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Stable Isotope Ratio to Identify Potential Food Chain Transfer of ^{137}Cs in Fish with Special Focus on Rudd (*Scardinius Erythrophthalmus)*

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It is a lesson you should heed, try, try again. If at first you don't succeed, try, try, and try again.

Thomas H. Palmer.

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Ås, May, 2022

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Summary

The uptake of radionuclides such as radiocaesium (^{137}Cs) in fish occurs mainly through diet. Generally, it is assumed that the transfer of ^{137}Cs in predatory fish is higher than in non-predatory fish. In the present work food chain transfer of ^{137}Cs in fish was studied in the Lake Glubokoye in Chernobyl Exclusion Zone (ChEZ) by analysis stable carbon ($\delta^{13}C$), stable nitrogen ($\delta^{15}N$) and ^{137}Cs in fish, bentic organisms, water plants and insects collected in and at shore of the contaminated lake.

Results show that the assumed non-predatory fish rudd (Scardinius Erythrophthalmus) had average of 10.74 $kBq\,kg^{-1}$ of ^{137}Cs, at least similar activity concentration of ^{137}Cs as in species of fish more associated with predatory behavior such as pike and perch. Roach was measured to a overall lower activity concentration of ^{137}Cs with average measured of 2.79 $kBq\,kg^{-1}$ while pike, perch and rudd have all individuals with over 10.0 $kBq\,kg^{-1}$ activity concentrations.

The activity concentration of ^{137}Cs in organisms at Lake Glubokoye was highly variable. Five plants collected in October 2021 was in range from 0.83 to 48.9 $Bq\,g^{-1}$ dry weight. In the Benthic predator (*Odonata*) ^{137}Cs was measured to 8.42 $Bq\,g^{-1}$. Snails collected showed a ^{137}Cs level of 20.89 $Bq\,g^{-1}$ in the body, and 1.66 $Bq\,g^{-1}$ in the shell. Several of the terrestrial insects had to low biomass possible to detect ^{137}Cs. Samples possible to detect had activity concentration of ^{137}Cs up to 7.49 $Bq\,g^{-1}$. The millipedes(*Diplopoda*) had an ^{137}Cs of 22.10 $Bq\,g^{-1}$. ^{137}Cs was detected in all groups of feed organisms studied, except in the low biomass samples.

The $\delta^{13}C$ in rudd had an average of 28.93 $\pm$ 1.24 ‰ and was found to be significantly higher than for other fish species. It is a clear difference in $\delta^{13}C$ signature of aquatic insects that had an average of 34.56‰ and terrestrial insects with an average of 26.43‰ being more similar to rudd. Results suggest that ^{137}Cs could be transferred from feeding of terrestrial-insects by rudd, as this seems to be a significant part of the diet to rudd. This is supported by the fact that analysis of intestine content contains different fractions of exoskeleton from invertebrates.

The activity concentration of ^{137}Cs was significantly higher in rudd collected during

June in 2017, with an average of 19.77 $kBq\,kg^{-1}$ ^{137}Cs, than the rudd collected during autumn in 2021. While the $\delta^{13}C$ was found to be significantly lower in June of 2017, with an average of (-30.01‰), compared to autumn 2021. This indicate a move towards an aquatic food source as a source for explaining high ^{137}Cs. The $\delta^{15}N$ was found to be significantly higher in June 2017 with a average of 7.12‰ compared to October 2021 (6.26 ‰), still significantly lower than for the predatory fish, but a move towards an isotope signature less different to the predatory fish could be observed. Predatory behavior in rudd could not be excluded, as gill covers were found in intestine samples.

Results indicate that terrestrial insects can be a major diet source for rudd and that ^{137}Cs in terrestrial insects can be a main source of ^{137}Cs in rudd, but aquatic sources as predation of other fish can contribute to higher levels during times of the year when availability of terrestrial food sources are scarce.

The findings highlight that information about the food chain is important to understand the dynamic transfer of radiocaesium.

Sammendrag

Opptak av radionuklider som radiocesium (^{137}Cs) i fisk er i hovedsak gjennom matinntak. Generellt er det antatt at overføring av ^{137}Cs i predatorisk fisk er høyere enn i ikke-predatoriske fisk. I dette arbeidet ble overføring av ^{137}Cs i næringsnettet til fisk studert i innsjøen Glubokoye inne i ekslusjonssonen rundt Chernobyl ved å analysere den stabile karbon-isotopen (^{13}C), stabil nitrogen isotop (^{15}N) og ^{137}Cs i fisk, bunndyr, vannplanter og akvatiske insekter samlet i, eller terrestriske insekter samlet på bredden av, den kontaminerte innsjøen.

Resultatene indikerer at den antatt ikke predatoriske fisken sørv (Scardinius Erythrophthalmus) var minst like høyt som aktivitetskonsentrasjonen av ^{137}Cs ($10.74\,kBq\,kg^{-1}$) som predatoriske arter, slik som gjedde og abbor. Mort ble funnet til å ha en lavere aktivitet av ^{137}Cs, $2.79\,kBq\,kg^{-1}$ men, gjedde, abbor og sørv alle hadde individer med over $10.0\,kBq\,kg^{-1}$ aktivitets konsentrasjon.

Aktivitetskonsentrasjonen til ^{137}Cs i organismer i og rundt innsjøen varierte. Fem planter samlet i oktober 2021 hadde ^{137}Cs mellom 0.83 og $48.9\,Bq\,g^{-1}$ tørr-vekt. I den bentiske predatoren øynestikkeren, *Odonata* ble målt til $8.42\,Bq\,g^{-1}$ ^{137}Cs. Snegler hadde et ^{137}Cs nivå på $20.89\,Bq\,g^{-1}$ i bløtdel og $1.66\,Bq\,g^{-1}$ i skallet. Flere av de terrestriske insektene hadde for lav biomasse til at det var mulig å måle ^{137}Cs, i prøver der deteksjon var mulig ble aktivitetskonsetrasjoner opp til $7.49\,Bq\,g^{-1}$ funnet. I tusenbein (*Diplopoda*) ble ^{137}Cs analysert til $22.10\,Bq\,g^{-1}$. ^{137}Cs ble påvist i alle grupper av for organismer til fisk med untak av i prøvene med svært lav biomasse.

Nivået av $\delta^{13}C$ i sørv var (-28.93‰) og signifikant høyere enn for andre arter av fisk i innsjøen. Det var signifikant lavere $\delta^{13}C$ i akvatiske insekter (-34.56‰) enn i terrestriske insekter (-26.43‰) som var i samme størrelsesorden som tilsvarende signatur i rudd. Resultatene indikerer at terrestriske insekter nær Glubokoye er en viktig del av dietten til sørv i innsjøen. Denne antagelsen ble støttet av funn i mageinnhold der fragmenter av exoskjelett fra insekter ble funnet.

Konsentrasjonen av ^{137}Cs var signifikant høyere i sørv innsamlet i juni i 2017, med et gjennomsnitt på $19.77\,kBq\,kg^{-1}$ ^{137}Cs, enn i sørv innsamlet på høsten i 2021, noe

som viser forskjell i aktivitetskonsentrasjonen av ^{137}Cs i fisk mellom årstid/år. $\delta^{13}C$ i sørv var signifikant lavere i juni 2017 med et gjennomsnitt på (-30.01‰), sammenlignet med høsten 2021. Dette indikerer en bevegelse mot en mer akvatisk diett som kilde for sørv når den var mest kontaminert tidlig på sommeren sammenlignet med høsten. Samtidig ble $\delta^{15}N$ funnet til å være signifikant høyere i juni 2017 (7.12‰) sammenlignet med oktober 2021 (6.26‰), dette viser en bevegelse oppover i trofisk nivå. Resultatene indikerer en forskyvning av isotopsignaturen mot de predatoriske fiskeartene i Juni 2017. Endring av $\delta^{15}N$ og funn av gjellelokk i mageinnhold til sørv gjør at predatorisk adferd i sørv ikke kan utelukkes.

Resultatene indikerer at terrestriske insekter kan være en betydelig diettkilde for sørv og at ^{137}Cs i terrestriske insekter derfor kan være en hovedkilde til ^{137}Cs i sørv, men akvatiske kilder som predasjon på andre fisk kan bidra til høyere nivåer i perioder av året hvor sørv har lavere tilgjengelighet av terrestriske matkilder.

Funnene fremhever at informasjon om næringsnettet er viktig for å forstå overføring av radioceasium i fisk.

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List of Acronyms

CERAD	Center for Environmental Radioactivity
ChEZ	Chernobyl Exlusion Zone
CR	Concentration Ratios
IRMS	Isotop ratio mass spectrometer
NMBU	Norwegian University of Life Sciences
NUBiP	National University of Life and Environmental Sciences of Ukraine
UIAR	Ukrainian Institute of Agricultural Radiology
UIO-CLIPT-lab	University of Oslo, CLimate Interpretation of Plant Tissue

1. Introduction

Atropogenic radinuclies have been released to the environment e.g., by the accidents in Chernobyl (1986) and Fukushima (2011) (Steinhauser et al., 2014) or nuclear weapon tests (Prăvălie, 2014). Exposure to radionuclides represents a potential risk to a number of aquatic organisms, due to radiological toxicity as well as chemical toxicity of the elements themselves(Pentreath, 1988; Copplestone et al., 2000; Linsley, 1996; Iryna, 2017). The radiation released to the environment by the Chernobyl accident is estimated to have been 400 times the amount released by the Hiroshima bomb. This made the area surrounding the Chernobyl power-plant among the most radioactive terrestrial ecosystems on earth and an Exclusion Zone of 4700 km^2 was established to stop people from inhibiting the area (Orizaola, 2020).

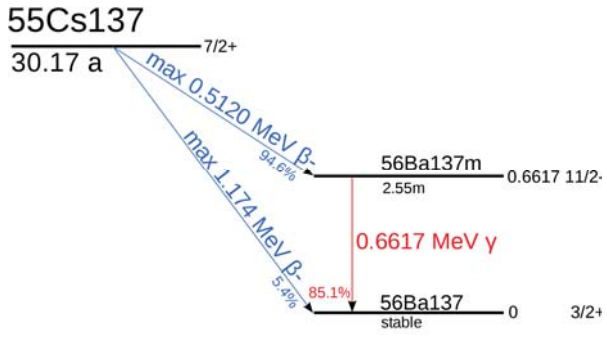


Figure 1.1: ^{137}Cs decay, radiation at 661 keV. It has a half-life 30years and 80days (Podgoršak et al., 2006).

The radioactive isoptope of caesium (^{137}Cs), is a common fission product of nuclear fission and an important radionuclide for the contribution of radiation dose to living organisms. It emits high energy beta radiation, and its daughter ^{137m}Ba emits gamma. A half-life of 30 years and 80 days makes ^{137}Cs in the environment a long term challenge. ^{137}Cs is highly reactive and a chemical analog of potassium (K).

Freshwater fish is a potentially important link in the transfer of radionuclides from polluted ecosystems to humans. Consumption of fish was one of the main sources for internal doses (40-50%) to inhabitants in some villages in the Ukraine after the Chernobyl accident (Travnikova et al., 2004). Freshwater fish was also found to contribute significantly (20-40%) to the radiocaesium intake by rural populations in Norway post the Chernobyl accident (Strand et al., 1989).

In lakes within the ChEZ, levels of radiocaesium in fish were reported to be in the order $100\,kBq\,kg^{-1}$ wet weight in the immediate proximity of the nuclear power plant in the years after the accident. These levels declined (Kryshev and Ryabov, 1990), but fish in the lakes of ChEZ is still characterized by high concentrations of ^{137}Cs. Levels of ^{137}Cs have thereafter been found to be $0.44 - 31.86\,kBq\,kg^{-1} - wet$ in pray fish (Rudd) and $1.73 - 22.03\,kBq\,kg^{-1} - wet$ in predatory fish Pike (*Esox lucius*) (Kaglyan et al., 2015). The ^{137}Cs average activity concentration were found in perch (*Perca flavescens*) $7.85\,kBq\,Kg^{-1} - wet$ and i roach(*Rutilus rutilus*) $2.91\,kBq\,Kg^{-1} - wet$ (Lerebours et al., 2018) In Scandinavia levels of radiocaesium in brown trout (*Salmo trutta*) and arctic char (*Salvelinus alpinus*) were found to be 10.5 *and* $3.1\,kBq\,kg^{-1}$ respectively after the Chernobyl accident and it is suggested that the levels might approach a steady state only being influenced by the half-life of ^{137}Cs (Jonsson et al., 1999). This is supported by a study in a high altitude lake in Scandinavia where ^{137}Cs was found to have declined to approximately $1.0\,kBq\,kg^{-1}$ in 1999 and did not show to have declined substantially in 2008 (Brittain and Gjerseth, 2010). In comparison, Norway and EU levels for maximum permissible radiocaesium is $370\,Bq\,kg^{-1}$ in foodstuff intended for infants, and $600\,Bq\,kg^{-1}$ for other foodstuff (Andric and Gajic-Kvascev, 2021). The level permitted in freshwater fish in Norway is $3000\,Bq\,kg^{-1}$ (Helse og Omsorgsdepartemente, 2022)

The level of ^{137}Cs in fish can be influenced by water chemistry and food organisms and varies between different species of fish and size of the fish (Smith et al., 2002).

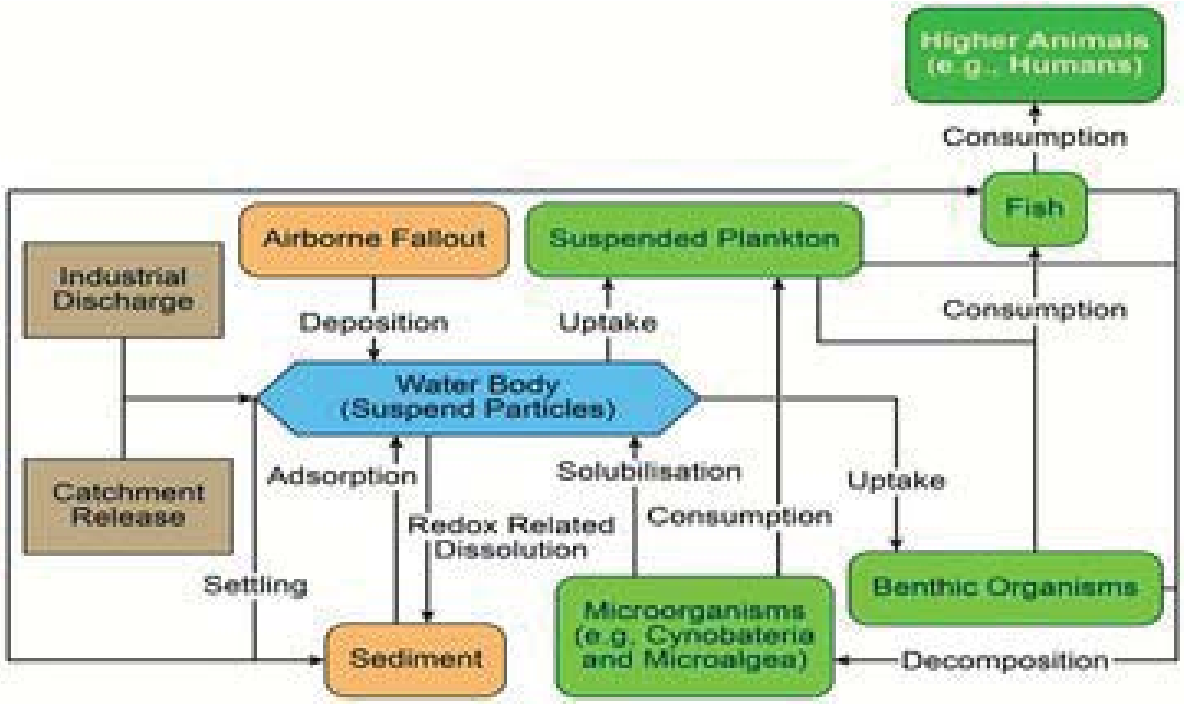


Figure 1.2: Suggested model of ^{137}Cs recycling in aquatic ecosystems (Ashraf et al., 2014)

The transport of ^{137}Cs in aquatic ecosystems is complex as seen in figure 1.2 and is influenced by many factors. It has been shown that ^{137}Cs re-circulate in biological systems for many years (Ashraf et al., 2014). ^{137}Cs can be taken up in fish directly from water. The uptake is reported to be dependent upon concentration of potassium with decreased uptake by increased water concentration of potassium (Smith et al., 2000). However the uptake from water through the gills is relatively low (Hewett and Jefferies, 1976) compared to the uptake by diet. The main pathway of caesium into fish is through food consumption (Teien et al., 2021; Metian et al., 2019; Smith et al., 2002; Kashparova et al., 2020; Haque et al., 2017). The dietary pathway dominated the ^{137}Cs accumulation in the omnivorous and carnivorous fish (Pan and Wang, 2016) and there has been reported increased accumulation of ^{137}Cs in predatory fish (Kryshev, 1995). Studies have predicted the activity concentrations of ^{137}Cs in predatory fish to be approximately two times higher than in non predatory fish (Smith et al., 2000 ; Rowan and Rasmussen, 1994). Other studies have showed a factor of approximately five times higher levels of ^{137}Cs in predatory fish, compared to the non-predatory species (Koulikov, 1996). The increased uptake of ^{137}Cs in predatory fish compared to non predatory fish have been implemented in existing models predicting ^{137}Cs in fish (Balonov et al., 2010).

Moreover differences exist also between non-predatory fish species, but the information is scarce and mechanism not known. Comprehensive databases exist internationally (IAEA, 2010), but information on underlying processes of uptake and early response is insufficient. In the field of radiological protection, robust models are required to predict the transfer through food chains. Knowledge of uptake and distribution of ^{137}Cs is needed.

Rudd and perch from Lake Glubokoye have shown a dependency of ^{137}Cs accumulation with size and age (Kaglyan et al., 2015). The activity concentration of ^{137}Cs in fish has been reported to change between seasons. Uptake of ^{137}Cs has been reported to be dynamic and higher during summer than during the winter season (Teien et al., 2021) and biological half-life higher during winter. The difference has been attributed to seasonal change in metabolic activities, but differences in food organisms and contamination of food organisms cannot be excluded.

Rudd *(Scardinius erythrophthalmus)* is a common freshwater fish in Eastern and Central Europe, included in Lake Glubokoye. The rudd feeds predominantly on plankton, terrestrial insects and plant materials. A preference towards plant food has been reported with age (Freyhof, 2008; Nurminen et al., 2003; Zapletal et al., 2019; Revnne and JeuErl, 1991).

Figure 1.3: Rudd *(Scardinius erythrophthalmus)* (www.fischlexikon.eu, 2021)

There are several potential food biota for fish, that can be a source of transfer of ^{137}Cs.

^{137}Cs has been found in terrestrial invertebrates. ^{137}Cs identified in a majority samples of invertebrates collected in the ChEZ from 1995 to 2004, with ants (*Formica Cynerea*) having the highest activity concentration at approximately $1.52\,MBq\,kg^{-1}$ ash weight. It is suggested that high radiocaesium activity concentrations in animal bodies can be expected mainly for relatively small creatures living on the litter surface (Mietelski et al., 2010). This is supported by another study finding that detritivorous invertebrates and ants have been found to be among the highest in ^{137}Cs compared to other groups of invertebrates. The levels of radionuclides have been shown to vary between species and on a timescale for invertebrate populations (Copplestone et al., 1999). The activity concentration of ^{137}Cs in plants has been reported to be in the range of $1.2-36.5\,kBq\,kg^{-1}$ in macrophytes and highly variable between plants. Mollusces have been reported to have an activity in the range of $3.2\,-\,27.2\,kBq\,kg^{-1}$ also variable between individuals and species (Gudkov et al., 2005).

It has been shown a differences between the transfers of radionuclides to different groups of aquatic species and between radionuclides. Overall, higher concentration ratio (CR) was observed to aquatic plants compared to aquatic animals. In freshwater animals, the highest CR is for insect larvae, followed by gastropods, bivalve molluscs, zooplankton,tadpoles and fish. Even though data were collected in lakes located in the same region, the CR values ranged from 12 to 600 for ^{137}Cs. (Fesenko et al., 2011). Thus ^{137}Cs activity concentrations within the same ecosystem can vary between organisms.

Stable isotope rations of nitrogen and carbon ($\delta^{15}N$ and $\delta^{13}C$) are widely used to identify trophic levels and food sources for aquatic organisms (Nielsen et al., 2018). The $\delta^{15}N$ isotopic ratio changes with a enrichment of the ^{15}N to the ^{14}N isotope through the food chain and typically a change on about 3‰ represents one trophic level (Post, 2002). The $\delta^{13}C$ isotopic ratio in aquatic organisms gives information about terrestrial and aquatic food sources. Terrestrial levels are normally lower in ^{13}C with an enrichment

of ^{13}C in aquatic systems. This is seen as competition for ^{12}C increases. $\delta^{13}C$ show a trophic fractionation (0.4‰) much lower than $\delta^{15}N$ (Post, 2002), but its ratio ^{13}C to ^{12}C varies in the ecosystem and thus it can be used as an indication of the carbon source (DeNiro and Epstein, 1978; Nielsen et al., 2018).

The aim of this study is to identify ^{137}Cs in fish species and in different food biota and using stable isotope signature of ^{13}C and ^{15}N as a tool, with a focus on rudd and also include other fish species, collected in contaminated Lake Glubokoye in ChEZ, to identify differences in ecosystem position of fish. Comparing isotope signature and ^{137}Cs activity concentration in fish species and biota were used to link possible groups of food organism as main contributor for ^{137}Cs to rudd. Investigation of intestine content was done to identify diversity of food.

The following hypothesis have been put forward:

- H_1 : There is a difference in stable isotope signature of $\delta^{15}N$ and $\delta^{13}C$ within the species of fish in the Glubokoye.
- H_2 : Rudd in the Glubokoye pray on both terrestrial and aquatic food organisms.
- H_3 : There is caesium in aquatic and terrestrial food organisms
- H_4 : ^{137}Cs in rudd in the Lake Glubokoye originates from both terrestrial and aquatic food organisms.

2. Methods

Organism used in this study were collected at several occasions, several times during 2021, but also stored samples from previous field experiments to Lake Glubokoye were used (Table 2.1).

Table 2.1: Overview of samples included in this work, sampling times, sampling type, sample preparation and place of performed analysis is presented.

Sample type	Collection date	Sample preparation	Analysis Preformed
Fish	2016-2021	NMBU	^{137}Cs at UIAR Stable isotopes at CLIPT
Intestine content	Summer and autumn 2021	NMBU	Mikroscope analysis at NMBU ^{137}Cs at NMBU
Bentic evertebrates	Autumn 2021	NMBU	^{137}Cs at NMBU Stabil isotop at CLIPT
Gastropoda	Autumn 2021	NMBU	^{137}Cs at NMBU Stabil isotop at CLIPT
Insects	Summer 2021 by UIAR	NMBU	^{137}Cs at NMBU Stabil isotopes at CLIPT
Plants	Autumn 2021	NMBU	^{137}Cs at NMBU Stabil isotop at CLIPT

2.1 Study Area

Lake Glubokoye is located in a highly contaminated area within the ChEZ, see figure 2.1, and is one of the most contaminated lake in ChEZ (Murphy et al., 2011).

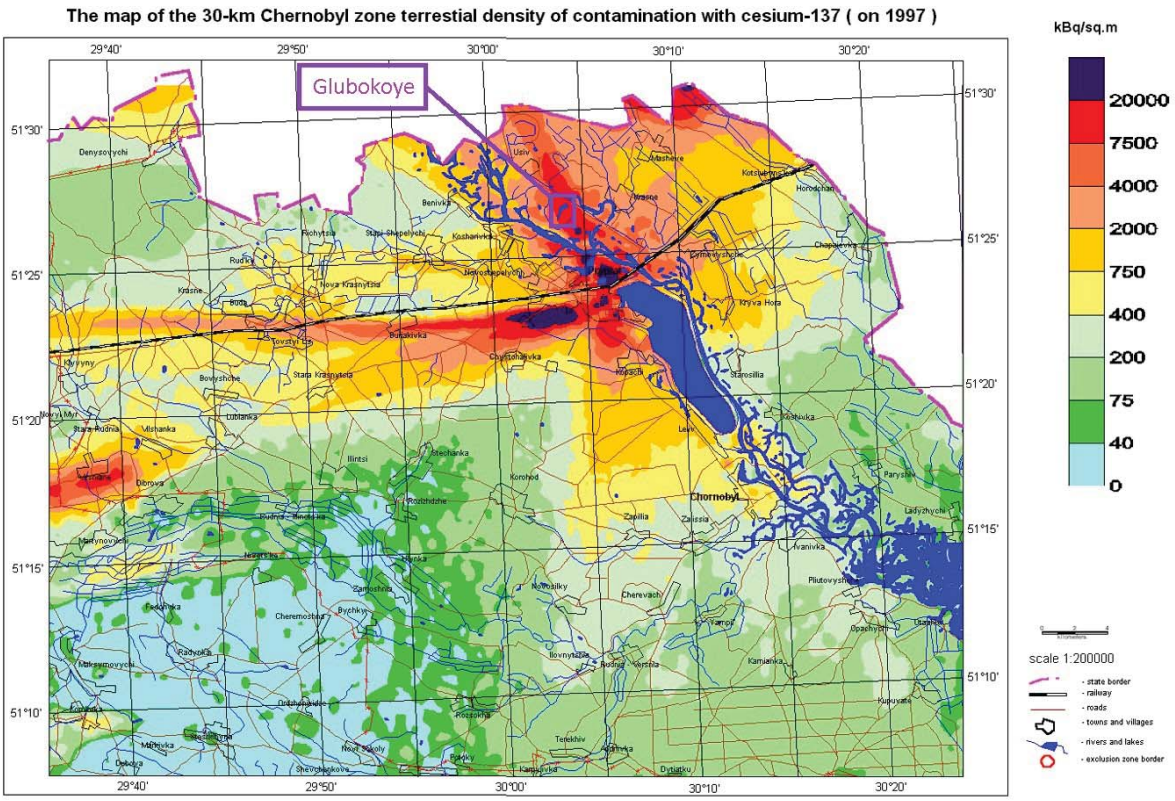


Figure 2.1: Deposition map of ^{137}Cs within the Chernobyl exclusion zone (UIAR map of ChEZ)

The characteristic of the lake water and activity concentration of radionuclides have been analysed by (Teien et al., 2021) and have not been found to change significantly over the years. The water is characterized as hard water. The pH 7.4 − 7.6, [Ca] $30 \pm 2\, mg\, L^{-1}$, [K] $1.2 \pm 0.1\, mg\, L^{-1}$, conductivity of $201 \pm 10\, \mu Si\, cm^{-1}$. The lake has a moderate concentration of DOC at $12 \pm 2\, mg\, L^{-1}$. The concentration of stable strontium and caesium was [Sr]$106 \pm 2\, \mu g\, L^{-1}$ and [Cs] $4.9 \pm 1.6\, ng\, L^{-1}$. The temperature of the water has reported a seasonal variation from a low at $2°C$ and a high at $28°C$. The ^{137}Cs has been reported with an average activity concentrations of $3.6 \pm 1.0\, Bq\, L^{-1}$ in Glubokoye lakewater and not found to change significantly between seasons (Baloga et al., 2011).

2.2 Collection of Samples

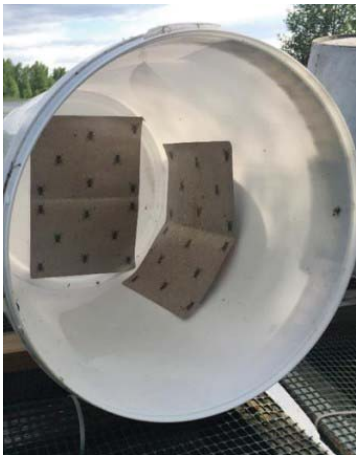
Fish were caught using a fishing rod and gillnet. The fish were killed by a blow to the head. Length(l) and weigth(w) was recorded. Samples of scales and gillcover was taken for age determination. Muscle sample without skin and samples of intestine content in fish were collected. Muscle samples were stored in a plastic bag and freezed until analysis. Since the rudd stomach is morphologically not evident, and thus gut content is collected from the first third of the intestine (Revnne and JeuErl, 1991). The intestine content collected in 2021 was transferred into 70-86% ethanol in June or freezed as it was in October (Tabel 2.1).

Perch was collected in May 2016 $[n = 9, l = 22.9 \pm 7.11\,cm, w = 185 \pm 183\,g]$. Pike was collected in November 2017 $[n = 11, l = 49 \pm 4.4\,cm, w = 724.5 \pm 208.7\,g]$ and March 2019 $[n = 6, l = 46.2 \pm 17.4\,cm, w = 916.8 \pm 605.58\,g]$. Prussian carp (*Carassius gibelio*)(Carp) was collected in March 2019 $[n = 12, l = 37 \pm 4.7\,cm, w = 987.5 \pm 331\,g]$. Rudd was collected in June 2017 $[n = 8, l = 16.17 \pm 1.7\,cm, w = 53 \pm 23\,g]$, June 2021 $[n = 7, l = 23.3 \pm 2.25\,cm, w = 143.6 \pm 29.89\,g]$ and October 2021 $[n = 10, l = 14.8 \pm 4.75\,cm, w = 48.8 \pm 61.55\,g]$.

A selection of bentic invertebrates and gastropodes were collected in Lake Glubokoya on the 26-28th of October 2021 using a kick net before transferred to a petri dish and then transferred to a sample tube and freezed. The following aquatic insects were collected Damselfly (*Zygoptera*) $[dryweight\ total = 0.063\,g, n = 1]$, Alderflies (*Megaloptera* $[dwt = 0.021\,g, n = 2]$, Dragonflyes(*Anisoptera* $[dwt = 0.06\,g, n = 7]$, Glassworms (*Chaoborus*) $[dwt = 0.012\,g, n = 2]$, nonbiting midges *Chironomidae* $[dwt = 0.0009\,g, n = 1]$, nonbiting midges *Chironomidae* $[dwt = 0.0047\,g, n = 2]$, Snail (*Gastropoda* $[dwt = 0.30\,g, n = 8]$ were collected.

During the summer of 2021 terrestrial insects were trapped in several traps. Two types of Malaise Trap (Figure 2.2c and Figure 2.2d) (Gressitt and Gressitt, 1962; Malaise, 1937), Glue traps on water (Figure 2.2b) and glue traps inland (Figure 2.2a). Terrestrial insects were also caught using a soil trap.(Figure 2.2e).

(a) Glue trap inland



(b) Glue trap on water

(c) Malaise trap

(d) Malaise trap 2

(e) Soil trap

Figure 2.2: Traps used for collecting insects

The Malaise was constructed with a tent with a cone upwards. Insects that came under the tent moved upwards to the top of the tent. At the top there was an opening in the end of the cone with a bottle that collected the insects. The bottom of the bottle was filled with 86% ethanol. Rainwater was prevented from entering the bottle (Figure 2.2c and 2.2d). The soil-trap was a container with cover (Figure 2.2e) to prevent rainwater, and provide shade, the bottom was filled with 86% ethanol. Two glue-traps were used, one freestanding inland (Figure 2.2a) and one placed on floating objects in the water inside a bucket for protection (Figure 2.2b). Glue traps used store bought adhesive glue traps for pest control. A total of 373 insects from the sampling arrived at NMBU, specified in Appendix, table A.

The following insects were selected for further analysis. Nine-spotted moth (*Amata phegea*) [dwt=0.021 $n = 21$], mosquito-small collection 1 (*Culicidae*) [$dwt = 0.0066\,g,\ n = 20$], mosquito-small collection 2 (*Culicidae*) [$dwt = 0.0071\,g,\ n = 15$], mosquito-large collection (*Culicidae* [$dwt = 0.135\,g,\ n = 19$], Flies (*Delia Platura*)[$dwt = 0.05\,g,\ n = 15$], millipedes (*Diplopoda*)[$dwt = 0.96\,g,\ n = 28$].

A selection of five plants was collected in October 2021 in the littoral zone of the lake. These samples were photographed, roughly rinsed and frozen. Prof. Susanne Schneider at the Norwegian Institute for Water Research was helpful in determining the species of plants. *Potamogeton sp* [$dryweigth = 0.095\,g$], *nymphaea sp* [$dw = 1.87\,g$] and *Cartophyllum demersum* [$dw = 0.22\,g$] were collected. In addition two plant samples containing plants were difficult to determine the species from and thus denoted as uknown plant 1 [$dw = 0.54\,g$] and 2 [$dw = 0.13\,g$].

2.3 Analysis

2.3.1 Analysis of Caesium

Determination of ^{137}Cs activity concentration in fish muscle samples was carried out using Marinelli vessels $20\,cm^3$, weight was recorded on a laboratory scale and a gamma measurements were performed using a low-background $\gamma - spectrometric$ complex with a multi-channel analyser $ASPEC - 927$ (software GammaVision 32). Samples of fish muscle were analysed at the UIAR. Activity concentrations in fish muscle are presented as $kBq\,kg^{-1}$ wet weight. Comparing wet weight and dry weight of muscle sample, the dry weight of fish was about 20% of wet weight. The assumption of 20% dry weight is based on a experiment done at the isotope-laboratory at NMBU (Teien, 2022).

After visual inspection of the intestine content, all the intestine content was freeze-dried in in $20\,cm^3$ HDPE plastic vial, before weight was recorded and the sample was analysed using a Perkin Elmer 2480 automatic gamma counter with (software wizard 2) with a counting time of two hours. Activity concentration of ^{137}Cs in intestine content is presented as $Bq\,g^{-1}$ dry weight as this is equal and comparable with $kBq\,kg^{-1}$ used in fish.

Poled samples of invertebrates of species or groups (Table 3.3) were analysed in a 50 ml poly-carbonate tubes, a cotton-ball was used to fix the sample and ensure that the sample did not move due to static electricity. In snails, the shell and the body were analysed separately. The samples only filled a small volume of the tube and a geometry for 10 ml in a 20 ml liquid scintillation vial was used. The samples were analysed on a Germanium Ortec HPGe Coaxial Detector, with counting time to ensure

sub 10% uncertainties or 48 hours. Activity concentration of ^{137}Cs in insects and snails is presented as $Bq\,g^{-1}$ dry weight. The same species or group of invertebrates used in stable isotope analysis.

The plants were analysed for ^{137}Cs in a $20\,cm^3$ HDPE plastic vial with all available freeze-dried plant-material or as much sample as the vial could contain. The sample weight were recorded. A Germanium Ortec HPGe Coaxial Detector (Software: Ortec gammavision) was used to analyse activity of ^{137}Cs with counting time to ensure sub 10% uncertainties. Activity concentration of ^{137}Cs in plant is presented as $Bq\,g^{-1}$ dry weight.

2.3.2 Stable Isotopes

To identify stable isotope signature in fish, analysis were based on frozen muscle samples analysis. Prior to stable isotope analysis approximatly $2.0\,g$ of muscle sample was taken into 2 ml of distilled water in a 5 ml tube, homogenized with a laboratory blender and freez-dried. $0.9 - 1.1\,mg$ of each fish freez-dried and homogenized samples were packed into 5.5x5 mm tin cups for IRMS-analysis. One replicate of each sample of fish was analysed for stable isotopes. The samples were stored as homogenized freeze-dried fish-muscle in containers with lid, the samples were stored in room temperature. Samples collected in June 2021 were not analysed for stable isotopes.

Collected plants were rinsed with distilled water, freeze-dried and homogenized by crushing. Three replicates of each plant were prepared by packing 4.0 mg of sample into 5.5x5 mm tin cups for IRMS-analysis.

All insects were dried in a drying chamber with $40°C$ and ambient humidity. The insects with a sample weight below 1.0 mg were packed as whole individes into 5.5x5 mm tin cups without any treatments, individual insects exceeding 1.0 mg were homogenized with a laboratory blender in 2 ml of distilled water in a 5 ml tube, and freeze-dried before subsamples of $0.9 - 1.1\,mg$ were packed in 5.5x5 mm tin cups for IRMS-analysis. Three samples of each species or pool of insect were analysed as replicates, independent whole individ analysis or homogenized prior to measurements.

Snails were freeze-dried to hinder the sample decomposing during preliminary analysis. For isotope analysis the snail were crushed to collect the body of the snail, three individual snails were selected as replicates and the soft tissue was taken into 2 ml of distilled water in a 5 ml tube, homogenized with a laboratory blender and freeze-dried. $0.9 - 1.1\,mg$ of each freez-dried snail was packed into 5.5x5 mm tin cups for IRMS-analysis.

The IRMS analysis were preformed at the CLIPT-lab stable isotope biogeochemistry lab at UiO.

2.3.3 Analysis of Age and Content of Intestine

The age of fish was determined using the opecular bone of the gill cover of the sampled fish. The sampled gill cover was put into boiling water for a few minutes until the skin was loose, the skin was removed, and winter zones were identified in a microscope and counted as a measure of age (Hansen, 1980). Age verification was done under supervision of Professor Emeritus Reidar Borgstrøm at the Faculty of Environmental Sciences and Natural Resource Management at NMBU.

All intestine content was inspected visually and pictures of identifiable fragments were taken on a stereo microscope. Objects that needed verification was verified by correspondence with Professor Thrond Oddvar Haugen at the Faculty of Environmental Sciences and Natural Resource Management at the NMBU.

2.4 Quality Assurance

Reference material [$IAEA\ 300, calculated = 0.7\,Bq$, $measured = 0.8 \pm 0.04\,Bq$] were analysed to calculate efficiency of the Germanium Detector. Blank samples were analysed to determine limits of detection (LoD) and limits of quantification (LoQ) according to (Currie, 1968).

Reference material [$IAEA300[calculated = 5.7,\ measured = 1.19\,cps\ efficiency = 19\%$] and [$IAEA\ 373\ calculated = 42.3\,Bq,\ measured = 6.71 cps,\ efficiency = 16\%$] were analysed to calculate efficiency on the Sodium Iodine Detector. An efficiency was set to 17% in conference with Senior Lab Engineer, Marit Nandrup Pettersen. Blank samples were analysed to determine LoD and LoQ.

Inhouse standard for stable isotopes of fish at NMBU were measured and values [$-19,29 \pm 0.047‰\ \delta^{13}C$ and $13.22 \pm 0.048‰\ \delta^{15}N$] show good accuracy compared to earlier measured values [$-19,27 \pm 0.07‰\ \delta^{13}C$ and $13.48 \pm 0.41‰\ \delta^{15}N$]. In addition Internal Reference and Quality Assurance Material [$Internal\ code : JALA\ \delta^{13}C = -20,58 \pm 0.08‰, \delta^{15}N = -3.19 \pm 0.08‰$] was used at the CLIPT-lab to ensure good accuracy. Internal references and quality assurance at the CLIPT-lab are described in Appendix B.

2.5 Statistical Analysis

Statistical analyses were performed in R [vR-4.1.2 (2021-11-01)]. Differences in isotope signature, $\delta^{13}C$ and $\delta^{15}N$ for the fish species were assessed using a MANOVA with a Pillai's Trace Statistical Test, Partial Eta Squared for effect size measurement, and using linear discriminant analysis [LDA] to evaluate differences between species. The data failed the multivariate normality test, but on evaluating the QQ-plot, the MANOVA was still used as it is still considered robust (Ntoumanis and Myers, 2015). A Pearson correlation coefficient with $\delta^{13}C$ and $\delta^{15}N$ respectively against ^{137}Cs was preformed in R to investigate if a linear relationship was present. A Welch Anova with a non parametric Games Howell Post Hoc Test was used was done to compare the measured $\delta^{13}C$, $\delta^{15}N$, ^{137}Cs and length in the samplings of rudd and the levels of $\delta^{13}C$ and $\delta^{15}N$ respectively in feed organisms and fish.

2.6 Data Handling

The samples of rudd collected in June 2021 were only analysed for ^{137}Cs, in addition three intestine content from fish collected in June were analysed. As several of the fish analyzed from earlier experiments at Lake Glubokoye did not have data regarding age, length were used as a parameter for size and age in the the statistical analysis. Due to labeling error, one fish collected in October 2021 was excluded from the data set.

On analysis of the data set, it became clear that the stable isotope values for individuals of roach did not match the values for the species. Roach and rudd at young age are easy to misinterpret with regard to determining species. It was decided to change two of the observations of roach to rudd of fish from Lake Glubokoye. The fish were collected in 2016 and it is not possible to recheck the determination of the species. It is also known that hybridisation can occur between roach and rudd (Pitts, 1994), and that this also might give origin to individual fish that are difficult to determine the species of.

On viewing the results of the isotope analysis of the insects that had been analysed, it was apparent that of a total of 6 supposed Diptera Culicidae species pooled as mosquito-small, two had an isotop signature matching a terrestrial origin and four had a aquatic origin. These insects were very small and determination of species was not easy, and thus they were grouped into a category of mosquito-small. Because of this, insects were grouped into Aquatic and terrestrial insects on the basis of their isotop signature. The two samples of mosquito-small had levels of ^{137}Cs below detection limits.

3. Results

3.1 ^{137}Cs Activity Concentration

3.1.1 Fish

Results show that the activity concentration of ^{137}Cs in fish varies between species and between individuals. Roach was measured to an overall lower activity concentration of ^{137}Cs, with the highest measured values of $2.79\,kBq\,kg^{-1}$ while pike, perch and rudd have all individuals with over $10.0\,kBq\,kg^{-1}$ activity concentrations (Table 3.1 and Figure 3.2).

The activity concentration in pike, perch, carp and rudd is not significantly different to each other, but activity concentrations in pike and carp are significantly higher than roach [$p = 7.9e^{-6}$ and $p = 7.65e^{-7}$ respectively] (Table 3.1).

Pike were collected on two different occasions in November 2017 and March 2019, both during the winter season. It was not found a significant difference in activity concentration of ^{137}Cs between these sampling dates, but the sampling in March had the individuals with over $10.0\,kBq\,kg^{-1}$ activity concentrations ^{137}Cs (Table 3.1, Figure 3.2).

Comparing rudd sampled at different times, results show that rudd sampled in June 2017 is significantly higher than rudd from October 2021 [$p = 1.5e^{-2}$], rudd from June 2021 [$p = 1.9e^{-2}$] (Table 3.2). Rudd sampled in June 2017 is also significantly higher than carp [$p = 3.50e^{-2}$], perch [$p = 5.80e^{-2}$] and roach [$p = 8.0e^{-3}$] but not significantly higher than Pike (Table 3.1). Overall, the sampling of rudd in 2017 has fish with activity concentrations exceeding known predatory fish such as pike and perch and is significantly higher than for all other sampling dates for rudd (Figure 3.3 and Table 3.2).

Table 3.1: ^{137}Cs and $\delta^{13}C$ and $\delta^{15}N$ in species of fish analysed from the Lake Glubokoye for all parameters. Average values with Standardeviation (SD) and min and max values are given.

	Species		^{137}Cs				$\delta^{13}C$				$\delta^{15}N$			
		n	$kBq\,kg^{-1}$	SD	min	max	Mean	SD	min	max	Mean	SD	min	max
1	Carp	12	6.73	1.24	4.06	8.92	-33.58	0.50	-34.21	-32.48	6.65	0.71	-34.21	-32.48
2	Perch	9	7.75	4.64	3.53	14.87	-30.98	0.79	-31.89	-29.36	8.56	0.28	-31.89	-29.36
3	Pike	17	9.80	3.68	5.08	20.59	-31.51	1.04	-33.77	-29.90	7.97	0.34	-33.77	-29.90
4	Roach	5	2.50	0.29	2.06	2.79	-32.39	1.30	-33.96	-30.60	5.07	0.54	-33.96	-30.60
5	Rudd	27	10.74	9.21	2.64	32.10	-28.93	1.24	-30.57	-26.83	6.73	0.61	-30.57	-26.83

3.1.2 Length and Age

The fish collected was different in size with pike ($l = 48 \pm 11.4\,cm$) and carp ($l = 37 \pm 4.7\,cm$) beeing larger than perch ($l = 22.9 \pm 7.11\,cm$), roach ($l = 23.76 \pm 2.66\,cm$) and rudd ($l = 17.60 \pm 4.92\,cm$). Pike and rudd had individuals collected on different dates.

Pike collected in November 2017 and March 2019 were not significantly different in length, but the sampling in 2019 had some large individuals over 60cm in length. Samples of rudd collected in June 2021 were significantly larger than rudd [$p < 4.00e^{-4}$] collected on the other collection dates (Tabel 3.2).

Correlation of size and ^{137}Cs was not found in the fish population overall [correlation coefficient = 0.07].

The age of the rudd was found to be two seasons for fish at 9 cm of length, four seasons for fish for 13.5 to 19.5 cm and five seasons for fish at 25 cm. The age of roach was determined to four seasons for fish 21.5 cm and 23.5 cm, and five seasons for fish with a length of 25.5 cm.

Table 3.2: Rudd sampled in 2016, 2017 and 2021 in the Lake Globokoya. Length of fish and ^{137}Cs, $\delta^{15}N$ and $\delta^{13}C$in fish muscle on different sampling dates.

Sample date	Length cm	^{137}Cs $kBgkg^{-1}$	$\delta^{13}C$	$\delta^{15}N$
n	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
	Min-Max	Min-Max	Min-Max	Min-Max
2016.05.12	12.2 ± 0.28	5.95 ± 0.92	-29± 0.76	7.40 ± 0.94
n=2	12-12.4	5.31-6.60	-29.5-(-28.5)	6.74-8.07
2017.06.13	16.2 ± 1.70	19.77 ± 8.37	-30.0 ± 0.541	7.12 ± 0.278
n=8	14-19	8.22-32.10	-30.6-(-29.0)	6.74 - 7.56
2021.06.21	23.3±2.25	5.00±0.25	na	na
n=7	19.5 - 24.5	4.65-5.16	na	na
2021.10.29	14.8±4.75	4.47±1.79	-28.0±1.01	6.29±0.415
n=10	8.5 - 25	2.65-8.48	-29.7-(-26.8)	5.71-7.19

3.1.3 Food Organism

The activity concentration of ^{137}Cs in five plants collected in October 2021 was highly variable and in range from 0.83 ± 0.08 to $48.9 \pm 4.8\,Bq\,g^{-1}$ dry weight. In the Benthic

Predator (*Odonata*), a collection of nymphs analysed, they had a level of ^{137}Cs at $8.42 \pm 0.8\, Bq\, g^{-1}$. Snails collected showed a ^{137}Cs level of $20.89 \pm 3.32\, Bq\, g^{-1}$ in the body, and $1.66 \pm 0.22\, Bq\, g^{-1}$ in the shell. Several of the terrestrial insects had to low biomass possible too detect ^{137}Cs. Samples possible to detect had activity concentration of ^{137}Cs up to $7.49 \pm 1.0\, Bq\, g^{-1}$. The millipedes (*Diplopoda*) had ^{137}Cs levels of $22.10 \pm 1.3\, Bq\, g^{-1}$. ^{137}Cs was detected in all groups of feed organisms studied except in the low biomass samples (Tabel 3.3).

Table 3.3: Activity concentrations of ^{137}Cs in selected samples of organisms and biota in and around the Lake Glubokoye.

	Species	$Bq\, g^{-1}$	σ
Terrestric-insects	Mosquito-small collection1	nq	na
	Amata phegea	2,19	0,16
	Delia Platura	4,92	1,11
	Mosquito-large collection	7,49	1,00
Detritivores	Diplopoda	22,11	1,28
Aqautic-insects	Odonata	8,42	0,80
Aqautic-snail	Snail-body	20,88	3,32
	Snail-shell	1,66	0,22
Aqautic-plants	Potamogeton sp.	12,36	1,42
	Unidentified plant 1	23,70	0,62
	Nymphaea sp.	4,18	0,61
	Cartophyllum demersum	0,83	0,08
	Unidentified plant 2	48,87	4,79
Aqautic and terrestric	Mosquito-small collection 2	nd	na

3.1.4 Intestine Content

The amount of intestine content in the ten fish collected in October was from $0.002 - 0,066\, g$ dry weight with a average of $0.014 \pm 0.019\, g$, and was lower than the stomach content in fish sampled in June 2021 with an average of $0.25 \pm 0.086\, g$.

In rudd collected in October activity of ^{137}Cs was only identified in two of 10 samples as the activity was below limits of detection in four samples and below limits of quantification in the other four samples. The two samples with quantifiable results had an activity of 23.42 ± 7.85 and $127.6 \pm 13.73\, Bq\, g^{-1}$ dry weight. Measured activity concentration of ^{137}Cs in intestine content collected in June 2021 was ^{137}Cs $12.76 \pm 1.9 - 17.0 \pm 2.47\, Bq\, g^{-1}$ dry weight.

3.2 Stabil Isotopes - Food Chain Identification

The results from the stable isotope analysis is presented graphically in figure 3.1 and indicate relative positioning in the ecosystem.

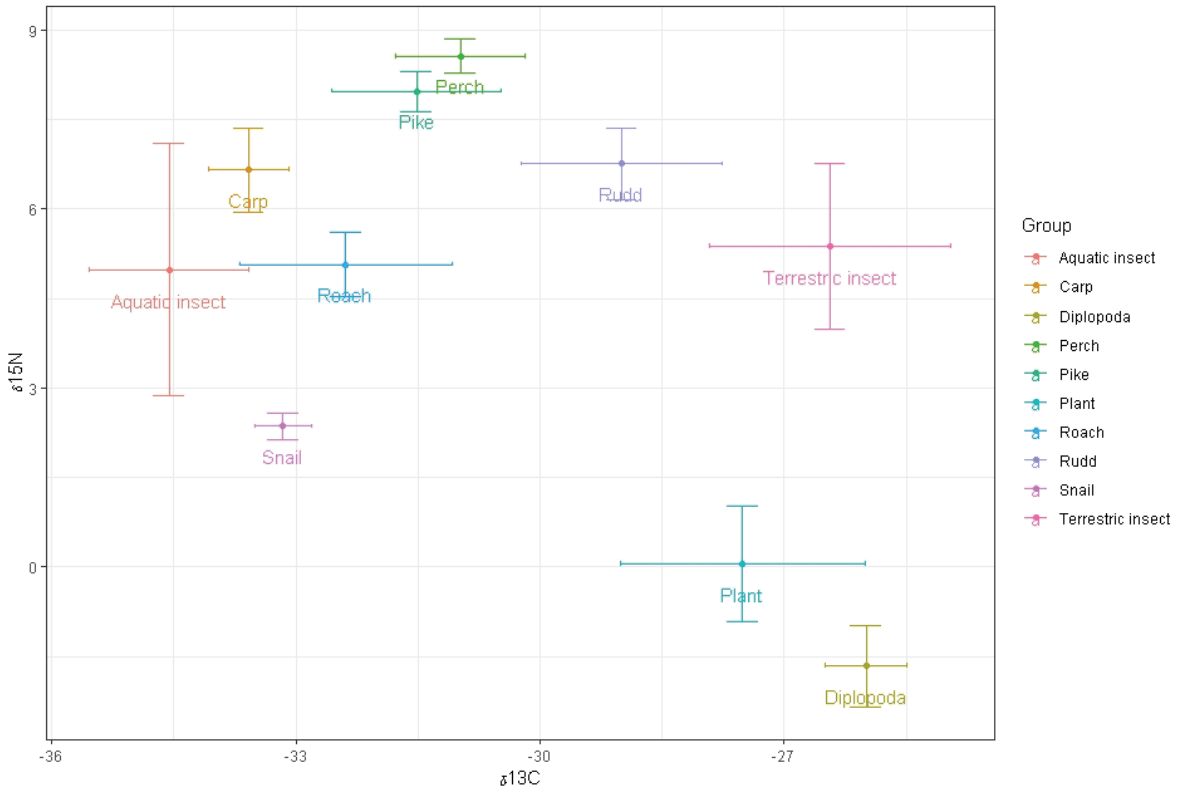


Figure 3.1: $\delta^{13}C$ and $\delta^{15}N$ in sampled organisms from the Lake Glubokoye within the ChEZ. Average values are presented and also shown as numeric values in 3.4. Standard-deviation is used to present an errorbar.

3.2.1 Fish

Analysing each variable individually with a Welch ANOVA test the results show that there is a difference between the species with respect to $\delta^{15}N[p = 2e^{-16}]$ and $\delta^{13}C[p = 2e^{-16}]$. The Games Howell Post Hoc Test shows that perch has the highest $\delta^{15}N$ (8.56 ± 0.28‰) significantly higher than pike (7.97 ± 0.34‰) $[p = 5.00e^{-3}]$, carp (6.65 ± 0.71‰) and rudd (6.75 ± 0.60‰) were not significantly different in $\delta^{15}N$ but significantly lower than pike $[p = 9.50e^{-4}$ and $p = 2.33e^{-7}$ respectively]. Roach samples has the lowest $\delta^{15}N$ value (5.07 ± 0.54‰), which is significantly lower than rudd and carp $[p = 1.10e^{-2}$ and $p = 1.33e^{-2}$ respectively]. Perch and pike are more than 3 ‰ over roach in $\delta^{15}N$ levels (Figure 3.2 and Table 3.4).

With respect to $\delta^{13}C$ the results show that carp (-33.58 ± 0.5‰) have the lowest value, not significantly lower than roach(-32.59 ± 1.3 ‰) but significantly lower than than for

perch (-30.98) $\pm$ 0.79 ‰) and pike (-31.51 ± 1.04 ‰) [$p = 4.19e^{-5}$ and $p = 9.94e^{-6}$, respectively]. Rudd (-28.93 ± 1.23 ‰)has a significantly higher value for $\delta^{13}C$ compared to all the other fish [$p < 1.00e^{-3}$]. Perch, pike and roach is not significantly different in $\delta^{13}C$ (Figure3.2, Figure 3.1 and Table 3.4).

Analysing $\delta^{13}C$ and $\delta^{15}N$ in simultanious with a multivariate MANOVA-test the results show differences between fish species on the response parameters $\delta^{13}C$ and $\delta^{15}N$ [Pillai's Trace = 1.6618, F(10, 108) = 53.073, p < 2.2e-16]. A measurement of effect size (Partial Eta Squared; $\eta p2$ 0.83) suggests that there is a large effect of fish species on both $\delta^{13}C$ and $\delta^{15}N$ combined. Based on both $\delta^{15}N$ and $\delta^{13}C$ a LDA show that the fish can be divided into four groups; roach, carp, pike-perch and rudd. All being different from each other, were carp and roach being less different to each other compared to the other species. Rudd sampled in 2021 is different from all the other species of fish, also different from the rudd sampled in 2017. Rudd sampled in 2017 is less different to pike and perch compared to rudd sampled in 2021 (Figure 3.2, Figure 3.3 and Table 3.4).

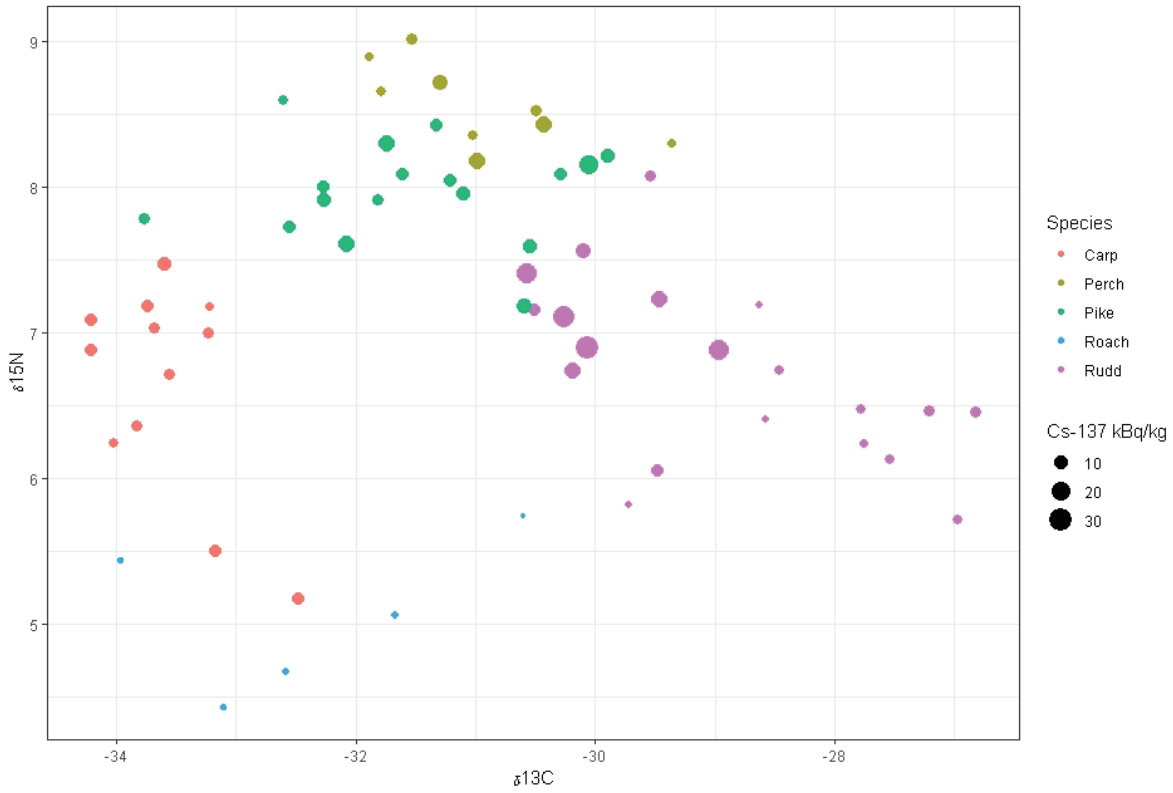


Figure 3.2: Stabil isotope $\delta^{15}N$ and $\delta^{13}C$, with ^{137}Cs as point-size in goldfish, perch, rudd, roach and pike from the Lake Glubokoye in Chernobyl.

3.2.2 Relationship Stable Isotopes, Caesium, and Lenght of Rudd from Lake Glubokoye

Overall, neither $\delta^{13}C$ or $\delta^{15}N$ show a large correlation in the level of ^{137}Cs in the fish population as a whole, $\delta^{15}N$ [correlation coefficient = 0.24] and $\delta^{13}C$ [Correlation coefficient = 0.12]. Comparing ^{137}Cs, $\delta^{13}C$, $\delta^{15}N$ and length of rudd at different times, there is a relationship between ^{137}Cs and $\delta^{13}C$. Length of rudd was not found to be significantly different in the rudd collected in 2017 compared to rudd collected in October 2021, but the observed size of the fish is significantly larger in June 2021, as stated in **??**. However this does not result in a increase in the levels of ^{137}Cs.

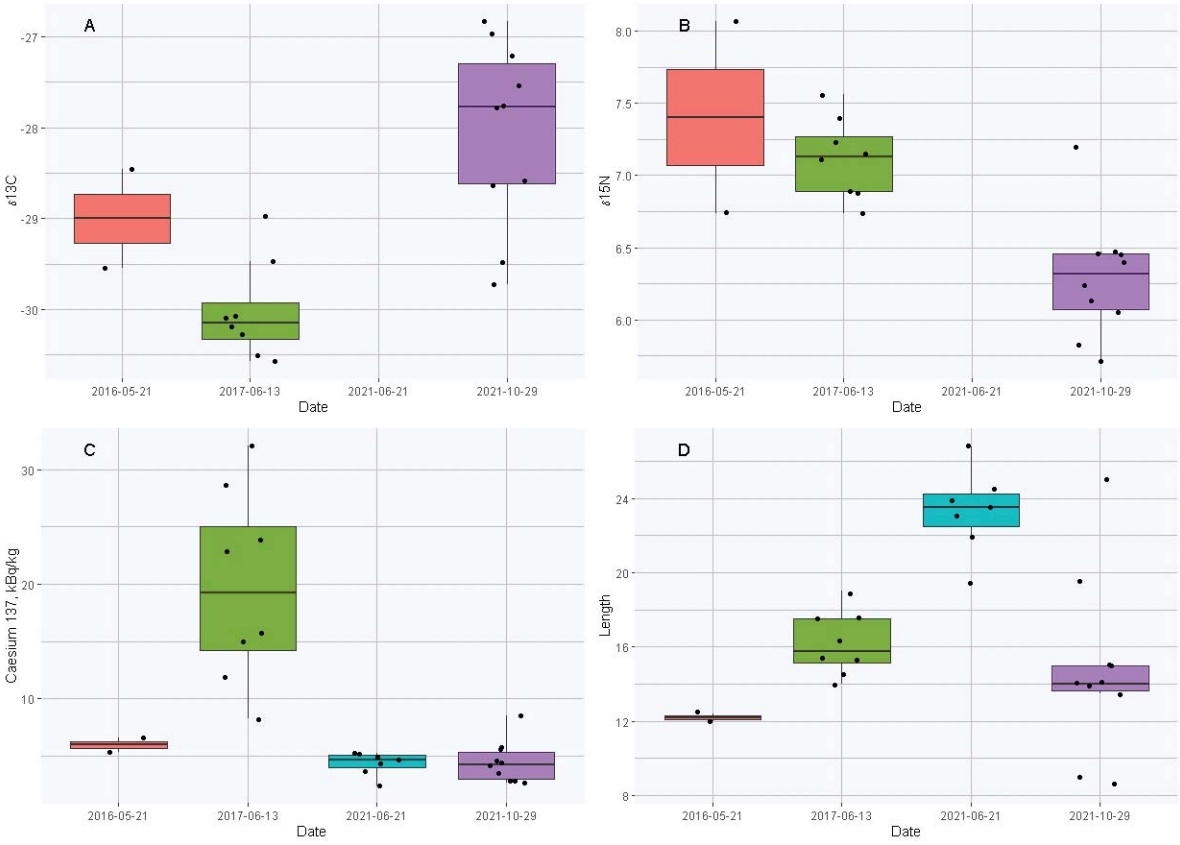


Figure 3.3: $\delta^{13}C$ (fig A),$\delta^{15}N$ (fig B),^{137}Cs (fig C) and length (cm) (fig D) in rudd from Lake Glubokoya on different sampling dates.

As there are only two samples collected in May 2016, statistics for this group was not possible.

The level of $\delta^{13}C$ was found to be significantly lower in 2017 (-30.01 ± 0.54‰) than in the samples collected in 2021 (-28.20 ± 1.01‰) [p=$7.06e^{-5}$](Table. 3.2 and Figure 3.3).

$\delta^{15}N$ had a average of 7.12 ± 0.28‰ for the rudd collected in 2017, it was significantly higher [$p = 7.3e^{-3}$] that in the fish collected in 2021 with a average of 6.26 ± 0.42‰, but

the two observations in 2016 are at the same level and makes conclusions on relationship uncertain (Table 3.2, Figure 3.3).

3.2.3 Stable Isotopes Fish and Food Organism

The results show that the response parameters $\delta^{15}N$ and $\delta^{13}C$ are different between the different potential food organisms, and that there is a difference in the isotope signature of aquatic and terrestrial insects (Figure 3.1 and Table 3.4). The snail, plants and millipedes also have different stable isotope signatures and are different compared to terrestrial and aquatic insects. Overall millipedes has the lowest $\delta^{15}N$ $(-1.66 \pm 0.68‰)$ indicating detritivores position and a $\delta^{13}C$ $(-25.99 \pm 0.50‰)$ indicating a carbon of a terrestrial origin.

Terrestrial and aquatic insects have the highest $\delta^{15}N$ among the studied food organisms with similar $5.37 \pm 1.39‰$ *and* $4.98 \pm 2.12‰$ respectively. However, Terrestrial insects $\delta^{13}C$ $(-26.43 \pm 1.48‰)$ is significantly higher than aquatic insects $(-34.56 \pm 0.98‰)$ $[p = 5.62e^{-10}]$ and snail $(-33.16 \pm 0.35‰)[p = 5.98e^{-10}]$. Terrestrial insects, plants and millipedes are not significantly different on $\delta^{13}C$, but terrestrial insects are significantly higher in $\delta^{15}N$ than plants $(p = 1.22e^{-12})$ and millipedes $(p = 3.74e^{-4})$ (Figure 3.1 and Table 3.4) .

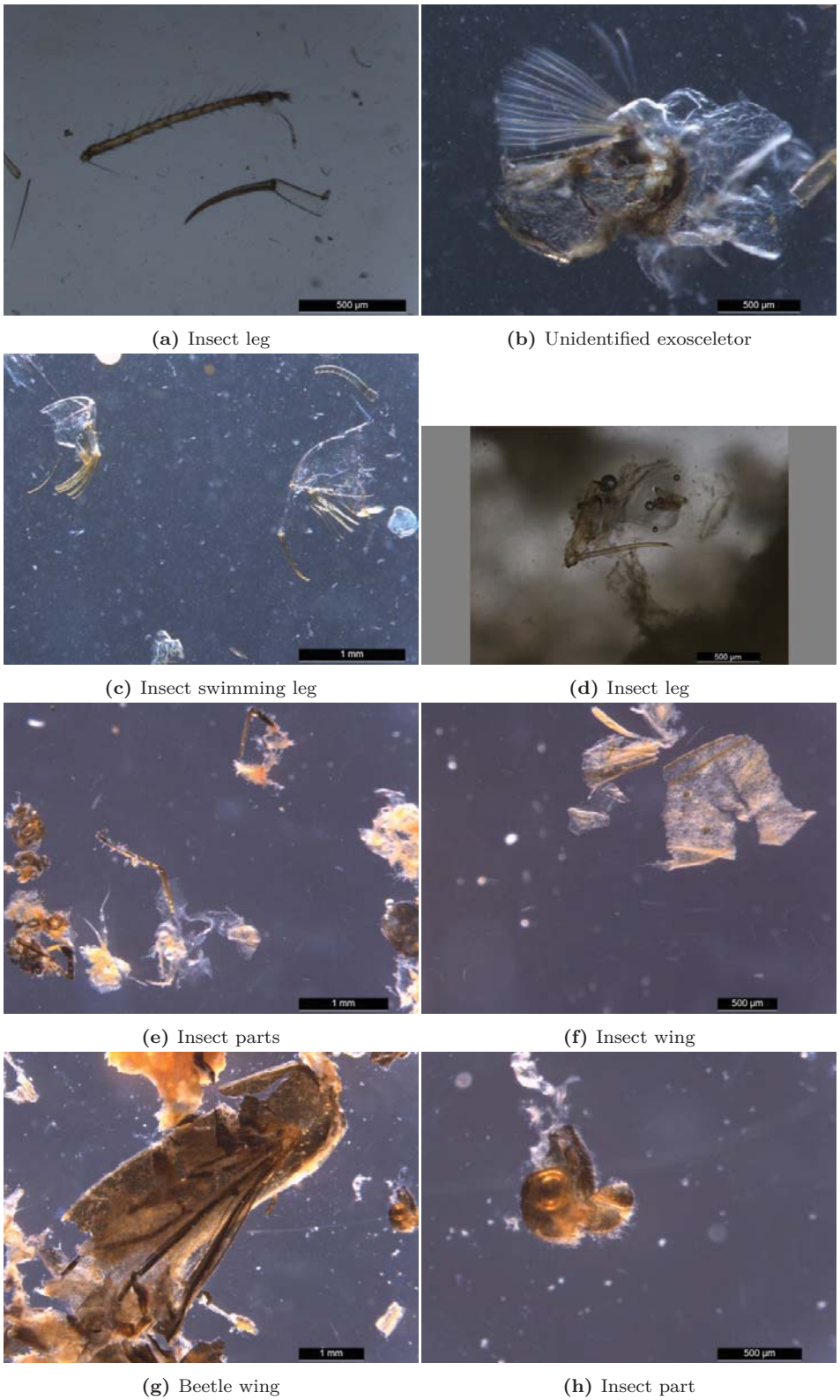
Table 3.4: Stabil isotopes of $\delta^{15}N$ and $\delta^{13}C$ in fish and organisms in and around the Lake Glubokoye.

	Group	$\delta^{13}C$ mean	$\delta^{13}C$ sd	$\delta^{15}N$ mean	$\delta^{15}N$ sd
1	Aquatic insect	-34.56	0.98	4.98	2.12
2	Diplopoda (millipedes)	-25.99	0.50	-1.66	0.68
3	Carp	-33.58	0.50	6.65	0.71
4	Perch	-30.98	0.79	8.56	0.28
5	Pike	-31.51	1.04	7.97	0.34
6	Plant	-27.51	1.50	0.05	0.97
7	Roach	-32.39	1.30	5.07	0.54
8	Rudd	-28.99	1.24	6.75	0.60
9	Snail	-33.16	0.35	2.36	0.22
10	Terrestrial insect	-26.43	1.48	5.37	1.39

3.3 Analysis of Intestine Content in Rudd

The intestine content collected in June was dominated by plant material, in October the intestine was dominated by insects or more or less empty except for one sample that was dominated by plant material.

In the ten rudd caught in October 2021, five fish were more or less empty, with only some mucus in the collected sample. In five of the fish, insect parts were found, invertebrate eggs were found in one sample, and in one sample plant material was abundant. Possible gill cover was also found (Figure 3.4 and 3.5).



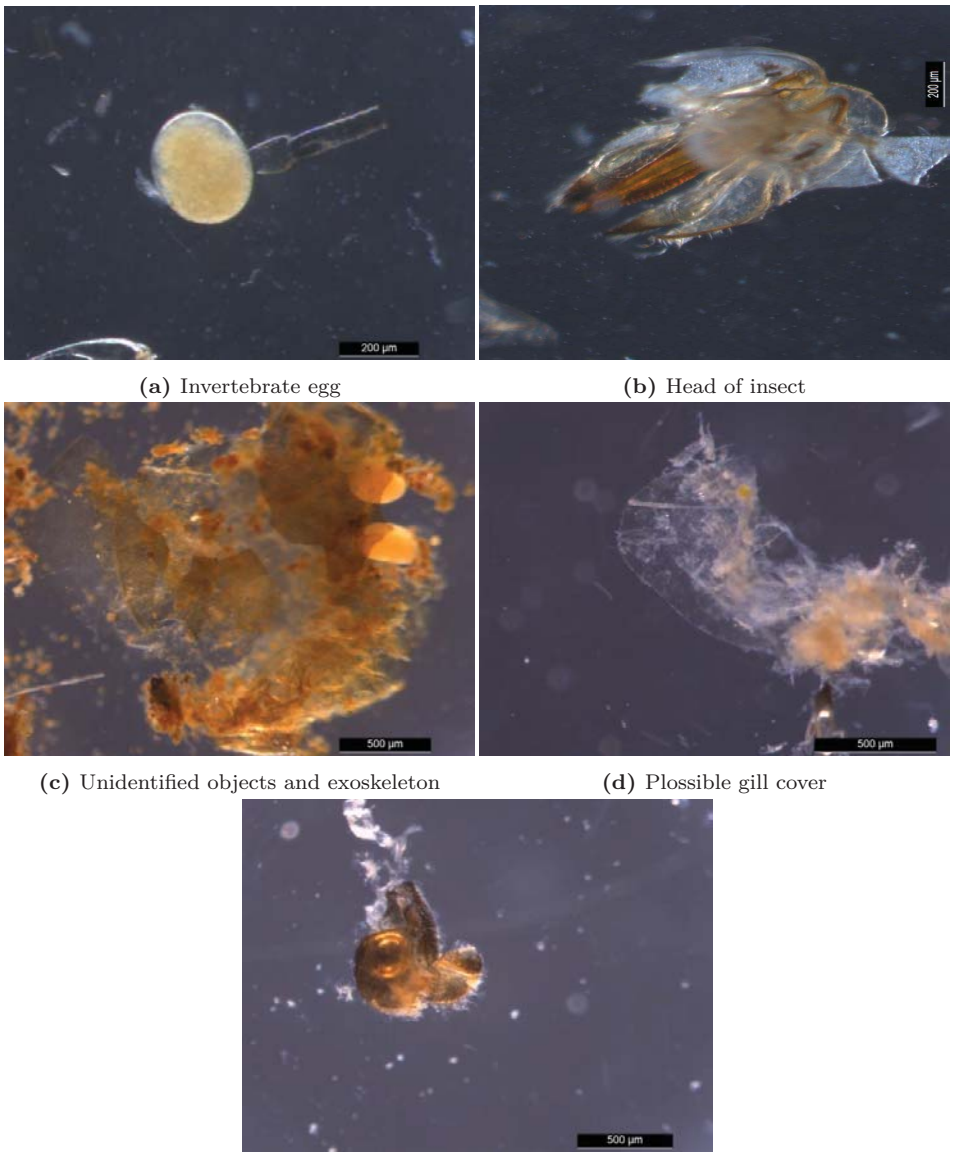
(a) Insect leg **(b)** Unidentified exosceletor

(c) Insect swimming leg **(d)** Insect leg

(e) Insect parts **(f)** Insect wing

(g) Beetle wing **(h)** Insect part

Figure 3.4: Combined intestine content in 10 sampled rudd from the Lake Glubokoye within ChEZ from October 2021. Analysed visually for identifiable fragments. Insect parts were found. Continuation in figure 3.5



(a) Invertebrate egg

(b) Head of insect

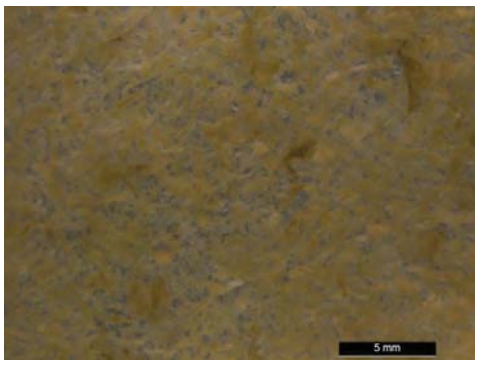
(c) Unidentified objects and exoskeleton

(d) Plossible gill cover

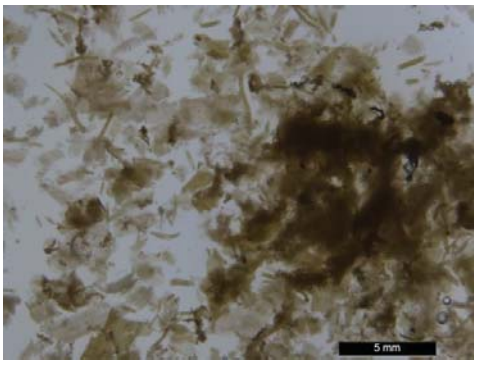
(e) Insect part

Figure 3.5: Combined intestine content in 10 sampled rudd from Lake Glubokoye within ChEZ from October 2021. Continuation from figure 3.4. Analysed visually for identifiable fragments. Insects, invertebrate egg and possible gill cover of small fish were found

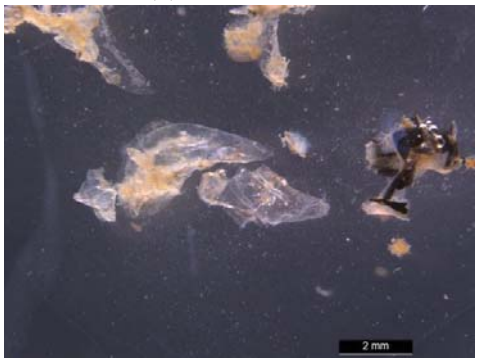
In the intestine content samples collected in June 2021, there was a domination of green plants i all the samples, but one sample had fragments of insects and also several probable gill covers of small fish, seen figure 3.6c (Tarkan et al., 2007). No other hard parts of fish were identified.



(a) Plant material

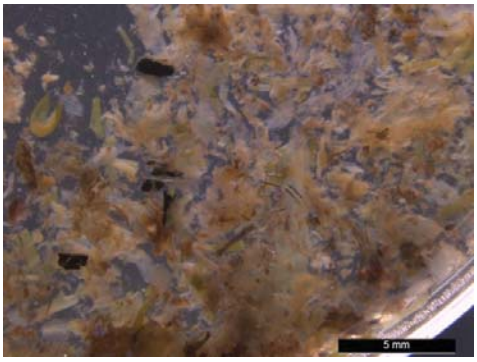


(b) Plant material



(c) Gill cover from fish

(d) Insect wing



(e) Overview plant domination

Figure 3.6: A representative visalisation of intestine content in three sampled rudd from the Lake Glubokoye in Chernobyl from June 2021, visually analysed for identifiable fragments. Plants, Insects and several gill cover of small fish were indentified

4. Discussion

4.1 Activity Concentrations of Caesium(^{137}Cs)

The presence of ^{137}Cs in and around Lake Glubokoye is not limited to a single group of biota, and there is a large variation in the activity concentration beween individes and between species. This has been shown in a multitude of studies within the ChEZ (Lerebours et al., 2018; Kaglyan et al., 2015; Gudkov et al., 2005; Mietelski et al., 2010; Copplestone et al., 1999; Fesenko et al., 2011), and other areas contaminated with ^{137}Cs (Iwasa et al., 2020; Wada et al., 2016; Jonsson et al., 1999).

The activity concentration of ^{137}Cs in fish was variable between fish species and individuals and it was highest in predatory fish species and rudd. This has also been shown in other studies from Lake Glubokoye (Kaglyan et al., 2015). In studies from other lakes levels of ^{137}Cs in rudd are among the lowest of the fish species (Koulikov, 1996). High transfer of ^{137}Cs to predatory fish species are in agreement with literature. However the high activity concentration in rudd is not according to transfer models (Smith et al., 2000; Rowan and Rasmussen, 1994; Koulikov, 1996).

The activity concentration of intestine content in rudd shows that ^{137}Cs enter the fish through dietary intake and support that dietary uptake is most likely is from food, and the highest concentration of ^{137}Cs found in a sample was in one of the intestine contents. Studies has shown that levels of ^{137}Cs in intestine and muscle have been found to correlate (Teien et al., 2021), and this also indicates that uptake of ^{137}Cs is related to feed. Also, the analysis of intestine content show that the levels of ^{137}Cs in some of the intestine, were higher than in the muscle of the fish, This indicates a net push of caesium into the fish. The intestine sample from October witch had the highest levels of ^{137}Cs could have eaten a hot particle. Intestine analysis gives a short integral of the feed eaten by the fish. Intestine content represents feeding over the last 6-48 hours (Nielsen et al., 2018). As ^{137}Cs in intestine content is variable, and uptake to the fish muscle is later on a timescale, the present levels of ^{137}Cs in the fish will represent a integral value of the intestine content through the last days, weeks or months, depending on the biological half-live of caesium in the organism, typically 30 days (Teien et al., 2021). Uptake and

exposure to Cs through feed may relate to calorie density of feed organisms, depending on how uptake of caesium occurs. It was observed that the intestine sampled in June had a lower specific activity concentraion than the samples collected in October. The samples in June had a higher content of plant material.

It has not been possible to identify the main source of ^{137}Cs transfer to rudd. However, results exclude snails and aquatic insects as major food items and as an important food source in transfer of ^{137}Cs to rudd, because of the ecosystem position of rudd shown by the stable isotope signature discussed further in chapter 4.2.

Comparing groups of food organisms results does not support a conclusion on significant differences in activity concentrations between the different groups of organisms. Nevertheless the results show that plants had the highest measured activity, with a large variation between the plants. In the invertebrates the highest activity concentrations was measured in the soft tissue of the snail and in millipedes. The activity concentration of snails was found to be this is within the range found in other studies (Gudkov et al., 2005). One of the collection of Mosquitos-large showed the highest activity of the flying terrestrial insects, higher than Delia Platura and Amata Phegea (Table 3.3). ^{137}Cs is present in terrestrial insects in a range from not detectable levels to 22.11 $Bq\,g^{-1}$ dry weight. The levels found in this study are within the range found in other studies (Beresford et al., 2020), where levels were found in the range of 1.07 $Bq\,g^{-1}$ to 66.8 $kBq\,kg^{-1}$ in fresh weight of insects. Comparing fresh weight and dry weight depend on species. Studies have shown a range of water content in insects from 50% to 75% (Straus and Aviles, 2018). Of the analyses of invertebrates considered available as pray for fish, snails had the highest activity but the terrestrial insects, a collection of Mosquito-large had a activity concentration of 7.49 $Bq\,g^{-1}$ comparable to the levels found in the aquatic predator Odonata 8.42 $Bq\,g^{-1}$.

The aquatic plants also had levels of ^{137}Cs within the range found in other studies (Gudkov et al., 2005), except for one unidentified plant that measured a higher value of 48.9Bqg^{-1}. When comparing plants to the estimated dry weight activity concentrations of the fish samples assuming a 20% dry weigth of fish muscle it is observed that the highest measured plant is on the same level as the average concentration in rudd. Rudd is known to eat plants and has been considered one of the most plant eating fish species in the European fauna (Dorenbosch and Bakker, 2012). Feeding on the plants with high activity concentrations can contribute to transfer of ^{137}Cs to rudd. Plants are less calorie dense as food, compared to fish and insects, thus feeding on plants more material needs to be consumed, and mechanisms for uptake of ^{137}Cs will affect how much is transferred to the fish. The high level of ^{137}Cs in one plant could be caused by the presence of a hot particle with high ^{137}Cs, contributing to the high measurement.

Comparison of roach and its potential food, based on isotope signature (discussed further in chapter 4.2) , show that roach would have a estimated activity concentration in dry weight of $12.5\,kBq\,kg^{-1}$. This is lower compared to the dry weigth of snail ($20.88\,Bq\,g^{-1}$).

Literature show that some species of terrestrial insects show a high contamination (Mietelski et al., 2010), and if these were feed for fish this could cause elevated levels of ^{137}Cs. The highest levels are found in terrestrial insects living on the litter surface, and these are not necessarily insects that represent a potential food for fish. This is supported by the findings in this study where millipedes showed a high activity concentration of ^{137}Cs compared to other terrestrial insects.

The activity concentration of ^{137}Cs in the water of the lake is lower than the biota analysed. The lake water has been shown to have $3.6 \pm 1.0\,Bq\,L^{-1}$ ($0.0036\,kBq\,kg^{-1}$) of ^{137}Cs, orders of magnitude under the lowest measured value for biota. This indicates a low transfer of ^{137}Cs to fish from water as studies have shown i.e. (Hewett and Jefferies, 1976).

The main finding in literature is that there is a large variation in activity concentrations in parts of the Glubokoye ecosystem and that transfer of ^{137}Cs is mainly through food consumption. This was also seen in this study.

4.2 Stable Isotopes

Stable isotopes of nitrogen and carbon presents an image of the organisms position within the ecosystem. The method of isotope signature is widely used to understand organisms trophic position and carbon source in an ecosystem. It is well established in literature that stable isotope analysis can be used to investigate ecosystem relations (Larsen et al., 2009). The $\delta^{15}N$ has been used for decades as a measure to identify the trophic position of the organism (Peterson and Fry, 1987) and $\delta^{13}C$ as an indication of the carbon source (DeNiro and Epstein, 1978). As observed in this study, the isotope signature gives a picture of the relative position of the species in Lake Glubokoye. Stable isotopes signature mixing models has been used to interpret a multitude of factors regarding the ecosystem(Fry and Sherr, 1989).

The relative positions of pike and perch high in $\delta^{15}N$ has been found in other studies (Moseby, 2011), and is expected as these fish are known predator species. Roach is know as a species consuming detritus, and its position fits the expected values found in this study. Rudd is known for eating higher plants (Dorenbosch and Bakker, 2012), and it is seen in this study that these plants are depleted in $\delta^{13}C$ compared to the aquatic isotope signal. This is reflected in the rudd sampled, but it is also clear that it must be

influenced by another food source as the $\delta^{15}N$ levels does not match a tropich level of 3‰. It is clear that rudd is more influenced by more terrestrial carbon source than the other fish, and this is as expected as litterature (Freyhof, 2008) and this study support the assumption that rudd eats plants and insects. Carp is known to feed on plankton, benthic invertebrates, plant material and detritus (Kottelat and Freyhof, 2007), thus the values found analysing stable isotopes in this study is as expected. It is to note that carp and rudd do not seem to be competitors with regard to food in the Lake Glubokoya. Sampling has not been able to established organisms on the lowest trophic position in the aquatic ecosystem. It is shown that rudd has different isotope signature on different sampling times. There is not enough data in this study to determine if this is related to a natural variation in rudd through the seasons, or if it can be attributed to a period of differences in food choice as rudd has been shown to change diett (Hicks, 2003). But it is a natural assumption that the influx of terrestrial feed is larger during the summer than in the winter, and this can be seen through the $\delta^{13}C$ values of rudd in spring.

Literature supports that isotop ratio can change in fish over time. This has been shown in Sockeye salmon(*Oncorhynchus nerka*) with a turnover of $\delta^{15}N$ of 40 days in fish two years of age, and that isotopic turnover time increases with the age of the fish(Sakano et al., 2005). Furthermore it has also been found that isotopic ratio had a turnover in muscle of 49-107 days in summer flounder (*Paralichthys dentatus*) (Buchheister and Latour, 2010). In other studies, it has been shown that vertebrates, isotopic signatures of blood, muscle, skin, hair, feathers, and bones represent time scales from days to months or years (Post, 2002). This implies that stable isotope ratio in the fish will change with a change in the stable isotope of feed, but factors may influence the rate of isotopic turnover. Insects has a shorter turnover time(5-10days), and show difference if measures as hole or if exoskeleton is analysed, whereas signatures in whole body invertebrate samples can experience rapid change (Ostrom et al., 1996).

4.3 Food Chain for Fish in Glubokoye

Taken into account that $\delta^{15}N$ change 3‰ between one tropic level, but $\delta^{13}C$ is normally similar with its feed. The results from this study gives an image of possible food chains in the Lake Glubokoye. Carp and roach have a main food source with an aquatic $\delta^{13}C$ and rudd has a $\delta^{13}C$ signature indicating a main food source of terrestrial origin.

Pike and perch has a $\delta^{15}N$ levels establishing them as predators in the Lake Glubokoye, and the $\delta^{13}C$ is positioned between the aquatic and terrestrial level and this imply a mixed input of carbon. Of the fish analysed, roach is a likely food-source for the predatory fish, but carp and rudd do not fit the expected 3‰ change expected for

a change in trophic level. The carp sampled in the present study was large and not expected to be a major food source for pike, however it is surprising that rudd does not seem a significant food source for pike.

Roach can eat snails having similar $\delta^{13}C$ and 3‰ lower $\delta^{15}N$, but is not likely to have aquatic insects as a large part of the diet, as the levels of $\delta^{15}N$ do not correspond to a shift in trophic position. Roach is a known detrivourus fish and mixed feeding can explain the $\delta^{15}N$ levels. Feeding on aquatic insects cannot be excluded as food items for Prussian carp and roach, as having $\delta^{13}C$ in similar level, and could explain higher levels of $\delta^{15}N$ in individuals.

Although rudd has a terrestrial signal of $\delta^{13}C$, it is also influenced by aquatic food source even though isotope signature for indicates a mainly terrestric $\delta^{13}C$ input, as seen in the shift in $\delta^{13}C$ in spring compared to autumn. The $\delta^{15}N$ in rudd indicated that it has a substantial part of its diet from other sources than plants, even though it has been show to have a preference for plants with age. Thus it can be assumed that isotopic signature results from a mixed input of food sources. This is a natural assumption taking into account the omnivorous nature of rudd. Terrestrial insects and aquatic plant seems to be important feed for rudd as $\delta^{13}C$ are similar level and $\delta^{15}N$ lower. However, the terrestrial insects are more similar $\delta^{15}N$ compared to fish and thus not a major feed source but how the mixed input manifests itself in isotope signature remains uncertain. This is supported by the fact that fraction of insects and plant were identified in intestine samples. The observation that rudd feeds on terrestrial insects can effect how ^{137}Cs is recycling in aquatic ecosystems (1.2), terrestrial input of ^{137}Cs can be a source of ^{137}Cs to aquatic ecosystems, with the subsequent distribution in aquatic food chains.

It is shown that rudd move towards a isotopic signature more similar to the predatory species, this can be caused due to pisivorius behavior. Pisivorius behavior in rudd has been shown in North America, where a change in trophic position towards predatory species was observed when other food sources was scare(Guinan Jr et al., 2015).This assumption is strengthend by the identified of structures that was identified as gill cover of a small fish, but no other hard parts of fish was identified. The reason for the lack of other fish parts is not known.

The ecosystem transfer of ^{137}Cs is complex and influenced by many factors (Figure 1.2). As mentioned it is well established that ^{137}Cs has been shown to bio-accumulate in piscivorous fish (Zhao et al., 2001; Rowan and Rasmussen, 1994) which has also been shown for Chernobyl-contaminated regions (Smith et al., 2000). Studies have also established transfer-factors indicating a bio-accumulation of ^{137}Cs from prey to predator (Haque et al., 2017). Also naturally insect eating species such as trout, char and also

bass species show a larger increase in the levels of caesium with regard to length than plankton feeders such as carp (Wada et al., 2019), It has been shown that ecosystem composition can have an effect on the transport in the ecosystem, the introduction of *Mysis relicta* was found to enhance the transfer of ^{137}Cs from zooplankton and settling particles to Arctic char and brown trout (Hammar et al., 1991). In Scandinavia, it has been reported that if perch and trout coexist in a lake the observed ^{137}Cs was 2-4 times higher in perch (NRPA and NFSA, 2013). These observations may also be attributed to predatory behavior as the species are known to eat fish. All in all these studies implies that variations within the ecosystem can change transport to fish.

4.4 Strength and Weaknesses

It is a challenge to identify ^{137}Cs in insects due to low sample weight. This results in measurements below detection limits within the measuring time available, even though the concentration of ^{137}Cs potentially can be high. To get results from the insects with low body-weight, there is a need for more sample material to get better results representative for the species analysed.

The used reference material IAEA 300 is a sediment reference material, and thus has a different matrix composition than the analysed insects and plants with regard to density and geometry. For measuring on Germanium Detector a geometry 10 ml in 20 ml liquid scintillation vial was used. Even if samples of insects did not fill 10ml and a different container than the specified geometry was used. Some plants was more than 10ml of volume. In addtion the filling of the vials containing the reference material also deviated from correct geometry, as the IAEA 300 was measured with a 10ml in a 20ml liquid scintillation vial geometry, even if the reference material filled sub 10ml of the vial. These deviations were not judged to have a large influence on the analytical results, but it is a compromise with regard to matrix and geometry, and can contribute to the security of the measurement. Studies have emphasised that Gamma Spectrometry requires homogenised material packed within a well-defined geometry. Also that the shape of invertebrates is not regular, and radionuclides can be distributed unevenly within invertebrate bodies. In addition the weight of insects has been shown to change in dry conditions (Mietelski et al., 2010), these factors can contribute to the security of the measurement. This could be improved by using a fixed volume liquid standard and a decomposition of the Insects and subsequent dissolving in a fixed amount of fluid would ensure equal geometry in all analysed samples. An effort to ensure that standards and samples have a equal matrix could also help improve the precision of the ^{137}Cs analysis.

For the complete overview regarding stable isotopes, some samples are missing, like fytoplankton, terrestrial vegetation, detritus, other fish and amphibians. Also the missing

analysis of stable isotopes in rudd collected in June 2021 is unfortunate. It is an obvious weakness that samples have been collected on different sampling dates with years apart and then compared. Nevertheless, with a aim to investigate if stable isotope analysis can give valuable information on the species in the Glubokoye with regard to ^{137}Cs, these weaknesses does not overshadow the results of this work. Ideally, a collection of organisms on regular intervals through the year, would give a clearer picture of what occurs in Lake Glubokoye. If there are annual variations in prey availability there might be a need to do it several years do fully understand the reason for the observed values.

Another challenge is to collect a representative selection of insects due to large seasonal variation in insect population and also a difference between species on how easy the insects are easy to catch.

A strength regarding this theses must be the relative security of the measurement of stable isotopes, the show little variation in in-house materials, and make for good analytical results. There was sent one replicate of each individual fish for stable isotope analysis, this attributes to that the measurement on each fish is uncertain, but on a group level the results are viewed as good.

Studies have found that Levels of $\delta^{13}C$ og $\delta^{15}N$ can change with storage, 3months, especially conservation in ethanol with regard to $\delta^{13}C$. $\delta^{15}N$ does not change significantly in ethanol. " Samples intended for isotope analysis should be frozen, freeze-dried or oven-dried, preservatives may be used only for $\delta^{15}N$ analysis and organisms should be sacrificed immediately after collection."(Kaehler and Pakhomov, 2001). This could effect some of the collected insects collected in this study. In this study analysis of a house standard of fish muscle show little effect on storage long term.

4.5 Future Work

An exact source for elevated levels of ^{137}Cs in rudd could not be established. Moving forward with a goal to discover what makes rudd show activities higher than the predatory fish on some samplings, more info is needed.

More samples with stable isotope analysis and ^{137}Cs analysis in rudd would give more information and improve on the image on what is occurring. A time series of rudd including analysis of ^{137}Cs and stabil isotopes could give information about co-variation of ^{137}Cs and stable isotopes. In addition, sampling and analysis of liver can give a timescale image with higher resolution of the variation on stable isotopes from food as liver has been shown to have a shorter turover(10-20days) in isotopic ratio than muscle(49-107day) in summer flounder (*Paralichthys dentatus*) (Buchheister and Latour, 2010)

It would be possible to analyse $\delta^{13}C$ in amino acids as $\delta^{13}C$ in amino acid are more able to distinguish between carbon sources compaired to analysis of bulk carbon. $\delta^{13}C$ patterns in consumers is largely matched to primary producers in each system. Using carbon from amino-acids instead of bulk carbon may be used to overcome limitations in complex environments with mixed inputs (Larsen et al., 2013). Isotope analysis of fatty acids have shown a potential on investigating bio-synthetic origins (Twining et al., 2020). There are thus several methods that might increase the resolution of the image given by stable isotope analysis, and consideration may be given on evaluation further of investigations.

Stable isotope analysis of intestine content was also suggested. But the mixed nature of intestine content in rudd, and the mix of sources with a large variation in carbon and nitrogen content as seen in plants versus insects, can pose difficulties in sampling and interpretation of results.

Surveillance of the lake with the aim to discover episodes with a large abundance of a specific food, and sampling of these, i.e, hatching of insects. Whereas ants known for potentially high levels of ^{137}Cs have a phase in the life-cycle where they are flying insects, and thus available as feed for fish such as rudd. Benthic organisms can also hatch in number, and if high in ^{137}Cs can contribute to rudd. Amphibians is also a species that may contribute a abundance of food in some parts of the year, with eggs and young in abundance in spring.

Information on the effect of stable isotope composition in fish when having mixed inputs of food is scarce, how do a mainly a plant based diet in fish influenced by occasionally eating insects of another isotope composition. Investigation of this theme can also give valuable information.

5. Conclusions

Viewing the the results in light of the hypothesis set for this study, the following conclusions can be made.

Stable isotope signature can be used to differentiate fish in the Lake Glubokoye within the ChEZ and gives valuable information on trophic positioning and indications on feed preferences. This supports the main hypotheses that there is a difference in stable isotope signature of $\delta^{15}N$ and $\delta^{13}C$ within the species of fish in Lake Glubokoye (H_1) .

Stable isotope signature for rudd shows that it is mainly influenced by a terrestrial food source but a drift towards a more aquatic food source is observed in the spring. In addition findings of terrestrial and aquatic organisms in intestine content support that rudd in Lake Glubokoye pray on both terrestrial and aquatic food organisms (H_2) .

There is contamination of ^{137}Cs in all measured groups of food sources in this study, with large differences between individuals and species. The findings in this study show that there is caesium in aquatic and terrestrial food organisms (H_3).

Since ^{137}Cs is present in aquatic and terrestrial food organisms (H_2) and rudd in the Glubokoye pray on both terrestrial and aquatic food organisms (H_3), ^{137}Cs in rudd in Lake Glubokoye originates from both terrestrial and aquatic food organisms (H_4).

Predatory behavior in rudd could not be excluded. Findings of gill covers in intestine samples collected in 2021 and a move towards a isotope signature more similar to predatory fish in spring of 2017 are indecations of predatory behavior. The samples with a isotope signature similar to predatory fish was the same samples associated with high levels of ^{137}Cs.

The findings highlight that information about the food chain is important to understand the dynamic transfer of ^{137}Cs.

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Appendix A. Terrestrial insects

Table A.1: Sampled terrestric insects

Sample	Date	Order	Family	Species	Number	Weight_g
1	30.06.2021	Hymenoptera	Formicidae	Ant	4	0,0197
2	30.06.2021	Lepidoptera	Arctiidae	Amata phegea	16	0,2893
3	30.06.2021	Odonata	Coenagrionidae	Zygoptera	4	0,0474
4	30.06.2021	Hymenoptera	Formicidae	Wasp	3	0,0301
5	30.06.2021	Lepidoptera	Heterocera	Moth	2	0,0243
6	30.06.2021	Diptera	Syrphidae	Hover fly	2	0,0413
7	30.06.2021	Diptera	Culicidae	Moskito small fly	7	0,0034
8	30.06.2021	Unknown	Unknown	non spesific	NA	0,0267
9	22.06.2021	Hymenoptera	Formicidae	Vespa crabro	1	0,3208
10	22.06.2021	Diptera	Anthomyiidae	Delia platura	7	0,0199
11	22.06.2021	Diptera	Culicidae	Moskito small fly	20	0,0066
12	02.06.2021	Odonata	Aeshnidae	Libelle	1	0,2201
13	02.06.2021	Diptera	Tabanidae	Horse-fly	6	0,2215
14	02.06.2021	Odonata	Aeshnidae	Libelle	1	0,0754
15	12.07.2021	Odonata	Coenagrionidae	Zygoptera	5	0,0451
16	12.07.2021	Unknown	Unknown	Larva	1	0,0282
17	12.07.2021	Lepidoptera	Arctiidae	Amata phegea	3	0,0372
18	12.07.2021	Lepidoptera	Heterocera	Moth	3	0,0178
19	12.07.2021	Diptera	Uknown	div small flys	6	0,0257
20	12.07.2021	Odonata	Coenagrionidae	Zygoptera	3	0,0242
21	12.07.2021	Lepidoptera	Heterocera	Moth	2	0,011
22	12.07.2021	Diptera	Culicidae	Moskito small fly	5	0,0083
23	22.06.2021	Odonata	Aeshnidae	Libelle	1	0,0866
24	22.06.2021	Odonata	Coenagrionidae	Zygoptera	5	0,0562
25	22.06.2021	Hymenoptera	Formicidae	Wasp	1	0,0198
26	22.06.2021	Lepidoptera	Arctiidae	Amata phegea	2	0,0627
27	22.06.2021	Diptera	Culicidae	Moskito large fly	2	0,0213
28	22.06.2021	Diptera	Culicidae	Moskito small fly	4	0,0014
29	22.06.2021	Diptera	Syrphidae	Hover fly	3	0,0695
30	22.06.2021	Lepidoptera	Heterocera	Moth	3	0,0105
31	22.06.2021	Diptera	Culicidae	Moskito small fly	15	0,0071
32	22.06.2021	Diptera	Unknown	Fly small	7	0,0048
33	22.06.2021	Hymenoptera	Formicidae	Wasp	1	0,0041
34	22.06.2021	Unknown	Unknown	parts	NA	0,0188
E1	12.07.2021	Arenea	Unkown	Spider	38	0,2891
E2	12.07.2021	Lepidoptera	Heterocera	Moth/spinner	6	0,0712
E3	12.07.2021	Odonata	Aeshnidae	Libelle	1	0,0359
E4	12.07.2021	Coleoptera	Geotrupidea	Dung beetle	3	0,5547
E5	12.07.2021	Coleoptera	Unkown	Beetle	19	0,5058
E6	12.07.2021	Diptera	Culicidae	Moskito large fly	17	0,1141
E7	12.07.2021	Hymenoptera	Formicidae	Ant	19	0,0013
E8	12.07.2021	Diptera	Unkown	Fly small	16	0,0647
E9	12.07.2021	Diptera	Unknown	Beetle	2	-0,0011
E10	12.08.2021	Diplopoda	Millipedes	Millipedes	28	0,9607
E11	12.08.2021	Hymenoptera	Formicidae	Ant	5	0,0041
E12	12.08.2021	Diptera	Unkown	Fluer	2	0,0039
E13	12.08.2021	Arenea	Unknown	Spider	46	0,3665
E14	12.08.2021	Hymenoptera	Apoidea	Bee	1	0,0165
E15	12.08.2021	Hymenoptera	Formicidae	Ant	5	0,1193
E16	12.08.2021	Hymenoptera	Symphyta	Planteveps	10	0,05
E17	12.08.2021	Coleoptera	Geotrupidea	Dung beetle	2	0,3592
E18	12.08.2021	Coleoptera	Unknown	Beetle	3	0,0303
E19	07.08.2021	Unknown	Unknown	Unknown		0,1167
E20	07.08.2021	Hymenoptera	Formicidae	Wasp	2	0,1572
				total	371	

Appendix B. IRMS Analysis

Text specified by the CLIPT-lab

Text for crediting CLIPT lab in publications/presentations/theses:
"Samples were analyzed at the CLIPT stable isotope biogeochemistry lab at UiO, funded by the Research Council of Norway through its Centers of Excellence funding scheme #223272 (Centre for Earth Evolution and Dynamics)"

Instrumentation:
For organic stable isotope analysis of bulk organic samples, we use a Thermo Fisher Scientific EA IsoLink IRMS System, which consists of a Thermo Fisher Scientific Flash Elemental Analyzer and a Thermo Fisher Scientific DeltaV Isotope Ratio Mass Spectrometer.

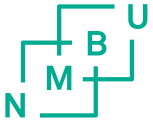
Analytical Sequence:
Samples containing between 0.3 and 0.7 mg carbon and 0.1 to 0.2 mg nitrogen are sealed in tin capsules, and loaded into a Costech Analytical Zero-Blank Autosampler configured with the Flash Elemental Analyzer. Within a continuous flow of helium, samples are dropped into an oxidation reactor held at 1000 degrees Celsius. The reactor is packed with chromium oxide and silvered cobaltous/ic oxide. A pulse of oxygen is timed to arrive as the sample drops into the hottest zone of the reactor, reacting with the tin, which is an exothermic reaction that instantly elevates the combustion temperature to 1800 degrees Celsius. The chromium oxide acts as a catalyst and provides an oxidizing environment, and the silvered cobaltous/ic oxide removes any halogens and sulfur generated from the combustion. The combustion products then flow through a reduction column packed with elemental copper wires, held at 650 degrees Celsius. This removes excess oxygen not used in the combustion, and reduces NOx to N_2 gas. Water generated via combustion is removed with a chemical trap containing magnesium perchlorate. The CO_2 and N_2 are then separated via a Thermo Scientific Isolink Ramped GC Oven operated in an isothermal state at 70 degrees Celsius. The gases then flow to a Delta V stable isotope mass spectrometer for analysis. Carbon and Nitrogen elemental composition is determined from mass spectrometry peak areas.

Reference and Quality Control Materials:
Within each batch run, between 3 and 9 reps of (depending on size of run) of two internal lab reference materials (JGLUT, POPPGLY) are analyzed and used to normalize the data to the VPDB scale for $\delta^{13}C$ analysis, and the AIR scale for $\delta^{15}N$ analysis:

1) JGLUT: L-glutamic acid obtained from Fisher Scientific, $\delta^{13}C$ = -13.43‰, $\delta^{15}N$ = -4.34‰

2) POPPGLY: glycine obtained from Fisher Scientific, $\delta^{13}C$ = -36.58‰, $\delta^{15}N$ = 11.25‰

Additionally, between 3 and 9 reps (depending on size of run) of a quality control material (JALA) are incorporated into every batch run and analyzed as an unknown to assess precision and accuracy of the measurement:



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