delta^{13}C$ closer to the fish farm (table 4.6) when focusing only on sampling locations A, B and C. The mean of $\delta^{15}N$ in samples from location B was significantly higher than in C (control group). The study by Woodcock et al. (2018) found the opposite than the present study, that urchins and brown crab feeding on organic waste from fish farm was found to have lower values of $\delta^{15}N$ at the farm site than at sites further away. This difference could be due to use of different fish feed, or maybe different migration pattern between the brown crab and nephrops. On the other hand, Yokoyama et al. (2006) observed reduced values of $\delta^{13}C$ and elevated values of $\delta^{15}N$ in seawater and sediment near fish farms in Japan, while Cutajar et al. (2022) reported the same trend in sea cucumbers near fish farms in Malta. The trend of increasing $\delta^{13}C$ in the present study could be explained by the observed trend of a higher $\delta^{13}C$ when located closer to the shore (Cutajar et al., 2022), which might be the explanation for the trend in the present study as well.

One potential explanation for enrichment of $\delta^{15}N$ is fish feed containing fish-oil and possibly containing more $\delta^{15}N$ enrich plant-material (Grefsrud et al., 2024), either through consumption of organic waste directly or indirectly via the sediment or other species feeding on the organic waste. The low range of diversity and enrichment from organic waste in the feed available in the benthic fauna near the fish farm can result in a few opportunistic species being extremely abundant in this area, as demonstrated by Pearson and Rosenberg (1978). The reason for the lack of significant reduction of $\delta^{13}C$ in the present study (as observed in other studies) is not known, but may be explained by the fish feed used on the nearby fish farms having similar $\delta^{13}C$ to that in the natural background. The fish farm near A and B, and the fish farm near D are run be the same company, meaning it is likely that these two fish farms were using the same fish feed giving these sampling locations the same isotopic baseline. Unfortunately, due to time limitations and lack of data on fish feed used in the area, further investigation into this aspect was not achievable within the scope of this study. However, the assessment of sampling location and stable isotope analysis of samples from the sampling locations does establish the fact that the diet of nephrops close to a fish farm (A, B and D) are more dominated by organic waste effluent from the facilities, making the findings of this study relevant for assessing impact of aquaculture.

5.3.2 Trace element concentrations

The most distinct finding in the present study is the significant decrease of Cu in the hepatopancreas samples as the distance from a fish farm increases (table 4.4), where the highest levels are found closest to a fish farm (sampling location A) at 538.7 ± 106.4 mg/kg, followed by sampling location B at 414.9 ± 93.7 mg/kg, and lastly C at 341.0 ± 130.1 mg/kg. These results are likely connected to the use of Cu as an antifouling agent. This finding supports previous research who linked elevated levels of Cu to aquaculture activities (Falk, 2014). Given the elevated levels of Cu found in the hepatopancreas even as use of Cu_2O has not been reported since 2022 (Grefsrud et al., 2024), there is a necessity to further investigate the effect of these alternative antifouling agents, copper pyrithione (as well as zinc pyrithione) and tralopyril, recently being used (Hoddevik, 2024).

The mean concentration of Pb in hepatopancreas (table 4.4) was found to be significantly higher in D (0.15 ± 0.09 mg/kg) than in the other sampling locations and is likely the reason for D being clustered in a different placement than the other sampling locations in the PCA plot (violet, figure 4.7A). The reason for this high level of Pb is not likely to be a result of fish farm activities, since this location was assumed to have moderated effects of the previously active fish farm. The sampling location A (much closer to a fish farm than D) was also found to have significantly lower mean concentration (0.08 ± 0.04 mg/kg), corroborating that this elevation in location D was likely not due to impact of fish farm activities. One possible explanation for the high concentration in D could be linked to the size of the nephrops in this sampling location, since the mean of carapace length in the males are smallest (41.60 ± 5.94 mm) in this sampling location. The mean size of the males in this group was found to be significantly lower than the mean carapace length of group B. Sampling location B was found to have the lowest mean of Pb concentration at 0.07 ± 0.04 mg/kg, further suggesting that this elevation of Pb found in hepatopancreas might be due to nephrops size and likely not a result of the vicinity to a fish farm. Another possible explanation for the difference between B and the other locations might be B contains more resources with higher levels of Pb. Alternatively, it could be the high volume of boat traffic in vicinity of sampling location B possibly contributing to this high Pb-concentration.

The findings in the effect of sex in the present study, as previously discussed, did not show any difference in Pb concentration (table 4.3) between the sexes. Therefore it might be due the

nephrops in D being smaller and more active, as seen in the findings of previous studies of Hg (Canli & Furness, 1993). This might be due to increased physical activity increasing the rate of the detoxification process due to increased metabolic rate and energy expenditure. However, further research is needed to fully understand how physical activity might affect the accumulation of toxins in nephrops. It is also possible that this finding is due to other geochemical differences in the area, however this falls beyond the range of the present study. Zn showed a trend of decreasing levels as the distance to the fish farm increased. Increase of both Cu and Zn closer to fish farms was previously suggested as a trend by Falk (2014). As previously discussed, elevated Cu-levels closer to the fish farm are found in the present study, but only a trend of increase in Zn is found which is not statistically significant. The lack of significance in the results of Zn concentration might be due to the large variation between the individuals (figure 4.4), or the sample size being too small. The PCA plot of hepatopancreas (figure 4.7A) indicate that Cu and Zn are correlated, possibly supporting the suggestion that Zn in addition to Cu could be found in elevated levels near fish farms. If focusing solely on group A and B, the same trend of decreasing concentration with increasing distance might be suggested in Cd and As. However, the control group C exhibited similar concentrations to group A in both instances, indicating that fish farm activities are less likely to impact the levels of Cd and As. The opposite trend could also be suggested for Fe, where concentration increase as the distance to the fish farm increases.

In the muscle tissue, there were some trends regarding effect of fish farms. As, Pb, Se and Zn (figure 4.5) showed a decreasing tendency within the sampling locations A, B and C as the distance to a fish farm increased. Out of these trends, the only statistically significant finding was for As in nephrops from sampling location A (9.7 ± 2.2 mg/kg) and B (9.7 ± 2.3 mg/kg), both being significantly higher than in location C (7.4 ± 1.9 mg/kg) (table 4.5). One possible explanation could be that the elevated levels of As is an effect of fish farming activities. On the other hand, it could be due to the nephrops sampling locations A and B being somewhat larger (though not significantly) than the nephrops in sampling location C. The lack of increased concentration of Cu close to the fish farm in the muscle tissue, as was found in the hepatopancreas, suggests that the regulatory function of metallothionein in the hepatopancreas prevents the muscle tissue from containing elevated levels of Cu. Given the similar concentration of Cu in all sampling locations, the release of Cu from aquaculture seems to have little effect on the muscle tissue of nephrops. The same might be concluded for the concentration of Zn in muscle tissue when looking at sampling location A, B and C. While Zn did show

significantly lower concentration in sampling location D than A and B, this might be due to the males in D being smaller of size. The male nephrops in D are significantly smaller than those in B, and smaller (but not significantly) than those in A (table 4.1).

Previous studies have focused on the concentration of Cd, due to its toxicity. Averina et al. (2022) speculated that fish farm activities resulted in higher concentrations of Cd, Hg and Pb in crabs. This does not appear be the case with nephrops collected in the fauna surrounding of fish farms in Romsdalsfjorden, Norway. In the present study there were no significant differences in mean concentration of Cd in the groups collected in different vicinity to farms (group A, B and C), not in the hepatopancreas samples (table 4.4) or in the muscle tissue samples (table 4.5). In fact, the concentrations of both Cd and Hg in muscle tissue were significantly higher in the control group C than the sampling location B, which is 3 kilometres closer to a fish farm. Other unidentified local sources may be responsible for the higher concentrations of Cd in sampling location C compared to B. The same can be concluded for the other suggested elevations of elements Hg and Pb by Averina et al. (2022), where none of them show trends or significant elevations near the fish farm (table 4.4, table 4.5).

The sampling locations A and B were found to have higher trophic levels than the sampling location C. Cd was one of the elements found to significantly decrease (R^2 = -0.46) with increasing trophic level (increasing $\delta^{15}N$) (figure 4.9). This finding is consistent with the study on benthic and pelagic fish (Signa et al., 2017), where they indicate a more efficient transfer of cadmium in lower trophic levels. The same phenomena were seen for As and Pb as well. The narrower niche of nephrops from sampling location A, might suggest an abundance of similar type of resources readily available for the nephrops close to the farm. Consequently, the possibly reduced need for scavenging in this sampling location might lead to minimized uptake of Cd. Nevertheless, considering that nephrops tend to passively ingest Cd from the sediment while creating burrows (Cristo & Cartes, 1998) and the affinity of Cd to accumulate in the nephrops' available resources, they might still take up Cd closer to the fish farm if it did serve as a release source of Cd. Concentration of Cu and Zn in the muscle tissue was found to have a significant positively correlation to $\delta^{15}N$ (figure 4.10). Zn was the most correlating with $\delta^{15}N$, which also can be seen in the PCA plot (figure 4.7B). As previously discussed, the organic waste from fish farms could result in $\delta^{15}N$-enrichment. This appearing correlation (figure 4.10) could therefore corroborate a connection between the consuming of fish farm effluent and

accumulation of Cu and Zn, since the nephrops near the fish farm are likely eating organic waste (seen by the $\delta^{15}N$-enrichment) and containing higher levels of Cu and Zn (effluent from antifouling agents).

5.4 Food safety

Out of the non-essential elements As was found to have the highest mean concentration in the hepatopancreas, followed by Cd, Pb and Hg (table 4.2). However, As in marine species is present in the non-toxic form arsenobetaine, indicating no food safety issue. The mean concentration of Cd in hepatopancreas at 6.15 ± 2.9 mg/kg is much higher than the concentration of in the tail muscle (0.0037 ± 0.001 mg/kg). However, being the part of the nephrops usually consumed, muscle tissue is more relevant part regarding food safety. In addition, the maximum levels only apply to muscle tissue of abdomen or appendages. None of the muscle samples exceeded the maximum level of the undesirable elements Cd, Pb and Hg set by EU (0.5 mg/kg ww) (EC, 2023). For Cd, concentrations in the hepatopancreas exceeded in the maximum level for muscle, which could be of importance when hepatopancreas is consumed, which is not unlikely, as also crab brown mean is commonly consumed (Maulvault et al., 2013). Another perspective to discuss is regarding the preparation of nephrops for consumption. It has been shown earlier that Cd concentrations in claw muscle meat of crabs were elevated during cooking and thawing due to a transfer of Cd from hepatopancreas (Wiech et al., 2017). The present study found that raw samples of tail muscle were well below the maximum level when the carapace and tail were separated directly after death. Given the significantly higher concentration of Cd in hepatopancreas, investigating the possible transfer from hepatopancreas to muscle tissue in nephrops is an important issue for future research regarding food safety.

5.4.1 Assessment of maximum recommended amount of weekly intake of nephrops

Even though none of the samples of muscle tissue exceed the maximum levels of Cd, Hg or Pb applied to muscle tissue of abdomen, it is further interesting to investigate the number of nephrops tails a 70 kg person would have to eat to exceed the established PTWI for the non-essential elements Cd, Pb and Hg (methylmercury) (table 2.2). The effect of fish farming activities was found to have no impact on the concentration of Cd, Pb and Hg, therefore it is more relevant to discuss the concentration of different sexes, which was shown to be significantly different. The calculated amounts of are shown below (table 5.1).

Table 5.1: Calculated weight of nephrops tissue tolerable per week (TPW, g) in the undesirable elements Cd, Hg and Pb in females and males of the organs hepatopancreas and muscle tissue.

		Hepatopancreas		**Muscle tissue**	
		Females	**Males**	**Females**	**Males**
Weight of nephrops tissue TPW (g)	Cd	20.3	31.8	3977	5000
	MeHg	948	1282	1936	2600
	Pb	14 583	18 229	176 767	175 000

In the calculations of hepatopancreas it was shown that regarding Cd the TPW was 20.3 g of female nephrops hepatopancreas and 31.8 g of male. A person would have to eat over 3900 g of nephrops tail muscle (if eating females) to exceed the PTWI for Cd, assuming one tail weighs around 10 g that would mean well over 300 tails a week which is improbable. The amounts one would have to consume to exceed TPW for the elements (table 5.1), except for Cd in hepatopancreas, seem to be unlikely to be exceeded. The hepatopancreas can weigh up to 4 g, and regarding Cd it is equivalent to eating 5 hepatopancreases a week. Since consumption of the hepatopancreas might be occurring, as is true for the brown crab, this could possess a danger regarding food safety. One therefore might consider recommending consumption of males rather than females when considering the levels of Cd in hepatopancreas. There are uncertainties to consider as the samples analysed are raw muscle tissue, whereas the tail (and eventually hepatopancreas) of nephrops is typically consumed after being cooked. Further investigation including studying human consumption patterns of nephrops should be conducted to address this properly. This assessment is assuming nephrops' are the only sources of exposure to Cd, Hg and Pb in the human diet. However, this is implausible as there are other known alternative sources of exposure to the elements from other key food staples.

5.5 Quality assessment of sampling and analysis

The nephrops were caught using traps with the bait inside perforated plastic containers to prevent them from consuming other food than naturally found in the surrounding fauna. Some of the containers in group C opened while the nephrops were inside the trap. It is possible that this could have influenced the trace metal concentrations in the hepatopancreas. During dissection, the tail was first separated from the carapace before the freezing of the muscle sample, thus minimizing the effect of transfer of Cd from hepatopancreas to muscle previously found to occur in brown crabs (Wiech et al., 2017). As we were five people working on the same individuals while dissecting, there may be some uncertainty arising. At some point, there

were several lobsters in line to be dissected after being stunned. Some of them were potentially mixed up, but we assume that we were able to fix this using the physical parameters. In addition to the potential mix-up, there was a concern of the effect of the extra time between the stunning and the dissection. The degeneration of hepatopancreas could occur rapidly, and leak into the tail muscle, in line with the transfer found when thawing and cooking (Wiech et al., 2017). No evidence of this was found in the results, and it is assumed that this had little effect on the concentration. After the mix-up, the nephrops were stunned and immediately dissected before the next nephrops was stunned.

On of the biggest contributor to high uncertainty of measurement is lack of homogenization (IMR, 2023a). The samples of muscle tissue were of larger quantity than the samples of hepatopancreas and were thoroughly grounded after the freeze-drying. During the subsampling of muscle tissue samples for both stable isotope analysis and the ICP-MS, efforts were made to avoid the largest grains. Regarding the hepatopancreas samples, they were roughly homogenized during initial sampling. During subsampling after freeze-drying, most of the hepatopancreas samples required utilizing all available material. For the few samples of hepatopancreas above 0.25 g (which was necessary for the acid digestion), different sections of the container with the most thoroughly mixture of small grains were subsampled. When analysing trace elements by ICP-MS, control cards of CRM were evaluated before each analysis as a part of quality assurance process. For samples within the 2SD, the ICP-MS analysis was run. There were two elements outside 2SD (Mn in Tort-3 and Cr in OT, appendix VII). However, Mn was deemed acceptable as it fell within the measurement uncertainty range and as Mn was accepted for Mn in OT for the same sequence run. The Cr in OT was also accepted because it fell just outside the 2SD-range, and within the normal spread.

Some of the elements did not pass the Shapiro Wilks test for normality (appendix I). The dataset was then tested after log-transforming it, but the Q-Q plots (appendix I) did not show a large difference. The log-transforming of the dataset was therefore presumed to have little effect, and it was assumed that ANOVA would be robust enough to handle the data. The carapace length (mm) was the only variable used for the size of the nephrops, but a further age determinations could have been useful in further investigations. The PCA plots explained 40-50% of the variance at the highest. These plots have therefore been used only to examine the data, finding possible correlations of the variables. It is also as a way of presenting the clustering of sexes and groups and exploring explanations for the clustering.

6 Conclusion

The main aim of the study was to investigate the concentration of trace elements in different locations with different impact of fish farm activities. Additional aims were to study the distribution of trace metals in sampled organs (hepatopancreas and tail muscle), and differences in concentration between females and males.

The most significant finding of this study is the increased concentration of Cu in hepatopancreas closer to fish farms. There were additional trends of Zn also being higher, but this was not statistically significant. The stable isotope analysis, as well as the distance from aquaculture facilities are strong indications that the nephrops close to the fish farm indeed are consuming organic waste effluent directly or indirectly. Another finding was the significantly large difference between concentration in the hepatopancreas and the muscle tissue. In addition to this, some elements (Cd, Hg, Cu and Mn) were found to have significantly higher concentration in the females than the males. As well as some elements (As, Zn, Fe and Se) which had significantly higher concentration in the males than the females. Considering food safety none of the undesirable elements exceeded the maximum legal limit of muscle tissue set by the European commission (0.5 mg/kg ww). The assessment of PTWI could suggest being cautious about consuming the hepatopancreas of nephrops due to high amounts of Cd.

6.1 Further perspectives

- Given that nephrops are a seasonal delicacy, it could be interesting to analyse trace elements over several months/seasons of the year.
- Many of the trace elements have a significantly higher concentration in the hepatopancreas than the muscle. Further studies could include freezing and/or cooking with and without the tail severed from the carapace.
- To gain a better understanding of the tissue variation in concentration other organs such as claws, and gill should be investigated.
- It could be interesting to investigate the possible effects of metabolic rate, size and age on the detoxification process, given the difference in female and male concentration (possibly due to size and age) found in this study.
- Finally, further investigation of the function of metallothionein in nephrops would help to understand how nephrops process trace elements once ingested.

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8 Appendices

Significant values ($p < 0.05$) are throughout the appendices coloured by green.

Appendix I: Testing for normality

Table 8.1: Shapiro Wilks test for the nine most relevant elements.

	Hepatopancreas	**Muscle**
As	0.039	0.61
Cd	0.0009	4e-05
Hg	0.31	0.51
Pb	5e-05	0.00052
Cu	0.15	0.053
Zn	0.13	0.59
Fe	0.052	1e-05
Mn	0.15	3e-10
Se	0.026	0.78

Example of comparing of QQ plots (As in muscle) using the code below in R.

#8.1

```
fit.lme <- lme(As ~ group, random=~1|organ, data=muscle)
residuals <- resid(fit.lme)
qqnorm(residuals)
qqline(residuals)
```

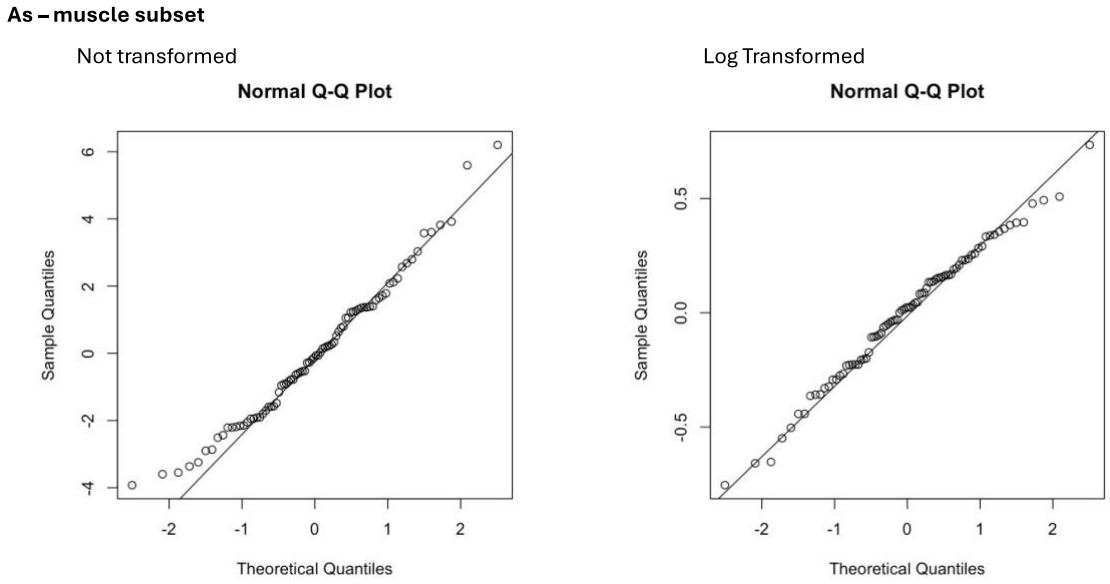

Figure 8.1: Example of QQ-plot

Appendix II: Linear mixed effects model

Table 8.2: Overview of all the models in this study.

M1	lme(carapace_length_mm ~ sex, random=~+1|group, data=muscle)
M2	lme(carapace_length_mm ~ group, random=~+1|sex, data=muscle)
M3	lme(carapace_length_mm ~ group, random=~+1|sex, data=muscle_m)
M4	lme(carapace_length_mm ~ group, random=~+1|sex, data=muscle_f)
GM1	lme(Cd ~ group, random=~+1|sex, data=muscle_m)
GM2	lme(Hg ~ group, random=~+1|sex, data=muscle_m)
GM3	lme(Pb ~ group, random=~+1|sex, data=muscle_m)
GM4	lme(As ~ group, random=~+1|sex, data=muscle_m)
GM5	lme(Cu ~ group, random=~+1|sex, data=muscle_m)
GM6	lme(Zn ~ group, random=~+1|sex, data=muscle_m)
GM7	lme(Fe ~ group, random=~+1|sex, data=muscle_m)
GM8	lme(Mn ~ group, random=~+1|sex, data=muscle_m)
GM9	lme(Se ~ group, random=~+1|sex, data=muscle_m)
GH1	lme(Cd ~ group, random=~+1|sex, data=hp_m)
GH2	lme(Hg ~ group, random=~+1|sex, data=hp_m)
GH3	lme(Pb ~ group, random=~+1|sex, data=hp_m)
GH4	lme(As ~ group, random=~+1|sex, data=hp_m)
GH5	lme(Cu ~ group, random=~+1|sex, data=hp_m)
GH6	lme(Zn ~ group, random=~+1|sex, data=hp_m)
GH7	lme(Fe ~ group, random=~+1|sex, data=hp_m)
GH8	lme(Mn ~ group, random=~+1|sex, data=hp_m)
GH9	lme(Se ~ group, random=~+1|sex, data=hp_m)
O1	lme(Cd ~ organ, random=~+1|group, data=clean_nephrops)
O2	lme(Hg ~ organ, random=~+1|group, data=clean_nephrops)
O3	lme(Pb ~ organ, random=~+1|group, data=clean_nephrops)
O4	lme(As ~ organ, random=~+1|group, data=clean_nephrops)
O5	lme(Cu ~ organ, random=~+1|group, data=clean_nephrops)
O6	lme(Zn ~ organ, random=~+1|group, data=clean_nephrops)
O7	lme(Fe ~ organ, random=~+1|group, data=clean_nephrops)
O8	lme(Mn ~ organ, random=~+1|group, data=clean_nephrops)
O9	lme(Se ~ organ, random=~+1|group, data=clean_nephrops)
SM1	sm1 <- lme(Cd ~ sex, random=~+1|group, data=muscle)
SM2	sm2 <- lme(Hg ~ sex, random=~+1|group, data=muscle)
SM3	sm3 <- lme(Pb ~ sex, random=~+1|group, data=muscle)
SM4	sm4 <- lme(As ~ sex, random=~+1|group, data=muscle)
SM5	sm5 <- lme(Cu ~ sex, random=~+1|group, data=muscle)
SM6	sm6 <- lme(Zn ~ sex, random=~+1|group, data=muscle)
SM7	sm7 <- lme(Fe ~ sex, random=~+1|group, data=muscle)
SM8	sm8 <- lme(Mn ~ sex, random=~+1|group, data=muscle)
SM9	sm9 <- lme(Se ~ sex, random=~+1|group, data=muscle)
SH1	sh1 <- lme(Cd ~ sex, random=~+1|group, data=hp)
SH2	sh2 <- lme(Hg ~ sex, random=~+1|group, data=hp)
SH3	sh3 <- lme(Pb ~ sex, random=~+1|group, data=hp)
SH4	sh4 <- lme(As ~ sex, random=~+1|group, data=hp)

SH5	sh5 <- lme(Cu ~ sex, random=~+1|group, data=hp)
SH6	sh6 <- lme(Zn ~ sex, random=~+1|group, data=hp)
SH7	sh7 <- lme(Fe ~ sex, random=~+1|group, data=hp)
SH8	sh8 <- lme(Mn ~ sex, random=~+1|group, data=hp)
SH9	sh9 <- lme(Se ~ sex, random=~+1|group, data=hp)
Iso1	iso1 <- lme(iso1 ~ group, random=~1|community, data=isotopes_clean)
Iso2	iso2 <- lme(iso2 ~ group, random=~1|community, data=isotopes_clean)
HI1	hi1 <- lme(Cd ~ iso2.y, random=~+1|group, data=hp_iso_clean)
HI2	hi2 <- lme(Hg ~ iso2.y, random=~+1|group, data=hp_iso_clean)
HI3	hi3 <- lme(Pb ~ iso2.y, random=~+1|group, data=hp_iso_clean)
HI4	hi4 <- lme(As ~ iso2.y, random=~+1|group, data=hp_iso_clean)
HI5	hi5 <- lme(Cu ~ iso2.y, random=~+1|group, data=hp_iso_clean)
HI6	hi6 <- lme(Zn ~ iso2.y, random=~+1|group, data=hp_iso_clean)
HI7	hi7 <- lme(Fe ~ iso2.y, random=~+1|group, data=hp_iso_clean)
HI8	hi8 <- lme(Mn ~ iso2.y, random=~+1|group, data=hp_iso_clean)
HI9	hi9 <- lme(Se ~ iso2.y, random=~+1|group, data=hp_iso_clean)
MI1	mi1 <- lme(Cd ~ iso2, random=~+1|group, data=muscle_clean)
MI2	mi2 <- lme(Hg ~ iso2, random=~+1|group, data=muscle_clean)
MI3	mi3 <- lme(Pb ~ iso2, random=~+1|group, data=muscle_clean)
MI4	mi4 <- lme(As ~ iso2, random=~+1|group, data=muscle_clean)
MI5	mi5 <- lme(Cu ~ iso2, random=~+1|group, data=muscle_clean)
MI6	mi6 <- lme(Zn ~ iso2, random=~+1|group, data=muscle_clean)
MI7	mi7 <- lme(Fe ~ iso2, random=~+1|group, data=muscle_clean)
MI8	mi8 <- lme(Mn ~ iso2, random=~+1|group, data=muscle_clean)
MI9	mi9 <- lme(Se ~ iso2, random=~+1|group, data=muscle_clean)

Appendix III: Detecting outliers and following AIC

#8.2 Example of R input for detecting the extreme outliers.

```
ub = (quantile(muscle$Cd, 0.75)) +
  3 * (quantile(muscle$Cd, 0.75)-quantile(muscle$Cd, 0.25))
lb = (quantile(muscle$Cd, 0.25))-
  3 * (quantile(muscle$Cd, 0.75)-quantile(muscle$Cd, 0.25))
ggplot(muscle, aes(x=Cd)) +
  geom_boxplot() +
  geom_vline(xintercept = ub) +
  geom_vline(xintercept = lb)
```

Table 8.3: AIC values for the different models, the subsets without outliers are the ones used in the list in table 8.2 (all without outliers, and some without females as well).

Model name	All data	Without outliers
M1	639	484
M2	634	478
M3	460	376
M4	157	157
GM1	-895	-558
GM2	-548	-376
GM3	-555	-323
GM4	467	262
GM5	403	242
GM6	300	183
GM7	267	55
GM8	-108	-163
GM9	-135	-74
GH1	510	281
GH2	-354	-221
GH3	-241	-140
GH4	720	420
GH5	1233	691
GH6	921	560
GH7	630	391
GH8	235	97
GH9	457	284
O1	938	645
O2	-887	-722
O3	-636	-500
O4	1386	999
O5	2519	1870

O6	1799	1349
O7	1203	914
O8	334	120
O9	822	638
SM1	-919	-765
SM2	-567	-507
SM3	-575	-439
SM4	471	350
SM5	404	310
SM6	301	230
SM7	261	67.0
SM8	-122	-238
SM9	-145	-118
SH1	511	351
SH2	-374	-295
SH3	-247	-190
SH4	725	520
SH5	1251	909
SH6	929	693
SH7	637	483
SH8	232	112
SH9	463	356

Appendix IV: ANOVA p-values

Table 8.4: p-values for all the models listed in table 8.2, where all p<0.05 are coloured green.

Model	p-value
M1	3e-04
M2	6e-04
M3	0.0034
M4	0.5815
O1	<.0001
O2	<.0001
O3	<.0001
O4	<.0001
O5	<.0001
O6	<.0001
O7	<.0001
O8	<.0001
O9	<.0001
Iso1	0.2687
Iso2	0.8171

Model	Muscle p-value	Model	Hepatopancreas p-value
GM1	0.0024	GH1	0.244
GM2	1e-04	GH2	0.7686
GM3	0.3832	GH3	7e-04
GM4	2e-04	GH4	0.3114
GM5	0.6267	GH5	<.0001
GM6	0.0017	GH6	0.6081
GM7	0.0248	GH7	0.1015
GM8	0.4965	GH8	0.2353
GM9	0.0768	GH9	0.0084
SM1	0.0424	SH1	8e-04
SM2	<.0001	SH2	0.0257
SM3	0.2568	SH3	0.8068
SM4	0.0074	SH4	0.0558
SM5	0.2701	SH5	<.0001
SM6	0.003	SH6	0.658
SM7	0.2991	SH7	0.0151
SM8	0.0439	SH1	0.1082
SM9	0.0459	SH2	0.4736
MI1	0.995	HI1	5e-04
MI2	0.9286	HI2	0.8354
MI3	0.0879	HI3	0.0086
MI4	0.95	HI4	6e-04
MI5	0.011	HI5	0.0398
MI6	0.0014	HI6	0.774
MI7	0.748	HI7	0.609
MI8	0.5731	HI8	0.078
MI9	0.6313	HI9	0.1281

Appendix V: Tukey contrasts

Table 8.5: Pr(>|z|) output from the Multiple Comparisons of Means Tukey Contrasts

		B – A	**C – A**	**D – A**	**C – B**	**D – B**	**D – C**
Carapace length	Male	0.55	0.95	0.16	0.16	<0.001	0.34
	All	0.487	0.721	0.078	0.026	<0.001	0.349
HP	Cd	0.25	0.98	0.75	0.40	0.80	0.92
	Hg	0.96	1.00	0.98	0.90	0.73	0.99
	Pb	0.96	0.99	0.0075	0.82	<0.001	0.0115
	As	0.24	0.84	0.72	0.70	0.83	1.00
	Cu	0.010	<0.001	<0.001	0.199	0.016	0.814
	Zn	0.83	0.96	0.56	0.99	0.94	0.82
	Fe	0.409	0.128	0.094	0.839	0.768	1.00
	Mn	0.53	0.97	1.00	0.19	0.59	0.89
	Se	0.0409	0.988	0.469	0.0071	0.6056	0.226
Muscle tissue	Cd	0.903	0.264	0.280	0.022	0.547	<0.001
	Hg	0.937	0.199	0.052	0.018	0.090	<0.001
	Pb						
	As	0.999	0.0215	0.0043	0.0060	<0.001	0.939
	Cu	0.59	0.96	0.97	0.86	0.83	1.00
	Zn	0.9146	0.5209	0.0015	0.8203	0.0014	0.0627
	Fe	0.955	0.952	0.132	0.638	0.011	0.280
	Mn	1.00	0.69	1.00	0.51	1.00	0.57
	Se	0.92	0.12	0.22	0.24	0.43	0.99
isotopes	C	0.999	0.999	0.0077	1.00	0.0026	0.0075
	N	0.777	0.449	0.909	0.036	0.306	0.846

Appendix VI: SEA_b density plot

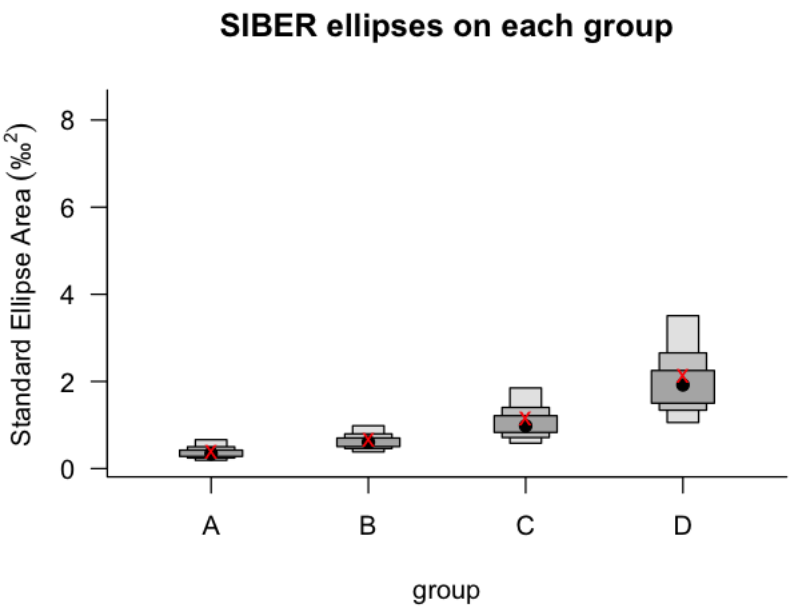

Figure 8.2: SEA_b density plot

Appendix VII: CRM

	Tort (lobster hepatopancreas) reference material Element concentration (mg/kg ww)											IMR (mg/kg ww)	
Date	**05.03.2024**		**06.03.2024**		**07.03.2024**		**10.04.2024**				**2RSD**		
Parallel	1	2	1	2	1	2	1	2	**Mean**	**2SD**	**(%)**	**Mean**	**2SD**
V	9.07	9.35	9.21	9.14	9.48	8.85	8.85	8.71	9.08	0.27	5.87	8.93	0.88
Cr	171	1.98	1.92	1.66	1.89	1.77	1.70	1.81	1.81	0.12	12.79	1.85	0.43
Mn	13.66	13.59	12.58 [a]	12.71	13.35	12.42 [a]	12.79	12.53 [a]	12.95	0.50	7.71	13.82	1.21
Fe	162.35	163.49	154.77	152.72	163.26	151.82	155.76	154.56	157.34	4.88	6.20	158.82	14.76
Co	1.05	1.05	1.01	1.01	1.07	1.00	1.00	1.00	1.02	0.03	5.82	1.02	0.09
Ni	4.68	4.89	4.64	4.55	4.91	4.64	4.66	4.72	4.71	0.13	5.31	4.67	0.62
Cu	444.15	446.41	435.59	438.51	476.32	448.07	438.88	438.75	445.84	13.06	5.86	449.80	39.95
Zn	130.36	131.56	123.57	122.70	132.02	123.41	127.02	125.93	127.07	3.81	5.99	126.33	13.35
As	64.47	66.33	62.75	62.05	66.39	62.71	64.38	64.20	64.16	1.62	5.05	64.87	5.70
Se	10.66	10.97	9.88	9.85	10.48	9.75	10.64	10.39	10.33	0.45	8.66	10.53	1.06
Mo	3.51	3.58	3.59	3.52	3.81	3.57	3.56	3.54	3.59	0.09	5.21	3.59	0.32
Ag	2.17	1.86	1.96	2.26	2.22	2.01	2.17	2.35	2.12	0.17	15.58	2.84	2.55
Cd	39.57	39.89	39.92	39.53	42.44	39.76	41.22	41.26	40.45	1.06	5.26	40.55	4.04
Hg	0.24	0.24	0.23	0.23	0.25	0.23	0.24	0.23	0.24	0.01	5.43	0.27	0.04
Pb	0.19	0.19	0.18	0.18	0.20	0.18	0.19	0.19	0.19	0.01	5.36	0.20	0.03

a) Values outside 2SD.

Oyster tissue reference material Element concentration (mg/kg ww)										IMR (mg/kg ww)	
Date	**06.03.2024**		**07.03.2024**		**16.05.2024**						
Parallel	1	2	1	2	1	2	**Mean**	**2SD**	**2RSD (%)**	**Mean**	**2SD**
V	0.56	0.56	0.55	0.54	0.54	0.56	0.55	0.01	3.28	0.54	0.05
Cr	0.38	0.34	0.33	0.33	0.55 [a]	0.38	0.39	0.08	43.97	0.38	0.15
Mn	16.87	16.84	16.96	16.77	16.91	17.02	16.90	0.09	1.06	17.21	1.40
Fe	186.23	187.63	181.22	172.40	189.55	191.20	184.71	6.93	7.50	187.46	15.81
Co	0.34	0.34	0.34	0.33	0.34	0.35	0.34	0.00	2.85	0.34	0.03
Ni	0.95	0.92	0.91	0.91	1.04	0.94	0.94	0.05	10.10	0.93	0.14
Cu	66.83	64.95	67.94	67.31	64.49	67.70	66.54	1.46	4.40	62.18	7.46
Zn	1409.20	1406.63	1441.88	1434.50	1392.34	1440.85	1420.90	20.88	2.94	1339.21	141.42
As	7.38	7.27	7.39	7.34	7.16	7.52	7.34	0.12	3.27	7.53	0.63
Se	1.94	1.91	2.01	1.96	2.12	2.13	2.01	0.09	9.33	2.08	0.22
Mo	0.19	0.18	0.18	0.18	0.18	0.19	0.18	0.00	4.78	0.18	0.02
Ag	0.56	0.59	0.55	0.56	0.54	0.57	0.56	0.02	5.41	0.59	0.06
Cd	2.49	2.41	2.50	2.48	2.41	2.52	2.47	0.05	3.90	2.52	0.21
Hg	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	11.46	0.03	0.01
Pb	0.28	0.27	0.28	0.28	0.28	0.28	0.28	0.00	2.56	0.30	0.03

a) Values outside 2SD.