Recreational fishing for Nephrops in Hordaland, Norway catches and potential mercury intake

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Thesis for the Partial Fulfillment of the Degree Master of Science in Marine biology



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June 2018

Acknowledgments

First and foremost, I would like to thank my supervisors Keno Ferter, Jeppe Kolding and Martin Wiech for essential feedback throughout this entire year. A special thanks to Keno for always being available to answer questions through Facebook, even during fieldwork. Next, I would like to thank Jon Helge Vølstad, for letting me take part in the Fisheries Dynamics research group and conduct this study. My gratitude also extends to Knut Helge Jensen and Edvin Fuglebakk for their help with the statistical parts of my thesis, and to Berit Solli for her help with the DMA-analyses. Thanks to Marian Kjellevold for help with the questionnaire, and to Martin Ohldieck for helping me with ArcGIS collector and making the polygon maps.

Thanks to Hanne Trømborg for helping me stay focused and positive this entire year. Thanks to Henry Seal for his friendship and writing advice. Also, thanks to Ina Nilsen and Hanne Trømborg for helping me with some of the sample preparation.

Finally, I would like to thank all the participants who took the time to complete the questionnaires, and a special thanks to those providing samples for Hg analyses. Without their co-operation, it would not have been possible to conduct this study.

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Abbreviations

CPUE	Catch per unit effort
CRM	Certified Reference Material
dw	Dry weight
EFSA	European Food Safety Authority
EU	European Union
GLM	Generalized linear model
Hg	Mercury
LM	Linear model
LME	Linear mixed effects model
LOD	Limit of detection
LOQ	Limit of quantification
MeHg	Methylmercury
NIFES	National Institute of Nutrition and Seafood Research
P95	95 th percentile
PCB	Polychlorinated bisphenyl
POPs	Persistent organic pollutants
Tort-3	Lobster Hepatopancreas
TWI	Tolerable weekly intake
WW	Wet weight

Abstract

Few studies provide information about recreational fishing in Norway. As recreational fishing often occurs in areas close to high-density surroundings, possibly affected by environmental pollutants, it is important to ensure the food safety of recreationally captured species. Recreational fishing for Nephrops has increased in popularity in recent years. However, limited information is available on the fishery in Hordaland, Norway. Standing gears were mapped in several fjords in Hordaland, and 2-25 buoys where confirmed to be from recreational Nephrops fishing in each of the surveyed fjords, which confirmed that recreational fishing for Nephrops is popular and widespread. The recreational fishers reported an average catch of 2.5 Nephrops per pot and haul, and 15 fishers reported eating Nephrops twice a month or more. The respondents also reported frequent consumption of fish, where 25 of 33 recreational fishers ate fish 2-3 times a week or more. Mercury (Hg) concentrations were also analyzed in tail muscle samples of Nephrops (N=235) using DMA-80. None of the measured Nephrops exceeded the maximum legal limit for commercially sold seafood (0.5 mg/kg wet weight) at any location. Twenty-three of 235 Nephrops exceeded 0.2 mg/kg wet weight, which is above the limit set for pregnant and lactating women. The study revealed significantly higher Hg concentrations in female Nephrops than in male Nephrops at the same size, and a difference in Hg concentration between the locations. The lowest Hg concentrations for both sexes were measured in Radfjorden, and the highest in Fanafjorden (outer station). The results of the consumption data and the measured Hg concentrations indicate no need for dietary guidelines for the consumers of recreationally captured Nephrops. However, the risk assessment revealed that some consumers might exceed TWI for MeHg with their total intake of seafood. Moreover, the consumers of recreationally captured Nephrops should be aware of the difference in Hg concentration between the sexes. As a precautionary approach, pregnant and lactating women should not consume female Nephrops above 50 mm carapace length as they may exceed 0.2 mg/kg (ww).

Summary in Norwegian

Få studier har fokusert på fritidsfiske i Norge. Ettersom fritidsfiske ofte foregår i områder påvirket av menneskelig aktivitet og dermed mulig forurensning, er det svært viktig å sørge for mattrygghet rundt populære fritidsfiskearter. Fritidsfiske på sjøkreps har økt i popularitet de siste årene, men lite informasjon er tilgjengelig om sjøkrepsfisket i Hordaland. Faststående redskap ble kartlagt i flere fjorder i Hordaland, og 2-25 blåser ble bekreftet å tilhøre fritidsfisket på sjøkreps i hver fjord, noe som bekreftet at fritidsfiske på sjøkreps er både populært og utbredt. Fritidsfiskerne på sjøkreps rapporterte gjennomsnittlig fangst på 2.5 sjøkreps per teine, og 15 fiskere rapporterte at de spiste sjøkreps to ganger i måneden eller oftere. Sjøkrepsfiskerne rapporterte også at de spiste fisk til middag ofte, der 25 av 33 rapporterte at de spiste fisk til middag 2-3 ganger i uken eller mer. Konsentrasjon av Kvikksølv (Hg) i prøver av halemuskel ble analysert ved hjelp av DMA-80 (N=235). Ingen av sjøkrepsene oversteg EUs grenseverdi for kvikksølv satt for kommersielt salg av sjømat (0.5 mg/kg våtvekt) uansett lokasjon. Tjuetre av 235 sjøkreps oversteg derimot grenseverdien satt for gravide og ammende (0.2 mg/kg våtvekt). Analysene avslørte signifikant høyere kvikksølvkonsentrasjon i hunnkreps enn hannkreps på samme størrelse, i tillegg til forskjeller mellom lokasjonene. De laveste kvikksølvkonsentrasjonene ble målt i kreps fra Radfjorden og de høyeste fra Fanafjorden (ytre stasjon). De målte kvikksølvkonsentrasjonene og de rapporterte spisevanene tilsier at ingen umiddelbar oppdatering av kostholdsrådene er nødvendig for å beskytte fritidsfiskerne på sjøkreps, men risikovurderingen avslørte at noen av fritidsfiskerne i studien kan overskride tolerabelt ukentlig inntak for metylkvikksølv (MeHg) på grunn av høyt totalinntak av sjømat. Alle som spiser sjøkreps bør være klar over forskjellen i kvikksølvkonsentrasjon mellom kjønnene, og for å være på den sikre siden bør ikke gravide og ammende spise hunnkreps over 50 mm ryggskjoldlengde, ettersom de kan overskride grensen satt for spesielle grupper.

1. Introduction

1.1 Marine recreational fishing in Norway

Marine recreational fishing is a popular activity worldwide, however, few studies provide harvest estimates from the marine recreational fishing sector. Several studies have reached the conclusion that marine recreational fishing can significantly impact fish stocks (Cooke & Cowx, 2004; Hyder et al., 2018; Ihde et al., 2011; Schroeder & Love, 2002). Hyder et al. (2018) estimated that 8.7 million Europeans participate in marine recreational fishing. Schroeder & Love. (2002) compared landings between commercial and recreational fishing on 17 nearshore species outside California and revealed that recreational fishing was the main source of fishing mortality in 16 of 17 species. The popularity and potential impacts of recreational fishing highlight the importance of more studies to get more accurate estimates.

Marine recreational fishing is also a popular activity in Norway, with one-third of the population fishing in the sea at least once a year (Vaage, 2015). Despite the popularity, there is no statistically accurate national estimate of total harvest by the whole marine recreational fishing sector. Only a few local studies on selected species have estimated recreational fishing effort relative to the commercial fishing effort (e.g., recreational lobster (*Homarus gammarus*) and Atlantic cod (*Gadus morhua*) fishing in the Skagerrak (Kleiven et al., 2016; Kleiven et al., 2012).

Recreational fishing is defined as "the capture or attempted capture of living aquatic resources mainly for leisure and/or personal consumption. This covers active fishing methods including line, spear, and hand–gathering and passive fishing methods including nets, traps, pots, and set–lines" (ICES, 2013). Although fishing motivations differ between recreational fishers, it can be expected that many recreational fishers consume their catches (Cooke & Cowx, 2004; Cooke et al., 2017). Fishing for personal consumption has been a tradition in Norway for centuries. In contrast to commercially sold fish or shellfish, self-caught seafood is often captured in local areas close to high-density surroundings (Aas, 2010), which can lead to increased intake of pollutants and toxic substances if the area is polluted (Cooke et al., 2017; VKM, 2006).

Even though there are many nutritional benefits with consumption of fish, contamination in fish is also a well-known issue both in Norway and worldwide. Seafood can be contaminated with different chemical compounds that can lead to health risks related to the consumption of recreationally caught fish (Cooke et al., 2017). The compounds can enter aquatic food webs naturally or through anthropogenic activities. Despite health advisories existing in many areas to protect consumers, they might be ignored as they may limit angling opportunities, or the risk associated with fish consumption is underrated (Cooke et al., 2017; Dawson et al., 2008; Pflugh et al., 1999). Given that recreational fishing is highly popular along the coastline of Norway, and fishing for personal consumption is common, more attention should be directed towards gaining knowledge about intake of contaminants through self-caught fish in Norway.

1.2 Environmental contaminants in Norway

Environmental pollutants are chemicals that can adversely affect living organisms. In terms of food safety, pollutants that bioaccumulate are of particular concern, as this can lead to high concentrations in food items. Pollutants can also be acutely toxic in high doses, however, regarding environmental toxicology, long-term exposure at relatively low individual doses (through food, air, water) is of more concern as they may accumulate in the body of humans (Baird & Cann, 2012). Bioaccumulation happens when the rate of absorption of a chemical substance is higher than the rate of catabolism or excretion (Baird & Cann, 2012). Intake of polluted food items can lead to toxic effects in humans. The characteristics of pollutants which tend to bioaccumulate are that they are persistent, mobile, often soluble in fats and biologically active (Baird & Cann, 2012). However, some contaminants, like methylmercury, are not lipophilic and will therefore distribute evenly throughout the body of the higher organisms like fish (Mieiro et al., 2009).

Norwegian authorities have a goal of reducing the release of contaminants and emissions into the environment towards the lowest possible level by 2020 (Klima- og miljødepartementet, 2015). However, preexisting pollution in sediments is a well-known problem in fjords in Norway, because of local pollution from industry and ports that traditionally have been located deep into the fjord (Miljødirektoratet, 2017a). Water exchange differs between Norwegian fjords. In some fjords, water exchange is limited close to the sediment, because of a threshold limiting the horizontal water connection with the open-ocean (Kaartvedt & Svendsen, 1995). The type of pollution mostly depends on the previous or present industry in the respective area. Some of the most common pollutants are persistent organic pollutants (POPs) such as PCBs and dioxins, and heavy metals like lead (Pb), mercury (Hg) and cadmium (Cd) (Miljødirektoratet, 2016).

1.3 Mercury (Hg) as a problematic environmental pollutant

Hg in nature comes from both anthropogenic and natural sources, which are roughly equal in magnitude globally (Fitzgerald & Clarkson, 1991). Hg is one of the pollutants mentioned on the Norwegian authority's priority list, and the total yearly pollution to air, water, and soil has successfully been reduced with an order of magnitude over the past 30 years, from 6 tons in 1985 to 0.63 tons in 2015 (Miljødirektoratet, 2017b). However, Hg can also be transported over long distances, as it can circulate in the atmosphere for up to a year (Berg et al., 2003). In 2012, it was estimated that 0.2 tons of Hg were deposited in Norway through atmospheric transportation from abroad (Klima- og miljødepartementet, 2015). In 2013, close to 140 countries signed the Minamata Convention on Hg; a global plan to reduce use and emissions of Hg to mitigate its effect on the environment (Kessler, 2013). In addition to the reduction of emissions, approximately 2615 kilos of Hg have been covered under clean sediments and shielded through remediation measures in Norwegian fjords between 2002-2017 (The Norwegian Environment Agency, 2017).

All types of Hg in the environment can be transformed into organic Hg by methylation performed by microorganisms, and Hg is then retained in these organisms and passed on to their predators (Morel et al., 1998). Organic Hg is mainly present as MeHg, which is easily taken up by microorganisms and zooplankton. This is how Hg first enters the food chain and bioaccumulates in organisms (Clarkson, 2002). Fish can also absorb MeHg directly from the seawater, but the accumulation of MeHg in higher trophic levels is mainly from food intake (Morel et al., 1998). Many trace metals bioaccumulate efficiently at microbial levels, but do not increase in concentration higher up in the food chain (biomagnification) like for example MeHg. Moreover, the biomagnification of MeHg explains why the highest concentrations of MeHg are usually found in longer living predatory fish or marine mammals (Clarkson, 2002). As humans are at the top of the food chain, people with frequent consumption of seafood may be at risk for elevated exposure to MeHg. High concentrations of MeHg in fish can also be produced by local pollution (Clarkson & Magos, 2006), so a possible increased risk can therefore not be dismissed for consumers of locally caught seafood in specific areas.

After ingestion, approximately 95% of MeHg in food is absorbed in the gastrointestinal tract in humans (Clarkson, 2002). Between 1-10% is distributed to the blood where roughly 90% bind to red blood cells. The majority of MeHg (>90%) is evenly distributed throughout the body since MeHg is water soluble mainly in complexes attached to the sulfur atom of thiol ligands, and because MeHg-Cysteine complexes can transport MeHg

into the cells (Clarkson, 2002). MeHg in the body is slowly demethylated to mercuric Hg (Hg2+). However, the mechanism of the demethylation process in humans is unknown (National Research Council, 2000). The daily excretion is about 1% of the body concentration, mainly through the bile and feces as MeHg and mercuric Hg. Breast milk is also considered a route of excretion, as lactation increases the clearance from blood (Grandjean et al., 1994). The half-life of MeHg is approximately 50 days in the blood, and 70-80 days in the body (National Research Council, 2000). However, the rate can vary substantially between individuals, which means that some individuals might have a higher risk from the same exposure (Tollefson & Cordle, 1986). MeHg rapidly crosses blood-brain and placental barriers. The brain, including the central nervous system, is the critical target organ, but MeHg can also cause damage in kidneys, liver and reproductive organs. Evidence from studies on rats suggests that the rapid transport into the brain is a consequence of the formation of the mentioned MeHg-cysteine complexes (Kerper et al., 1992), where it then accumulates and is slowly converted to inorganic Hg. It is still debated whether central nervous system damage is due to the toxicity of MeHg or its biotransformation to inorganic Hg (National Research Council, 2000). A Finnish study linked dietary Hg intake in fish with an increased risk of cardiovascular disease (Salonen et al., 1995), and animal studies have also revealed negative effects of MeHg on the immune system (Ilbäck et al., 1996). Although the impact of MeHg on the human immune system has not been studied. Humans exposed to MeHg poisoning show symptoms such as numbness, lack of coordination and loss of vision, speech, and hearing. However, there might be an extended latency period (weeks-months) from ingestion before any symptoms appear (Clarkson, 2002). The toxicology of Hg remains complex due to the biotransformation of MeHg and elemental Hg (Hg(0)) to inorganic Hg in the brain (National Research Council, 2000).

1.4 Potential exposure of recreational fishers to mercury

In Norway, all marine recreational fishers (Norwegian citizens and legal residents) can use several fishing gears, such as hand-held tackle, pots, gillnets, and long-lines (FISKERIDIREKTORATET, 2017a). According to regulations, one fisher or boat can use a maximum of 20 pots to catch Nephrops. When fishing, it is impossible for the fisher to know the contamination levels of the seafood they catch, as these levels are not readily visible. High concentrations of Hg in fish captured in areas with industrial pollution have been reported in several studies, for example on demersal fish species in Sørfjorden (Ruus et al., 2010) or outside Bergen (Måge & Frantzen, 2008). For humans, the primary source of Hg is consumption of fish, marine mammals and crustaceans (Clarkson & Magos, 2006; National Research Council, 2000). Furthermore, Hg contamination is the primary factor in recommendations against eating fish (Bank et al., 2007; Ruus et al., 2005).

Hg in seafood is predominantly present in the organic form methylmercury (MeHg), which is the most toxic form for humans (Baird & Cann, 2012; Hammerschmidt & Fitzgerald, 2006). More than 90% of total Hg in fish muscle was estimated to be MeHg (Bloom, 1992; Davidson et al., 1998; Grieb et al., 1990; Lockhart et al., 1972). In contrast to many organic environmental pollutants, which are predominantly found in the fatty tissues of fish, MeHg binds to proteins in muscle tissue and is evenly distributed throughout the fish filet (EU, 2006; Harris et al., 2003).

Several studies have examined fish contamination and assessed the implications for human health ((Boischio & Henshel, 1995; Lincoln et al., 2011; Mieiro et al., 2009; Olmedo et al., 2013). Boischio et al. (1995) indicated that young children in a riverside population in the Amazon basin might be ingesting Hg from fish in doses that have been correlated with neurological damage. Both Mieiro et al. (2009) and Olmedo et al. (2013) concluded that a potential risk associated with Hg intake from ingestion of fish could not be dismissed, as it depends on individual consumption rates and type of fish species consumed.

In the last few years, several research projects have focused on environmental pollutants in seafood in Norway as well (Måge & Frantzen, 2008; Måge & Frantzen, 2009, 2016). Most of the commercially important wild-caught seafood is well monitored for the most common contaminants in Norway, and a database of contaminants in seafood is available online [sjomatdata.no]. The maximum legal limits of contamination in commercially sold fish and seafood used in Norway have been set by the European Commission. The maximum legal limit for Hg is set at 0.5 mg/kg wet weight¹ (EU, 2006). The maximum legal limits are implemented to protect consumers from heavy exposure when consuming commercially caught fish. It is illegal to sell fish that exceeds these limits (EU, 2006).

In the context of recreational fishing, the concept of Tolerable Weekly Intake (TWI) is more relevant (VKM, 2006). TWI is defined as "an estimate of the average quantity of a

¹ Except for several longer living species with a limit of 1.0 mg/kg wet weight. Muscle meat of the following fish has an upper limit of 1.0 mg/kg wet weight: anglerfish (*Lophius* species), eel (*Anguilla* species), grenadier (*Coryphaenoides rupestris*), halibut (*Hippoglossus* hippoglossus), marlin (*Makaira* species), pike (*Esox lucius*), shark (all species), sturgeon (*Acipenser species*), swordfish (*Xiphias gladius*), tuna (*Thunnus* species, *Euthynnus* species, *Katsuwonus pelamis*) and several others (EU, 2006).

chemical contaminant that can be ingested weekly over a lifetime without posing a significant risk to health" (EFSA, 2012). This means that it is possible to evaluate individual risk if consumption data and measurements of the respective pollutant are present. TWI values are set by the European Food Safety Authority (EFSA) to protect the most vulnerable groups of the population, such as women of reproductive age, and children. The TWI for inorganic Hg is 4 μ g/kg body weight per week, while the TWI for MeHg is set to 1.3 μ g/kg body weight per week (EFSA, 2012). As MeHg represents over 90% of the Hg in seafood (EU, 2006; Grieb et al., 1990), the TWI for MeHg is particularly important. A review by EFSA (2012) concluded that the average consumer in Europe (within country and across all age groups) is unlikely to exceed the TWI for MeHg, even though amount and type of fish consumed varied by country. However, consumers with frequent fish consumption (P95) were close to or exceeded the TWI for MeHg across all age groups (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012). The same conclusion was also reached in a risk assessment from Spain (Olmedo et al., 2013), which indicated that fish and shellfish was safe for the average consumer, however, a potential risk could not be dismissed for regular consumers of some fish species.

Hg concentrations in humans are mostly determined using hair or blood, or both in combination (Agusa et al., 2005; Airey, 1983; Renzoni et al., 1998). However, scalp hair is considered the best indicator of human exposure to MeHg (Malm et al., 1995). Moreover, the Hg concentration in hair is 300 times higher than blood which makes analysis easier to conduct (Phelps et al., 1980). Several studies have determined Hg concentrations especially in fishers (Al-Majed & Preston, 2000; Cheng et al., 2009; Gaggi et al., 1996; Kosatsky et al., 1999; Lebel et al., 1998; Lincoln et al., 2011). Gaggi et al. (1996) investigated total Hg in scalp hair from fishers and their families in Portugal. The study concluded that the fishermen and their families on average had higher total Hg concentrations compared to other populations also consuming high amounts of seafood (Gaggi et al., 1996). Kosatsky et al. (1999) investigated intake of several contaminants through fish consumption among sports fishers in Canada. The study revealed that frequent consumption of fish was correlated with significantly higher tissue contamination levels (Kosatsky et al., 1999). Lebel et al. (1998) also concluded that fishers had significantly higher hair Hg concentrations than non-fishermen in a study from the Amazonian basin.

Lincoln et al., (2011) surveyed and collected hair samples from recreational anglers in Louisiana, USA, and questioned species-specific consumption rates three months prior. The study revealed elevated hair Hg concentrations for the recreational fishers, and that approximately 74% of the estimated Hg intake came from recreationally caught fish. Consequently, there is a general need for more regional studies, to evaluate if newer and more regionally specific health advisories should be established. Recreational fishers in Norway may also represent a highly exposed subgroup, with consumption of locally caught seafood. Fishing at polluted localities, possibly in combination with more frequent fish intake, might lead to an increased risk for recreational fishers.



1.5 Contaminants and recreational fishing around Bergen

Figure 1.1 Areas with dietary advice for all consumers are represented by the dashed lines on the map in Byfjorden and outside Håkonsvern. In Byfjorden should consumers avoid eating bottom-feeding fish such as tusk and ling, or brown meat of crab. Outside Håkonsvern should no seafood be consumed. The red area represents where pregnant and lactating women in addition to other advice, should avoid consumption of cod. Retrieved from: http://www.miljostatus.no/bergen

Bergen, located on the west coast, is the second largest city in Norway (Thune & Thorsnæs, 2014). The marine recreational fishing effort is presumably high, as Bergen is a coastal city, and its surroundings offer numerous shorebased, near-shore and offshore fishing spots, including many fjords suitable for standing gears. However, the extent of marine recreational fishing (catch and effort) in and around Bergen is largely unknown. The Norwegian food safety authority established dietary advice for the coastal areas around Bergen (Frantzen & Måge, 2011; Måge & Frantzen, 2008). This advice was based on measurements of different contaminants in important seafood species by NIFES in 2007-2008. Hg concentrations above 0.2 mg/kg were measured in cod, and Hg concentrations

exceeded the maximum legal limits (0.5 mg/kg) in both, tusk (*Brosme brosme*) and ling (*Molva molva*) (Måge & Frantzen, 2008). According to the advice, people should not consume bottom feeding fish due to heavy metals, or brown meat of brown crab (*Cancer pagurus*) due to POPs, captured in certain areas around Bergen (Mattilsynet, 2017). The geographical areas were determined by the Norwegian Food Safety Authority and extend from the Askøy bridge in the south, and the line between Bakarvågneses (Askøy) and

Helleneset in the north, and additionally includes the area around Håkonsvern (areas colored red/black in Figure 1.1) Pregnant and lactating women are also discouraged from eating cod from an extended area (red colored area in Figure 1.1) due to results from a follow-up study in 2011 (Frantzen & Måge, 2011). In 2008-2009, another study was conducted addressing specifically the food safety of recreationally fished species around Bergen (Måge & Frantzen, 2009). The study analyzed PCB levels in fish liver, and Hg content in fish filet from several species assumed to be landed when fishing recreationally from the shore in Bergen. It was concluded that it was not necessary to extend the local advice for cod filet to other fish species. However, the advice against consuming fish liver was extended to all gadoid fish species. In 2009, NIFES re-examined the factual basis for the dietary advice from 2008 by measuring pollutants in fish liver and eel, to evaluate if the advised geographical area in the port of Bergen should be extended (Frantzen & Måge, 2011). While the advised area for pregnant and lactating women was slightly enlarged, the boundaries for the dietary advice regarding fish liver was kept, as it was not possible to conclude on other specific geographical boundaries. More data is necessary to conclude on the geographic area included in the dietary advice, especially since there are multiple known sources of contamination around Bergen and no continuous decrease in contaminants with distance from the city center (Frantzen & Måge, 2011; Måge & Frantzen, 2008). In 2011, a national advisory against the consumption of fish liver emerged (any species self-caught in the archipelago in Norway) due to high levels of dioxins and PCB, except cod (Gadus morhua) caught in the open ocean, outside the archipelago (Mattilsynet, 2011). All dietary advice regarding the port of Bergen is available to the public online [http://www.miljostatus.no/Bergen].

The sediments around Bergen are known to be contaminated from ships and industry (Kringstad, 2015). For example, the sediments in Puddefjorden inside the city, contain approximately 940 kg Hg, 64 000 kg lead and 30 kg PCB (Kvisvik, 2015). Sediment concealment started in 2017 to reduce the bioavailability. Another example of Hg pollution in Hordaland is outside the island of Fedje, which is the location of a submarine wreck from the second world war containing up to 65 tons of metallic Hg. The area has been closely monitored by analyzing Hg concentrations in fish and seafood every year since 2004 (Sylvia Frantzen, 2018; Uriansrud et al., 2005). According to the Coastal administration, the Hg concentrations in fish and brown crab around the wreck are low, possibly due to little organic material in the sediment, and consequently, low rates of methylation (Kystverket, 2015). In 2015, The Norwegian Food Safety Authority's previous warning against eating seafood from this area was lifted (Miljødirektoratet, 2017b). Based on the known contamination around

Bergen and considering that some species have been proven to contain environmental toxins above maximum legal limits (Måge & Frantzen, 2009, 2016), more studies are needed to ensure the food safety of several recreationally fished species.

1.6 Nephrops around Bergen, delicious or dangerous?

Nephrops is a popular seafood in Norway, with a commercial catch of 195 tons and a total value of 23 million NOK along the coast of Norway in 2015 (Bakketeig et al., 2017). In general, few studies provide information about contaminants in Nephrops in Hordaland and fjords in Norway. Lately, recreational fishing for Nephrops seems to have increased in popularity in Norway (Bakketeig et al., 2017). Nephrops are sedentary species and may therefore be targeted efficiently by fishers (Johnson et al., 2013). The popularity has been suggested to result from the lack of restrictions in this type of fishery, compared to the strict regulations in the European lobster fishery, and as a consequence of the declining catch rates in the recreational European lobster fishery (Bakketeig et al., 2015). The restrictions include a maximum number of pots (20 pots per boat or fisher) and a minimum landing size of 13 centimeters total length (Fiskeridirektoratet, 2017b).

In 2013-2014, data was collected on recreational Nephrops fishing in Skagerrak through interviews and catch diaries (Kleiven et al., 2015). Sixty percent of fishers (n=131) believed that there had been an increase in recreational Nephrops fishing within the last five years. Forty percent of the respondents also believed that their catch rates had been reduced in the previous five years.

Nephrops are benthic predators and scavengers that live in burrows in the sediment found between 20-800 meters depth (Bakketeig et al., 2017). They are highly light sensitive, and are thus night-active in shallow waters, and active by day in deep waters (Arechiga & Atkinson, 1975). Sediments can absorb metals and organic pollutants efficiently, and the concentration in sediments can therefore be several orders of magnitude higher than the surrounding seawater (Hart, 1982). As Nephrops are sediment-burrowing animals, they might be susceptible to exposure to pollutants from the sediment when burrowing, in addition to exposure from food and sediment intake (Eriksson et al., 2013). This may cause concern for food safety, as pollutants bioaccumulate in the body of Nephrops (Katoh et al., 2013).

Several factors may affect Hg concentrations in Nephrops. There is usually a correlation between size and Hg concentrations in marine species (Burger et al., 2007; Storelli

et al., 2007). Size is usually used as a surrogate for age in crustaceans, where larger individuals have been exposed longer and therefore have bioaccumulated higher Hg concentrations over time (Elahi et al., 2012). However, size and location do not always explain Hg concentrations. Elahi et al. (2012) reported that variation in Hg could be explained by several individual variations, in both size, age, and sex, including feeding habits. Barrento et al. (2009) studied the accumulation of metals in brown crab and reported higher Hg concentrations in females than males. A study on size-related Hg increase in several edible marine species in the Tyrrhenian sea reported a sharper increase of Hg for female Nephrops beyond three centimeters compared to males (Barghigiani et al., 2000). Other studies have found similar sex-related differences which have been explained by differences in growth rate between the sexes (Baldi, 1984; Canli & Furness, 1993; Minganti et al., 1990). The females' growth rate decreases after sexual maturity, and differences in Hg concentration between the sexes of similar size can therefore be explained by a difference in age. The difference in growth rate also affects the sex proportion in the different length classes, where females are most abundant at smaller sizes, compared to a higher proportion of males in the larger length classes (Bell et al., 2006; de Figueiredo & Thomas, 1967).

A recent study on Hg in crustacean species from the Italian fishery revealed Hg concentrations exceeding the maximum legal limit (0.5 mg/kg ww) in 10% of Nephrops in the Tyrrhenian sea, and all individuals captured in the Adriatic Sea (Di Lena et al., 2018). Di Lena et al. (2018) reported that the habitat of the species might influence the high Hg concentrations, as the upper layer of deep water sediments also is the site of Hg methylation. Twenty-three percent of Nephrops measured in the Northwestern Mediterranean slope also exceeded the maximum legal limit (Cresson et al., 2014). As contaminants bioaccumulate in crustaceans as well as other marine organisms, it is essential to ensure that Nephrops captured recreationally for consumption in Norway do not contain contaminants above the limits considered safe regarding human health. The previously mentioned contamination in sediments outside Bergen and studies on Hg contamination in the species (Cresson et al., 2014; Di Lena et al., 2018) justifies further research.

Food safety risk assessments are conducted to assess whether the exposure to environmental pollutants in food presents any health risk for the consumers. Consumption data is combined with measured concentrations of pollutant in the food items. According to the National Service Center for Environmental Publications (NSCEP) in the US, the objective of a risk assessment is "to estimate the probability of adverse health effects from exposure to a toxic agent" (Pastorok, 1987). The hazard around Hg in general has been widely known for decades, especially since the large-scale poisoning accidents in Minamata in the 1950s and Iraq in the early 1970s (Kojadinovic et al., 2006). MeHg is, as mentioned, even more toxic and represents most of all Hg in seafood (Llull et al., 2017). A study in Norway assessed total dietary Hg intake in selected Norwegian municipalities (Mangerud, 2005). Additionally, several studies have assessed the risk of MeHg exposure associated with seafood intake (Carrington & Bolger, 2002; Grandjean et al., 2003; Llull et al., 2017; Spada et al., 2012; Steuerwald et al., 2000; Ström et al., 2011). Grandjean et al. (2003) and Steuerwald et al. (2000) both assessed the exposure to MeHg and associated risks for children related to maternal seafood intake. Spada et al. (2012), measured MeHg in marine organisms in Italy, and evaluated the risk associated with consumption. A risk assessment on the population of the Balearic Islands revealed that both adults and children exceeded TWI for MeHg (Llull et al., 2017). Ström et al. (2011) conducted a risk assessment for MeHg on women in childbearing age in Sweden. In contrast, Carrington & Bolger (2002) conducted an exposure assessment for MeHg from seafood for the entire U.S population, including two subpopulations, one with children 2-5 years old and one of women in reproductive age. NIFES has conducted analyses on a few recreationally fished species in Hordaland, among these, 20 Nephrops captured in Hardangerfjorden (Måge et al., 2012). The study revealed Hg concentrations exceeding the maximum legal limit for commercially sold seafood (0.5 mg/kg ww) in several individuals. However, a risk evaluation combining analyses on contaminants with catch from recreational fishing and consumption by recreational fishers has not been done in Norway.

1.7 Objectives of this thesis

This master thesis combines catch data of Nephrops in Hordaland with eating habits from recreational fishers and Hg concentrations of recreationally caught Nephrops to evaluate the risk for recreational fishers in Hordaland, Norway. The study had four primary objectives; 1) Verify the popularity of recreational Nephrops fishing and map the activity and catches in some selected areas in Hordaland.

2) Analyze Hg concentrations in recreationally captured Nephrops.

3) Identify factors influencing Hg concentrations in Nephrops caught in Hordaland.

4) Assess if the recreational Nephrops fishers are at risk of exceeding TWI for MeHg (risk evaluation).

A survey was conducted to map standing gears (objective 1), and Nephrops were collected from several recreational fishers in Hordaland to analyze Hg concentrations in tail muscle samples (objective 2). Capture location and biological data on size and sex of the sampled Nephrops were examined as potential factors influencing Hg concentrations (objective 3). Interviews on seafood eating habits were conducted and combined with the results on Hg concentrations in Nephrops to assess the risk of exceeding the TWI for MeHg (objective 4).

2. Materials and Methods

2.1 Mapping and identification of standing gear

2.1.1 General information about the study

The study area consisted of fjords in both urban areas, industrial areas and less inhabited areas in the county of Hordaland, Norway. A sampling system made by Jon Helge Vølstad divided the fjord areas in Hordaland into smaller sampling units (polygons), each of them covering an area of approximately 4 km². A survey was conducted to map standing gears in three rounds, the first two rounds as part of a recreational fishing project at the Institute of Marine Research (IMR) (Hauge, 2017). The polygons in round one and two were randomly selected for mapping recreational fishing activity in Hordaland. Only polygons with buoys observed in deep waters (>50 m) were included in this thesis. In round three, conducted explicitly for this thesis, the polygons were selected based on expert knowledge on popular fishing spots for Nephrops (Ferter & Bjelland, 2017), and observations of buoys in deep water areas (see section 2.1.3 for details).

2.1.2 Survey

A survey was conducted to identify areas with buoys in deep waters, i.e., possible Nephrops fishing spots. The polygons were created by first selecting random points on the map, and then generating randomly shaped polygons of approximately 4 km² around them. The sizes of the polygons range between approximately 3-4 km², due to the randomly selected boundaries between them. Therefore, some smaller polygons might appear between larger polygons.



Figure 2.1: Map of sampled polygons where buoys were observed in deep waters outside Bergen, Hordaland, Norway. Polygon numbering is not visible for all polygons. From north to south; Radfjorden (1178 and 1242), outside Knarvik (1796), Hauglandsosen (1019 (two polygons) and 1421), Byfjorden (1361), Raunefjorden (1358 (two polygons)), Fanafjorden (1250 and 1267 (three polygons)), Bjørnafjorden (1142 (two polygons) and 1278), and Austevoll 1518 (three polygons). The green colored polygons show surveyed areas that were not included in the selected sample because of a limited number of recreational Nephrops fishers registered.

In total, eight fjords were surveyed between May and July 2017 (May 8th-9th, June 1st, and July 24th-25th). The surveyed polygons (Figure 2.1) were examined individually using the boat "KV TOR" of the Norwegian coast guard (Forsvaret, 2014) as a base. Fast boats of the type "*Sjøbjørn*" (Maritime Partner, 2015) or "*HPB*" were used for fast and thorough examination, including close-up registration of all observed buoys using the mapping program ArcGIS Collector on an iPad. All buoys within each polygon were registered with GPS coordinates and notes with information from the buoys. An effort was put into avoiding registering buoys that were not attached to fishing gear, especially in shallow waters. This was done by checking for eventual chains and potential attachments for a boat in connection to the buoys, or by pulling the rope to feel if it was movable, as fishing gear would be.

In Norway, all recreational fishing gears must be marked with the owner's name and address (FISKERIDIREKTORATET, 2017a), while commercial fishing gear has to be marked with a commercial fishing registry number (H-XXX-xx²). Observed buoys were categorized into four categories. All buoys with a visible "H" for the county Hordaland or a

² H-XXX-xx: H describing the county Hordaland, XXX for the number, xx describing municipality.

complete fishing registry number were classified as commercial fishing gear. If the buoy did not have a visible H, but contained any other information (i.e., partial name, telephone number and/or address), it was categorized as recreational fishing gear. One buoy included a number possibly from the recreational small boat registry (Småbåtregisteret) and was also categorized as recreational fishing gear. If the buoy did not contain any information or the information was completely unreadable, it was classified as unidentifiable. Some buoys were not possible to register as the boat could not get close enough due to shallow waters, and these buoys were put in a fourth category (not possible to register).

In May and June, randomly selected areas were screened for buoys to retrieve information about all standing gears from the recreational fishing sector, including recreational Nephrops fishing. Areas where buoys were observed in deep waters were added to the sample for this thesis. In July (24th-25th), areas recommended by experts (Ferter & Bjelland, 2017) were targeted to increase the number of possible participants in the study, without spending too much time and money. The Norwegian coast guard also provided some expert knowledge, in addition to the previous boat surveys from the recreational fishing project at IMR. The temporal differences are due to the recreational fishing projects goal of sampling in multiple seasons, including when the coast guard had time available.

In May, the inner part of Hauglandsosen (1019 Figure 2.1³), outer parts of Fana (1250 Figure 2.1), Byfjorden (1361 Figure 2.1), Bjørnafjorden (1142⁴ and 1278 Figure 2.1) and one polygon south of Knarvik (1796 Figure 2.1) were surveyed. In June, Raunefjorden (1358⁵ Figure 2.1) was examined. On the sampling days in July, Radfjorden (1178 and 1242 Figure 2.1), Hauglandsosen (1421 and 1019 Figure 2.1), Fanafjorden (1250, 1014 and 1267 Figure 2.1) and Austevoll (1058,1831 and 1518 Figure 2.1) were surveyed.

2.1.3 Telephone survey to retrieve information about the owners of the buoys

Between 6 and 23 days after retrieving information from the recreational fishing buoys observed in the field, the owners of each gear were attempted contacted via telephone. Personal information from the buoys, such as name and address, was used on the websites *"www.1881.no"* or *"www.gulesider.no"* to obtain the owner's phone numbers, if the phone number was not already obtained from the buoy. The primary goal of the phone call was to

³ 1019 is two polygons. A closer map is available in Appendix I, Figure 1.B.

⁴ 1142 is two polygons. A closer map is available in Appendix I, Figure 1.D.

⁵ 1358 is two polygons. A closer map is available in Appendix I, Figure 1.C.

get information about the gear (i.e., type of equipment, number of pots or fishing nets in meters, soak time, number of buoys), and catch data (Appendix II). After attempting to contact the owners of each recreational fishing gear, the buoys were divided into four new categories; recreational Nephrops fishing gear, other types of recreational fishing gear, no response recreational fishing gear, and buoys where it was not possible to retrieve the owner's contact information on the websites using information from the gear. The recreational Nephrops fishers in each fjord were counted. The goal was to get at least three fishers from each fjord to cooperate in this study.

In total, 38 recreational fishers reported fishing for Nephrops in the areas surveyed. In Bjørnafjorden and the polygon south of Knarvik, only one recreational Nephrops fisher was registered during the survey. Therefore, these areas were not included in the final sample, as the goal of three cooperative recreational Nephrops fishers was not reached. Additionally, the locations were not particularly relevant regarding environmental toxins. Byfjorden contained only one recreational fisher on Nephrops. However, due to the known contamination and the mentioned dietary advice in the area, it was deemed necessary to retrieve muscle samples from Nephrops for Hg analysis and information about eating habits from this fisher.



Figure 2.2: Map of sampled polygons included in the final sample outside Bergen, Hordaland, Norway. From north to south; Radfjorden (1178 and 1242), Hauglandsosen (1421 and 1019), Byfjorden (1361), Raunefjorden (1358), Fanafjorden (1250, 1014 and 1267), Austevoll (1058, 1831 and 1518).

The final sample (Figure 2.2), included 16 polygons from six different fjords. A closer look at the polygons is available in Appendix I.

2.1.4 Assessment of contamination in the study area

Radfjorden (polygons 1178 and 1242 Figure 2.2) is sparsely inhabited, and the only identified source of possible Hg contamination was salmon farming (3 aquacultures distributed throughout the fjord). A risk report for Norwegian fish farming estimated pollution from fish farms to 7.2 grams Hg per fish farm per year (Svåsand et al., 2017). Therefore, low contamination was assumed for Radfjorden. Austevoll (polygons 1058, 1831 and 1518 Figure

2.2) is an island municipality located southwest of Bergen with one salmon farm within the sampled polygons. Due to low population density, proximity to the open ocean, and one salmon farm within the sampled polygons, Austevoll was assumed to be non-contaminated.

Hauglandsosen (1421 and 1019 Figure 2.2) includes sea access to the industrial areas Hanøytangen and Horsøy. Hanøytangen includes the firm Nordscrap West AS, with a boundary limit of 0,001 mg/l Hg into water per 6 hours (Relling, 2009). The area has also been used for industrial activities before Nordscrap, and Kollevågen, a former waste disposal area is in close distance. Kollevågen was used for waste disposal from 1930-1975, but due to a threshold at the inlet, these water masses are not in complete circulation with the water in Hauglandsosen (Vassenden & Johannessen, 2009). As industrial areas are close by, including the former waste disposal area, several environmental studies have been conducted. The sediments around Horsøy on average had heavy metal concentrations equivalent to environmental class II (moderate contamination) (Johnsen et al., 2007). In Johansen et al. (2004), the sediments around Nordscrap West AS were classified into environmental class II. On the other hand, Hauglandsosen has good water exchange west towards Hjeltefjorden and is therefore assessed with some degree of contamination.

Fanafjorden (1250 and 1267 Figure 2.2) is one of the study areas known to be contaminated, due to a former waste landfill with runoff through Pålamyrsbekken into the fjord (Nilsen, 2017). Pålamyrsbekken is a freshwater stream with a 0,1 μ g/L Hg concentration, which is assessed as severe contamination in fresh water (Hansen & Danielsberg, 2009). However, from the data available, it is not possible to determine the degree of polluted waters transported into Fanafjorden, and to date no environmental effects have been documented in the fjord (Fedje et al., 2009). Fanafjorden was therefore assessed as possibly contaminated.

Byfjorden (1361 Figure 2.2), in the immediate vicinity to Bergen, is the most urban and densely populated area in the sampled areas. Seafood from the fjord area is well known to be contaminated with dioxins, PCB and Hg which led to the above mentioned dietary advice in the area (Mattilsynet, 2009). Raunefjorden (1801 and 1358 Figure 2.2) is not directly associated with pollution, but the area is in close contact with Håkonsvern and Fanafjorden. Hg concentrations in environmental class III or higher have been reported in 65% of sediment samples outside Håkonsvern (Konieczny, 1994). In addition, it has some run-off from Bergen Airport Flesland, but this is not especially related to Hg (Johnsen & Sundfjord, 1999). Raunefjorden was therefore assessed as possibly contaminated.

2.2 Follow-up interviews of recreational Nephrops fishers

A secondary telephone survey was conducted on the recreational Nephrops fishers. In this follow-up survey, they were asked to participate in the project by answering a questionnaire about their fishing on Nephrops and related eating habits. Some fishers (maximum three in each fjord) were also asked to provide at least 15 Nephrops for Hg analysis. The first fishers asked to provide samples were selected randomly based on achieved time of contact. However, when a fisher agreed to participate in the study, it was attempted to contact other fishers that were observed fishing in other parts of the fjord. This was done to avoid retrieving Nephrops samples from the same locations to get a better areal distribution, and to assess for a potential gradient from known contaminated areas.

The final sample of recreational Nephrops fishers included 36 people, and 35 of these were contacted, and 33 interviews were conducted successfully. The interviews were conducted on the phone between 10.08.17-11.10.17. One fisher did not want to participate in the project, and another fisher was impossible to get in touch with after six phone calls.

The questionnaire (Appendix III) used in the follow-up study contained 20 questions relating to gender, age, nationality, education, number of fishing trips per year and fishing motivation. Multiple answers per fisher were possible on the question regarding fishing motivation. Additionally, the questionnaire included questions about the fishing gear (the type of pot, number of pots and buoys, soak time), information about the latest catch, eating habits on Nephrops and habits regarding fish consumption for lunch and dinner in the last three months. The question on fish eating habits for lunch was included as frequent consumption has been reported both in Sweden (Björnberg et al., 2005; Björnberg et al., 2003) and Norway (Mangerud, 2005).

Questions about the fishing gear also included a question about the fishing location to assess whether the fishers mainly fish near where their buoys were observed. The reason for this is that some Norwegian fjords or harbors are more polluted than others and may, therefore, contain higher amounts of Hg. Questions regarding last catch also included questions on release and release motivations. The release motivations included alternatives such as "Minimum length size", "Too small", "Too many", "Too big" or "Females with roe". A differentiation was made between "Too small" and "Minimum landing size" to assess whether the release motivation was voluntary or a result of the management regulations in the fishery. Multiple answers were possible regarding release motivations. On the question regarding eating habits, the participants were asked to describe how often they had eaten fish or other seafood the preceding three months. The format of response included the options "Never", "Less than one time per month", "1-3 times per month", "1 time per week", "2-3 times per week" or "4 times a week or more". The questionnaire also included a question on the participant's perception on the degree of pollution in Nephrops in their fishing area, from a low degree of contamination (1) to high degree of contamination (9). The middle point (5) was described as some degree of pollution, but safe to eat 1-2 times per month for those not pregnant or lactating. It was also possible to answer, "I don't know" (0).

The participants were not asked to report the size of the portion of fish they usually ate for dinner or lunch, as this is known to be associated with a high degree of day-to-day variations (Haraldsdottir et al., 1994). Additionally, a comparison of standard portions to individually reported portions show marginal differences (Johansson & Solvoll, 1999). Considering these findings, it was decided not to include questions about portion sizes in the food questionnaire but to use standard portions when converting the eaten fish frequencies into grams consumed per week. Standard portions were based on the average portion size for lean fish in Matvaretabellen (Dalane et al., 2015).

The participants were asked to report the average number of Nephrops eaten per meal, which parts they consume and how often they eat Nephrops. The question regarding the frequency of eating Nephrops included the options: "once a week", "Several times a week", "Several times a week (during the summer)", "twice a month", "once a month" or "less than 12 times a year". When the respondent answered with ranges, like for example 4-6 Nephrops per meal, the mean value (5 Nephrops) was used for the analysis.

2.3 Mercury analysis

2.3.1 Direct mercury analyzer (DMA-80)

The direct mercury analyzer (DMA-80) is a stationary instrument for analyzing Hg concentration without the need for any sample preparation before analysis. Once the sample is weighed directly into a sampling boat and put in the autosampler (Figure 2.3), each boat is retrieved and moved individually into a furnace where the sample is dried (if necessary) and then burned. Different heating times for dry samples and wet samples can be chosen. In the furnace, the Hg is vaporized, and oxygen gas transports the vapor into a catalyst tube where the various states of Hg are reduced to elemental Hg. The elemental Hg is then trapped onto a gold amalgamator, which is heated and releases the Hg into two cuvettes with atomic

absorption spectrophotometers (Milestone, 2013). Light with a wavelength of 254 nm is sent through both cuvettes, and the amount of light absorbed is proportional to the amount of Hg in the cuvette. The cuvettes have different shapes to cover high and low concentrations. One long and thin cuvette for higher sensitivity in low concentrations, and a thicker one for higher concentrations (NIFES, 2015). Certified reference material (CRM) is analyzed with the samples to assess the quality and the accuracy of the method. In DMA-80, all values within 2SD for CRM are considered acceptable. The detection limit (LOD) for the DMA-80 is 0.02 ng, and the limit of quantification (LOQ) is 0.08 ng. For samples measured in the linear area (1.5-1000 ng), the measurement uncertainty is 20%, and therefore the accuracy between 80-120% (NIFES, 2015). For calibration of the instrument, different reference materials were used covering the whole measurement range (TORT-3, Bovine Liver 1577, Skimmed Milk Powder, Fish muscle 422, Dolt-4 and Tuna 464)⁶.



Figure 2.3 Components of the direct mercury analyzer (DMA-80) (NIFC, 2016).

2.3.2 Sample preparation

Nephrops samples were obtained from 11 different recreational fishers, and therefore, the Nephrops were obtained from several locations within some fjords. Nephrops from three different fishing locations in Austevoll and Hauglandsosen were obtained, and from two

Bovine Liver SRM1577 Sigma-Aldrich, St. Louis, USA.

⁶Tort-3 (Lobster hepatopancreas) National Research Council, Ottawa, Canada.

Skimmed Milk Powder (ERM-BD 150) National Institute of Standards and Technology, Gaithersburg, USA. Fish muscle (ERM-BB 422) National Institute of Standards and Technology, Gaithersburg, USA. Dolt-4 National Research Council, Ottawa, Canada.

Tuna fish ERMCE464 Sigma-Aldrich, St. Louis, USA.

locations in Fanafjorden (see results, Appendix VII). There was only one fishing location in the other fjords (Byfjorden Raunefjorden, Radfjorden). As there were no known differences regarding contamination sources, the three locations in Austevoll were pooled for the statistical analysis. In Fanafjorden, the two fishing locations were treated separately as inner and outer location based on distance to the known contamination source at Pålamyrsbekken. The three sampling stations in Hauglandsosen were treated as two locations, Hauglandsosen Ågotnes (n=30) and Hauglandsosen Hetlevik (n=15), to assess potential differences based on the distance to the former waste disposal area and the current industrial area.

In total, 235 Nephrops were caught with baited pots in six different fjords from eleven different fishers (fishing locations) and frozen directly after catch. For each Nephrops, carapace length was measured from the eye socket to the back of the carapace in millimeters, using a digital caliper. For weighing, claws and legs were removed to get comparable numbers, as many of the Nephrops had already lost limbs during storage and transport. The tail was removed in frozen condition and thawed separately before dissection, as it is known, that freezing and thawing can alter the tissue distribution of trace metals (Wiech et al., 2017). The tail muscle was sampled by cutting the tail open with a scissor. The tail muscle was weighed, after excluding eventual inside roe, gonads and the intestines. For some Nephrops, also claw muscle samples were taken. All muscle samples were homogenized (Polytron 2100, Kinematica AG, Switzerland). The equipment used was cleaned at least twice between each sample using clean water and paper.

Sex determination was conducted by inspecting the basal segments of the pereiopods. While females have oviducts on the basis of the third pereiopods, the male opening of the vasa deferentia is paired on the basis of the fifth pereiopods (Powell & Eriksson, 2013). A descriptive image is available in Appendix IV.

2.3.3 Mercury analysis using DMA-80

For the Hg determination using DMA-80 (Milestone, Sorisole, Italy), approximately 0.1 g (0.095 - 0.125 g) of thawed and homogenized wet sample of Nephrops tail muscle or claw muscle was weighed into nickel boats. The certified reference material Tort-3 (Lobster Hepatopancreas), 292 ± 22 ng/g dry weight (Mean \pm SD) (National Research Council, Ottawa, Canada) was used to assess the accuracy of the analysis on the given calibration. To control for eventual contamination of the instrument (*carry-over effect*), each sample series started with two blank samples, followed by two samples of CRM (Tort-3). If a set included

more than 20 samples for analysis, two samples of CRM were also inserted in the middle, in addition to two more samples at the end of the series. Between each sample series, the nickel boats were cleaned by heating them at 650°C using a muffle oven for 30 minutes. Sixteen pairs of tail and claw muscle samples were freeze-dried to calculate dry matter. Dry matter content was calculated as "dry matter weight" divided by "total weight before freeze-drying" to ensure that Hg concentrations in claw and tail muscle were comparable.

2.4 Statistical methods and calculations

2.4.1 Catch calculations

Mean value was used when typing data from the interviews for the analyses if a respondent answered in a range. Two recreational fishers could not remember last catch, and CPUE was therefore estimated using data from 31 of 33 fishers. CPUE was calculated using all catch, including released or discarded Nephrops. Soaking time was assumed not to affect catch rates, and CPUE was calculated per haul. Furthermore, ten fishers stated their harvest in kilograms and released Nephrops in number of individuals, while the twenty-one others stated entire catch in number of Nephrops. Conversion from kg to number of Nephrops caught was conducted before CPUE was calculated. When catch data was provided in kilograms, mean carapace length for all locations was used on a length-weight key (Appendix V) to predict mean weight. The key was developed utilizing 2016 catch data from the Norwegian Reference Fleet at the Institute of Marine Research (more information available at www.imr.no/temasider/referanseflaten/en). Mean weight was estimated to 112 grams.

2.4.2 Factors influencing mercury concentrations

Statistical analyses were conducted using R (version 3.4.3, R Development Core Team, 2017). The hypothesis was that location, size and sex possibly could influence Hg concentration in Nephrops. As the response variable (Hg concentration in μ g/kg) is continuous, a linear model assuming constant variance was used (normal distribution). Carapace length was used as a measure of size instead of weight, to get results comparable with other studies. Firstly, a linear mixed effects (LME) model with one continuous predictor and one categorical predictor was used to look at the effect of size and sex on Hg

concentrations (Appendix VI, 6.1). In this case, location was considered a random effect factor to account for dependency due to samples clustered within locations.

Secondly, location was included in the model to evaluate its influence on Hg concentrations. Eight different fishing locations were included in the model, one location in Raunefjorden, Radfjorden, Austevoll and Byfjorden, and two locations in Hauglandsosen (Ågotnes and Hetlevik), and in Fanafjorden (Inner and Outer station). A linear model with two categorical predictors (location and sex) and one continuous predictor (size) was tested.

The first linear model included all possible interactions (Appendix VI, 6.2). The nonsignificant interactions were after that removed using the ANOVA output. The linear model with the significant predictors was thereafter tested (Appendix VI, 6.3). The diagnostics plot (Appendix V, 6.4) showed that the line in the Scale-Location plot is not entirely horizontal, and the points are not entirely randomly spread around the line. This indicates that the assumption of equal variance might be broken. Although, no observations were outside Cook's distance in the Residual vs leverage in the diagnostics plot, and no observations are therefore severely influential on the model. A log transformation would have improved the Scale-Locations plot by making the line more horizontal (Appendix VI, 6.5). However, the same conclusions were reached when testing the log-transformed model (Appendix VI, 6.6), and therefore, the original data was used to avoid interpreting the results on a log scale.

A Tukey test was used to compare Hg concentrations in the two sexes from the different locations (Appendix VI, 6.7). The underlying assumption was that the catch was a representative sample of the population at each location, and therefore the model compared Hg concentrations based on the sizes that were available at the time of sample at each location for females and males separately.

2.4.3 Analysis of correlation between age, education and perception of pollution

To analyze for correlations between age or education, and the participants' perception on the contamination status of the Nephrops in their area, the values from the questionnaire had to be rescaled into binomial values to include an upper and lower boundary. Education was also put into new categories; Low education (Primary and High school), Vocational college (Intermediate) and High education (University) due to low n. A GLM tested the hypothesis with three categories, and with two categories. Vocational college was regarded as low education when using two categories in the test. Both models were specified with "family=quasibinomial" and an F test to test the two hypotheses (Appendix VI, 6.8).

2.4.4 Risk assessment

To calculate Hg intake from dinner, a portion size of 200 grams was used, according to Dalane et al. (2015). For fish consumed for lunch, 20 grams of fish spread per slice of bread was used as the standard. For this study, two slices of bread for lunch were assumed to be average, and therefore 40 grams of fish spread was used for calculating Hg intake from fish for lunch. Both high and low values of Hg concentration in fish were used for calculating Hg intake from fish for dinner. Minimum and maximum concentrations were calculated as the average value of concentrations reported in saithe, cod, haddock and pollack (Sjømatdata, 2017). Concentrations from these species were selected as they were reported to be the most frequently consumed fish species in a group of high consumers of seafood (Mangerud, 2005). The present study did not provide species-specific consumption rates other than Nephrops, and it was assumed that the Hg concentrations in the species mentioned above were representative for the species consumed by the respondents. Maximum values of the same species were used as Hg concentrations can be higher in fish caught inside the archipelago (Måge & Frantzen, 2009), where recreational fishers often fish. The estimated mean value was 86 µg/kg and estimated max value was 257.5 µg/kg⁷ for fish for dinner.

Calculating Hg in fish for lunch was done by using concentrations from [sjomatdata.no] from a selection of species associated with fish for lunch, assuming a similar consumption of the species by calculating an average concentration. For lunch, only the mean value was used because it was assumed that fish consumed for lunch is mostly store-bought and not self-caught. The lunch value was estimated to 44.3 μ g/kg⁸,

Total Hg intake was calculated using personal consumption rates reported by 33 recreational Nephrops fishers. When the respondents answered using their own range, like for example 1-2 times per week rather than the mentioned alternatives, they were put in the category representing the lowest reported eating frequency. Conversion of eating frequency to numerical value was done as reported by Markhus et al. (2013). When a respondent answered consuming fish for dinner or lunch "2-3 times per week", 2.5 times per week was used for the

⁷ Calculated using measured Hg concentrations in: Saithe (*Pollachius virens*) filet 2016 (mean 0.059 mg/kg, max 0.22 mg/kg), Atlantic Cod (*Gadus morhua*) filet 2016 (mean 0.069 mg/kg, max 0.28 mg/kg), Haddock (*Melanogrammus aeglefinus*) filet 2016 (mean 0.076 mg/kg, max 0.18 mg/kg), and Pollack (*Pollachius pollachius*) filet 2014 (mean 0.14 mg/kg, max 0.35 mg/kg). (Sjømatdata, 2017)

⁸ Calculated using measured Hg concentrations in: Shrimps wild 2016 (0.07 mg/kg), Sardines 2010 (0.02 mg/kg), smoked trout filet 2007 (0.053 mg/kg), mackerel wild 2016 (0.03 mg/kg), farmed salmon 2016 (0.017 mg/kg) (Sjømatdata, 2017) and canned Tuna (0.076 mg/kg) (Nilsen & Måge, 2016).

calculations. When a respondent answered between "1-3 times per month", 0.5 times per week was used for the calculations. When a respondent answered, "Less than one time per month", 0.15 times per week was used for the calculations. Consumption frequency for Nephrops was scaled up to a full year even if a fisher had been fishing for less than a year. Two fishers reported Nephrops eaten per meal in grams, which was converted to number of Nephrops using the predicted mean weight of 112 grams, estimated utilizing data from the Norwegian reference fleet (Appendix VI). The frequency measurements used for the calculations of intake from Nephrops are (weekly); Less than once a month (0.15), once a month (0.25), twice a month or twice weekly in the summer (0.5), once a week all year (1), and twice a week all year (2). Whether or not they consumed only tail meat from Nephrops or tail meat including claw meat was accounted for. Mean weight for a Nephrops tail muscle was calculated to 24.1 gram (n=235), and muscle meat in the claws was calculated to be approximately 36% of tail meat (n=43). Total Hg intake from seafood was calculated using average and high consumption rates from the respondents. Average intake rates were calculated using the mean intake from all consumers, while high consumption is defined as the mean intake of the five consumers with the highest consumption frequency.

The results of the risk assessment were compared to the Tolerable Weekly Intake (TWI) of $1.3 \mu g/kg$ body weight set for MeHg (EFSA, 2012), as 90-100% of Hg in fish muscle have been shown to be MeHg (Bloom, 1992; Grieb et al., 1990; Lockhart et al., 1972). In Nephrops, 87% of total Hg has been shown to be MeHg in an industrially polluted area and 100% of Hg shown to be MeHg in a control area (Buzina et al., 1989). Hg concentrations in Nephrops and seafood are therefore assumed to be MeHg in this thesis. Assuming a total body weight of 80 kg, the tolerable weekly intake represents 104 μ g MeHg

3. Results

3.1. Results of the survey mapping standing gears

3.1.1 Registered buoys in different fjords divided into categories

Fishing buoys were found between 5-300 meters spread all over the fjords, in all investigated areas (Appendix VII). In round one (May), five buoys were registered in Knarvik, seven in Byfjorden, 21 in Bjørnafjorden, 24 in the outer parts of Fanafjorden, and 29 in Hauglandsosen (Figure 3.1A). The percentage of recreational fishing buoys was lowest in Bjørnafjorden (19%) and highest in Fanafjorden (42%). The phone survey revealed that the areas in Knarvik and Bjørnafjorden did not include more than three recreational Nephrops fishers, and thus, were not included in the final sample (Figure 3.1B).

Only two buoys were registered from recreational fishing in Byfjorden (Appendix VII, Figure 7.1) In the inner parts of Hauglandsosen (Appendix VII, Figure 7.2), recreational Nephrops fishing represented 83% of the recreational fishery. In comparison, recreational Nephrops fishing represented 40% of all recreational fishing in Fanafjorden (Appendix VII, Figure 7.3). In total sixteen buoys were confirmed to be from recreational Nephrops fishing in survey round one, and nine different Nephrops fishers were registered within the final sample area.



Figure 3.1A Number of registered buoys in round one (May) divided into categories registered during the survey in May; recreational fishing (blue), commercial fishing (orange), unidentifiable (grey) and not possible to register (yellow).


Figure 3.1B Number of recreational fishing buoys in round one (May) divided into categories after attempted contact; recreational Nephrops fishing (orange), and other recreational fishing (grey).

In round two (June), 30 buoys were registered in the surveyed area in Raunefjorden, and 18 (60%) of these were recreational fishing gear (Figure 3.2). Buoys were registered between approximately 5-200 meters distributed throughout the fjord (Appendix VII, Figure 7.4). Half of the recreational fishing buoys (50%) were from recreational Nephrops fishing. These nine buoys were owned by four different recreational fishers.



In round three (July), 44 buoys were registered in Radfjorden (Appendix VII, Figure 7.5), 79 in Hauglandsosen (Appendix VII, Figure 7.6), 136 in the area in Austevoll (Appendix

VII, Figure 7.7), and 142 in the area in Fanafjorden (Appendix VII, Figure 7.8). The percentage of recreational fishing buoys was lowest in Austevoll (18%) and highest in Hauglandsosen and Radfjorden (48%) (Figure 3.3A).



Figure 3.3A

Number of registered buoys in round three (July) divided into categories registered during the survey in July; recreational fishing (blue), commercial fishing (orange), unidentifiable (grey) and not possible to register (yellow).

Of the twenty-one, recreational fishing buoys registered in Radfjorden, five buoys (24%) were from recreational Nephrops fishing owned by three different fishers (Figure 3.3B). It was not possible to retrieve contact information using information from eight buoys on the websites; however, these were owned by only two fishers. In Hauglandsosen, nine (24%) of the 38 recreational fishing buoys registered were from recreational Nephrops fishing and from five different fishers. It was not possible to retrieve contact information for two fishers (two buoys). Fanafjorden contained 52 recreational fishing buoys, and 25 of these (48%) were from Nephrops fishing. Ten different recreational Nephrops fishers were registered in Fanafjorden. It was not possible to retrieve contact information for two fishers (two buoys). Austevoll contained 24 recreational fishing buoys, and 16 of these (67%) were from Nephrops fishing. Eight different recreational Nephrops fishers were registered in Austevoll. It was not possible to retrieve contact information for two fishers (three buoys). In round three, other types of recreational fishing were highest in Hauglandsosen, representing 23 of 38 recreational fishing buoys (61%) from twelve different fishers. The no-response category was highest in Fanafjorden (12%), which also was the location with the most fishers (28 fishers in total).



Figure 3.3B Number of recreational fishing buoys in round three (July) divided into categories after attempted contact; not possible to retrieve contact info (blue), no response (orange), recreational Nephrops fishing (grey), and other recreational fishing (yellow).

3.1.2 Density of buoys in the selected sample

The highest fishing effort from standing gears was observed in Austevoll, with an estimated 15 buoys per km² (Table 3.1). The lowest fishing effort from standing gears was observed in Byfjorden with one buoy per km². Recreational fishing varied between 29% (Byfjorden) and 65% (Raunefjorden). Recreational Nephrops fishing seems to represent a substantial part of the recreational fishery in the selected areas, for example, 24% in Hauglandsosen and Radfjorden, 67% in Austevoll and 100% in Byfjorden. Maps with results from the survey rounds are available in Appendix VII.

In total, 100 recreational fishers were registered within the total surveyed area, and 95 were registered in the selected sample area. However, five fishers were registered twice, which made the total number of individual fishers 95 in the total survey area and 90 in the selected sample. Thirty-six (40%) of the 90 registered recreational fishers in the selected sample were recreational Nephrops fishers.

Location	Size of	Total	Estimated	%	% of	Estimated	
(Survey round)	the	buoys	density	recreational	recreational	density	
	total		(total	fishing of	fishers	(total	
	selected		number of	total	targeting	number of	
	survey		buoys per	registered	Nephrops (n)	Nephrops	
	area		km ²)	buoys (n)		buoys per	
	(km ²)					km ²)	
Fanafjorden (1)	3.44	24	7	42% (10)	40% (4)	1	
Hauglandsosen	8.77	29	3	41% (12)	83 % (10)	1	
(1)							
Byfjorden (1)	6.33	7	1	29% (2)	100% (2)	< 1	
Raunefjorden	9.50	30	3	60% (18)	50% (9)	1	
(2)							
Radfjorden (3)	8.40	44	5	48% (21)	24% (5)	< 1	
Hauglandsosen	13.52	79	6	48% (38)	24% (9)	< 1	
(3)							
Fanafjorden (3)	11.30	142	13	37% (52)	48% (25)	2	
Austevoll (3)	9.16	136	15	18% (24)	67% (16)	2	

Table 3.1. Estimated density of buoys in the selected sample areas in the three different survey rounds including the number of registered recreational fishing buoys and confirmed recreational Nephrops fishing buoys.

3.1.3 General information about the recreational Nephrops fishers

Except for one female, the interviewed fishers were all males. The mean age was 49 years, and the number of Nephrops fishing trips ranged from 2 to 100 per year (mean 39 fishing trips per year). Ten fishers had been fishing for Nephrops for only a year or less, while five fishers had fished Nephrops recreationally for over ten years (mean 3.83 years). Eighty-five percent of the fishers (28 of 33) reported fishing exclusively in the observed area, and four reported staying between 10 to 20 km away from where their equipment was observed. One fisher reported fishing more than 30 kilometers away from the point of observation, especially in the summer, when bringing his pots on vacation with his boat.

3.1.3.1 The recreational fishers' perception of contamination in Nephrops

Eight fishers perceived the Nephrops in their area as somewhat contaminated, but safe to eat 1-2 times a month for all consumers except pregnant and lactating women (Figure 3.4).

Moreover, none of the recreational fishers perceived the contamination above five on the scale from one (low) to nine (high), and nine recreational fishers believed that the Nephrops in their area was not contaminated at all. Education level in the group of recreational fishers ranged from vocational college as the most common (55%), to high school education (21%), primary school (9%), University 1-3 years (9%) and university more than four years (6%). No correlation was found between their age and beliefs regarding pollution (p>0.22), or between their education and beliefs regarding pollution, concerning two (High/Low) educational categories (p>0.45) or three (High/Intermediate/Low) educational categories (p>0.33).



Figure 3.4 The recreational Nephrops fishers' perception of contamination in Nephrops in their area. Scale from 1-5 visible.

3.1.3.2 Fishing motivations

The most common reported motivation for fishing Nephrops was either fishing for consumption or as a leisure activity (Figure 3.5). Fishing for tradition was mentioned by three fishers, and two fishers reported other reason for fishing, where one of them specifically reported sale as the motivation for fishing Nephrops.



Figure 3.5 Fishing motivations, multiple answers for some fishers.

3.1.3.2 CPUE in the different fjords

All fishers reported using pots especially developed for catching Nephrops, and the pots should therefore be about the same size. However, three fishers reported using them in combination with two-chamber fish pots. The fishers used between 3 to 24 pots (average 14 pots), with 2 to 30 days soak time (average 7 days). The was an indication that Raunefjorden had higher estimated CPUE than the other locations, but only two registrations (Figure 3.6). Byfjorden had the second highest estimated CPUE, but only one registration. Three locations had more than seven catch registrations. Fanafjorden had the highest median CPUE of these three, followed by Hauglandsosen and Austevoll.



Figure 3.6. Mean catch of *Nephrops norvegicus* per pot and haul (CPUE) for the different locations. The black horizontal line shows median value, while vertical lines show maximum and minimum value. The box is the interquartile range. The number of observations (n) is displayed at the top. Soaking time is assumed not to have any effect on catch rates.

3.1.3.3 Release motivations

Twenty-three fishers reported releasing Nephrops during last catch, and the answers ranged from 1 to 25 Nephrops released (mean 6 Nephrops released). Release motivations varied from the most mentioned reason "Too small" (45%), to "Females with roe" (33%) and "Minimum length size" (21%) (Figure 3.7).



Figure 3.7 Release motivations, multiple answers for some fishers.

3.2 Nephrops norvegicus; size, sex and mercury concentrations

3.2.1 Size and sex determination for the different locations

The largest Nephrops were found in Byfjorden (Table 3.2), but these were all males. For the other locations, both sexes were present in the catch. The smallest mean size Nephrops were captured in Radfjorden. The widest size range was measured in Austevoll, which was also the locations with the highest number of individuals.

Nephrops norvegicus	Carapace le	Sex (N)				
Location (N total)	Range	All	4	8	9	8
	(min-max)	$Mean \pm SD$	$Mean \pm SD$	Mean \pm SD		
All locations (235)	37 – 78	54 ± 9	48 ± 5	57 ± 8	74	161
Austevoll (47)	41 - 78	55 ± 10	48 ± 4	60 ± 10	17	30
Byfjorden (30)	51 - 69	60 ± 5		60 ± 5	0	30
Fanafjorden Outer	46 – 71	59 ± 7	54 ± 3	62 ± 7	5	10
station (15)						
Fanafjorden Inner	37 – 69	53 ± 8	48 ± 5	56 ± 9	12	22
station (34)						
Hauglandsosen Ågotnes	40 - 72	53 ± 8	49 ± 8	51 ± 6	11	19
(30)						
Hauglandsosen Hetlevik	45 - 67	50 ± 6	48 ± 6	56 ± 7	4	11
(15)						
Radfjorden (34)	38 - 67	47 ± 6	45 ± 5	49 ± 8	20	14
Raunefjorden (30)	45 – 75	57 ± 6	51 ± 4	58 ± 6	5	25

Table 3.2 Carapace length (mm) of Nephrops for eight different locations. Range, mean and standard deviation are shown, for females and males.

3.2.2 Mercury concentration in Nephrops

The content of total Hg in the certified reference material Tort-3 was measured to 254 \pm 16 µg/kg dry weight (Mean \pm SD, n=54), and falls within the range of 2 SD of the certified value for Tort-3 of 292 \pm 22 µg/kg dry weight. The Hg concentrations in the Nephrops tail muscle samples were considered validated as the results of the CRM was acceptable (within range of 2 SD) when assessing the accuracy of the method.

The mean Hg concentration in tail muscle across all locations was measured to $81 \pm 32 \mu g/kg$ wet weight in males (n=161), and $140 \pm 69 \mu g/kg$ wet weight in females (n=74) (Table 3.3). Results from freeze-dried samples of tail muscle revealed an average dry matter content of 21% (n=16).

The Hg concentration in claw muscle was measured in 43 animals, with a mean value of $21 \pm 9 \ \mu g/kg$ wet weight corresponding to an average 24% (SD=9%) of the Hg

concentration in the corresponding tail muscle. The dry matter content in samples of claw muscle was on average 18% (n=16).

Nephrops norvegicus	Hg concent	Sex (N)				
Location (N)	Range All	All	9	3	9	2
	(min-max)	Mean \pm SD	$Mean \pm SD$	Mean \pm SD		
All locations (235)	26-290	100 ± 50	140 ± 69	81 ± 32	74	161
Austevoll (47)	35 - 240	120 ± 50	160 ± 47	92 ± 36	17	30
Byfjorden (30)	59 - 130	80 ± 20		80 ± 20	0	30
Fanafjorden (Outer	73 – 250	160 ± 72	230 ± 14	120 ± 60	5	10
station) (15)						
Fanafjorden (Inner	33-200	90 ± 40	130 ± 48	67 ± 19	12	22
station) (34)						
Hauglandsosen Ågotnes	53 - 290	130 ± 73	200 ± 71	81 ± 23	4	11
(30)						
Hauglandsosen Hetlevik	60 - 220	100 ± 53	180 ± 44	85 ± 27	11	19
(15)						
Radfjorden (34)	26 - 130	60 ± 20	67 ± 27	43 ± 8	20	14
Raunefjorden (30)	57 - 240	100 ± 50	200 ± 26	84 ± 24	5	25

Table 3.3 Hg concentration (μ g/kg ww) in homogenized tail muscle of Nephrops for eight different locations.Range, mean and standard deviation are shown, for females and males.

The linear mixed effects model revealed a significant difference in mean Hg concentration between the different sexes (Appendix V, 6.1, p < 0.001, Figure 3.8). A clear relationship between carapace length (mm) and Hg (μ g/kg wet weight) was observed across all locations, for both females and males (Appendix V, 6.1, interaction between CL and sex, p < 0.001, Figure 3.8). This means that the sexes have significantly different slopes.



Figure 3.8 Mercury concentration $(\mu g/kg)$ in the tail muscle versus carapace length (mm) *Nephrops norvegicus* in females and males for all locations. The points in the figure are the raw data, while the lines are the predicted line from the linear mixed effects model.

The linear model which included location revealed a significant difference in mean Hg concentration between the different fishing locations depending on sex (Appendix V, 6.3, interaction between Location and Sex, p < 0.001, Figure 3.9). The interaction between sex and carapace length was also significant (Appendix V, 6.1, interaction between Sex and Cl, p < 0.001, Figure 3.9). p < 0.001), meaning that the Hg concentrations were increasing with size with significantly different slopes for the sexes, but the difference was not significant between the locations (no three-way interaction). The best model included both size, sex, and location, meaning that all three variables affected Hg concentrations in the individual Nephrops. The scale in Figure 3.9 is the same along both axes, and the plot can therefore be used to predict Hg concentration if size and sex are available for individual Nephrops at the given locations.



Figure 3.9 Mercury (μ g/kg ww) versus carapace length (mm) of *Nephrops norvegicus* in females and males for the different locations. The points in the figure are the raw data, while the lines are the predicted lines from the linear model. The scale is the same for all figures, on both axes. The figure can therefore be used to predict Hg concentration if size and sex are available for individuals at a given location.

Assessing which locations differed was done by comparing mean Hg concentrations at the eight locations for males and females. For the females, individuals in Radfjorden had significantly lower Hg concentration than in Austevoll, Hauglandsosen Hetlevik, Hauglandsosen Ågotnes, Raunefjorden and Fanafjorden (outer station) (p<0.001, Tukey's multiple comparison test), including Fanafjorden (inner station) (p<0.003). Females from Fanafjorden (outer station) were higher in Hg concentration than Fanafjorden (inner station) (p<0.003) and Austevoll (p<0.03). Females from Hauglandsosen Ågotnes were significantly higher in Hg compared to Fanafjorden (inner station) (p<0.02).

For the males, individuals in Radfjorden were significantly lower in Hg concentration than those from Raunefjorden, Hauglandsosen Ågotnes, Fanafjorden (outer station), Byfjorden and Austevoll (p<0.001, Tukey's multiple comparison test). Males from

Fanafjorden (outer station) were higher in Hg concentration than Fanafjorden (inner station) (p<0.001), Byfjorden (p<0.002), Hauglandsosen Hetlevik (p<0.004), Raunefjorden (p<0.006) and Hauglandsosen Ågotnes (p<0.02). Males from Austevoll also had significantly higher Hg concentrations than males in Fanafjorden (inner station) (p<0.03).

3.3 Consumption pattern and risk assessment

3.3.1 Consumption pattern

Consumption frequency of Nephrops for the interviewed fishers is shown in Figure 3.10. The average number of Nephrops eaten per meal was approximately seven, with answers ranging from one to 15 Nephrops per meal. High consumers ate on approximately 12 Nephrops per meal. When consuming Nephrops, 18 recreational fishers reported eating both the tail and the claws, eight people reported eating only tail meat, and seven fishers reported eating tail, claw meat and the brown meat of the head.



Figure 3.10 Consumption frequency of Nephrops (N=33 total).

Eating fish for dinner was common among the recreational Nephrops fishers. 73% ate fish for dinner 2-3 times per week or more (Figure 3.11A). Fish for lunch was not equally popular. Twenty people ate fish for lunch less than once a week (60%) (Figure 3.11B).



Figure 3.11A Consumption frequency of fish for dinner the preceding three months.



Figure 3.11B Consumption frequency of fish for lunch the preceding three months.

3.3.2 Risk assessment

Mean intake from all consumers was approximately 433 grams fish for dinner weekly and 42 grams fish for lunch weekly. The five highest consumers ate on average 620 grams fish for dinner weekly and 112 grams fish for lunch weekly. Mean concentration used for calculating intake from Nephrops was 100.9 μ g/kg (n=235), and high concentration used was 250.6 μ g/kg (n=10, mean of 10 highest concentrations). Hg concentration in claw meat was calculated to 24% of the concentration in tail meat. When considering total intake, including the contribution of MeHg from other seafood than Nephrops, there was no risk of exceeding the TWI for MeHg using average concentrations for Nephrops and other seafood, even considering high consumption rates with an intake of 78.6 μ g Hg/week. Considering an average consumption of seafood with average concentration, it would be possible to consume up to 27 Nephrops tails weekly without exceeding TWI. If the Nephrops were high in Hg, it would be possible to consume up to ten Nephrops tails weekly without exceeding the TWI for MeHg.

None of the recreational Nephrops fishers were at risk of exceeding the TWI for MeHg when only considering Hg from Nephrops consumption. Considering Hg intake from Nephrops only, 42 tails with average concentration or 17 with high Hg concentrations could be consumed weekly, without exceeding the TWI.

Total seafood intake with high Hg concentrations in Nephrops and other seafood would exceed the TWI both with average consumption (intake of 135.6 μ g Hg/week) and high consumption (intake of 216.9 μ g/week). A combination of high consumption of other seafood with average concentrations (intake/exposure of 57.0 μ g Hg/week), and high consumption of Nephrops with high Hg concentrations (intake/exposure of 53.5 μ g Hg/week), would also exceed the TWI with approximately 6.5 μ g (not shown in Table 3.4), with a weekly intake of 110.5 μ g. According to the estimates, the average respondents in this study would only be in danger of exceeding TWI when consuming other seafood with high concentrations. Consumers with the highest intake of Nephrops may also exceed the TWI when consuming other seafood with average concentrations, if the Nephrops consumed have high Hg concentrations.

Table 3.4 Calculated total weekly mercury intake (µg) from consuming Nephrops, other seafood for lunch and dinner, including total intake from all seafood. Exposure considering both average and high concentration for Nephrops and other seafood, including average and high consumption rates is shown. Bold values indicate an exceeding of the TWI for a person of 80 kg $(104 \mu g \text{ weekly})^9$.

	Hg intake from Nephrops		Hg intake from	other seafood	Total Hg intake		
	norvegicus		for dinner and	lunch	(Nephrops + other seafood)		
µg weekly	Average	High	Average	High	Average	High	
	concentration ¹⁰	concentration ¹¹	concentration ¹²	concentration ¹³	concentration	concentration	
Average	8.9	22.1	39.2	113.5	48.1	135.6	
consumption							
High	21.6	53.5	57.0	163.4	78.6	216.9	
consumption ¹⁴							

⁹ Calculated using TWI for MeHg (1.3 µg/kg body weight weekly).

¹⁰ Calculated using the mean of all Hg concentrations measured in Nephrops (100.9 μ g/kg). ¹¹ Calculated using the mean of ten highest Hg concentrations measured in Nephrops (250.6 μ g/kg). ¹² Calculated using mean dinner (86 μ g/kg).

¹³ Calculated using max concentrations for dinner (257.5 μ g/kg).

¹⁴ Defined as the mean of the five consumers with the highest consumption.

4. Discussion

This study found that recreational fishing for Nephrops is very popular in Hordaland, that the catches can be relatively high, and that recreational fishers consume their catch. None of the sampled Nephrops exceeded the maximum legal limit for Hg in commercially sold seafood (0.5 mg/kg wet weight) at any location. Even though Hg concentrations in Nephrops in Hordaland are generally low, some groups can be exposed to MeHg exceeding the tolerable weekly intake (TWI) with their total seafood intake. There were significantly higher Hg concentrations in female Nephrops compared to male Nephrops at the same size, including a difference in Hg concentrations between the locations. Some Nephrops exceeded 0.2 mg Hg/kg wet weight and should thus not be consumed by pregnant and lactating women (VKM, 2006). Higher Hg concentrations in females explained by sex and size is supported by other studies on Nephrops (Barghigiani et al., 2000; Canli & Furness, 1993; Minganti et al., 1990). No immediate update in dietary guidelines is required to protect consumers. However, the consumers of recreationally captured Nephrops should be aware of the difference in Hg concentration between the sexes, and pregnant and lactating women should not consume large female Nephrops.

4.1 Recreational fishing for Nephrops is popular

Recreational fishing represented 18%-60% of all registered buoys in the surveyed fjords, and the recreational Nephrops fishery represented 24%-100% of recreational fishing in the surveyed fjords, which indicate that the fishery is popular and frequent. Although, the areas were selected based on water depth (>50 m) including information about buoys observed in deep waters (Ferter & Bjelland, 2017). Thus, the areas are not representative for all areas. The highest density of recreational Nephrops buoys (2 buoys per km²) was reported in Fanafjorden and Austevoll, both in round three of the surveys (July).

4.1.1 Catch data and CPUE estimations

In this study, the catch rates were relatively high, a broad size range was present in the catch (37-78 mm carapace length) and 70% of the recreational fishers reported releasing parts of their catch. The CPUE estimations indicated that Raunefjorden had a higher CPUE than the other locations, with a median catch of almost nine Nephrops per pot and haul. However, the small sample (n=2) is highly skewed due to an outlier where one fisher reported very high

catch (15 kg). Byfjorden had the second highest CPUE, of nearly 3 Nephrops per pot and haul, but only one observation. The fishing location in Byfjorden is in an area with low effort, most likely because it is within the area with specific dietary advice against consumption, near the port of Bergen (Figure 1.1), and it is likely not a popular fishing spot for Nephrops. The lowest median CPUE was estimated in Austevoll (n=7), which can possibly be explained, as this is also one of two locations with the highest density of recreational Nephrops buoys (2 per km²). However, it is not possible to conclude whether there is an actual difference in CPUE between the different fjords due to low sample size and potential bias issues, like recall bias and prestige bias. The CPUE estimations are therefore purely indications. To test for potential differences in CPUE between fjords in Hordaland, more data is needed.

In this study, the mean CPUE across all locations was 2.5, and the median 2.1 Nephrops per pot. Results from 17 fishers reporting catch between 2012-2014 in Skagerrak estimated CPUE to 1.7 Nephrops per pot within the same time-period (August-September) (Kleiven et al., 2015). In other words, the CPUE estimated based on 31 fishers in the present study in Hordaland is likely higher.

Another difference compared to the study in Skagerrak is the sex ratio. On average, 58% of the catch over the entire sampling period in Skagerrak were males (Kleiven et al., 2015), compared to 69% males in the present study. The difference in sex ratios can possibly be explained by seasonal variations. The sampling frame in this thesis was August-September, compared to an average sex ratio reported for the entire study period in Skagerrak. Average years spent fishing Nephrops was similar in Hordaland (3.83 years) and Skagerrak (4 years), which is low compared to average fishing years for European lobster (26 years) reported in the Skagerrak study (n=131). This suggests that the popularity of recreational Nephrops fishers reported that they only recently started to fish Nephrops recreationally.

Considering the popularity for marine recreational fishing for Nephrops, further monitoring may be warranted. Catch diaries (log books) could provide information on catches, size distributions, sex ratios and percentage of egg-bearing females, to create a time series to follow CPUE between years.

4.1.2 Release motivations: voluntary or regulatory

Observations from this study suggest that recreational fishing for Nephrops using pots (creel fishing) is a highly species-specific fishery, as only one fisher reported catch of a

different species (*Galathea strigosa*). The results revealed that three-quarters of the fishers released a part of their last catch. An investigation of the release motivations revealed that seven of the fishers reported minimum legal length size as one of their reasons for releasing Nephrops. The most frequently reported release motivation was either "Too small" or "Females with roe", which both are assessed as voluntary release motivations. Voluntary release motivations seem to be the most common, compared to regulatory release motivations such as minimum length size. Several fishers expressed concerns for the population and reported releasing females with roe as they rarely encountered them in their catch. However, it is known that the catch is dominated by males, as males dominate the largest size classes (Bell et al., 2006; de Figueiredo & Thomas, 1967), and egg-bearing females rarely leave their caves (Bakketeig et al., 2015; Bakketeig et al., 2017).

A possible factor when releasing catch is possible unaccounted mortality. In general, however, the unaccounted mortality for Nephrops fishing is considered low for creel fisheries, especially as there is no harm inflicted on the animal itself, at least by the fishing gear. Additionally, the stress on creel-caught animals is reported to be limited (Ridgway et al., 2006). However, some studies suggest that Nephrops might experience light-induced eye damage after as short as five minutes of exposure to light (Shelton et al., 1985) and recapture studies showed no recovery from retinal damage with time (Chapman et al., 2000; Gaten et al., 2013; Shelton et al., 1985). On the other hand, there was no evidence of lower growth rates, reproductive rates or survival rates in blind individuals, and they appeared to function equally well as fully-sighted individuals (Gaten et al., 2013).

Several studies have evaluated the survival rate of discarded Nephrops from both creel and trawl fishery (Harris & Ulmestrand, 2004; Wileman et al., 1999; Ziegler & Valentinsson, 2008). There is a consensus that Nephrops caught by creel fishing have a higher survival rate than those caught by trawl fishing (Bernasconi & Uglow, 2008; Méhault et al., 2016; Valentinsson & Nilsson, 2015; Ziegler & Valentinsson, 2008). Wileman et al. (1999) reported a 99% survival rate of Nephrops discarded from creel fishery. Similarly, Valentinsson & Nilsson (2015) reported a 97% survival rate of Nephrops discarded from creel fishery. However, survival studies usually take place in simulated environments (tanks) and do not account for natural predation, for example when sinking down to the seabed after release.

4.1.3 Recreational fisher's perception of pollution in Nephrops in Hordaland

All the respondents in this study believed the Nephrops had low contamination, and 27% perceived the Nephrops as not contaminated at all. As the recreational fishers generally believed the Nephrops had low contamination and was safe for consumption, the perception of pollutants most likely did not influence the frequency of consuming Nephrops. This is a similar attitude as was found to be displayed by anglers in Lake Ontario, of whom 50% were slightly or not at all concerned that fish consumption could lead to potential health risks (Knuth et al., 2003), while 47% of urban anglers from a New York study reported that they thought fish from local waters were safe to eat (Pflugh et al., 1999). Fifteen percent of recreational fishers answered "I don't know" regarding whether or not the Nephrops from their local area is polluted, which is the same as respondents reporting "I don't know" regarding the safety of consuming fish in New York (Pflugh et al., 1999).

No correlations were observed between age or education and perception on pollutants in the present study. Contradictory, a study on general food awareness and consumer concerns in Norway revealed differences in how various sub-groups relate to food and health, and that these differences depend on their heritage, education, age, sex or social status (Wandel, 1994). However, Wandel (1994) concluded that men were less concerned than women, and this study only included one female recreational fisher.

4.2 Mercury concentrations in Nephrops tail muscle

The results revealed significantly higher Hg concentrations in female Nephrops compared to male Nephrops at the same size, and there was a clear connection between size and Hg concentration, both for females and males (Figure 3.8). A significant interaction term between sex and size means that size and sex cannot be treated separately. The results also revealed statistically significant differences in Hg concentrations between locations (Figure 3.9). However, as the Hg concentration at all locations were low, there is no reason to establish fishing recommendations regarding area.

None of the analyzed Nephrops exceeded the maximum legal limit for Hg (0.5 mg/kg ww). The results of the consumption data and the measured Hg concentrations indicate no need for dietary guidelines for the consumers of recreationally captured Nephrops.

The mean Hg concentrations for males $(0.08\pm0.03 \text{ mg/kg ww})$ and females $(0.14\pm0.07 \text{ mg/kg ww})$ were lower than observed in Hardangerfjorden, both in Kvam $(0.5\pm0.13,$

n=10) and in Rosendal (0.2 ± 0.03 , n=10). However, Hardangerfjorden is known as an area with an exceptionally high Hg contamination (Måge et al., 2012). The measured Hg concentrations were also low compared to Nephrops from the Adriatic Sea, where 46% of individuals exceeded the maximum legal limit (Perugini et al., 2009), and in the Northwestern Mediterranean Sea where 23% exceeded the maximum legal limit (Cresson et al., 2014).

The approximately four times higher Hg concentrations in the tail muscle compared to the claw muscle of Nephrops is a rather surprising finding. One possible reason could have been a lower dry matter content in the claws, but since the measured dry matter content in the tail muscle (21%) was comparable to the dry matter content in claw muscle (18%), this cannot be the explanation. Måge et al. (2012) reported Hg concentrations in claw muscle to 45% of Hg concentration in tail muscle in European Lobster in Hardangerfjorden (n=5). However, the study did not propose an explanation for the findings (Måge et al., 2012). No other studies have been found to address this phenomenon, and further research is warranted.

The increase in Hg with size was significantly steeper for females compared to males (Appendix V, 6.1, interaction between carapace length and sex, p < 0.001, Figure 3.8). The same patterns have been observed in Nephrops in the Ligurian sea (Minganti et al., 1990), outside Scotland (Canli & Furness, 1993) and in the Tyrrhenian sea (Baldi, 1984; Barghigiani et al., 2000). The steeper increase of Hg with size in females can be explained by the biology of the species. After maturity, the female's growth rate decreases, and moulting reduce, from three or four times a year to approximately one per year (Bell et al., 2006). This means that females are generally older than males at the same size.

Differences in Hg concentrations by sex have also been observed in other crustaceans (Barrento et al., 2009; Bu-Olayan et al., 1998; Elahi et al., 2012). Elahi et al. reported significantly higher Hg concentrations in females in a species of shrimp (*Penaeus semisulcatus*) in the Persian Gulf. Bu-Olayan et al. (1998) reported significantly higher muscle Hg concentration in female individuals compared to males of a species of lobster (*Thenus orientalis*) in Kuwait. Barrento et al. (2009) studied the accumulation of several elements in different tissues of brown crab captured outside Scotland and in the English Channel. The study concluded that Hg concentrations were generally higher in all tissues of female crabs compared to males.

The linear model which included location revealed a location-dependent difference in mean Hg concentrations between sexes. For example, the distance (difference in intercept) between the regression lines for males and females in Radfjorden is smaller than in Raunefjorden (Figure 3.9). However, the different slopes for males and females are the same for all locations (Figure 3.9), i.e., there was no statistically significant three-way interaction between location, sex, and size. The regression lines in Figure 3.9 fits the data nicely at all locations, except the outer station at Fanafjorden. The variation in Hg concentration in males in Fanafjorden (outer station) is considerably larger than other locations, which leads to a higher degree of uncertainty if the regression line is used for predictions in Fanafjorden (outer station). However, the residuals are small for all the other locations, and the regression can therefore be used to predict Hg concentrations in captured individuals at the different locations, if sex and size of the Nephrops are known.

The Nephrops in Radfjorden had the lowest mean Hg concentrations. This finding fits well with our assessment of Radfjorden as a low contamination area. Hauglandsosen was assessed as somewhat polluted based on distance to industrial areas and former waste disposal areas. No difference was found between Hauglandsosen Ågotnes and Hauglandsosen Hetlevik, even though Hauglandsosen Hetlevik is closer to Hanøytangen, Horsøy, and Kollevågen. Fanafjorden was assessed as possibly contaminated by a freshwater stream with high Hg concentrations with run-off into the inner part of the fjord. However, Hg concentrations were significantly higher in Nephrops from Fanafjorden (outer station) compared to Fanafjorden (inner station). This suggests that Hg concentrations are not necessarily explained by distance to the contamination sources, but the methylation rate of Hg might play a role. Hg methylation is influenced by the speciation and biochemical availability of Hg, but also several, possibly interrelated, environmental variables (Ullrich et al., 2001). The methylation process and production rate of MeHg are not clearly understood, however, it is suggested to be determined by several complex interactions between nutrient availability, temperature, pH, biological activity and redox potential (Ullrich et al., 2001).

4.3 Risk assessment of methylmercury intake from Nephrops including other seafood

The present study confirmed that recreational fishing for Nephrops is popular and that the catch rates are high (mean 2.5 Nephrops per pot). The gathered consumption data confirmed that consuming Nephrops is common, with an average of 7 Nephrops consumed per meal, and on average two Nephrops meals per month. However, the mean Hg concentration in the measured tail muscle and claw muscle were generally low, and the risk of exceeding TWI for MeHg by consuming Nephrops is low. Some large female Nephrops exceeded 0.2 mg/kg (ww), including two males, and should not be consumed by pregnant and lactating women (VKM, 2006). The study confirmed that many of the recreational fishers are frequent consumers of other types of seafood, and fish for dinner 2-3 times per week was the most common frequency of consumption. Fish for lunch was not equally popular, and the most common eating frequency of fish for lunch was less than once a week. Even though the questionnaire on fish consumption for dinner and lunch did not provide information about species-specific consumption patterns, it indicated that some recreational fishers could exceed the TWI for MeHg considering their total consumption of seafood.

4.3.1 Consumption pattern and risk assessment

The obtained Hg concentrations in Nephrops and the reported consumption habits showed that none of the recreational Nephrops fishers exceed the TWI for MeHg by consuming Nephrops. However, considering intake from other seafood sources might put some consumers at risk of exceeding TWI for MeHg. Moreover, the risk is associated with frequency and species consumed. The recreational Nephrops fishers ate on average 62 grams of fish for dinner daily, compared to 54 grams daily in the high consumer group in the Norwegian Fish and game study part C (Mangerud, 2005). Consumption of fish for lunch was on average 6 grams daily, which is lower than reported in Mangerud (2005) (mean 33 grams daily). However, Mangerud (2005) included a total of eight questions regarding fish for lunch compared to only one question in this study, and studies show that people tend to overestimate when too many choices are available in food frequency questionnaires (Cade et al., 2002). Mangerud (2005) also discussed the possibility of this value being overestimated. As no other data is available for recreational Nephrops fishers, it is not possible to assess if 6 grams daily is over- or underestimated. However, it is evident that fish for lunch contributes very little to MeHg intake in almost all recreational Nephrops fishers in the sample. Eating fish for dinner was more common than eating fish for lunch among the recreational Nephrops fishers, and recreational Nephrops fishers seem to have a higher, but similar intake of fish for dinner compared to a high consumer group of seafood (Mangerud, 2005).

Jenssen et al. (2012) estimated dietary Hg exposure among fish-consumers in Norway using food frequency questionnaires and total Hg concentrations in marine and freshwater fish compiled from other studies. Samples expected to be impacted by local pollution or from areas with known point sources were excluded. The study also measured Hg in blood and urine in the fish-consumers (Jenssen et al., 2012). The mean dietary intake was estimated to be 74 grams daily, which is slightly higher than in the present study. Using the estimated dietary Hg intake, Jenssen et al. (2012) modeled blood Hg concentrations based on dietary seafood intake and compared them to the measured values. The highest blood Hg concentrations were found in consumers with a high portion of recreationally obtained seafood. For these consumers, the modeling of blood Hg concentrations underestimated exposure, perhaps because these consumers ate fish captured closer to the shore with possibly higher Hg concentrations (Jenssen et al., 2012). Locally captured seafood often has higher Hg concentrations near harbors, and values may differ substantially from the same species captured in open water (Måge & Frantzen, 2009). The amount of self-caught seafood was deemed an important determinant in Hg exposure (Jenssen et al., 2012). This demonstrates the importance of using Hg concentrations in species from the local area when estimating Hg intake for recreational fishers. The unique thing about the present study is that it analyzed actual catch from recreational Nephrops fishers, which provides confidence in the estimates and the conclusion that the consumption of recreationally captured Nephrops is safe in the reported eating frequency. More uncertainty is related to the estimates of total MeHg intake from fish for dinner and lunch as the Hg concentrations used in the calculations are not from locally captured fish. However, the mean and high concentrations used provide information that can be used by recreational fishers to assess their own risk of exceeding TWI. For example, the high concentration used for fish for dinner is close to mean Hg concentrations reported in cod (0.3 mg/kg ww) (Måge & Frantzen, 2008) and ling (0.22 mg/kg ww) outside Bergen (Måge & Frantzen, 2009). More information regarding species consumed and proportion of self-caught fish is needed to assess the risk of exceeding TWI for MeHg from total seafood intake more accurately for recreational fishers in general. For example, if the recreational Nephrops fishers also consume bottom-feeding fish such as tusk and ling associated with high Hg concentrations (Beylich & Ruus, 2011; Måge & Frantzen, 2009) or capture fish within the area for the dietary advice in the port of Bergen, the risk of exceeding TWI will increase significantly.

Based on the present findings, the recreational Nephrops fishers seem to be part of a group of high consumers of fish and other seafood. More data is needed on the recreational fishery and associated consumption patterns on other species, especially consumption patterns for bottom-feeding fish such as tusk and ling. Ideally, hair or blood Hg samples should be sampled to assess the risk more accurately for other recreational fishers. A study similar to Lincoln et al. (2011) would provide more information about the risk of exceeding TWI by consumption of self-caught seafood in Hordaland, by evaluating hair Hg concentrations.

4.3.2 Consumption pattern and risk assessment regarding the families of the recreational fishers

Although none of the Hg concentrations measured in Nephrops exceeded the maximum legal limit of 0.5 mg/kg (ww), 23 of 235 Nephrops exceeded the limit set for pregnant and lactating women (0.2 mg/kg ww). Pregnant consumers may also exceed TWI when consuming Nephrops in combination with other seafood with Hg concentrations considered safe.

Consumption of 200 grams of Nephrops meat with high Hg concentrations (250 µg/kg, or 0.25 mg/kg) would contribute with 64% to the TWI for a person of 60 kg. Exceeding the TWI on a regular basis is therefore likely if the consumer also eats other types of seafood. As the fetus and infants are particularly sensitive to potential damages to the central nervous system, it is especially important to stay below TWI for pregnant and lactating women. Studies on low doses of prenatal MeHg exposure suggested that it might lead to long-term negative health impacts (Debes et al., 2006; Debes et al., 2016; Grandjean et al., 1997; Lam et al., 2013; Oken et al., 2005). Debes et al. (2006, 2016) and Grandjean et al. (1997) studied MeHg exposure in the same birth cohort in the Faroe Islands at age 7, 14 and 22. The most severe deficits were observed in motor speed, attention and language at 7 and 14 years of age (Debes et al., 2006), but cognitive deficits associated with prenatal MeHg from maternal seafood remained detectable 22 years after birth (Debes et al., 2016). Oken et al. (2005) also concluded that higher Hg concentrations during pregnancy were associated with lower cognition in infants. However, the study also reported improved cognition in infants associated with increased fish consumption (Oken et al., 2005).

Consumers should bear in mind that tolerable weekly intakes have safety margins and several studies have not found evidence of any adverse cognitive effects from MeHg even in populations with frequent fish consumption. For example, several studies from the Seychelles showed no evidence of adverse effects in children whose mothers consumed on average 12 fish meals per week with mean Hg concentrations of 0.3 mg/kg (Matthews, 1983; Myers et al., 2003; Shamlaye et al., 2004; Van Wijngaarden et al., 2013). Van Wijngaarden et al. (2013) rather suggested improved performance for some cognitive endpoints possibly associated with essential nutrients in fish. Nutrients in fish have been suggested to mask effects of Hg, as omega-3 fatty acids, vitamin D, and iodine might have beneficial effects (Mergler et al., 2007). Selenium might additionally help neutralize Hg toxicity (Oken et al., 2016). A possible interaction between selenium and Hg has been suggested to partly account

for the conflicting results from the Faroe Islands and Seychelles regarding cognitive deficits associated with prenatal Hg intake (Raymond & Ralston, 2004).

Several studies assessing Hg concentration in seafood also investigated Hg intake for fishers and their families (Al-Majed & Preston, 2000; Cheng et al., 2009; Gaggi et al., 1996). Al-Majed & Preston (2000) concluded that fishers in Kuwait had significantly higher hair Hg concentrations than a control group. Cheng (2009) did not compare against a control group but revealed higher hair Hg concentrations in fishers in China compared to the Kuwaiti fishers. As TWI is based on body weight, children may have increased risk of exceeding TWI by having a lower body weight. If a child of 40 kg consumes 200 grams of Nephrops tail meat with high Hg concentrations weekly, it will likely exceed the TWI.

Another important aspect when considering the risk associated with Hg in seafood is cooking methods. Perugini et al. (2016) showed increased Hg concentrations in cooked samples of Nephrops compared to raw samples. Increased Hg concentrations after cooking have also been shown in other fish and shellfish (Costa et al., 2016). According to Perugini et al. (2016) it is possible to underestimate Hg concentrations in shellfish due to a thiol-group and protein affinity, as Hg is known for its strong affinity to proteins (Harris et al., 2003). Ouédraogo & Amyot. (2011) indicated that cooking did not significantly increase Hg concentrations in fish tissue. However, the study concluded that cooking methods reduced Hg bioaccessibility with 40-60% compared to raw fish (Ouédraogo & Amyot, 2011). It has been suggested that boiling or frying processes could change Hg bioaccessibility by altering mineral content and protein structure (Burger et al., 2003; Maulvault et al., 2011). How recreational fishers prepare Nephrops might, therefore, affect the Hg concentrations in the Nephrops. In this thesis, the muscle samples were analyzed raw. In future studies, attention should be raised to gain knowledge about how cooking processes affect Hg concentration, as this could have significant implications for the risk from Hg (Perugini et al., 2016).

The highest Hg concentrations were measured in female Nephrops, and although sex is rarely included in dietary advice (Gewurtz et al., 2011), consumers should take notice of sex when consuming Nephrops. The present study also revealed that recreational fishers are frequent consumers of other types of seafood, which can lead to exceeding TWI for MeHg with their total consumption of seafood. Even if the risk of exceeding the TWI for MeHg by Nephrops consumption is low, other pollutants must also be considered when assessing the risk for consumers. Several studies have conducted analyses for other pollutants in Nephrops (Bodin et al., 2007; Förlin et al., 1996; Måge et al., 2012; Perugini et al., 2004). Måge et al. (2012) reported values below EUs maximum legal limit for cadmium and lead in Nephrops

from Kvam and Rosendal. Bodin et al. (2007) investigated PCB, PCDD/F and PBDE values in several crustacean species and concluded that none exceeded the maximum limits. The same conclusion was reached by Perugini et al. (2004), where no crustacean samples exceeded the maximum legal limit for PCBs. Forlin et al. (1996) reported higher concentrations of the neurotoxin manganese in gill and hemolymph of Nephrops from Kattegat and Skagerrak compared to the Faroe Islands. All in all, the studies suggest that other contaminants seem to be of minor importance for Nephrops.

4.4 Survey choices and data bias issues

4.4.1 Survey mapping standing gears

In the survey mapping standing gears, some anchor buoys or warning buoys in shallow waters may have been sampled and assumed to be unidentifiable fishing gear. However, effort was put into avoiding this, and these errors should be limited. Additionally, some recreational fishers use multiple types of gear at the same time and may not remember exactly the kind of gear they had in use on a specific location and date in retrospect. This may have led to some fishing gear being put in the wrong category in the field (maps, Appendix VII). However, the goal of the follow-up phone survey was mainly to get in touch with recreational Nephrops fishers, and therefore, it did not necessarily matter which type of fishing gear that was observed in the survey mapping standing gears. Errors regarding wrong identification of fishing gear by the fisher in the follow-up phone survey are assumed to be limited in this survey, as the phone call was conducted at the latest 23 days after observation in the field. However, for one of the recreational Nephrops fishers, it was later noticed that the buoys observed in the field were of a gillnet, rather than Nephrops pots.

4.4.1.1 Recreational Nephrops fishers: sample representativeness

The short sampling timeframe might have affected the representativeness of the participants in the study, especially since those who fish more frequently have a greater possibility of being in the sample, a term called avidity bias (Pollock et al., 1994). Thus, respondents with a high degree of experience might be overrepresented in the sample of recreational fishers. Ideally, sampling should have been conducted several times over an entire year to ensure representativeness of the sample. However, as no information about recreational Nephrops fishers is available, it is impossible to assess the representativeness of

the sample. Although, a larger sample size would have increased confidence in the sample of recreational Nephrops fishers and perhaps made generalization of the findings possible.

The sample of recreational Nephrops fishers is also affected by possible bias from the people not possible to get in touch with during the first phone-survey. Although, the lowest percentage of achieved contact was 83% (round 3), which still can be considered high. Out of 95 recreational fishers in the final sample, it was not possible to retrieve contact information for eight fishers using information from the buoys on the websites. Additionally, 12 fishers did not answer the phone during the entire project. In other words, the sample does not seem profoundly affected by this type of bias. However, it is not possible to rule out a potential difference between the fishers that answered the phone, and the ones that did not. The sample of recreational Nephrops fishers is also affected by nonresponse bias (Pollock et al., 1994). However, considering that only one fisher did not want to participate in the project nonresponse bias is considered minimal in this study.

4.4.1.2 Catch and CPUE related limitations

According to Pollock et al. (1994), catch data collected using off-site methods is unlikely to provide accurate and representative data. In general, it is recommended to inspect catch, rather than rely on angler-reported information, as it is associated with a significant degree of uncertainty (Mallison & Cichra, 2004). However, inspection of the catch is impractical when dealing with recreational fishing from passive fishing gears, as it is unlikely to encounter hauling in field surveys by chance. Besides, Norway does not have a countable number of access-points where it is possible to inspect the catch after a completed trip. This makes off-site methods such as interviews or catch diaries the best alternative to gain information about the fishery. Catch diaries and interviews have been implemented with success in several studies (Kleiven, 2010; Kleiven et al., 2012; Strehlow et al., 2012).

The effects of recall bias and prestige bias should also be considered for the CPUE calculations in this survey. Recall bias occurs when respondents fail to recall their catch accurately (Tarrant & Manfredo, 1993). Prestige bias, on the other hand, occurs when respondents exaggerate number and size of fish caught (Pollock et al., 1994). In this survey, the respondents were explicitly asked for the last catch, rather than catch from a specific time-period in attempts of limiting recall bias. Furthermore, the catch data was reported by fishers' maximum six weeks after catch, most frequently within the last two weeks of catch. Consequently, problems with remembering catch should be limited. In addition, the likelihood

of remembering the number of landed individuals has been suggested to increase for species with a minimum length size, as that requires measuring of the catch (Mallison & Cichra, 2004). Prestige bias might have influenced the catch data to some degree. One fisher reported a catch significantly higher than all other catches (15 kg), which led to a CPUE estimation of 15.9 Nephrops per pot and haul, which is significantly higher than all other CPUE estimations.

In this study, all catch data was collected through phone interviews, except the one in Byfjorden. The landed catch in Byfjorden was inspected, and the fisher reported the release directly after the catch, as the reported catch was the same as the ones utilized for the Hg analyses. The catch in Byfjorden is validated, however, the small sample size for several locations makes the CPUE calculations vulnerable to possible outliers. Additionally, some inaccuracy is related to the conversion of the catch from kg to number of Nephrops caught, but this inaccuracy is assumed to be limited as there is no reason to believe that data from the Norwegian reference fleet (length-weight key, Appendix VI) is not comparable to our data.

4.4.2 Questionnaire

The primary challenge regarding food frequency questionnaires is an overestimation of consumption rates. If the questionnaire contains too many questions, the respondents might overestimate or get tired, which is called "respondent fatigue" (Hess et al., 2012). Additionally, the questions might be difficult to understand, or the survey might not contain an answer that fits their situation. However, respondent fatigue should not be of vital importance in this project as the questionnaire only included 20 questions. It was attempted to keep the questionnaire as short and precise as possible. Furthermore, the respondent did not have to answer the questionnaire on their own but had the possibility of asking questions and providing options that fit better with their situation if the option was not already available.

Overestimations in food frequency questionnaires can especially occur if the food item in question is considered healthy, and seafood is most likely considered healthy by the respondents (Birgisdottir et al., 2008). The term is called social desirability bias or social approval bias, where respondents answer to obtain approval or to avoid criticism (Kowalkowska et al., 2013). On the other hand, over-reporting is more frequent when asked about consumption frequency for several species and seafood products, as recall leads to overreporting on low intakes (Gersovitz et al., 1978; Madden et al., 1976). Estimating food frequency using summary questions like in the present study, have shown a strong correlation with estimations using more detailed questionnaires. Markhus et al. (2013) reported a strong correlation between seafood consumption frequencies from summary questions and more detailed food frequency questionnaires when accounting for over-reporting in the detailed questionnaire by using the lowest or middle point value. The uncertainty associated with the reported seafood consumption frequencies should therefore be minor. However, a question regarding fish consumption other than for lunch or dinner would have increased the confidence in the estimates even further, as several fishers reported eating crab or other seafood for example in the evenings, as a snack before bed (*kveldsmat*). This information was not used for the risk assessment, as just a few fishers gave the information by their initiative and not all participants were explicitly asked. In hindsight, information about fish consumption of consumed fish that is self-caught should have been included in the questionnaire, as that would have been useful when selecting fish species for the risk assessment.

4.4.3 DMA-80

The measured content of total Hg in the certified reference material Tort-3 was rather low, but acceptable in this study because the mean value of the reference material was within two standard deviations. Certified reference materials are used to assess the accuracy of a method as the concentration of a pollutant in the reference material is known to be within a certified range (Harris, 2010).

Another evaluation of the accuracy of the result is the methods measurement uncertainty (Menditto et al., 2007). The measurement uncertainty for the DMA-80 is 20% and provides information about how reliable the results are. In theory, the measured values can be 20% higher or 20% lower. However, as the results show relatively low Hg concentrations this uncertainty is of limited importance for the risk assessment. Furthermore, the measurement uncertainty of 20% is dependent on the homogeneity of the sample (NIFES, 2015). Homogenization is another aspect that possibly can contribute with uncertainty when using DMA-80. Poor homogenization can result in greater variability in measurements from the same sample (Bloom, 1992). Therefore, the samples were homogenized thoroughly, and considerable effort was spent to ensure that the weighed-in samples were as homogenous as possible, to not further add uncertainty to the measurement.

4.4.4 Risk assessment

As this study did not provide information about consumption rates of other seafood species, there is uncertainty with the estimates. Additionally, Hg concentrations from *sjømatdata.no* were used for the intake estimations of fish for dinner and lunch, instead of conducting analyses on other species captured by recreational fishers as well. Only the consumption rate of fish for dinner and lunch was obtained in this thesis, which leads to a large degree of uncertainty associated with the intake calculations as no information about consumption patterns for fish species or Hg concentrations in the consumed fish species are available. However, the goal was to evaluate if the recreational fishers were at risk for exceeding the TWI for MeHg by consumption of Nephrops, and additionally assess if they might be a highly exposed subgroup of the population with increased intake of seafood. Using the obtained data, it is not possible to provide accurate estimates of total Hg intake from all seafood. However, both mean and high values for fish for dinner were used, which provides information that can help recreational fishers assess their approximate Hg intake based on their intake of self-caught fish and possible intake of fish species known to be high in Hg around Bergen, such as tusk and ling (Måge & Frantzen, 2008).

Standard portion sizes were used in the risk assessment estimations to simplify the interview and because studies show that respondents have difficulties estimating accurate portion sizes themselves (Cade et al., 2002). A low degree of uncertainty is assumed to result from the standard portion size.

4.5 Conclusions and recommendations

Recreational fishing represented 18%-60% of all registered buoys in the surveyed fjords, while recreational fishing for Nephrops represented 24%-100% of recreational fishing, which indicates that this fishing is popular and widespread in Hordaland. The catches of Nephrops are relatively high (mean CPUE 2.5 Nephrops per pot and haul), and recreational fishers consume on average seven Nephrops, every other week. Hg concentrations in Nephrops were generally low, but 23 of 235 Nephrops exceeded 0.2 mg/kg (ww), which is above the limit for pregnant and lactating women (VKM, 2006). Consumers should be aware that female Nephrops have higher Hg concentrations than male Nephrops of the same size. Sex of species is usually not a part of fish consumption advisories, however, knowledge about how sex affects Hg concentrations might help recreational fishers assess Hg exposure risk, as shown in the present study. A difference in Hg between the locations was also observed, but there is no need for location specific guidelines due to the generally low Hg concentrations. Even though consumption rates are high, the recreational fishers in this study are not at risk of exceeding TWI for MeHg from consuming Nephrops. However, the risk of exceeding TWI for MeHg cannot be dismissed for high consumers of seafood. Seventy percent of recreational Nephrops fishers ate fish for dinner 2-3 times per week, which indicates that they are part of a group of high consumers of seafood. Information about consumption rates for fish species would make it possible to assess the risk of exceeding TWI for MeHg from total seafood intake more accurately. Future studies should attempt to evaluate the risk of exceeding the TWI for MeHg for larger groups of recreational fishers, possibly by conducting hair Hg analyses, as this study supports findings that suggest that recreational fishers might be a exposed subgroup with high consumption of seafood. Information about species-specific consumption rates, amount of self-caught fish consumed, fishing areas, perception of contaminants and knowledge regarding dietary advice is recommended for assessing risk in future studies.

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6. Appendices

Appendix I: Maps of surveyed polygons



Figure 1.A Map of polygons surveyed in Radfjorden (1178 and 1242), and one polygon close to Knarvik (1796, north of Bergen). Pink color for the polygons that were included in the final sample, and green color for the polygon not included in the final sample.



Figure 1.B Map of polygons surveyed in Hauglandsosen (1421, 1019 and 1627), and one polygon in Byfjorden (1361). Pink color as they were included in the final sample.



Figure 1.C Map of polygons surveyed in Raunefjorden (1358 and 1122), and polygons in Fanafjorden (1250, 1014 and 1267). Pink color as they were included in the final sample.



Figure 1.D Map of surveyed polygons in Bjørnafjorden, south of Bergen. Green color as they were not included in the final sample.



Figure 1.E Map of surveyed polygons in Austevoll, south of Bergen. Pink color as they were included in the final sample.

Appendix II: General questionnaire for the recreational fishing survey in Norwegian

Spørreskjema fritidsfiske faste redskaper

ID:

Posisjon: Dato registrert: Navn: Adresse: Telefonnummer: Oppringt dato: Type redskap: Antall av redskap: Beskrivelse av redskap (for eksempel type garn): Når ble redskapet trukket sist: Hvor lenge hadde det da stått i sjø (soaking time): Totalt hvor mange blåser har du til redskapet:

Art: Antall beholdt: Antall sluppet ut:

Appendix III: Questionnaire for the recreational Nephrops fishers in Norwegian

<u>Er du:</u>			a) Kvinne	b) Mann	c) Ønsker ikke oppgi		
Hvilke	t land er du født i?						
Hvilke	t land bor du fast ia	?					
Hvor g	ammel er du?						
Hvorfo	or fisker du sjøkrep	s?					
a)	Matauk	b) Rekreasjon	, hobby, sosiali	sering			
C)	Tradisjon	d) Annen årsa	k: spesifiser gje	erne.			
<u>Hvor n</u>	nange år har du fis	<u>ket sjøkreps?</u>					
Hvor n	<u>nange sjøkreps-fisk</u>	eturer har du i lø	pet av de siste i	12 månedene?			
Fisker	du utelukkende i o	mrådet hvor vi fa	nt blåsen?				
a)	Ja b) Hvis nei	; mer enn 10 km ur	nna, mer enn 20	km unna, 30 ki	n eller mer		
HVIIKE	<u>t redskap bruker d</u>						
Hvor n	nange teiner har du	<u>i ute?</u>					
Hvor le	enge har redskapet	statt ute?					
Hvor n	nange overflateblås	er er festet til red	skapet?				
<u>Siste</u> fa	angst?						
Art]	Landet	Sluppet u	ut	Hvorfor sluppet ut?		
Minimum lengdemål							
					TS – too small		
	TM – too many						
	TB – too big						
	Females with roe						
	·						

Har	du pla	aner (om å	spise	he	le/d	eler av fangsten?	a) JA	b) NEI	c) Vet ikke
Hvis	JA, h	nvor n	nange	e sjøl	kre	ps s	<u>piser du i gjennom</u>	ısnitt?		
	0									

Hvor ofte spiser du selvfisket sjøkreps?

- a) En gang i uken (omlag 50 ganger i året)
- b) Flere ganger i uken (Hele året)
- c) Flere ganger i uken (I sommerhalvåret)
- d) To ganger i måneden (omlag 24 ganger i året)
- e) En gang i måneden (omlag 12 ganger i året)
- f) Færre enn 12 ganger i året

Hvilke deler av sjøkrepsen har du planer om å spise? (Kryss av alle alternativene som gjelder)

- a) Klør
- b) Hale
- c) Brunmat

HVA MENER DU OM FORURENSNINGSGRADEN OG SPISELIGHETEN AV

SJØKREPSEN I OMRÅDET DU FISKER? Sett ett kryss i en skala fra 1 til 9, hvor 1 er Sjøkrepsen er ikke forurenset i det hele tatt og 9 Sjøkrepsen er ekstremt forurenset og uspiselig.

1 Sjøkrepsen er ikke forurenset i det hele tatt

- 2
- 3
- 4

5 Sjøkrepsen er noe forurenset, men trygg å spise 1-2 ganger i måneden for de som ikke er gravide/ammende

6 7

8

9 Sjøkrepsen er ekstremt forurenset og uspiselig

0. Vet ikke

Hvor ofte har du spist fisk, fiskeprodukter eller annen sjømat som måltid de siste tre månedene? (Kun ett kryss mulig på middag og ett på lunsj).

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1 gang/uke	2-3 ganger/ uke	4 ganger eller mer/uke
Middag						
	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1 gang/uke	2-3 ganger/ uke	4 ganger eller mer/uke
Lunsj						

Er det noe sjømat du ikke spiser som følge av miljøgifter?

Appendix IV: Determination of sex



To the left a female *Nephrops norvegicus* with oviducts visible at the basis of the third pereiopod. To the right a male *Nephrops norvegicus* with the paired opening of the vasa deferentia at the basis of the fifth pereiopods.

Appendix V: Length-weight key



Plot of carapace length and weight data for Nephrops data from the Norwegian reference fleet. The plot was utilized to estimate mean weight for use in converting catch from kg to number of Nephrops in this thesis.

Appendix VI: Statistical R codes

6.1# Linear mixed effects model

fit1.lme <- lme(kvikksolv~CL*Sex, random=~+1|Location3, data=kreps.df) anova(fit1.lme)

	Df	F value	P value
CL	224	0.2093	0.6878
Sex	224	431.2478	<.0001
CL:Sex	224	86.8701	<.0001

6.2# Linear model including all interactions fit1.lm <- lm(kvikksolv~Location3*CL*Sex, data=kreps.df) anova(fit1.lm)

Output:

	Df	F value	P value
Location3	7	36.6853	< 2.2e-16
CL	1	0.0774	0.78114
Sex	1	517.2692	< 2.2e-16
Location3:CL	7	1.9595	0.06214
Location3:Sex	6	14.1057	1.995e-13
CL:Sex	1	60.4845	3.553e-13
Location3:CL:Sex	6	1.2582	0.27825

6.3# Linear model with only significant interactions

fit2.lm <- lm(kvikksolv~Location3*Sex+CL*Sex, data=kreps.df) anova(fit2.lm)

Output:

	Df	F value	P value
Location3	7	34.393	< 2.2e-16
Sex	1	392.632	< 2.2e-16
CL	1	100.845	< 2.2e-16
Location3:Sex	6	12.920	1.752e-12
Sex:CL	1	58.730	5.880e-13



6.5# Diagnostics plot of the linear model with log transformed data



	Df	F value	P value
Location3	7	38.0385	< 2.2e-16
Sex	1	325.5610	< 2.2e-16
CL	1	116.3186	< 2.2e-16
Location3:Sex	6	3.9122	0.0009843
Sex:CL	1	19.3765	1.68e-05

6.6# Output for; lm(log(kvikksolv)~Location3*Sex+CL*Sex

6.7# Tukey test: which locations are different in mean Hg concentration Which locations differ females: sub.df <- subset(kreps.df, Sex=='F') fit2.lm <- lm(kvikksolv~Location3, data=sub.df) library(multcomp) mc <- glht(fit2.lm, linfct = mcp(Location3="Tukey"), data=sub.df) summary(mc)

Which locations differ males: sub1.df <- subset(kreps.df, Sex=='M') fit2b.lm <- lm(kvikksolv~Location3, data=sub1.df) library(multcomp) mc <- glht(fit2b.lm, linfct = mcp(Location3="Tukey"), data=sub1.df) summary(mc)

6.8# Testing for correlation between the participants perception on the contamination status of the Nephrops in their area, education and age (separately). fit.glm <- glm(Perception~Age, family=quasibinomial, data=utd.df) anova(fit.glm, test="F")

fit.glm <- glm(Perception~Education2, family=quasibinomial, data=utd.df) anova(fit.glm, test="F") fit.glm <- glm(Perception~Education3, family=quasibinomial, data=utd.df) anova(fit.glm, test="F")





Category

Unidentifiable

Commercial fishing Other types of recreational fishing Recreational Norway Lobster fishing

Figure 7.1 Byfjorden survey results round 1. The white X is the fishing locations in Byfjorden.



Figure 7.2 Hauglandsosen survey results round 1.



Figure 7.3 Fanafjorden survey results round 1. The two white Xes are the fishing locations in Fanafjorden named; Inner and Outer station.



Figure 7.4 Raunefjorden survey results round 2. The white X is the fishing location in Raunefjorden.

Other types of recreational fishing Recreational Norway Lobster fishing

Unidentifiable

Round 3



Figure 7.5 Radfjorden survey results round 3. The white X is the fishing location in Radfjorden.



Category

Commercial fishing

Unidentifiable

Other types of recreational fishing Recreational Norway Lobster fishing

Figure 7.6 Hauglandsosen survey survey results round 3. The three white Xes are the fishing locations in Hauglandsosen. The locations were treated as two locations in the analysis due to distance to known contamination; Hauglandsosen Ågotnes (the two Xes to the left), and Hauglandsosen Hetlevik (one X to the right).



Category

- Commercial fishing Not possible to register

- Other types of recreational fishing Recreational Norway Lobster fishing .
- Unidentifiable

Figure 7.7 Austevoll survey results round 3. The three white Xes are the fishing locations in Austevoll. However, due to low assessed contamination, the locations were treated as one.



Figure 7.8 Fanafjorden survey results round 3.