Abstract

Mercury is a toxic heavy metal that is found to bioaccumulate and biomagnify in food webs across ecosystems. Monitoring of mercury and creating background levels for different areas is an important part of conservation and understanding possible drivers behind increased mercury concentrations and potential risk. Selenium is found to have the ability to alleviate the toxic effects of mercury and monitoring selenium and mercury together as well as their molar ratio, can assess the potential risk of negative effects from mercury. Here I assessed the concentration of selenium and mercury and the molar ratio between them in liver and fur samples in mink from Innlandet, Norway. Additionally, data that served as a proxy for forestry intensity, and the amount of forest and water in proximity to the capture site, was collected and generalized linear models and generalized additive models were fitted to explore the potential effects on mercury levels in mink. 56 mink were collected from the valleys Østerdalen, Glomdalen and Gudbrandsdalen between 2022 and 2024. The mean mercury across all valleys in liver was found to be 4.33 µg/g dw (SD ± 5.39) with a range 0.31 – 28.94µg/g dw. In fur samples, the mean was 19.02µg/g dw (SD ± 22.12) with a range of 1.57 µg/g dw 123.18 µg/g. No significant differences between area or sex were detected. For selenium, a significant difference between Østerdalen and Glomdalen was detected and the overall mean in the liver was 2.76 ± 1.08 µg/g dw with a range of 1.40 – 7.88 µg/g dw and fur had overall lower concentrations with a mean of 0.99 ± 0.37µg/g dw and a range of 0.55 µg/g dw– 2.33 µg/g dw. the mean hepatic molar ratio detected was 3.29 but showed a large range between 0.55 to 12.23. no significant results were found associated with the collected forestry or habitat for hepatic samples, for fur samples the amount of forest cover was a significant predictor though the model showed poor explanatory power. Most mink in Innlandet do not have mercury levels associated with negative health effects, but a substantial part of the population (up to 30 %) could have symptoms related to chronic mercury toxicosis, because of high mercury or low selenium concentrations. This study is therefore a contribution to reference levels of mercury in wild mink from Innlandet and proposes that future monitoring should include seasonal, dietary and environmental variables in future studies to possibly understand the large variability in mercury exposure found in mink from Innlandet.

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Contents

Introduction

Mercury (Hg) is a toxic heavy metal that is monitored due to its possibility to bioaccumulate and biomagnify in food webs across ecosystems. Hg is a natural part of the earth's crust and emitted by volcanic activity contributing to naturally low concentrations in the environment, with certain geological areas having naturally higher concentrations. Because of Hg's properties, it is used in a wide range of products ranging from thermometers to larger industry and mining activities (Driscoll et al., 2013). Hg emissions can be transported long distances with air currents and deposited in water and forest habitats far away from where it was emitted (Pacyna et al., 2010). While Low-level exposure to Hg is at current times inevitable (Clarkson & Magos, 2006; Syversen & Kaur, 2012), small concentrations, of elemental or organic Hg are not generally associated with significant health risks (Ralston & Raymond, 2018). However, Hg does not have any known biological function in the physiology of warm-blooded animals. As a result, accumulation or high exposure can affect the nervous, immune, and reproductive systems, particularly the central nervous system, leading to symptoms ranging from mild cognitive and sensory impairments to severe motor dysfunctions and death (Lian et al., 2021; Ralston et al., 2007; Scheuhammer et al., 2015). Despite reductions in many regions, global anthropogenic Hg emissions remain with an estimated 2,220 tons emitted annually (UN Environment, 2019).

There is increasing evidence that certain micronutrients play an important role in mitigating the toxicity of heavy metals (Aaseth et al., 2020; Li et al., 2014; Peraza et al., 1998). In particular, studies on Hg toxicity show how Selenium (Se), an essential element involved in several protein functions, could have a protective role against toxic effects from Hg when total Se concentration ([TSe]) are relatively higher than total concentrations of Hg ([THg]) (Berry & Ralston, 2008; Bjørklund et al., 2017). Hg binds to Se with a strong affinity, and when [TSe] exceeds [THg], this excess can potentially mitigate Hg's toxic effects, though the exact mechanisms behind this effect are not yet understood. Ralston and Raymond (2018) suggest that the methylated Hg (MeHg) binding to Se inhibits selenoenzymes that normally prevent and reverse oxidative damage, this binding induces a conditioned Se deficiency, reducing the body's ability to manage oxidative stress. Excess of Se should therefore alleviate this toxic effect of Hg by compensating for this deficiency. Se is naturally occurring nutrient found in soils and bedrock, however, many regions in Europe including Norway is considered Se poor (Christophersen et al., 2013; X. Wu & Låg, 1988), which increases the risk of Se deficiency and, consequently, the toxic effects of Hg.

Forests, especially coniferous forests can capture and store Hg deposited from the atmosphere in soils (Graydon et al., 2008). Disturbances of forest soils by logging, can alter catchment hydrology, increase erosion and nutrient runoff, and possibly Hg concentrations (Kuglerová et al., 2021). In Norway, Forestry is a major industry and clear-cutting accounting for approximately 70% of logging practices (Landbruksdirektoratet, 2023). Previous studies in Scandinavia have shown that forestry practices can influence Hg contents in waterbodies located near recently logged sites (Bishop et al., 2009; Porvari et al., 2003). However, responses to forestry harvests and Hg concentration in both water and biota seem to vary and drivers behind the effect are poorly understood (de Wit et al., 2014). Innlandet County is one of the most forestry-intensive regions in Norway (SSB, 2021), but there is limited research investigating Hg runoff from industrialized forestry and no prior studies have specifically investigated this issue in Norway. Local industries such as pulp and paper mills, as well as wastewater treatment facilities along the two larger rivers in Innlandet, Gudbrandsdalslågen and Glomma have contributed to contamination in Lake Mjøsa and have previously been found to have relatively high levels of Hg. (NIVA, 2020). Given the possible low availability of Se in soils of Norway, combined with the extensive watershed of the two rivers, ecosystems, wildlife and human health in the region may be particularly susceptible to the harmful effects of Hg runoff from increased Hg runoff from forestry if this is transported into the terrestrial ecosystems and accumulates.

Monitoring Hg is an important part of conservation, and the potential risk of Hg for ecosystem and human health is only predicted to increase with a changing climate due to increased precipitation and thawing of contaminated glaciers and sea ice (Kozak et al., 2021; Stern et al., 2012). Therefore, establishing Hg concentrations and exposure in terrestrial and aquatic food webs, and identifying potential drivers of Hg runoff are important for human, wildlife, and ecosystem health. Relatively few studies are focusing on Hg and Se ratios in terrestrial systems, and studies from Se-deficient areas are needed (Kalisinska et al., 2017).

Monitoring Hg exposure in rivers and lakes by measuring Hg in water can be difficult due to the relatively low concentrations of inorganic Hg. However, anaerobic bacteria in aquatic ecosystems methylate Hg into methylmercury (MeHg), which is the most bioavailable form of Hg. MeHg now biomagnifies in the food chain, where concentrations can reach a million times higher in the apex predator than the surrounding water (AMAP, 2021). Innlandet's river systems are home to diverse fish populations, including carnivorous species like pike (*Esox lucius*) and brown trout (*Salmo trutta*) both of which are commonly harvested for human consumption and bioaccumulate and biomagnify mercury as long-lived apex-predators (VKM, 2019). Due to this biomagnification, apex predators are often used as sentinel species to measure Hg exposure (Basu et al., 2007; Kalisińska et al., 2019).

The Mink (Neovison vison), a small semi-aquatic carnivore, is commonly used as a sentinel species for Hg levels in the environment (Basu et al., 2007; Yates et al., 2005). Mink are mostly territorial (Dunstone, 1993; Palomares et al., 2017; Yamaguchi & Macdonald, 2003), widespread, and have little migration behaviour, and offers a localised glimpse into the contamination, bioavailability, and biomagnification of Hg and other nutrients in certain areas. The home ranges of mink might vary depending on habitat quality, such as food availability and competition (Halbrook & Petach, 2018; Yamaguchi & Macdonald, 2003). Although no specific study has looked at mink's home ranges in Norway, Innlandet rivers, which are rich in fish, likely provide good food access, even if competition with returning otter populations (NINA, 2020) may influence mink behaviour. Since the primary source of MeHg to mammalian species is through fish consumption (Clarkson & Magos, 2006), with the mink diet that can consist of up to 60% fish (Kalisinska et al., 2017), as well as sharing a sensitivity to Hg toxicity similar to humans, mink provide a good basis for examining the Hg exposure of mammals and possibly humans in their environment (Basu et al., 2005 a, 2005 b).

This study had three main objectives. The first and second were to assess the concentration and ratio between Hg and Se in a terrestrial and semi-aquatic carnivore and determine if these values vary across different regions within Innlandet. The Hg concentration serves as an indicator of biomagnification in the food web in this area, while the ratio with Se can predict if Hg is affecting the animals negatively or if they have an excess of Se to mitigate toxic effects. Two tissue samples were selected for analysis, fur, which can reflect long-term bioaccumulation of mercury, and liver, which provides insight into more recent exposure. The results from this study will be compared to two risk thresholds created by The Arctic Monitoring and Assessment Programme (AMAP) in 2011 and 2021.

The third objective was to explore whether Hg concentration related to forestry intensity and site-specific conditions of forest and waterbodies in the home ranges of mink. Forestry variables included the volume of trees removed (m^3), dominating tree species and the percentage of forest surrounding the mink. Habitat variables considered potentially influential for Hg concentrations were the distance to the nearest waterbody as well as the percentage of water in proximity to the mink.

Methods

Study area and sampling

Data were collected from Innlandet County, Norway, one of the few landlocked regions in the country. Innlandet is the most forestry-intensive area In Norway and has approximately 25, 900 km^2 of forest, covering around 50% of the total land cover (SSB, 2024). The geology in the county varies with some regions rich in clay and nutrient-rich sediments. However, the region is dominated by nutrient-poor soils that erode slowly, providing limited nutrients such as selenium to the surrounding environment and water (SNL, 2024)

The animals for this study were collected from three valleys within Innlandet: Østerdalen, Glomdalen and Gudbrandsdalen (see appencix C for map). Østerdalen and Glomdalen share a large proportion of the watershed of Norway's largest river Glomma. Østerdalen in the North and Glomdalen in the South are divided by the city Elverum in Innlandet. Glomma's watershed covers an area of 41 970 km^2 and runs 625 km from the Swedish border near Røros to Lake Øyeren before flowing into the ocean. The neighbouring valley Gudbrandsdalen, has the river Gudbrandsdalslågen which runs for 202 km with a watershed of 12 508 km^2 and drains into Norway's largest lake Mjøsa, before joining Glomma south of the lake.

Study population

Between 2022 and 2024, mink were collected from local trappers through a collaboration with the Norwegian Hunting and Anglers Association (NJFF) in Innlandet County. Considered an invasive species in Norway, mink are subject to year-round harvest. A total of 56 mink were collected from the three valleys: Østerdalen (n = 24), Glomdalen (n = 23) and Gudbrandsdalen (n = 9). For each animal GPS coordinates were recorded. The mink were stored in plastic bags and kept frozen at – 18 °C until necropsies. Most mink were caught in traps, a few were obtained after being killed by cars or dogs.

Necropsy procedures

The Animals were processed at the laboratory of Inland University of Applied Sciences. Each specimen was thawed before being weighed, sexed, and measured (length of animal and chest girth). Chest girth was measured around the thorax caudal to the front legs (measure tape under front legs into axilla), while total length was measured from the nose to the tip of the tail along a curved dorsal line. The condition of the animal was decided and put into three categories for both fat and muscle status. For fat, each animal was assessed and categorized into either "abundant fat/no atrophy", "reduced fat reserves" or "fat atrophy". If the subcutaneous fat was clearly visible it was considered in good condition, if the animal had reduced subcutaneous fat but still had omental fat deposits it was categorised with "reduced fat reserves" and lastly, if organ fat was visible (no/minor deposits on kidneys) it was considered with "fat atrophy".

Muscle condition was similarly categorized based on signs of muscle concavity, protruding bones or underdevelopment around the thighs and chest. Animals with no signs of decreased muscle mass were categorized as being in "good condition". If muscle appeared smaller and softer than normal, and the rib cage and spine could easily be felt through the skin, the animals were placed in the "moderate condition", category. Animals showing an emaciated appearance, with prominent rib cage and spine with sunken flanks, were categorized as having "atrophy".

Two tissues were collected from each mink:

- Fur sample: A caudo-dorsal fur sample collected with a clipper from the lumbar region of the back.
- Liver sample: The entire liver was harvested.

All samples were placed in sealed Whirl-Pak® bags (7.5 x 18.5cm 4 oz), refrozen at – 18 °C and subsequently sent to ALS Scandinavia AB in Luleå Sweden for mercury and selenium concentration analysis.

Laboratory analysis

Elemental concentration analyses were conducted at ALS Scandinavia AB Luleå, Sweden. Upon arrival, all samples were given a unique code to facilitate blind handling. Sample preparations took place in cleanroom environments rated as 10 15,000, personnel adhered to clean room attire protocols as well as contamination control measures (Rodushkin et al., 2010), were implemented. The Nitric acid (HNO3, Sigma-Aldrich Chemie Gmbh, Munich, Germany) used in the analysis was of Suprapur purity, and the water was de-ionized Milli-Q water (Millipore, Bedford, MA, USA) treated by reverse osmosis and passed through ion-exchange filters. For dilution purposes, the water was further purified with sub-boiling distillation in Teflon stills. Prior to sample preparation, all laboratory materials were immersed in in 0.7 M HNO3 for 24 hours at room temperature before it was rinsed with Milli-Q water.

The digestion of samples was performed using a MARS 5 microwave digestion system (CEM Corporation, USA). Each 0.5g sample was placed into a Teflon vial along with 5mL of HNO3. The vials together, with method blanks included for validation, were sealed and arranged into a carousel, which held up to 40 samples in numbered slots. The entire carousel was inserted into the microwave digestion unit and underwent a digestion cycle consisting of 30 minutes temperature increase up to 170̊C and held for another 30 minutes at this temperature. After digestion, samples were diluted with 10mL with deionized water, and a further dilution using 1.7M HNO3, achieving an overall dilution factor of approx. 400m/v

To determine the concentrations of the 71 elements an ICP-SFMS system (ELEMENT XR, Thermo Scientific, Bremen, Germany) was used. The system was equipped with a demountable quartz torch, a 1.5 mm i.d. sapphire injector, and a platinum capacitive de-coupling shield, along with a nickel sampler cone, high-sensitivity 'X-type' skimmer cone, and a PFA spray chamber with dual gas inlet. A micro-concentric PolyPro nebulizer and FAST SD2 auto-sampler (ESI, Perkin-Elmer, Santa Clara, CA, USA) with a six-port valve and a 1.5 mL sample loop (filled and rinsed by vacuum suction) were used for sample introduction. Further, methane was added to the plasma to limit the creation of oxide-based spectral interferences, enhance sensitivity for elements with first ionization energies, and mitigate matrix effects (Rodushkin et al., 2005). The matrix effect was further corrected adding 2.5 µg/l concentration of Indium to all measurement solutions.

Detection limits (LOD) were determined as three times SD of the element concentrations found in the preparation blanks ($n > 10$), with values below 0.002 mg/kg for Hg and below 0.04 mg/kg for Se. Quality control was performed with certified reference material (Dorm-2, Dogfish muscle from National Research Council of Canada, Ottawa, Ontario and a sample of Spirulina powder from own lab) The recovery of Hg an Se for the controls ranged from 95% to 108%.

Forestry and habitat variables

Forestry and habitat predictors were collected from two open data repositories: Kilden managed by Norwegian Institute of Bioeconomy Research (NIBIO) (NIBIO, 2023) and The Norwegian Water

Resources and Energy Directorate (NVE) (NVE, 2024). Data extraction was performed using R Studio software (R Core Team, 2022) version 4.2.1 using the “sf” (Pebesma E. & Bivand R., 2023) or “terra”(Hijmans, 2024) packages for spatial data manipulation.

Due to the uncertainty surrounding the habitat use of mink, with unknown habitat quality and competition, as well as only having the location of retrieval this could possibly not represent their actual home range. To account for this uncertainty and the unknown impact of forestry in the region, four circular buffers were created around each mink’s GPS location at increasing distances (1km, 3km, 5km, 10km). These buffers were designed to help determine the appropriate scale for the continued modelling of habitat factors. The forestry and habitat variables were extracted from within the varying buffers to determine if distance around the mink influenced the effect on mercury levels in the mink.

Data sourced from NIBIO were Skogressurskart (SR16 beta), SatSkog and Arealtypekart (AR50). From Skogressurskart we extracted data of volume forest removed over the past three years as a proxy for the amount of forestry within the buffers. This proxy was chosen due to the lack of knowing the exact year each mink was collected, as well as to quantify the amount of forestry over the time of the study. Given that more than 70% of forestry in Norway involves clear-cutting, this proxy was regarded to be able to capture potential impacts of these logged areas being of similar disturbance with the data of volume removed trees. Skogressurskart also provided information on the dominant tree species.

From the AR50 dataset we calculated the percentage of forest and water bodies within each buffer. Lastly two raster datasets form NVE, Elv (rivers) and Innsjø (lakes) made it possible to calculate the distance from each mink and the nearest body of water.

Statistical analyses

All statistical analyses were performed using R studio software (version 4.2.1). Mercury and selenium concentrations from ALS Scandinavia were reported in dryweight (dw) as µg/g. To investigate the molar ratio between selenium and mercury, we calculated µmol/L for each element using the atomic weight of mercury (200.59) and selenium (78.96) with the following formula:

$$Molar\ ratio\ (Se{:}Hg) = \frac{[THg]\ or\ [TSe]\ \times 1000}{Atomic\ Weight}$$

When studying Hg it differs if results are provided in dry weight (dw) or wet weight (ww). In this study dw concentrations were transformed to ww to allow comparison with a risk assessment for mammals and birds conducted by AMAP. The conversion from dw to ww were done using the following dry matter (dm) formula:

$$WW = DW \times \left(\frac{\%\ DM}{100}\right)$$

Although converting from dw to ww may cause the loss of some accuracy, as dry matter percentage was calculated individually for each sample, the loss of accuracy should be minimal.

The risk assessment created by AMAP used for hepatic samples was divided in the following categories: “no risk” (<4.2 µg/g ww), “low risk” (4.2–7.3 µg/g ww), “moderate risk” (7.3–22.7 µg/g ww), “high risk” (22.7–30.5 µg/g ww) and “severe risk” (≥30.5 µg/g ww) (AMAP, 2021). Fur samples were compared to the AMAP risk categories from 2011 (AMAP, 2011) with two suggested thresholds. A lower threshold of below 20 µg/g dw and a higher threshold of below 30 µg/g dw have been set as suggested thresholds for mammalian life, where symptoms of health responses to Hg may affect the animals

The Shapiro-Wilks test was used to determine if response variables were normally distributed. Since both [THg] and [TSe] in liver and fur deviated from normality ($W < 1$ and $p < 0.05$ see Table 1 for exact values), I continued with a Kruskal-Wallis (KW) test to analyse variance across the predictor variable Study area (Østerdalen, Glomdalen, Gudbrandsdalen) for [THg] and [TSe] in both fur and liver. Applied the same method to evaluate the molar ratio of Se and Hg.

In addition, a KW test was used to evaluate if there was a difference in [THg] based on dominating tree species (spruce and pine), and if there was a difference in forestry intensity based on the "volume removed" variable. For analyses where the KW-test proved significant, a post-hoc Dunn test was performed to distinguish the difference between the areas.

Finally, before modelling, a Spearman rank correlation test was used to determine whether there was a relationship between the [THg] in the different tissues.

Forestry and habitat modelling

All predictor variables were standardized using z-score standardisation (mean of 0 and SD of 1) to account for differences in scale. [THg]. Four Generalized linear models (GLMs) were constructed to compare the effect of different buffer sizes, to identify what buffer could have the best explanatory power. With one of the main objectives of the study being the effect of forestry, the predictor used for this selection were the proxy for forestry intensity "volume removed trees" and [THg] in the liver. Based on model performance, the buffer of 1 km was selected for the following analysis. All plots and visualisation of data were created using the "ggplot2" package (Wickham, 2016).

Using the "mgcv" package (Wood, 2017), we fitted a series of GLMs and Generalized additive models (GAMs) using [THg] in liver and fur as response variables, using a Gamma family with a log link. GLMs and GAMs were included to explore whether the relationship between predictor variables and mercury concentrations might be non-linear, as bioaccumulation, mercury deposition, or other habitat variables could have complex effects on [THg]. We examined the following predictors within the 1 km buffer; forest removed (m^3), distance to the nearest body of water (river or lake), percentage of forest and percentage of water.

Due to the size of the dataset, the knots in the GAMs were restricted to 3 or 5 to prevent overfitting to noise, and to simplify the models. One model was created for liver and fur samples that included a physiological trait of the mink and a forestry variable with the best fit to see if this added explanatory power. For hepatic [THg] the model was tried with body length and for fur to the best fitting predictor "percentage of forest

Missing data and outliers

When fitting the model with the proxy for forestry intensity, one outlier with extreme values were detected and removed to improve the model estimates and fit. Missing data were minimal in the dataset and in cases where missing values occurred in the response variables, the observations were excluded from the analysis. One mink was excluded from the habitat analysis due to missing GPS data, and another was excluded from [THg] analysis in the liver due to the inability to sample liver tissue.

Model diagnostics and selection

Model assumptions were checked using the “DHARMa” package (Hartig, 2022) for diagnostic plots including residuals vs. fitted values and Q-Q plots for normality. Model selection for all GLMs and GAMs were based on the Akaike Information Criterion corrected for small sample sizes (AICc), both because of relatively small sample (n =56) size and that comparisons between models with different numbers of parameters and complexity, AICc corrects to avoid overfitting. R^2 (coefficient for determination) and the models ability to explain the deviance in the response was used to evaluate the explanatory power of the models.

Initially, the method included fitting a GAMM (generalized additive mixed model) with the study area as a random effect, as some individuals were caught in the same trap. However, this approach was discarded when the KW test showed no significance across study areas for [THg] and with only three levels, the study area was deemed insufficient to capture meaningful variance and the effects of the individuals caught in the same trap were considered minimal.

Results

Descriptive statistics

Mercury

The mink were generally in good condition with 43 mink classified as having abundant fat reserves, 10 had reduced fat reserves and 2 showed signs of fat atrophy. In terms of muscle condition 46 mink were classified as being in good condition, and 9 in moderate condition.

No significant differences in [THg] were detected between study areas for either liver or fur samples *(*$p > 0.05$ see table 3 visualised in figure 1) nor were there differences between males and females (see table 3). The mean hepatic mercury concentrations across all valleys were 4.33 µg/g dw (SD ± 5.39) with a range between 0.31 – 28.94µg/g dw. Gudbrandsdalen had the highest mean and range THg (5.43 µg/g dw, SD ± 8.86, range 1.59–28.94 µg/g dw), while Østerdalen had a mean of 4.18 µg/g dw, and the lowest variability (SD ± 3.66). Glomdalen had similar mean [THg] of 4.06 µg/g dw, but with greater variability (SD ± 8.86).

The mean [THg] in fur was 19.02µg/g dw (SD ± 22.12), with values ranging from 1.57 µg/g in Glomdalen to a maximum of 123.18 µg/g in a female mink from Gudbrandsdalen with signs of recently having had kits. Østerdalen had the lowest mean [THg] in fur (14 µg/g dw, SD ± 9.04), but both Østerdalen and Glomdalen showed high variability, with SDs of 23.7 µg/g and 36.9 µg/g, respectively. (see Table 1 for additional details on dw concentrations and corresponding ww values). A significant positive relationship between [THg] in liver and fur was detected ($\rho = 0.584$, *p-value* = 0.2.91e-06)

Table 1 Summary statistics of [THg] with Mean, Median, SD values and range across all study areas and from each of the three valleys within Innlandet County. Values are given in both dw and ww

Studyarea	**n**										

		Mean (µg/g dw)	Mean (µg/g ww)	Median (µg/g dw)	Median (µg/g ww)	SD (µg/g dw)	SD (µg/g ww)	Min (µg/g dw)	Min (µg/g ww)	Max (µg/g dw)	Max (µg/g ww)
THg in liver											
All	55	4.33	1.30	2.47	0.739	5.39	1.64	0.31	0.09	28.9	8.60
Østerdalen	23	4.18	1.25	3.01	0.86	3.66	1.08	0.99	0.30	15.1	4.39
Glomdalen	23	4.06	1.22	1.94	0.64	5.35	1.69	0.31	0.09	22.1	7.11
Gudbrandsdalen	9	5.43	1.63	2.46	0.73	8.86	2.63	1.59	0.48	28.9	8.6
THg in fur											
All	56	19.02	14.05	14.90	10.39	22.12	16.6	1.57	0.80	123	97.31
Østerdalen	24	14.00	11.12	15.60	10.66	9.04	11.24	2.56	1.7	35.1	56.79
Glomdalen	23	20.5	14.67	13.59	10.76	23.7	14.66	1.57	0.8	111	58.39
Gudbrandsdalen	9	28.1	20.26	20.70	9.21	36.9	29.58	2.97	1.92	123	97.32

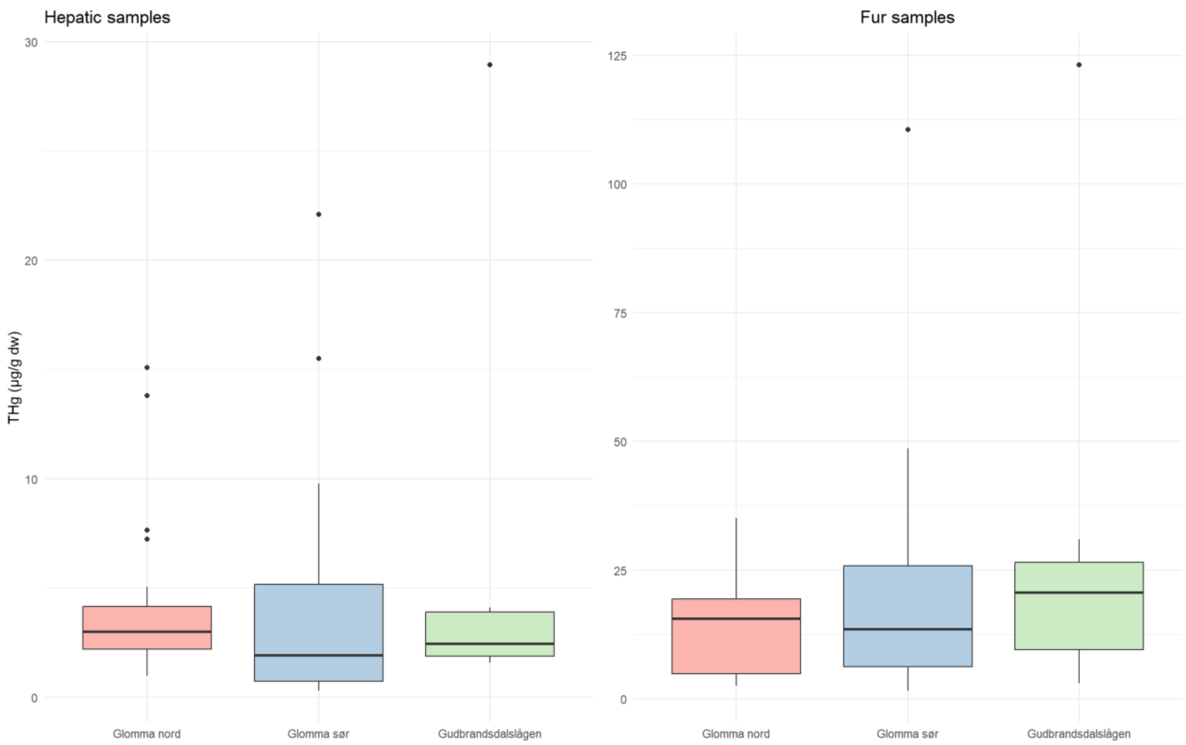


Figure 1 Boxplot with a side-by-side comparison of [THg] in hepatic and fur samples, note different scale on y axis. No significant differences between regions were detected by the KW test

Selenium

A significant difference in TSe was found between Østerdalen and Glomdalen for both liver and fur (*p-value* see table 3. The mean hepatic [TSe] across all valleys were 2.76 ± 1.08 µg/g dw with a large range from 1.40 – 7.88 µg/g dw (Table 2). Østerdalen had a mean of 2.94 ± 0.54 µg/g dw, Glomdalen the lowest mean 2.46 ± 1.05 µg/g dw, and Gudbrandsdalen the highest mean at 3.08 ± 1.89 µg/g dw.

[TSe] in fur were lower than those in liver but followed the trends with minor differences between the means. Across all study areas the mean was 0.99 ± 0.37µg/g dw and the range were from 0.55 µg/g dw– 2.33 µg/g dw. Østerdalen had the highest [TSe] mean in fur 1.14 ± 0.39 µg/g dw, Glomdalen had the lowest at 0.86 ± 0.20 µg/g dw and Gudbrandsdalen had a mean of 1.02 ± 0.53 µg/g dw.

Table 2 Summary statistics of Tse with Mean, Median, SD values and range across all study areas and from each of the three valleys within Innlandet County. Transformations into ww was not done for fur samples.

Study area	Mean (µg/g dw)	Mean (µg/g ww)	Median (µg/g dw)	Median (µg/g ww)	SD (µg/g dw)	SD (µg/g ww)	Min (µg/g dw)	Min (µg/g ww)	Max (µg/g dw)	Max (µg/g ww)
TSe in liver										
All	2.76	0.82	2.61	0.76	1.08	0.34	1.40	0.41	7.88	2.34
Østerdalen	2.94	0.88	2.97	0.89	0.536	0.16	1.89	0.51	4.38	1.27
Glomdalen	2.46	0.72	2.06	0.61	1.05	0.34	1.4	0.41	5.71	1.83
Gudbrandsdalen	3.08	0.92	2.33	0.71	1.89	0.56	1.92	0.55	7.88	2.34
TSe in fur										
All	0.99	-	0.92	-	0.37	-	0.51	-	2.33	-
Østerdalen	1.14	-	0.99	-	0.39	-	0.56	-	2.33	-
Glomdalen	0.856	-	0.82	-	0.20	-	0.51	-	1.35	-
Gudbrandsdalen	1.02	-	0.76	-	0.53	-	0.58	-	2.00	-

Figure 2 left: Boxplot visualizing Tse in fur measured in µg/g dw the differences and significant difference found between Østerdalen and Gudbrandsdalen in median values across the three valleys within Innlandet County. Right: Boxplot overlayed with the actual datapoints.

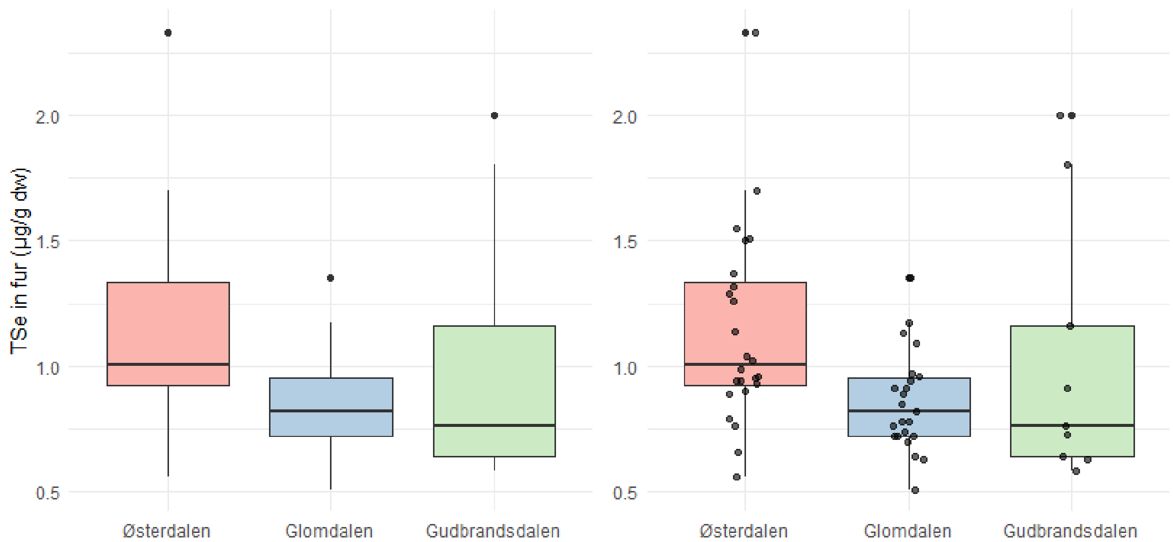
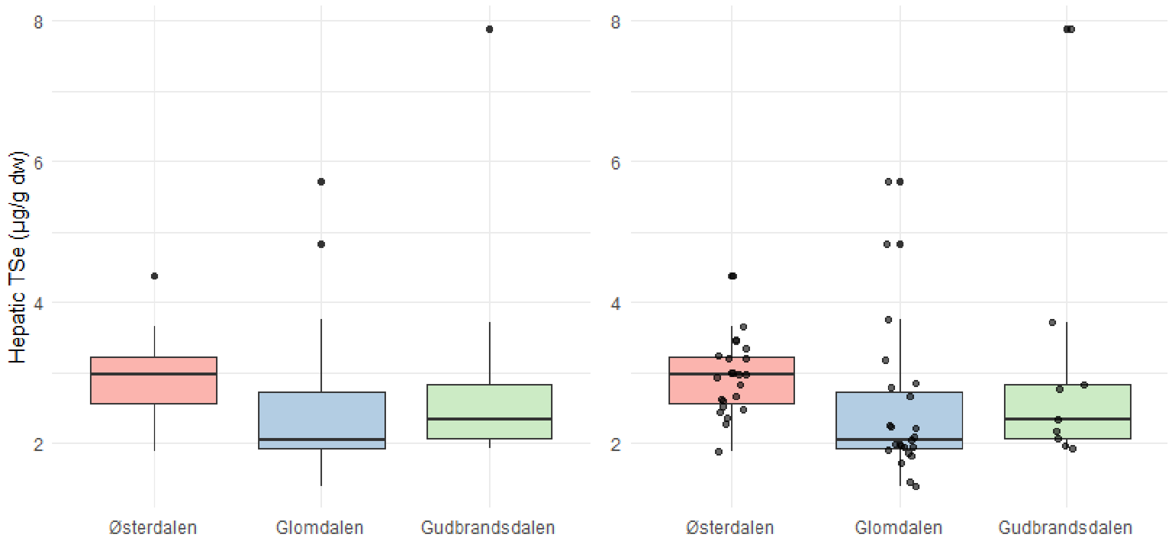


Figure 3 Boxplot visualizing hepatic TSe measured in µg/g dw the differences and significant difference found between Østerdalen and Gudbrandsdalen in median values across the three valleys within Innlandet County. Right: Boxplot overlayed with the actual data points



Mercury and Selenium modelling results

Table 3 All results from the Shapiro-Wilk test of normality for the response variables of this study. none of the responses were normally distributed, which resulted in the use of the KW test when running further analysis

Response	**W-value**	***p*-value**
Hg in liver	0.63869	2.233e-10
Hg in fur	0.62714	1.136e-10
Se in liver	0.78713	1.679e-07
Se in fur	0.87524	3.287e-05
Molar ratio liver	0.81349	7.228e-07
Molar ratio fur	0.76501	4.384e-08

Table 4 results from all KW tests with chi-squared, degrees of freedom, p-values and if the test proved significant

Kruskal-Wallis rank sum test	**Chi-squared**	**df**	**p-value**	**Significant**
Thgliver by studyarea	2.539	2	0.280	No
Thgfur by studyarea	1.207	2	0.546	No
THg liver by sex	0.003	1	0.950	No
Molar ratio by study area	0.737	2	0.691	No
Molar ratiofur by study area	3.074	2	0.215	No
TSeliver by study area	10.445	2	0.005	Yes
TSe fur by studyarea	8.425	2	0.014	Yes
TSe liver by treespecies	2.067	2	0.355	No
TSe in liver by sex	0.740	1	0.39	No

THgliver by tree species	1.039	2	0.594	No
THgfur by tree species	0.198	2	0.905	No
Volume removed by study area	19.463	2	5.937e-05	Yes

[TSe]:[THg] molar ratio

The mean hepatic molar ratio across all mink in Innlandet County was 3.29 (SD ± 2.53) with a wide range from 0.55 to 12.23 (Table 5). No significant differences in molar ratios were found between study areas (Table 4). The highest molar ratios were found in Glomdalen, where liver samples had a mean of 4.10 (SD ±2.75) and the highest observed ratio of 12.23. Østerdalen had a lower mean molar ratio of 2.79 (SD ±1.83) while Gudbrandsdalen had the lowest mean ratio (2.52±1.06) and had the smallest range.

In fur, molar ratios were generally lower compared to liver tissues. Østerdalen had the highest mean molar ratio in fur (0.36 ± 0.32), while Glomdalen and Gudbrandsdalen had slightly lower means at 0.25 ± 0.26 and 0.23 ± 0.30 respectively.

Table 5 Summary of mean, median, standard deviation and range of the molar ratios in both hepatic and fur samples between the three valleys within the study area.

Studyarea	Mean	Median	SD	Min	max
Liver					
All	3.29	2.53	2.65	0.55	12.23
Østerdalen	2.79	2.40	1.83	0.55	8.82
Glomdalen	4.10	2.75	3.52	0.65	12.23
Gudbrandsdalen	2.52	2.62	1.06	0.69	3.83
Fur					
all	0.29	0.17	0.29	0.03	1.13
Østerdalen	0.36	0.19	0.33	0.05	1.04
Glomdalen	0.25	0.14	0.26	0.03	1.13
Gudbrandsdalslågen	0.23	0.15	0.30	0.04	0.99

The spearman correlation test revealed a significant positive correlation between molar ratios in liver and fur ($\rho = 0.525$, $p = 4.881e\text{-}05$), indicating that higher molar ratios in liver tissues corresponded to higher molar ratios in fur.

When dividing hepatic molar ratios into categories, to use in risk assessment, a total of 18 mink (approximately 30% of our sample) had molar ratios below 2. Of these, a total of 7 individuals had ratios below 1. The mink in the lowest category (below 1) were evenly distributed between Glomdalen and Østerdalen with three individuals each, and then the last mink from Gudbrandsdalen. The largest group of mink (25 individuals) had molar ratios between 2 and 4, and 12 individuals had above 4.

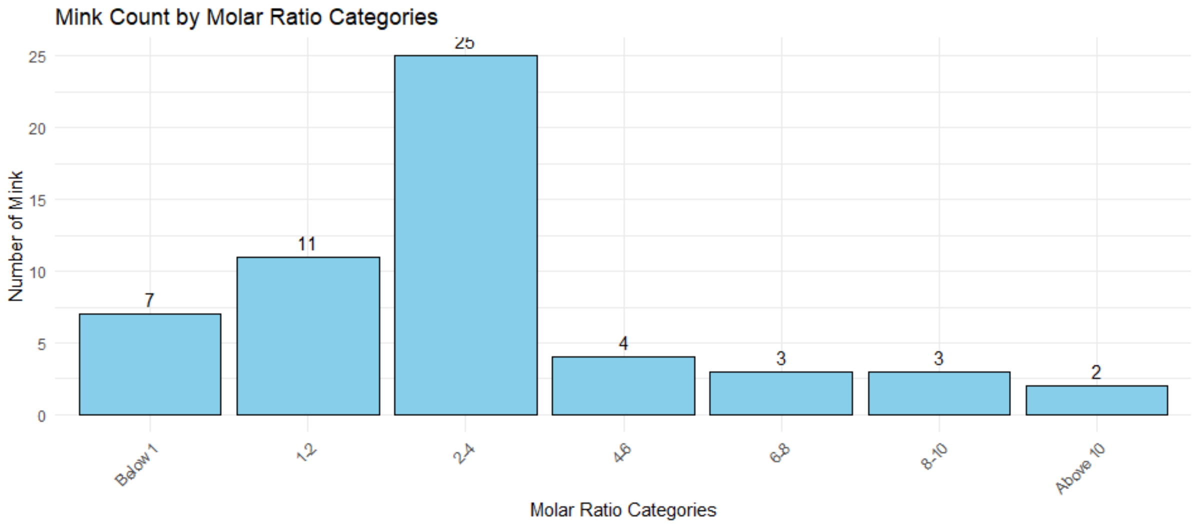


Figure 4 Histogram with molar ratios divided into categories and the number of individuals in each category. Molar ratios above 2:1 are considered a minimum to have enough Se to possibly alleviate toxic effects of Hg and have enough for other vital functions in the body.

Comparative risk assessment

Results from comparing the findings of [THg] with Hg thresholds created by AMAP from 2011 and 2018. The assessment from 2021 is for hepatic samples and result are in ww. AMAP 2011 were used for fur samples and results are given in dw.

AMAP 2021

Using the AMAP risk categories for hepatic Hg for Arctic biota, 50 of the 56 sampled mink were categorized as being at “no risk” for adverse health effects from mercury exposure based on liver [THg], as shown in Figure 5. Three animals fell into the “low risk” category, one animal was categorized as being at “moderate risk”, and lastly one individual could not be sampled.

In the “low risk” category, there were two males with a mercury concentration in the liver of 4.39 and 4.67 µg/g. The two animals with the highest value are both females. One female was collected in Elverum (Glomdalen) with 7.12 µg/g placing her in the “low risk” category. The last female, previously mentioned with higher [THg] in the liver was collected in Ringebu (Gudbrandsdalen) with 8.60µg/g ww placing her in the “moderate risk” category

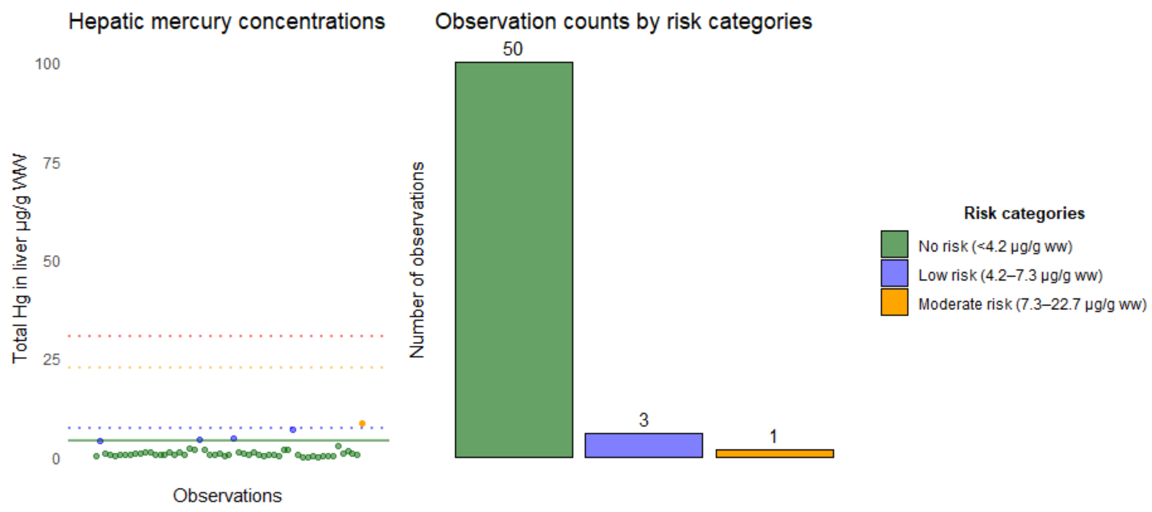


Figure 5 visualisation of hepatic samples categorized into AMAP's risk categories for wildlife. Left: Scatterplot for individual hepatic [THg] with colour signifying category and horizontal lines indicating the threshold between categories. Right: histogram visualising the number of individuals in each risk category. Higher categories were not included as no mink had higher concentrations.

AMAP 2011

Using the AMAP risk categories for Hg in fur samples two different thresholds were suggested. For the higher threshold of 30 µg/g dw, 48 out of the 56 sampled mink were below the threshold and 8 individuals having [THg] were considered to have negative health effects. When reducing the threshold to the lower limit of 20 µg/g dw this changed to 39 individuals being below this limit and 17 being above (Figure 6). The distribution of individuals above 30 µg/g dw were 5 from Glomdalen, 2 in Gudbrandsdalen and 1 in Østerdalen. With the lower threshold above 20(µg/g dw) Glomdalen had 7 individuals, while Gudbrandsdalen and Østerdalen had 5 each

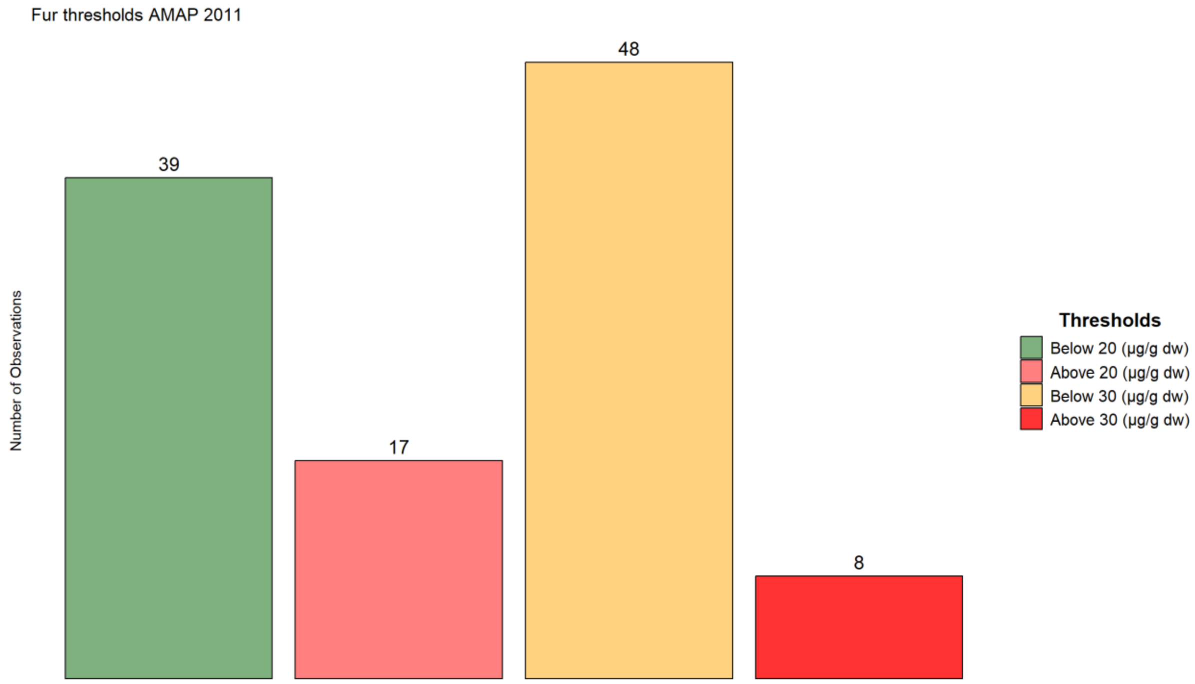


Figure 6 Barplot with the number of mink within each threshold category suggested by AMAP (2011) for any sublethal or negative health effects of Hg. Two lower thresholds were suggested. Mink can fall in both below 20µg/g dw and 30µg/g dw category.

Forestry and habitat

Forestry intensity

Data collection and analysis revealed a significant difference in forestry intensity between the three valleys, as indicated by the Kruskal-Wallis test (table 4). The post-hoc Dunn test confirmed the difference, with Glomdalen showing more forestry than both Gudbrandsdalen (Z = 4.21, $p < 0.001$ and Østerdalen (Z = 2.79, $p = 0.016$) after adjustments for multiple comparisons. There was initially a significance between Gudbrandsdalen and Østerdalen, but this difference became non-significant after adjustments (Z = - 2.12 p.adj= 0.102). This suggests that the forestry intensity in the study area is highest in Glomdalen, followed by Østerdalen, and Gudbrandsdalen having the lowest intensity.

Deciding buffer sizes

The exploratory modelling to decide the buffer size for further modelling showed 1 km buffer to be the best model. The 1 km buffer had an AIC weight of 0.545, indicating a 54% probability of it being the best-fitting model among the tested buffer sizes. Although the 3 km buffer model was close and just within 2 ΔAICc, it showed a slightly poorer fit as seen in Table 6.

Table 6 Model summary of the models used to determine the appropriate spatial scale for further analysing of forestry variables. 1 km buffer showed the best fit, of the buffer sizes and were usen in further analyses

Buffer size	AICc	ΔAICc	AICc Weight	Log-Likelihood	df estimated parameters
1 km	266.6	0	0.545	-132.772	3
3 km	268.5	1.9	0.22	-134.442	3
10 km	269.6	3	0.123	-136.233	3
5 km	269.8	3.2	0.112	-136.084	3

Habitat models for hepatic [THg]

None of the forestry or habitat models revealed significant predictors of hepatic [THg], with low deviance explained and low log-likelihood across all models. The low deviance explained by the models indicates that only a small proportion of the total variation in hepatic [THg] can be explained by the chosen predictors. The best-fitting model which has the amount of forest within the 1 km buffer, had the lowest AICcc (266.2 see Table 7) but explained only 6.01% of the deviance.

When GAMs were applied, some models showed modest improvements in explained deviance, suggesting potential non-linear relationships between forestry and habitat variables such as proximity to water, and hepatic mercury. Notably, the null model (AICc = 267.8) was within 2 ΔAICc of the best fitting model, indicating it is statistically equivalent of the more complex models. This suggests that the inclusion of the forestry and habitat variables did not provide a substantial improvement in explaining variation in hepatic mercury concentrations. The model that included the interaction between forestry intensity and distance to water increasing from 5.5% in the GLM to 19.3% deviance explained in the GAM. The interaction term was not found to be significant (p = 0.980 see appendix for full list of estimates).

The model including only forestry intensity, although non-significant, it demonstrated a negative effect size (effect size = -0.246, p = 0.125) This suggests a potential association where increased forestry might be linked with lower hepatic mercury concentrations. However, due to the lack of significance, further investigation is needed to confirm if this is indeed a trend. (see Figure 7 for visualization).

Table 7 Ranked model table based on AICc in hepatic [THg]. Showing the model outputs that were used when comparing model fit. Only showing GAMs that performed different than the initial GLMs in either AICc or deviance explained were included. s() indicating smooth term in GAM, te() indicating interaction term in GAM.

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Predictor(s)		AICc	AICc weights	ΔAICc	Deviance explained	log-likelihood	Df (parameters used)
Forest within 1 km	glm	266.2	0.107	0	6.01%	-131.30	3
Forestry intensity	glm	266.6	0.088	0.4	5.37%	-132.77	3
s(forestry intensity) + s(Forest within 1 km)	gam	267.2	0.067	1	8.16%	-130.48	4
none	glm	267.8	0.050	1.6	-9.47E-16	-136.05	2
Water within 1 km	glm	268.2	0.082	2	2.87%	-134.10	3
Forestry intensity + water within 1 km	Glm	268.4	0.037	2.2	6.30%	-132.19	4
Water within 1 km + Forest within 1 km	Glm	268.5	0.034	2.3	6.06%	-131.53	4
s(forestry intensity) + s(distance to water)	Gam	268.5	0.035	2.3	16%	-129.60	6.64

te(forestry intensity, distance to water)	Gam	268.9	0.028	2.7	19.30%	-128.11	7.66
Forestry intensity + distance to water	Glm	268.9	0.028	2.7	5.50%	-133.00	4
s(distance to water) + s(water within 1 km)	Gam	269.5	0.021	3.3	18.00%	-129.73	7.53
Distance to water, water within 1 km	Glm	269.9	0.017	3.7	3.99%	-133.78	4
Forestry intensity + study area	Glm	271.4	0.008	5.2	5.43%	-132.97	5
Forestry intensity * distance to water	Glm	271.4	0.008	5.2	5.50%	-133.24	5

Figure 7 Visualisation of a possible negative trend (effect size = -0.246) of forestry on hepatic mercury concentrations in mink. The effect size on the y-axis and standardized values for forestry on x-axis. Not-significant result and p = 0.125 more investigation is needed.

Habitat models [THg] in fur

When applied to the [THg] in fur, the AICc identified the model with the percentage of forest cover within 1 km as the best-performing model (Table 8). This model, which had forest cover as the only predictor, showed a significant effect (p = 0.004 see appendix for a complete list of predictor estimates). However, the best model explained only 15.57% of the deviance in [THg] levels in fur indicating limited explanatory power and had a negative log-likelihood reflecting a poor fit to the data. According to the DHARMa diagnostics, there were no significant issues with outliers or residual patterns.

Two other models, one combining forest cover with freshwater proximity (water distance) and the other including forest cover and forestry performed similarly. These models explained slightly more deviance (16.17% and 16.14% respectively) and were within 2 ΔAICc points of the best model indicating that they can be considered equally good.

The GAM that included forestry and distance to water improved deviance explained (up to 36%) showed no significant predictors ($p > 0.1$). Additionally, the model had higher AICc, lower log-likelihood and higher degrees of freedom (df = 11) indicating a potential overfitting the data with the small sample size.
Overall, most models explained only a small fraction of the variance in [THg] in fur, had little explanatory power and several non-significant predictors.

Predictors	AICc	AIC Weights	ΔAICc	deviance explained	log-likelihood	Df (Prameters used)
Forest within 1 km	421.7	0.252	0	15.57%	-207.60	3
Forestry intensity + water distance	423.6	0.097	1.9	16.17%	-207.42	4
Forest + water within 1 km	423.6	0.097	1.9	16.14%	-207.39	4
s(forestry intensity) + s(distance to water)	423.6	0.097	1.9	16.10%	-207.96	4
Water within 1 km	427.1	0.017	5.4	7.60%	-210.33	3
S(forestry intensity) + s(water within 1 km	428.3	0.009	6.6	9.31%	-211.03	4
forestry intensity + water within 1 km	428.3	0.009	6.6	9.30%	-209.76	4
Forestry intensity	428.7	0.008	7	5.13%	-211.13	3
Distance to water + water within 1 km	429.3	0.006	7.6	7.83%	-210.25	4
Forestry intensity + study area	429.4	0.005	7.7	11.40%	-210.67	5
s(forestry intensity) + s(distance to water)	429.4	0.005	7.7	36%	-199.99	11.73
Forestry intensity + study area	429.4	0.005	7.7	11.37%	-209.06	5
None	429.7	0.005	8	0.00%	212.75	2
Forestry intensity + distance to water	431.1	0.002	9.4	5.13%	-211.13	4
Forestry intensity * distance to water	433.5	0.001	11.8	5.22%	-211.10	5
Te(Forestry intensity, distance to water)	435.8	0.000	14.1	30.10%	-203.54	12.07

Table 8 Ranked model table based on AICc [THg] in fur showing the model outputs that were used when comparing model fit. Only showing GAMs that performed different than the initial GLMs in either AICc or deviance explained were included. s() indicating smooth term in GAM, te() indicating interaction term in GAM.

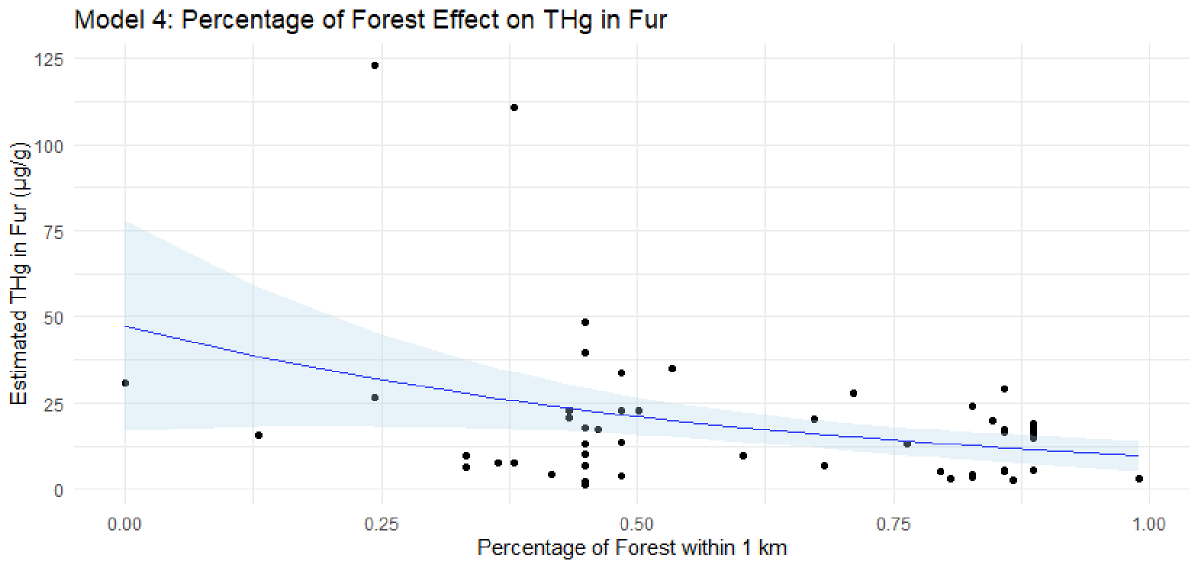

Figure 8 visualisation model predictions and confidence intervals of best model of [THG] in fur. Predictor is percentage of forest within a 1 km buffer around GPS location of where mink were trapped.

Discussion

Assessing and creating background levels for Hg in Innlandet is important to monitor Hg's possible effect on both animal and ecosystem health. This study revealed no significant differences in [THg] between the sampled valleys Østerdalen, Glomdalen, and Gudbrandsdalen in either hepatic or fur samples. The range in both fur and hepatic samples indicates that there are large individual differences between individual mink within Innlandet and some mink having concentrations associated with toxic effect of Hg. Selenium levels from Innlandet were significantly different between Østerdalen and Glomdalen and similarly to Hg results had a large range with individuals being more exposed to or being able to take up more Se. The large range in Hg and Se is reflected in the molar ratio having a similar large spread. The largest proportion of mink had a molar ratio between 2 and 4 and this result, together with the comparative risk assessments from AMAP several mink in Innlandet should have negative health effects of Hg exposure. Neither forestry intensity, forest cover nor water could explain the variability found in Hg in hepatic samples, and though the amount of forest cover within 1 km was found as a significant predictor in the best model, it explains little of the variation.

Mercury

The mean hepatic Hg concentration found in this study was lower than the mean concentrations found by Norheim et al. (1984), pointing towards a decline in hepatic Hg values in the region over the last forty years. However, similar to their findings, the large variation in the range of the Hg levels (0.31 – 28.9 µg/g dw), shows individuals with much higher Hg loads, which could suggest that unknown factors determining Hg contamination in these animals have not changed. Compared to a more recent study from Sweden (Ljungvall et al., 2017) the mean hepatic concentrations from Innlandet are twice as high. Even with an overlap in the range between these studies, the highest levels from Innlandet are higher than the maximum they found in Sweden. The methodology and background levels of Hg vary among areas, and several studies done on Hg from North America shows both higher, (Fortin et al., 2001; Martin et al., 2011) and similar (Yates et al., 2005) concentrations. However, it may be difficult to compare these directly in the same way as studies done in Scandinavia due to site-specific differences affecting background levels of Hg. The differences found between this and Norheim's study could add to the hypothesis of declining Hg levels across Europe (Kalisinska et al., 2021). Hence, the result from Sweden showing substantially lower concentrations, could also point towards site-specific conditions affecting Hg levels. Although no significant differences between study areas were detected in either this or Nordheim's study, it could mean that the scales are too small and both increased sample sizes, and including more regions could show significant differences as found in other studies (Haines et al., 2010; Wren et al., 1986). Further sampling is therefore needed to understand the background levels of Hg.

A Canadian study assessing Hg in areas of concern, with reference sites, applied a reference threshold of 5 µg/g dw in hepatic samples of mink for a site to be considered to have a high Hg load. (Martin et al., 2011). Using 5 µg/g dw as a limit for elevated Hg, the majority of individuals in Innlandet (78.18%, 43 mink) had [THg] below this threshold. Notably, even with a small sample size (n=9) The mean of Gudbradsdalen (5.43 µg/g dw) is slightly above this threshold. Lethal Hg concentrations in mink were found to exceed 20 µg/g ww, though the onset of symptoms of chronic exposure happens earlier (Aulerich et al., 1974; Wobeser et al., 1976; Wobeser & Swift, 1976) Chronic Hg toxicosis has symptoms affecting the animals general body condition, coordination and reproduction (Lian et al., 2021; Wobeser et al., 1976). With finding the highest hepatic [THg] measured to 8.6µg/g ww, none of the mink captured in this study should have any lethal intoxications of Hg. Uptake and demethylation of Hg vary by species, with mink more susceptible to the effect of Hg and demethylating Hg, slower or less than river otters (Evans et al., 2000; Haines et al., 2010). Finding that more than 20% of the animals exceeding 5 µg/g dw, and the high variability across the study area indicate that individual mink could already experience symptoms of chronic Hg toxicosis, but with the majority of [THg] below 5 µg/g dw indicates that there is no need for active measures of Hg mitigation in Innlandet.

Fur is known to be a primary route for Hg execration and with the time it takes to produce new fur and the mink having a distinct difference in summer and winter pelage (Maurel et al., 1986), it can reflect Hg exposure from the last 6 -12 months (Evans et al., 2000; Lieske et al., 2011; Magos & Clarkson, 2008). Fur samples showing a higher mean of Hg than the hepatic samples (19.02 µg/g dw) and with a similar large range (1.57 – 123 µg/g dw) suggests that the mink are able to excrete a considerable amount of Hg through the fur, though it also shows that there are mink having a high exposure to Hg. Mink that were exposed to MeHg in an experimental feeding study all showed a considerable weight loss during the treatment (Wobeser et al., 1976). Our general finding of animals in fairly good condition points towards the animals not being visibly affected by a higher Hg concentration. Notably, our categorisation of good condition is limited, not being able to measure weight loss as we only observed the animals once. The high variability in range and concentrations in Hg should be explored to understand the possible sources of elevated concentrations.

The high variability in [THg] in individual mink observed in Innlandet, confirms the complex drivers behind Hg in the environment. Hg accumulation is found to be species- as well as site-specific (Chan et al., 2003; Porvari & Verta, 2003) and affected by biotic and abiotic factors and individual differences such as parasitism load, contributing to higher Hg values (Klenavic et al., 2008). Environmental factors can be changes in temperature that may increase productivity in water and soil, precipitation can increase runoff, and combined they can influence the food-web dynamics (Kozak et al., 2021; Matilainen et al., 2001; Obrist et al., 2018) and affect the mink. Fish is the main source of MeHg in mammals, and with the opportunistic nature of the mink, this can change the accumulation of Hg depending on the proportion of fish in their diet. This will add to a variability in the Hg found in mink (Gamberg et al., 2005). Furthermore, the trophic level of the fish mink predate on will influence the amount of Hg that is transferred to the mink through diet (Chan et al., 2003). If the mink in Innlandet prefer smaller herbivorous fish, they would presumably be affected less by MeHg (Kalisinska et al., 2017) than with bigger carnivorous fish. Although no significant difference in Hg was found between male and female mink in this study, differences in Hg concentrations have been found in other areas, with females found to have a higher load (Gamberg et al., 2005), but this is not consistent and are also found without notable sex differences (Klenavic et al., 2008; Wren et al., 1986) The lack of difference could be caused by similar dietary preferences between sexes in Innlandet and little difference in habitat types, though the biased sample with 40 males to 14 females is limiting when creating assumptions based on sex. The female mink captured in Gudbrandsdalen, measuring the highest [THg] in both liver (28.94µg/g dw) and fur (123.18µg/g dw) while showing signs of recently having pups, is thought-provoking as her [THg] should have been reduced through the placenta to her pups (Yang et al., 1972). This can indicate that her Hg levels before whelping, could have been even higher and raises questions about site-specific exposure risks in Innlandet and if any areas are more at risk than others.

Besides the small sample size, lack of data concerning seasonal variation and the mink dietary preferences' possible effect on [THg], is a limiting aspect of this study. Mink can change both home range use as well as dietary preferences during a year (Gerell, 1967, 1970). Additionally, Norway is a country with four distinct seasons, this can cause significant differences in Hg exposure, with increased temperature or precipitation. Seasonality has been found to affect the uptake of MeHg with increased levels in fish in summer (Weis et al., 1986). However, this too, seems to depend on the species and diet of piscivorous animals (Pitoňáková, 2023). This highlights the importance of understanding the drivers behind Hg dynamics being both species and site-specific when determining background levels and risk assessments for Hg for future studies.

Selenium

A Significant difference in [TSe] was detected between the study areas Østerdalen and Glomdalen for both liver and fur samples, with Østerdalen having higher concentrations. This confirms the variability found in Se over small distances, depending on site-specific conditions such as soil type and vegetation (Christophersen et al., 2013). The lack of significant difference between Østerdalen and Gudbrandsdalen, despite the visual difference (Fig. 2 and 3), is due to the spread in the data and small sample size. With few observations the data points from Gudbrandsdalen overlap and there is no significant difference. This could mean that differences may emerge with more sampling.

Selenium is a micronutrient, a strong antioxidant and necessary for the protein function of mammals but in excess, it can itself become toxic (Schomburg et al., 2004). With soils in Europe usually considered Se poor, Se deficiency is a more common problem (Christophersen et al., 2013; Dos Reis et al., 2017). Compared to other studies with wild mink from Europe, our results show that there might be more Se than previously thought as the mean finding is higher than could be expected from Se-deficient areas (Christophersen et al., 2013) The mean hepatic samples from Innlandet is higher (2.76 ± 1.08µg/g) than previously measured in Norway (Norheim et al., 1984) and similar to a study from Poland 2.40 (Brzeziński et al., 2014) and British Columbia Canada (Harding et al., 1998). These results could indicate that there is more Se present in Innlandet in certain areas than previously thought. With burning of fossil fuels being responsible of atmospheric deposits of Se from anthropogenic influence (Christophersen et al., 2013) it could be increasing the background levels of Se in Innlandet. This hypothesis is only speculative, and notably the few observations from

Gudbrandsdalen could affect the overall mean with the one female with higher concentrations creating the highest mean of the study areas.

Though no universal limits of normal ranges of Se have been proposed (Kalisinska et al., 2017), a study with captive mink, found a normal range in fur of male mink to be between 0.158 and 0.363 µg/g dw (Staroverova et al., 2018). The mean of [TSe] in fur across all regions was measured to 0.99 making it higher than this range, but wild mink compared to farm-bred mink are exposed to different environmental factors and a mixture of pollutants and might not have the same base level. They did however find that Se correlated with age with sub-adults having higher levels of Se. Without knowing the age of our sampled mink, it could be that a substantial part of them were young mink dispersing, hence influencing the mean. The minimum range across study areas were 0.51-0.58 µg/g dw which is still higher, though closer to the normal range suggested by Staroverova. The closest animal group with suggested levels of Se were created in 1994 by Robert Puls for canines and used on arctic foxes. (Pilarczyk et al., 2019). The suggested categories based on [TSe] in hepatic samples were; deficient (0.1-0.3µg/g ww), marginal (0.3-0.5µg/g ww) and normal (0.5-1.5µg/g ww). Compared to canine categories it is interesting that none of our mink should be considered deficient and all 23 hepatic samples of mink from Østerdalen are within canine reference ranges. Glomdalen have 4 that are marginal, 18 within their normal range, and Gudbrandsdalen having 8 within normal range for canines. 2 females in this study from Glomdalen and Gudbrandsdalen are measured with higher concentrations of [TSe] one is above 2.34 the other at 1.83. The result of these categories should be interpreted with caution, with Se uptake from the diet being species-specific (Haines et al., 2010; Kalisinska et al., 2017) and the proportion of fish in the diet possibly influencing the Se levels measured in mink (Gamberg et al., 2005). The findings can signal that Se levels are not necessarily being deficient in the study area and rather underline the possibility that prey species could explain the variation found in Se in Innlandet.

Molar ratio

The molar ratio of Se to Hg in the environment is a key indicator to establish the extent of Se's ability to mitigate the toxic effect of Hg. The toxicity of Hg is theorised to originate in binding all available Se to Hg, leading to induced Se deficiency, increasing oxidative stress and not enough Se left to be used in vital protein functions (Ralston et al., 2016; Raymond & Ralston, 2020). The molar ratio of 1:1 is considered as an absolute minimum, though a 1:1 ratio, will leave no Se left for normal protein functions as well as dietary studies showing Se only being able to alleviate a certain amount of Hg even with higher molar ratios (Lian et al., 2020; Ralston et al., 2007). In Innlandet the mean molar ratio in liver tissue between all regions were 3.29, but a large range (0.55 – 12.23) suggesting uneven distribution similar to the findings of Se and Hg. With the assumption of a 1:1 ratio being too low and a minimum of 2:1 to not experience oxidative stress, the sorting into categories (Figure 4) revealed that approximately 30% (18 mink) had molar ratios below 2. Seven mink had ratios below 1, indicating that they do not have enough Se to alleviate Hg and are probably experiencing symptoms of chronic Hg toxicosis. The unknown factors and the ratio needed are speculative, with studies showing that a 4 ratio can show some signs of oxidative stress, a ratio of above 4:1 can be a guideline to assume minimal effect of Hg toxicity. The finding of most of the samples having a ratio above 2 is higher than a similar study found on mink in Europe (Kalisinska et al., 2017). This could point towards mink in Innlandet having a diet consisting of fish with more Se, and able to contain enough Se through their diet as was suggested from the US (Stafford et al., 2016).

The strong positive correlation between molar ratios in hepatic and fur samples (Table 3) emphasizes the possibility for fur or hair samples for studying Se and Hg dynamics, however with the molar ratios in fur being substantially lower, and overall showing a ratio below 2 it is possible that Se is being used up or bound in the body when the liver is demethylating Hg (Evans et al., 2000; Ralston et al., 2007). This hypothesis could explain why the difference in [TSe] in liver and fur varies between the three areas with some seemingly "using" up more Se in the body and these levels not showing in the excreted fur. Understanding how Se and Hg are used together to alleviate this effect from the liver and how these molar ratios are to be interpreted in fur is therefore important, before analysing only fur samples from a population.

Overall, these results together with the Se concentrations found in this study imply that the diet of mink in Innlandet possibly contains sufficient Se to alleviate the toxic levels of Hg in ca. 13% of the population (12 mink) with a molar ratio above 4. However, even if the population has a mean above 3 the range implies that several mink could be negatively affected by Hg.

Comparative risk assessment

Comparing AMAP's 2021 thresholds of Hg concentrations in the liver found the majority of mink (50 of 56) fell into the "no risk" category for adverse health effects. Two female mink had hepatic [THg] that put them in the low and moderate risk showing individuals having a larger risk. Comparing with the assessment created from 2011 which based their risk assessment on the concentration in fur used different thresholds with clinical effects thought to occur above 30 µg/g dw. This threshold gives a similar, though slightly lower, result with 85% (48 of 56) mink below the threshold. With the lower limit set to 20 µg/g dw which was created as a more sensitive limit for negative health responses (AMAP, 2011) the proportion goes down further to approximately 70% (see Figure 6 for counts) below the threshold. In total between 14% and 30% were above suggested thresholds. Notably, the thresholds created for 2018 were used for Arctic foxes, and the sensitivity of mink might be different as it is found to be more sensitive to the effects of Hg, and to use a cautionary approach, the lower limit might be considered for Hg impacting the mink. The distribution of the different mink with the higher concentrations is consistent with the finding of no difference in Hg load in the different valleys, and local contamination from unknown sources might be the source of the higher levels in certain individuals.

Two of the females of the study had the highest [THg] that even surpasses a suggested World Health Organisation limit of 70 µg/g ww that is set for humans where the risk of motor retardation in offspring is 30% (WHO, 1990). Some of these elevated concentrations could point toward a risk of reproductive health to parts of the population. These findings from Innlandet show that most individuals from our sampling should not have any major health effects of Hg however a substantial part can indeed be negatively affected. Continued monitoring as well as understanding possible sources of individual mink having higher concentrations is necessary to assess the current and future risk of Hg exposure in this area.

Forestry

With forestry having been identified as a potential driver of increased [THg] in both the environment and biota (Bishop et al., 2009; Eklöf et al., 2016; Porvari et al., 2003) it is important to understand its effects on terrestrial ecosystems and how forestry practices could influence Hg bioaccumulation and biomagnification. The objective in including forestry and some general habitat variables collected from open data repositories was to investigate if the Hg in mink measured in both hepatic and fur samples, varied based on forestry intensity. Other habitat variables that could be linked with intensity or the transport of Hg in the environment such as the amount of forest cover within 1 km, the amount of water and the distance to water could explain Hg levels.

There was a significant difference detected for forestry intensity with Glomdalen having the most forestry, but none of the models fitted for hepatic Hg found any significant predictors for [THg] or any significance of tree species. Additionally, they explain only a small proportion of the overall variance in [THg]. The best-fitting model based on AICc, was the model with the amount of forest cover within 1 km of the capture site of the mink, but the model had low explanatory power and did not fit the data well. Additionally, the model with no predictors added was within 2 ΔAICc of the best fitting model and should be considered statistically equivalent. This confirms that the chosen predictors were not equipped to explain the variation in hepatic [THg]. Interestingly, for the fur samples, the best model with the lowest AICc included the same predictor but the amount of forest within a 1 km radius was here a significant predictor of [THg] (see appendix A for full list of predictor estimates) showing less [THg] with an increasing amount of forest cover (Figure 8). The AICc additionally having more than 2 ΔAICc points between the best and the model with no predictors, suggests that the forest cover explains some of the variation in [THg] in fur, The model does however show low explanatory power and is in need of better predictors.

The relationship between forestry intensity and Hg concentrations in the environment is complex and previous studies have found varying results with increased Hg concentrations in water and fish following logging activities (Negrazis et al., 2022; Porvari et al., 2003; Skyllberg et al., 2009), or no significant changes measurable in nearby water (de Wit et al., 2014; Eklöf et al., 2014). These differences could result from site-specific conditions as well as combinations with management practices. The models with forestry intensity showing little explanatory power could imply that the forestry intensity from the last three years does not affect or bioaccumulate in mink within Innlandet. The reason for these results could be that the environmental restrictions implemented by forestry agencies in Norway (Forskrift om berekraftig skogbruk, 2006) could prevent increased Hg mobilisation as suggested by Ekløf et.al (2016) in Sweden. Certain forestry practices like logging on wet soil, stump removal or soil preparations could create favourable suboxic conditions, combined with increased runoff of dissolved organic carbon that add electron receptors for Hg methylation (Gilmour et al., 1992) resulting in higher Hg levels associated with forestry. These practises could be limited in the study areas and precautionary measures could be good enough, resulting in forestry intensity not increasing Hg in biota. This hypothesis should be tested further with more detailed environmental data from the capture sites of the mink.

Finding increasing amount of forest cover associated with decreasing amount of Hg could be explained by Hg naturally being found in higher concentrations in wetlands, such as bog areas (Chan et al., 2003; Martin et al., 2011), as well as possible point sources of Hg coming from industrial areas that would have lower amount of forest. Furthermore, there might be other unknown variables that could be interacting or dependent on the amount of forest cover within the 1 km radius of the captured mink, that is not included in this study. These possibilities could make the amount of forest cover significant in the model even if the models have low explanatory power. In theory the amount of forest cover could possibly serve as another proxy for previous forestry intensity, with areas having less forest cover potentially having previously been logged and having higher Hg. It was however not possible to figure this out with the current data, as forest cover was only collected up until 2022 and the data did not include a layer for differences between years. Finding another way of quantifying the amount of forest and change between years could prove that the amount of forest or forestry does affect Hg in mink in Innlandet but could not be distinguished in this study.

In the hepatic Hg model that included only forestry intensity as a predictor of [THg], a weak negative tendency, was observed. With increasing forestry leading to slightly decreasing [THg] in hepatic concentrations. Though as foresty intesity was not found to be a significant predictor for [THg] in hepatic samples, and could be noise in the data, this tendency must be interpreted with caution, but it raises some interesting questions as this might not be what could be expected based on the previous findings between forestry and Hg. With the studies previously mentioned where Hg runoff can increase downstream of logging sites without changing the concentrations in waterbodies nearby (Eklöf et al., 2014). A possible hypothesis could be that Hg is being transported downstream too fast to be measurable close to the logging sites and disappears into larger lakes in the systems, such as lake Mjøsa, which is contaminated by Hg (NIVA, 2020).

The time frame within which forestry could affect runoff, as well as the possible following biomagnification of Hg in the food web is not well understood. Studies have shown forestry practises having effects on Hg concentrations in the surrounding water or streams 1- 4 years after logging (Rask et al., 1998; Skyllberg et al., 2009; P. Wu et al., 2018). With the length of studies varying and samples collected once or monitoring for a short time (Negrazis et al., 2022; Sørensen et al., 2009), after logging it could be that the time frame and scale of monitoring make a difference when researching forestry intensity and Hg effects. With mink as an apex predator in the system, it could take longer to detect changes in Hg levels that could originate from forestry. A subtle hint toward this could be the slightly better fit of the model with the same predictor, the amount of forest, applied to fur compared to hepatic samples. With fur indicating previous rather than current exposure (Lieske et al., 2011) it could be that the exact date the mink is captured is important to better locate possible an effect, or distinguish if, or what, environmental variables play a role in Hg levels in mink. This underlines that the grouped variable as a proxy for forestry used for this study is not fine-scale enough to measure these differences and further research is needed to assess the impacts of forestry.

Lastly, regarding the possible effects of forestry on mink is the possible changes in the food web that can come from habitat disturbance. A study by Wu et al.(2018) found that logging practices could alter food web dynamics and increase Hg loads in fish following clear-cuts even when water concentrations remained unchanged. However from Norway, de Wit et al (2014) found the opposite result, where increased runoff from forestry decreased MeHg but also suggested as a result of food web shifts. This suggests that forestry possibly affecting food web shifts, rather than direct contamination, could play a role in Hg biomagnification and accumulation. Future monitoring should therefore include more fine-scale temporal analysis including, capture and forestry data together with dietary preferences of individual mink to understand how forestry could affect Hg concentrations in mink

Some limitations related to the data and methodology of this study should be noted. The sample size (n = 56) as mentioned was relatively small, and the male bias as well as an uneven distribution in sample size from the three valleys within Innlandet could limit the ability to detect significant variances in [THg] by sex or region. The data on the percentage of forest cover, water and volume removed, were collected from raster data, and the data showed limitations in variance due to the coarse resolution of the raster grid (Appendix B). As a result, several observations had the same value, potentially reducing the accuracy of the models, increasing the probability of overfitting, and the models being sensitive to outliers. Additionally, the data being open-source material, there were some limitations with the accuracy and reporting of logging activities. To address these issues, simpler models with few predictors that are easier to interpret were used to account for the small sample size and removing an obvious outlier in the forestry data that could affect the model. Using goodness-of-fit test and making sure that any major deviation in the model performances were detected were done to prevent these issues as much as possible. The models were not able to capture the complexity of Hg cycling in the ecosystem to forestry, and more explanatory variables are needed. Introducing non-linear terms with more predictors such as the weather patterns, prey species (diet), and different predictors for forestry might improve our understanding of the relationship between Hg and industrialized forestry as well as environmental factors and are recommended for future studies.

Conclusion

The mean Hg concentrations in mink from Innlandet do not indicate that the population of mink are in danger of Hg intoxication, with the majority of individuals below a threshold of 5 µg/g dw set as a high load. However, the broad range observed across all three regions within Innlandet, suggests that individuals are exposed to a higher contamination risk and areas potentially having higher background levels of Hg. Finding higher Se concentrations compared to a previous study from the area and an experimental feeding study, highlights the importance of creating background levels of Se in relation to Hg levels. Se availability is site-specific and could be higher than expected from a Se deficient area. The higher Se levels in some areas could be linked to the diet of mink, but further research is needed to confirm this. Even with the finding of higher Se than could be expected, most mink had molar ratios between 2 and 4. With a ratio of 2:1 theorized as a bare minimum to have any excess of Se to mitigate the toxic effect of Hg, and enough Se for vital functions, point towards there still not being enough Se compared to Hg. The result of 30 % of the sample from Innlandet having a lower ratio than 2 and 18% having a ratio above 4, that could be considered a safer, this result together with the comparative risk assessment shows that a part of the population could be affected by chronic Hg exposure and health effects.

The non-significant result and poor fit of the models with forestry and habitat variables explaining variability in Hg could indicate that Norwegian regulation for forestry practises is effective also in reducing the effect of Hg contamination. However, the limitations in the forestry variables, small sample size and possible unknown relation between variables should be noted. As there are few clear tendencies in the causal effects between forestry and Hg in mink, more studies are needed to confirm if forestry does or does not affect Hg in mink. These findings contribute to the broader understanding and possible background levels for Hg and Se in Innlandet and highlight the importance of continued research and monitoring to understand the potential drivers behind Hg accumulation. Future studies should aim to increase sample size and include finer-scale temporal variables as well as seasonality and dietary preferences in mink.

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Appendix A Model estimates and predictors

9 Predictors and estimates for forestry and habitat modelling with fur samples

MODEL	PREDICTOR	ESTIMATE	STD. ERROR	T-VALUE	P-VALUE
M1	volume removed	-0.2453	0.1575	-1.558	0.125
M2	volume_removed_1km	-0.2087	0.1483	-1.407	0.166
M2	studyareaGlomdalen	0.4616	0.3039	1.519	0.135
M2	studyareaGudbrandsdalslågen	0.5253	0.4018	1.307	0.197
M3	(Intercept)	2.9315	0.1502	19.516	<0.001
M3	volume_removed_1km	-0.2329	0.1668	-1.397	0.169
M3	water_distance	-0.0034	0.1668	-0.02	0.984
M4	(Intercept)	2.8859	0.1216	23.742	<0.001
M4	**Forest_bufdist1**	**-0.3703**	**0.1227**	**-3.018**	**0.0039**
M5	(Intercept)	2.8837	0.1233	23.387	<0.001
M5	volume_removed_1km	-0.0736	0.1364	-0.54	0.592
M5	Forest_bufdist1	-0.3452	0.1364	-2.531	0.0145
M6	(Intercept)	2.9571	0.1933	15.299	<0.001
M6	volume_removed_1km	-0.2599	0.207	-1.255	0.215
M6	water_distance	0.071	0.3729	0.19	0.85
M6	volume_removed_1km	-0.0634	0.293	-0.216	0.83
M7	(Intercept)	2.9133	0.134	21.738	<0.001
M7	volume_removed_1km	-0.1397	0.1467	-0.952	0.346
M7	Freshwater_bufdist1	0.2024	0.1467	1.379	0.174
M8	(Intercept)	2.9197	0.1354	21.567	<0.001
M8	water_distance	-0.0466	0.1389	-0.335	0.739
M8	Freshwater_bufdist1	0.2427	0.1389	1.748	0.087
M9	(Intercept)	2.9207	0.1347	21.676	<0.001
M9	Freshwater_bufdist1	0.2531	0.136	1.861	0.0684
MNULL	(Intercept)	2.9539	0.1581	18.681	<0.001

10 Predictors and estimates for forestry and habitat models for hepatic Hg

MODEL	PREDICTOR	ESTIMATE	STD. ERROR	T-VALUE	P-VALUE
M1	(Intercept)	1.4601	0.1564	9.337	<0.001
M1	volume_removed_1km	-0.246	0.1579	-1.558	0.125
M2	(Intercept)	1.4299	0.245	5.835	<0.001
M2	volume_removed_1km	-0.2443	0.1733	-1.409	0.165
M2	studyareaGlomdalen	0.0497	0.3542	0.14	0.889
M2	studyareaGudbrandsdalslågen	0.0547	0.4695	0.117	0.908
M3	(Intercept)	1.4595	0.158	9.239	<0.001
M3	volume_removed_1km	-0.229	0.1756	-1.304	0.198
M3	water_distance	-0.0414	0.1756	-0.236	0.814
M4	(Intercept)	1.4569	0.1472	9.895	<0.001
M4	Forest_bufdist1	-0.246	0.1487	-1.655	0.104
M5	(Intercept)	1.4463	0.1448	9.99	<0.001
M5	volume_removed_1km	-0.1614	0.1594	-1.013	0.316
M5	Forest_bufdist1	-0.1874	0.1594	-1.175	0.245
M6	(Intercept)	1.4627	0.2048	7.142	<0.001
M6	volume_removed_1km	-0.2325	0.2207	-1.053	0.297
M6	water_distance	-0.0323	0.3931	-0.082	0.935
M6	volume_removed_1km	-0.0078	0.3132	-0.025	0.98
M7	(Intercept)	1.4555	0.1539	9.455	<0.001
M7	volume_removed_1km	-0.2041	0.1677	-1.217	0.229
M7	Freshwater_bufdist1	0.1027	0.1677	0.613	0.543
M8	(Intercept)	1.4669	0.1609	9.119	<0.001

M8	water_distance	-0.1127	0.1651	-0.682	0.498
M8	Freshwater_bufdist1	0.148	0.1651	0.896	0.374
M9	(Intercept)	1.4725	0.1616	9.113	<0.001
M9	Freshwater_bufdist1	0.1704	0.1631	1.044	0.301
M10	(Intercept)	1.4567	0.1491	9.771	<0.001
M10	Freshwater_bufdist1	0.0313	0.179	0.175	0.862
M10	Forest_bufdist1	-0.2258	0.179	-1.261	0.213
MNULL	(Intercept)	1.4867	0.1698	8.756	<0.001

Appendix B Variability in predictors

Index plots of the variability in Forestry (volume removed) and habitat predictors used in the models showing limited variabity.

Appendix C Study area

All maps provided by Sharon Lustenberger

The valleys of Østerdalen, Gudbrandsdalen and Glomdalen (Glåmdalen)