Master's thesis

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

Ailin Østerås

Accumulation of Gadolinium in lumpfish (*Cyclopterus lumpus*)

Master's thesis in BI3910 Ocean Resources - Ecosystems
Supervisor: Tomasz Maciej Ciesielski (NTNU)
Supervisor: Julia Farkas (SINTEF)
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Abstract

Rare earth elements (REE) are contaminants of emerging concern as anthropogenic enrichment in natural environments has been reported, yet the knowledge of potential effects is limited. Gadolinium (Gd) is one of the REEs and is mainly used in the medical field, as a contrast agent in magnetic resonance imaging (MRI). Elevated Gd concentrations have been found in waters around urban areas. However, knowledge about the potential toxicity and bioaccumulation of Gd in marine species is still limited. This study aimed to investigate bioaccumulation and organotropism of Gd in lumpfish (*Cyclopterus lumpus)*. Organisms were exposed to (nominally) 1.3 µg/L (low) and 130 µg/L (high) of a chelated Gd in the form of a gadoliniumbased contrast agent (GBCA) and to $GdCl_3$ as ionic/inorganic Gd. Exposures were performed as pulsed exposure, with a daily exposure duration of 2 hours in static conditions, for 10 days. Following the exposure period was a 10–day recovery period to investigate changes in organotropism and excretion rates of Gd. Following each period (exposure and recovery), sampling of gills, kidneys, livers, and brains was conducted, and Gd concentrations were analysed. Results showed the highest Gd accumulation in fish exposed to $GdCl_3$ high, followed by GBCA high, considering all organs. Concentrations in organs were highest in gills, followed by kidneys, then livers, and least in brains. Results also indicated excretion of Gd during a period of recovery for all treatments and all organs, except for kidneys and brains of fish exposed to $GdCl_3$ high, where recovered groups had higher concentrations of Gd than exposed groups. This indicates a change in organotropism of ionic Gd over time. Results of this study show that Gd is bioavailable to marine fish in both inorganic and organic forms, highlighting that more research is needed to understand the effects of Gd on marine species.

Sammendrag

Sjeldne jordarter, også kjent som rare earth elements (REE) er grunnstoffer som vekker økende bekymring som forurensningskilder. Økt menneskelig bruk av REE har ført til rapportering av økte REE konsentrasjoner i naturen, mens kunnskapen om mulige effekter og toksisitet er begrenset. Gadolinium (Gd) er en REE, og benyttes hovedsakelig som kontrastmiddel i magnetresonansundersøkelser. Økte konsentrasjoner av Gd har blitt rapportert i flere urbane områder. Kunnskap om mulig toksisitet og bioakkumulering av Gd i marine arter er svært begrenset. Formålet med denne studien var å undersøke bioakkumulering, og mulige forskjeller i akkumulering, i ulike organer hos rognkjeks (*Cyclopterus lumpus).* Organismene ble eksponert for 1.3 µg/L (lav) og 130 µg/L (høy) av Gd i en uorganisk forbindelse ($GdCl_3$) og som en organisk forbindelse i form av en Gadolinium–basert kontrastvæske (GBCA). Eksponeringene ble gjennomført over en periode på 10 dager, med daglige eksponeringsintervaller på 2 timer under statiske forhold. Eksponeringsperioden ble etterfulgt av en restitusjonsperiode, for å undersøke endring av konsentrasjoner og utskillelse av organisk og uorganisk Gd. Prøvetaking ble gjennomført etter hver 10–dagers periode, hvor gjeller, lever, nyrer og hjerner ble tatt ut av organismene for å analysere Gd–konsentrasjonene. Resultatene viste høyest grad av Gd akkumulering i fisk som ble eksponert for høy konsentrasjon av $GdCl_3$, etterfulgt av høy konsentrasjon av GBCA, i alle organene. Konsentrasjoner av Gd var høyest i gjeller, etterfulgt av nyrer, så lever, og til slutt hjernen. Resultatene indikerer også utskillelse av Gd under restitusjonsperioden for alle eksponeringer og organer, med unntak i nyrer og hjernen til fisk som ble eksponert for $GdCl_3$ høy. Disse fiskene hadde høyere Gd– konsentrasjoner i nyrene og hjernen etter restitusjonsperioden, som indikerer endringer i bioakkumulering og organfordeling av uorganisk Gd over tid. Funnene i denne studien viser at både uorganisk og organisk form av Gd er biologisk tilgjengelig for marine fisker, samt understreker nødvendigheten av mer forskning for å forstå effekter av Gd på marine arter.

Foreword

This thesis is written as a part of the ELEMENTARY research project. This research project aims to find out whether rare earth elements (15 lanthanoid elements with yttrium) are emerging contaminants in the marine environment in Norway. My part in this study has been to help perform an exposure study of lumpfish *(Cyclopterus lumpus)* to different forms of Gd, sampling fish organs and doing laboratory work with both preliminary testing and the main experiments, sample processing, data processing and data analysis. This study consisted of two periods of work. One period was with my co-supervisor Julia Farkas at SINTEF Ocean and other personnel from SINTEF Ocean, conducting the exposure-part of the experiment and the sampling of organs. The other part was conducted with my supervisor, Tomasz Maciej Ciesielski, at NTNU and included organ sample digestion and analysis. My thesis aims to investigate the bioaccumulation and organotropism of Gd, specifically in brain, liver, kidney, and gill tissue.

I would like to give a special thanks to my supervisors, Julia Farkas (SINTEF Ocean) and Tomasz Maciej Ciesielski (NTNU), for all the feedback, patience, guidance, and encouragement they have given me throughout this thesis.

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Ailin Østerås

NTNU, Trondheim 30.06.23

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List of abbreviations

Ca = calcium

dw = dry weight

GBCA = gadolinium–based contrast agent

Gd = gadolinium

Groups = refers to the exposed and recovered group within each treatment

ICP-MS = inductively coupled plasma–mass spectrometry

Inorganic Gd = $GdCl_3$, which is soluble in liquids

Ionic Gd = Gd^{3+}

Organic Gd = GBCA

REE = rare earth elements, consisting of the 15 lanthanide elements

REY = the 15 lanthanide elements (REE), and including Yttrium (Y)

Treatment = refers to exposure treatments; Control (CTRL), DOTA, GBCA low/high and $GdCl_3$ low/high

ww = wet weight

1 Introduction

Gadolinium (Gd), together with the 14 other lanthanide elements (La, Ce, Pr, Nd, Pm, Sm, Eu, Tb, Dy, Ho, Er, Tm, Yb and Lu) and yttrium (Y) comprise the group of rare earth elements (REY). Despite their name, these elements are amongst the 15 most abundant elements in the crust of the earth (EPA, 2012). Due to the physiochemical properties of REY, these elements are applied in a variety of industries (Piarulli et al., 2021; Squadrone, Brizio, Stella, Mantia, et al., 2019a), and are considered as "technology critical elements" and raw materials of high strategic importance (Keersemaker, 2020; Piarulli et al., 2021; Squadrone et al., 2019). Industries utilizing REY include industries producing electronic products, renewable energy technology, the metallurgical–, automotive–, and nuclear industries (Charalampides et al., 2015; Garcia-Solsona et al., 2014; Guimarães et al., 2016; Ramos et al., 2016). REY are further abundant in raw materials used in fertiliser production and animal feed (He & Rambeck, 2000; Henderson, 1984; Krebs Greenwood & Bracken, 1999; Tommasi et al., 2021; Wen et al., 2001).

The application of REY has resulted in increased production and use of REY throughout the last decades (Zhou et al., 2017). Global demand for REY increased from approximately 75500 tonnes (t) in 2000 to 123100 t in 2016, with estimations suggesting that the demand will increase with a further 40% by 2030 (Services, 2013). The increased anthropogenic release of REYs to the environment has resulted in concerns of contamination (Gwenzi et al., 2018; Squadrone, Brizio, Stella, Mantia, et al., 2019a). Recent research reported anthropogenically derived REY concentrations in various environmental matrices to be magnitudes higher than natural geochemical background levels (Bau et al., 2006; Cao et al., 2000).

Gadolinium is one of the REYs and is used in the medical field, as Gd–based contrast agents (GBCA) for magnetic resonance imaging (MRI–scans) (Trapasso et al., 2021). GBCAs are used in relatively large quantities, with an estimated 50 metric t being administered annually worldwide (Brünjes & Hofmann, 2020; Wahsner et al., 2019). As GBCAs are not removed in wastewater treatment plants (WWTP), they enter the marine environment through rivers and wastewater outlets (Bau et al., 1997; Bau & Dulski, 1996; Ebrahimi & Barbieri, 2019; Farkas et al., 2020; Hissler et al., 2016; Klaver et al., 2014; Kulaksiz & Bau, 2013; Kulaksız & Bau, 2011; Kümmerer & Helmers, 2000; Lawrence et al., 2009; Lerat–Hardy et al., 2019; Song et al., 2017), causing them to be of emerging concern as it has been suggested that these are highly bioavailable and stable in the environment (Kulaksiz & Bau, 2013; Liang et al., 2014; Piarulli et al., 2021; Tyler, 2004). A significant increase in anthropogenic Gd concentrations has already

been reported near densely populated areas with a highly developed healthcare system in industrialized countries (Hatje et al., 2016; Kulaksiz & Bau, 2007; Nozaki et al., 2000).

Due to the toxic properties of Gd^{3+} (ionic Gd), Gd is chelated with ligands such as DOTA (1,4,7,10–tetraazacyclododecane–1,4,7,10–tetraacetic acid), when applied in MRI–scans, which significantly reduces the toxicity of Gd^{3+} as a contrast agent (GBCA). GCBAs are not metabolized and are secreted through urine within a short time following administration (Caravan et al., 1999; Rogowska et al., 2018; Trapasso et al., 2021; van der Molen & Bellin, 2008). However, the DOTA coating might break down over time after entering marine ecosystems, releasing the ionic Gd (Hanana et al., 2017; Henriques et al., 2019).

There is still limited knowledge of Gd bioaccumulation and potential toxicological mechanisms in aquatic organisms, and little is known about the internal distribution of Gd in marine biota (Lortholarie et al., 2021; Malhotra et al., 2020). Studies have quantified Gd and other REY, and found levels above natural background in benthic invertebrates, crustaceans, bivalves, echinoderms, macroalgae, fish and birds, causing concern regarding bioaccumulation and potential toxic effects (Bau et al., 2010; Espejo et al., 2023; Fu et al., 2000; Li et al., 2015; Lortholarie et al., 2021; MacMillan et al., 2017; Mashitah et al., 2012; Ponnurangam et al., 2016; Qiang et al., 1994; Reindl et al., 2021; Squadrone, Brizio, Stella, Favaro, et al., 2019; Squadrone, Brizio, Stella, Mantia, et al., 2019b, 2019a; Squadrone et al., 2017, 2020a; Wang et al., 2019; Yang et al., 2016). Organisms at lower trophic levels and benthic organisms seem to generally exhibit higher Gd concentrations, suggesting that species feeding near sediment and filter feeders have a higher intake of Gd and other REY (Bau et al., 2010; Piarulli et al., 2021; Ponnurangam et al., 2016; Squadrone, Brizio, Stella, Mantia, et al., 2019a; Squadrone et al., 2020a). Higher trophic organisms may have more effective metabolic mechanisms facilitating metal regulation (Liu et al., 2019) and therefore have a higher capacity to excrete Gd at higher rates compared to species at lower trophic levels (Piarulli et al., 2021; Squadrone, Brizio, Stella, Mantia, et al., 2019a).

Studies so far focus mainly on ionic Gd, and less is known about the effects and bioavailability of GBCAs in marine environments (Davies et al., 2022; Gulani et al., 2017; Parant et al., 2019; Pasquini et al., 2018). Impacts of ionic Gd such as decreased survival, growth rates and decreased reproduction have already been reported, as well as alterations of cardiac and neural activity and embryonic development in freshwater zooplankton, echinoderms, and fish (Blaise et al., 2018; Cui et al., 2012; Dubé et al., 2019; Lürling & Tolman, 2010; Zhao et al., 2021) (Lortholarie et al., 2021). Exposure studies (28 days) at concentrations above 120 μg/L resulted

in the accumulation of 2.5±0.50 μg/g in Mediterranean mussels (*Mytilus galloprovincialis)*, as well as impairment of processes involved in physiological performance and reproduction (Freitas et al., 2020; Henriques et al., 2019). Ionic Gd has also shown to inhibit cellular homeostasis and interfere with ionic calcium (Ca^{2+}) pathways in mussels (*Mytilus galloprovincialis*) (Henriques et al., 2019). A study conducted by Lortholaire et al., 2021, on wild European eels (*Anguilla anguilla*) from the Loire estuary showed Gd accumulation above background levels, with highest accumulation of Gd in gills (126.90±50.78 μg/kg dw) and liver (181.78±62.04 μg/kg dw for males; 203.79±111.86 μg/kg dw for female) (Lortholarie et al., 2021).

Hanana et al. (2017) conducted a comparison study between $GdCl_3$ and Omniscan (GBCA) exposure on adult zebra mussels (*Dreissena polymorpha)* at different concentrations for 28 days that showed a significant increase of Gd dependent on the exposure dose and type. There was a significant accumulation of Gd in the soft tissue of the mussels following $GdCl_3$ exposure, but only a small amount of Gd was accumulated in mussels exposed to Omniscan (Hanana et al., 2017). Due to a strong complexation between Gd and the diethylenetriaminepentaacetate ligand, the organic molecule might not dissociate significantly to liberate Gd^{3+}, causing a lower degree of bioaccumulation (Ghio et al., 2011). Low detection of free Gd^{3+} following Omniscan exposure indicates some breakdown of the Gd chelate in tissues, or Gd^{3+} displacement from the ligand by other metals (Mann, 1993). Therefore, exposures to inorganic and chelated forms (organic) of Gd might result in different organotropisms.

Aim of this study

The overall aim of this work was to study Gd uptake, depletion, and organotropism in lumpfish (*Cyclopterus lumpus).* We further wanted to compare the bioavailability of Gd in the form of $GdCl_3$ and Gd as medical contrast agent (GBCA) and the rates at which Gd accumulates in soft tissues and is subsequently eliminated.

In this study, we hypothesized that inorganic Gd ($GdCl_3$) would exhibit higher bioavailability compared to GBCA, primarily due to the dissociation of $GdCl_3$ and further transport of Gd^{3+} across ion channels. GBCA also contains DOTA coating, potentially impairing the release of Gd^{3+}, and thus reducing bioavailability and accumulation. As a result, we anticipated a higher rate of $GdCl_3$ accumulation, while expecting significantly lower accumulation of GBCA, regardless of concentration. Based on existing literature, concentrations were expected to be highest in gills, followed by livers, kidneys, and with brains exhibiting the lowest accumulation.

Additionally, we hypothesized that there would be differences between exposed and recovered groups, with Gd excretion in all organs expected to occur during the recovery period.

2 Materials and methods

2.1 Experimental design: a short overview

Lumpfish (*Cyclopterus lumpus*) was chosen as the test organism in this study, a commercial and ecologically important species in Norway. All individuals used in the experiment were first–year juveniles obtained from a hatchery, weighing between 190–400 grams (g) (average weight of 250 g) at the start of the experiment. To reach the target weight and acclimatize fish, the fish were kept in house for several weeks (see section 2.2). A visual representation of the experiment is shown in Figure 1. The experiment can be divided into two main periods; the exposure period (explained further in section 2.3.2) and the recovery period (explained further in section 2.3.3) Sampling was conducted twice, after the exposure period and the recovery period, followed by elemental analysis of Gd in the brain, liver, kidneys, and gills. These analyses are further explained in sections 2.4 and 2.5.

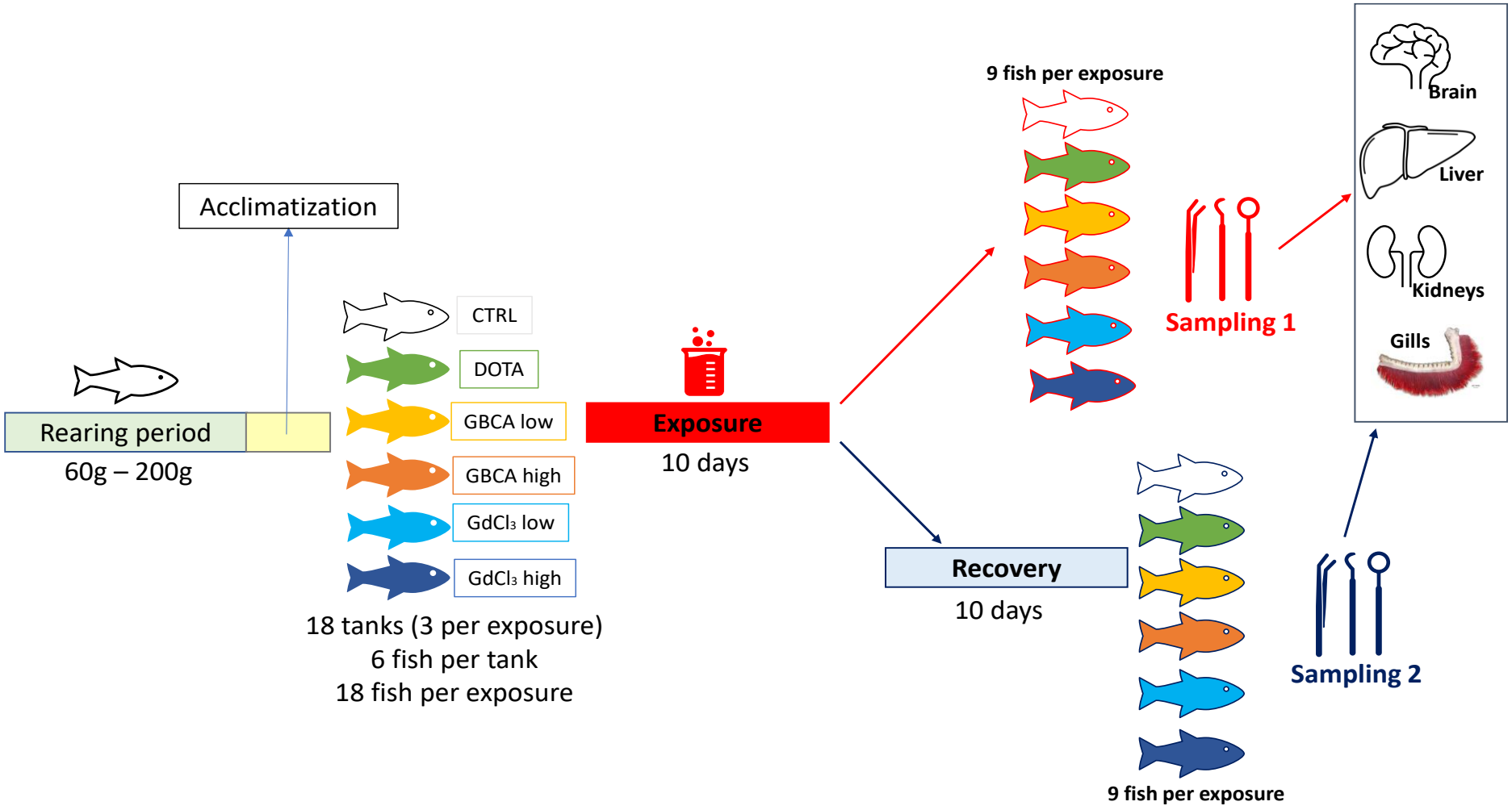


Figure 1. Visual representation of the experimental setup for this study, where lumpfish (*Cyclopterus lumpus*) was exposed to Gd.

2.2 Rearing period

In order to obtain fish with a large enough organ size (weight) for the determination of Gd in the target tissues, the fish were kept for a growth period of 82 days, in which the fish increased from an average of 54.6 g to an average of 250 g body weight. The fish were held in a room in 2 (later 3) tanks with a volume of 400 L and a water flow of approximately 350 L/min. The fish were kept in natural seawater from Trondheimsfjorden, pumped up from 40–90 m depth.

Feeding of the fish was conducted automatically 6 times a day, with fed amounts of approximately 1.25% of biomass per day, which were adjusted over time based on estimated and measured biomass growth in the tanks. Cleaning was conducted daily, during which feed and fecal matter accumulated at the tank outlet was flushed out, and larger particles and faces were removed using a fish hand net and a manual vacuum siphon tube. During the cleaning, the fish was also checked for development of cataract, and fish with signs of this disease were removed as test subjects.

Tagging of fish was conducted on day 68 to have control over the growth of each fish during the experiment. Tagging on day 68 was combined with measurements of the length and weight of each fish. Ethyl–3–aminobenzoate (MS222, 100 mg/L, CAS nr. 886–86–2) purchased from Sigma–Aldrich, was used as an anaesthetic for the fish during tagging to reduce stress. The fish were left in the MS222 solution for approximately 30 seconds–1 minute. Thereafter, the response of the fish was checked to see if they were fully sedated. When the fish did not respond to touch, the fish were tagged with an identification number, and then weighed and their length was measured. After tagging, the feeding was turned off for 2 days to the reduce risk of infection in the wound.

2.3 Experimental procedure

2.3.1 Experimental preparation

A total of 118 fish were assigned to size classes and then randomly assigned to tanks (n=18 per treatment, n=9 per group) and tanks (n=6 fish per tank), so the average biomass in each tank was 1103.5 ± 8.4 g.

Water supply during the experiment was from Trondheimsfjorden, the water was filtered through sand filters before entering the fish tanks. Water parameters were continuously measured, and no anomalies were detected. Prior to the exposure period, 6 fish were moved into a tank in the exposure room as a test to monitor their reaction and reduce stressors when moving the other fish, no behavioural abnormalities and signs of distress was observed. A test

of water flow stop in the tanks with the 6 fish was also conducted, to determine the length of the water stop during the exposure period. The water was stopped for a total of 2 hours, and measurements of salinity, dissolved oxygen (DO%), water temperature and dissolved ammonium (NH_4, mg/L) were conducted at the start and every 30 minutes throughout the water pumping pause. No change in salinity and water temperature was measured, but minor changes in dissolved oxygen and NH_4 were measured. Measured values can be found in the appendix, section A1, table A.1.1.

The chemical parameters of the water used for the rest of the experiment were as follows: salinity was between 34.77‰–34.9‰, with an average of 3.83‰±0.093‰. Dissolved oxygen had values ranging between 88%–90%, with an average of 87.8%±2.6%. Temperature had a range between 6.8 °C–7.5 °C, with an average of 7.1 °C±0.27 °C. All water measurements are shown in appendix, section A1, tables A.1.2–Table A.1.4.

For acclimatization, the fish were moved to the exposure tanks 4 days prior to exposure start. During the moving, the fish were weighed, and their length was measured before being put in the tank corresponding to their tag number.

The exposure setup consisted of 18 tanks with a total volume of 90 L that were filled with 80 L, with a water flowthrough of 0.5 L/min (720 L/day) so that the total water volume was replaced in 2 hours and 40 min. The 18 tanks were divided into treatments: CTRL, DOTA, GBCA low, GBCA high, $GdCl_3$ low, and $GdCl_3$ high (n=3 tanks per treatment).

Feeding was conducted manually, where ~6 g of fish feed was given to each tank at 08:00 and 16.00. Feeding time was 45 minutes per timepoint, and was followed by manual cleaning, using a siphon tube, to remove larger particles, faeces, and leftover fish food. Tissue paper was used to clean the edges of the tanks and around the drainage holes to avoid build–up of organic materials and formation of biofilm. Equipment was thoroughly rinsed in warm water and salt water between each treatment group to avoid cross–contamination.

2.3.2 Exposure period

Exposure was conducted over a period of 10 days and was followed by sampling (further explained in section 2.3.5). Exposure was conducted for 2 hours each day and occurred at the same time interval each day (11:00–13:00), with a stop of water flow during this period. Exposure solutions were prepared from stock solutions and seawater from the exposure room in 1 L bottles rinsed with HNO_3 (1M) ultrapure grade, purified from HNO_3 [AnalaR NORMAPUR®, VWR].

DOTA (1,4,7,10–tetraazacyclododecane–1,4,7,10–tetraacetic acid, ≥97.0% purity, CAS nr. 60239–18–1) was purchased from Sigma–Aldrich and was included as a background control.

This solution was used to make a stock solution (65.2 mg/mL in purified water, MilliQ; 18.2 MΩ), which was further used to make the DOTA exposure solutions (387 µg/L) to ensure similar DOTA concentrations in the DOTA treatment and GBCA treatments. GBCA high and low exposure solutions were made using a solution of GBCA chelated with DOTA (Gadoteric acid containing Gd: 78.6 mg/mL and DOTA: 202.46 mg/mL, pharmaceutical contrast agent formulation). $GdCl_3$ high and low exposure solutions were made from a solution of Gadolinium(III)chlorine hexahydrate ($GdCl_3 \times 6H_2O$, CAS nr. 13450–84–5), purchased from Sigma–Aldrich.

Fish were exposed in 80 L tanks, nominal target concentrations of Gd were: 1.5 µg/L (low exposure), and 150 µg/L (high exposure) for both $GdCl_3$ and GBCA treatments. Concentrations in this study were selected to investigate the movement of Gd between organs (high) and to reflect Gd concentrations found in marine environments (low). Measured exposure concentrations of Gd for each treatment are given in section 3.1. After stopping the water flow, exposure solutions were added to their respective tanks.

2.3.3 Recovery period

The exposure period and sampling 1 were followed by a recovery period of 10 days without any exposure (and without any stops of water flow), to investigate the potential excretion and/or movement of Gd within the organs of the organism. Feeding and cleaning routines, as well as water parameters were kept identical to the procedure during the exposure period (see section 2.3.1). The recovery period was followed by a final sampling (sampling 2, see Figure 1).

2.3.3 Recovery period

The exposure period and sampling 1 were followed by a recovery period of 10 days without any exposure (and without any stops of water flow), to investigate the potential excretion and/or movement of Gd within the organs of the organism. Feeding and cleaning routines, as well as water parameters were kept identical to the procedure during the exposure period (see section 2.3.1 Experimental preparation). The recovery period was followed by a final sampling (sampling 2, see Figure 1).

2.3.4 Water sampling

Throughout the experiment, filtered and unfiltered water samples from each tank were taken to measure the Gd exposure concentrations. The water samples were taken on exposure day 2 and 6 from all tanks at different timepoints; before exposure, at the start of the exposure (straight after adding exposure solutions), at the end of the exposure (before opening the water flow), after the exposure (after opening the water flow), after 1.5 hours and after 3 hours. A total volume of 10 mL was taken from the water column and stored in 10 mL vials. Filtered samples were taken using a syringe and then filtered through a polyethersulfone syringe filter (AVANTOR, 0.45 µm). Unfiltered samples were only taken from one tank per treatment on day 2 and 6, not all tanks. All samples were preserved using 3 drops of ultrapure HNO_3 (65% v/v).

2.3.5 Organ sampling

Sampling was conducted after the 10 days of exposure (n=9 fish per treatment, total of 59 fish) and after the 10 days of recovery (n=9 fish per exposure, total of 59 fish).

Before sampling, the fish were euthanized by keeping them for approximately 2 minutes in MS222 (500 mg/L). The fish were removed from the MS222 solution when they were unresponsive to touch and a blow to the head was applied to ensure unconsciousness. The gills were then severed to ensure death. Blood samples were then collected, and the fish were weighed, and their length was measured. Subsequently, samples of brains (n=6 per treatment, n=3 each for the exposed group and the recovered group), gills, livers, and kidneys were taken out (n=18 per treatment, n=9 each for exposed groups and recovered groups) and put into plastic bags, and frozen to -18 °C. The fish and organs were kept on ice throughout the entire procedure. During sampling, all equipment and surfaces were rinsed in ethanol (EtOH; C_2H_6O, 70%), and purified water (MilliQ) and disposable equipment was changed between each dissection. All samples were stored at -18 °C until further processing.

2.4 Element analysis

After thawing, the organs were cut and divided into smaller pieces with a titanium grade 2 knife, previously cleaned in HNO_3 (1 M) overnight and purified water (MilliQ). The cutting board was protected with plastic (food–grade) foil. Subsequently, all fish samples were freeze–dried and the water content in each sample was calculated.

Fish samples (n=40 for brains, n=118 for livers, kidneys, and gills) were microwave digested using a high–pressure microwave system (Milestone UltraClave, EMLS, Leutkirch, Germany).

Polytetrafluoroethylene (Teflon®) vials used for the digestion were soaked with ultrapure HNO_3 (50% v/v) in a sub–boiling distillation system, Milestone, SubPur, Sorisole, BG, Italy) for at least 12 hours prior to digestion. Samples were digested in ultrapure HNO_3 (50% v/v), using a temperature profile with a maximum temperature of 245 °C at 110 bar for 2.5 h. After digestion, all samples were diluted 10 times with ultrapure water (0.055 µs, Purelab® Chorus 1, ELGA Labwaters, UK). Determination of Gd was performed by inductively coupled plasma–mass spectrometry (Agilent–8800 ICP–MS Triple Quad, USA). Certified reference materials (BCR®–668, mussel tissue, Institute for Reference Materials and Measurements (Geel), European Commission, Directorate General, Joint Research Centre) and reference material (INCT–OBTL–5, Oriental Basma Tobacco Leaves from the Instytut Chemii i Techniki Jądrowej, Laboratory of Nuclear Analytical Methods), and blanks were processed during each analytical batch to verify the performance of the methods. Brains from fish exposed to DOTA, GBCA low and $GdCl_3$ low were not analysed using ICP-MS, and are therefore excluded from this study due to preliminary test measurements indicating no detection of Gd in brains for these treatments.

Filtered water samples were diluted 10 times using ultrapure water (ELGA) then 3 drops of concentrated ultrapure HNO_3 (65% v/v) were added to all samples. For the unfiltered water samples, 4 mL of the sample was transferred to a digestion vial, and 2 mL of ultrapure HNO_3 (50% v/v) was added. The digestion steps for the unfiltered water samples and blanks were identical to the ones applied to the fish organs. After digestion, samples were diluted to 50 mL using ultrapure water (ELGA). Determination of Gd presence was performed by Inductively Coupled Plasma–Mass Spectrometry (Agilent–8800 ICP–MS Triple Quad, USA).

2.5 Statistical analyses

Microsoft Excel (Office 365) was used to perform basic statistical analysis.

Organ–specific bioconcentration factors (BCF) were calculated using the average Gd concentrations in the different organs of lumpfish, and the average Gd exposure over 24 hours for each treatment, based on the average Gd concentration at the start of the experiment (highest exposure concentration**).** The average Gd exposure over 24 hours was calculated by integrating the concentration decrease over time (% decrease in 24 hours) based on tank volume and flow (dilution). The area under the obtained curve was then calculated and used to further obtain the average % exposure over 24 hours.

Factors were calculated between each treatment group for all organs using the average measured concentration of Gd in each treatment group ($GdCl_3$ low/high and GBCA low/high) and dividing it by CTRL for the same treatment group (CTRL was used as a reference).

Limits of detection (LOD) were calculated, based on measured Gd values in blank samples and detection limit values calculated from the ICP–MS machine. The highest value of LOD was used to determine the validity of the measured values for each individual sample. In addition, if the relative standard deviation (RSD) of 3 subsequent scans of m/z signal intensity was high RSD (> 20), the results were considered uncertain, and treated as below LOD.

In the case where the value of Gd concentration for a specific sample was below the limit of detection, the concentration of the element in question was set to be half of the detection limit (LOD/2) for further statistical analysis. Sample exposure groups containing >50% of values below LOD were removed from further statistical analysis. An exception was CTRL E+R for brains, where all 6 data points are based on LOD/2 to get a reference for comparing treatments in the brains. Treatment groups excluded from further statistical analysis due to high uncertainty of measured Gd concentrations were CTRL R in gills, DOTA R in gills, CTRL E in livers, DOTA E in livers, and DOTA R in livers (also shown in Table 2).

RStudio was used to perform further data analyses (R studio v. 0.99.903, R core Team, 2016). Homogeneity was checked using Q–Q plots from ANOVA. The normality was checked using the Shapiro–Wilk normality test. Bartlett's test and Levene's test were used to evaluate the homogeneity of variances among treatment groups. However, since the data did not meet the assumptions for parametric statistics, non–parametric tests were employed. The Kruskal–Wallis one–way analysis of variance was used to compare the treatments, followed by multiple group comparisons using the Wilcoxon rank–sum test (also known as Mann–Whitney U test) with Bonferroni adjustment for p–values to control family–wise error rates. Additionally, the Spearman method was applied to assess the correlation between weight, length and Gd accumulation.

3 Results

3.1 Exposure concentrations

Average exposure concentrations in the water during the 1–hour exposure were measured to be highest in GBCA high (131.43±5.36 µg/L), followed by $GdCl_3$ high (127.7±1.77 µg/L), GBCA low (1.37±0.039 µg/L) and $GdCl_3$ low (1.29±0.04 µg/L). Measurements of Gd concentration in DOTA (0.009±0.006 µg/L) and CTRL (0.022±0.047 µg/L) were both lowest. Gd concentrations determined for each treatment are presented in Figure 2. All values and standard errors are given in the appendix, section A2, Table A.2.

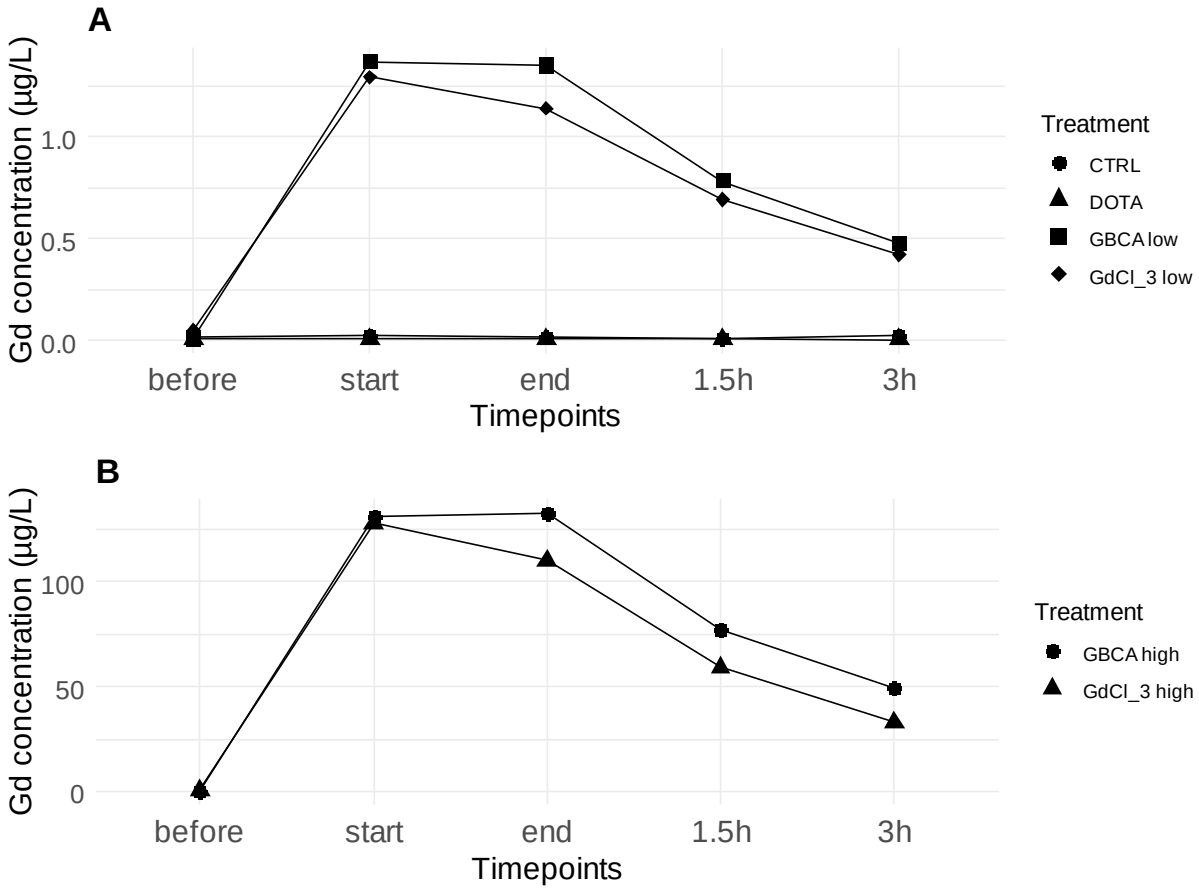


Figure 2. Measured Gd exposure concentrations (µg/L) for the treatments CTRL, DOTA, GBCA low, $GdCl_3$ low (A) GBCA high and $GdCl_3$ high (B) based on average measurements of Gd concentrations on day 2 and day 6 at the timepoints: before exposure (before), start of exposure (start), end of exposure (end), after 1.5 hours (1.5h) and after 3 hours (3h).

3.2 Mortality

There was no occurrence of mortality during the experiment in any of the exposure or recovery groups.

3.3 Water content in organs

Water contents in the organs of lumpfish were highest in the gills, followed by kidneys, livers, and lastly brains. An overview of average measured water contents in the different organs is given in Table 1.

Table 1. The average percent of water content (%) in gills, kidneys, livers, and gills of lumpfish (*Cyclopterus lumpus*).

Organ	n	Median	Avg (%)	SE	Min	Max
Gills	107	86.1	85.9	2.4	72.9	92.6
Kidneys	106	83.2	83.1	1.8	79.2	93.8
Livers	108	50.9	50.8	4.9	37.8	85.2
Brains	37	82.6	82.3	3.8	73.1	96.0

n represents the number of samples

3.4 Exposure uptake

Element analyses showed that the accumulation of Gd varied between different treatments and between exposed and recovered groups from each treatment. The concentrations of Gd in organs of lumpfish (dry weight, dw) were highest in gills, followed by kidneys, livers, and lastly brains. An overview of the Gd concentrations in the different organs and for each treatment and exposed/recovered groups is given in Table 2.

An overview over Gd accumulation in wet weight (ww) of kidneys, livers and brains can be found in the appendix, section A3, Table A.3.1.

Table 2. Concentrations of Gd (ng/g dry weight (dw)) in gills, kidneys, livers, and brains of lumpfish (*Cyclopterus lumpus*) for CTRL, DOTA, GBCA low, GBCA high, $GdCl_3$ low and $GdCl_3$ high and exposed and recovered groups (Gr) for each treatment.

Organ	Treatment	Gr.	n	Median	Average	SE	Min	Max
Gills	CTRL	E	9	2.012	2.741	2.429	0.007	6.093
		R	9	-	-	-	-	<LOD
	DOTA	E	9	1.460	2.936	4.133	0.416	13.42
		R	9	-	-	-	-	<LOD
	GBCA low	E	9	1.187	2.313	3.492	0.512	11.46
		R	9	0.251	0.288	0.110	0.191	0.558
	GBCA high	E	9	7.979	10.61	4.083	6.436	17.38
		R	9	3.037	3.013	0.423	2.436	3.789
	$GdCl_3$ low	E	9	5.046	5.913	4.025	2.458	15.86
		R	9	0.974	1.065	0.266	0.731	1.483
	$GdCl_3$ high	E	9	1429	1468	669.9	593.0	2547
		R	9	386.2	473.3	222.8	233.4	960.7
Kidneys	CTRL	E	8	0.227	0.267	0.161	0.094	0.551
		R	9	0.245	0.233	0.126	0.023	0.408
	DOTA	E	9	0.230	0.279	0.182	0.011	0.565
		R	9	0.191	0.176	0.109	0.011	0.305
	GBCA low	E	9	0.222	0.391	0.485	0.084	1.732
		R	9	0.154	0.226	0.271	0.004	0.879
	GBCA high	E	9	3.622	6.167	8.347	1.605	28.08
		R	9	2,915	2,883	0,623	1,931	3,660
	$GdCl_3$ low	E	9	0.841	0.856	0.338	0.319	1.481
		R	9	0.674	0.684	0.340	0.010	1.121
	$GdCl_3$ high	E	9	22.56	22.98	3.636	15.68	27.55
		R	9	28.50	28.54	11.32	16.72	54.96
Livers	CTRL	E	9	-	-	-	-	<LOD
		R	9	0.003	0.021	0.032	0.003	0.094
	DOTA	E	9	-	-	-	-	<LOD
		R	9	-	-	-	-	<LOD
	GBCA low	E	9	0.062	0.097	0.104	0.012	0.346
		R	9	0.154	0.226	0.271	0.004	0.879
	GBCA high	E	9	1.038	1.396	1.184	0.503	4.221
		R	9	0.886	0.929	0.308	0.399	1.478
	$GdCl_3$ low	E	9	0.181	0.174	0.103	0.012	0.370
		R	9	0.109	0.117	0.049	0.072	0.241
	$GdCl_3$ high	E	9	5.516	6.050	2.673	2.123	10.03
		R	9	3.982	4.208	1.596	2.580	8.092
Brains	CTRL	E	3	0.252	0.264	0.114	0.156	0.383
		R	3	0.157	0.161	0.015	0.149	0.178
	GBCA high	E	4	0.191	0.656	0.947	0.166	2.077
		R	3	0.744	0.734	0.584	0.145	1.312
	$GdCl_3$ high	E	3	1.136	0.905	0.804	0.010	1.569
		R	3	3.019	2.210	1.821	0.124	3.487

n represents the number of samples

3.5 Gd accumulation in gills

In gills, average Gd concentrations (in ng/g dw) were highest in $GdCl_3$ high, followed by GBCA high>$GdCl_3$ low>GBCA low>DOTA=CTRL. Concentrations were reduced after a period of recovery for all treatments. Gd concentrations determined in the gills of each exposure group are given in Table 2 and Figure 3.

Gd concentrations in gills for $GdCl_3$ high exposed (E) were significantly higher compared to that in CTRL and DOTA ($p=0.0027$; $p=0.0054$, respectively), with measured concentrations being on average 535 times higher in $GdCl_3$ high exposed (E) than in CTRL. In $GdCl_3$ high recovered (R) Gd concentrations were 172 times higher than in CTRL. $GdCl_3$ high (E+R) treatment had a significantly higher Gd concentration compared to all other treatments.

Gd concentrations in gills for GBCA high E were also significantly higher compared to DOTA and CTRL ($p=0.0027$; $p=0.0054$, respectively), with measured concentrations being on average 4 times higher than CTRL.

Comparing exposed and recovered groups within each treatment, concentrations of Gd were significantly lower after the 10–day recovery, compared to concentrations found in the gills of fish in the exposed groups for the treatments $GdCl_3$ high ($p=0.0326$), $GdCl_3$ low ($p=0.0027$) and GBCA high ($p=0.0027$). There was no significant difference ($p>0.05$) between CTRL, DOTA, and GBCA low.

A full overview of all p–values can be found in appendix, section A4, Table A.4.1. An overview of factors can be found in the appendix, section A5, Table A.5.1.

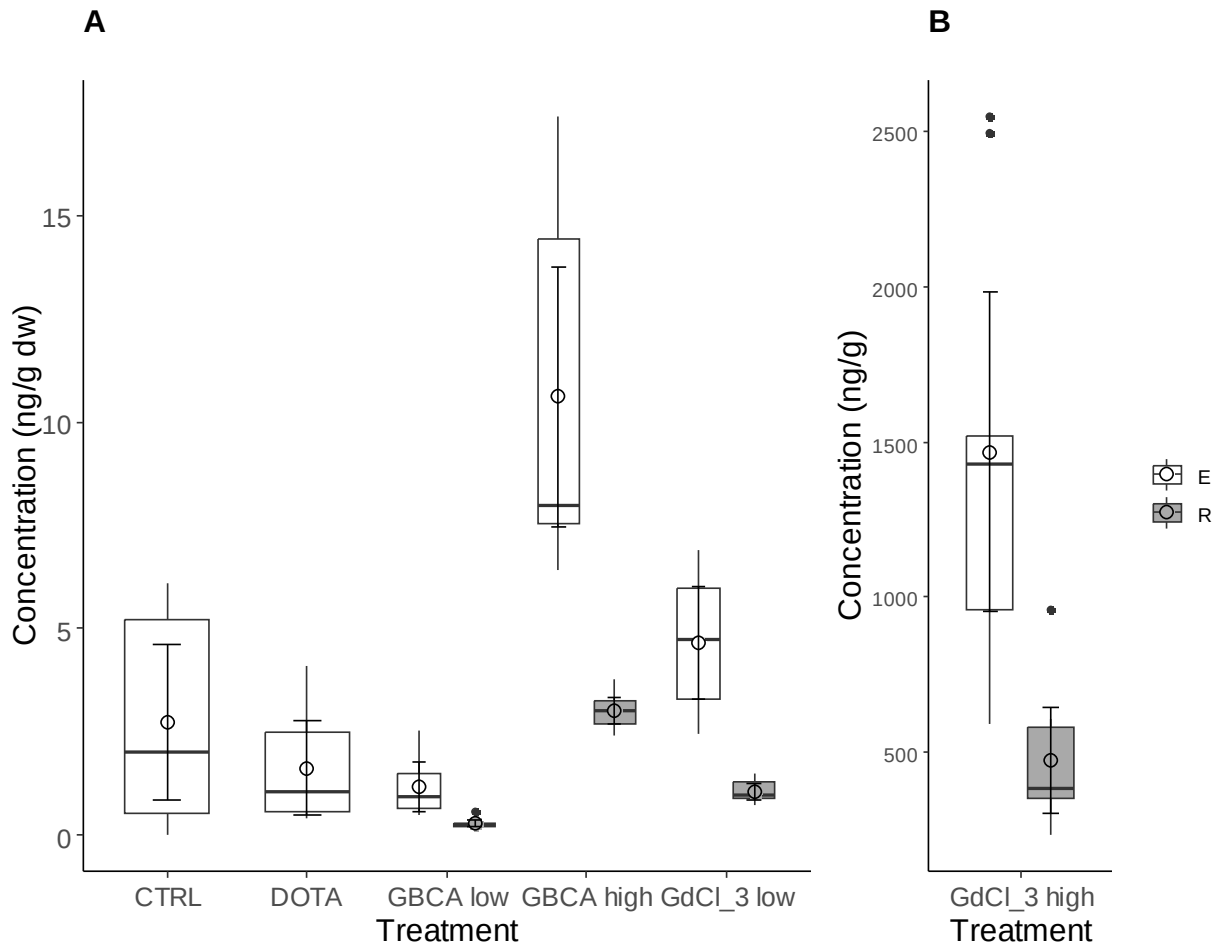


Figure 3. Gd concentrations (ng/g dw) in gills of CTRL, DOTA, GBCA low, GBCA high, $GdCl_3$ low (A) and $GdCl_3$ high (B) and the exposed (E, white) and recovered (R, grey) groups from each treatment (n=9 for exposed and n=9 for recovered groups). The line in each box represents the median, the circle represents the average concentrations, and the vertical whiskers with horizontal stop–lines are standard error bars, the vertical whiskers without horizontal stop–lines indicate the spread of the data points. Outliers are shown as dots.

3.6 Gd accumulation in kidneys

In kidneys, average Gd concentrations (in ng/g dw) were highest in $GdCl_3$ high>GBCA high> $GdCl_3$ low>GBCA low>DOTA=CTRL. Concentrations were reduced after a period of recovery for all treatments, except for $GdCl_3$ high, where the recovered group had an increased average concentration compared to the exposed group. Gd concentrations determined in the kidneys of each exposure group are given in Table 2 and Figure 4.

Gd concentrations in kidneys for $GdCl_3$ high E were significantly higher compared to that in CTRL and DOTA ($p=0.0027$; $p=0.0027$, respectively), with measured concentrations being on average 92 times higher in $GdCl_3$ high exposed (E) than in CTRL. In $GdCl_3$ high R, concentrations were also significantly higher than those in CTRL and DOTA ($p=0.0027$; $p=0.0054$ respectively), with measured concentration being 114 times higher than in CTRL.

Gd concentrations in kidneys for GBCA high E were significantly higher compared to DOTA and CTRL ($p=0.0054$; $p=0.0054$, respectively), with measured concentrations being 13 times higher than CTRL. GBCA high E treatment also had a significantly higher concentration of Gd than CTRL and DOTA ($p=0.0054$; $p=0.0027$ respectively), with the measured concentrations being 11 times higher than in CTRL.

$GdCl_3$ high (E+R) and GBCA high (E+R) treatments both had significantly higher Gd concentrations compared to all other treatments.

There was also a significantly higher Gd concentration in $GdCl_3$ low E compared to CTRL and DOTA ($p=0.0190$; $p=0.0190$ respectively), with concentrations being 3 times higher than CTRL.

No significant differences ($p>0.05$) were found between CTRL, DOTA, and GBCA low or between recovered and exposed groups for each treatment. A full overview of all p–values can be found in the appendix, section A4, Table A.4.2. An overview of factors can be found in the appendix, section A5, Table A.5.1.

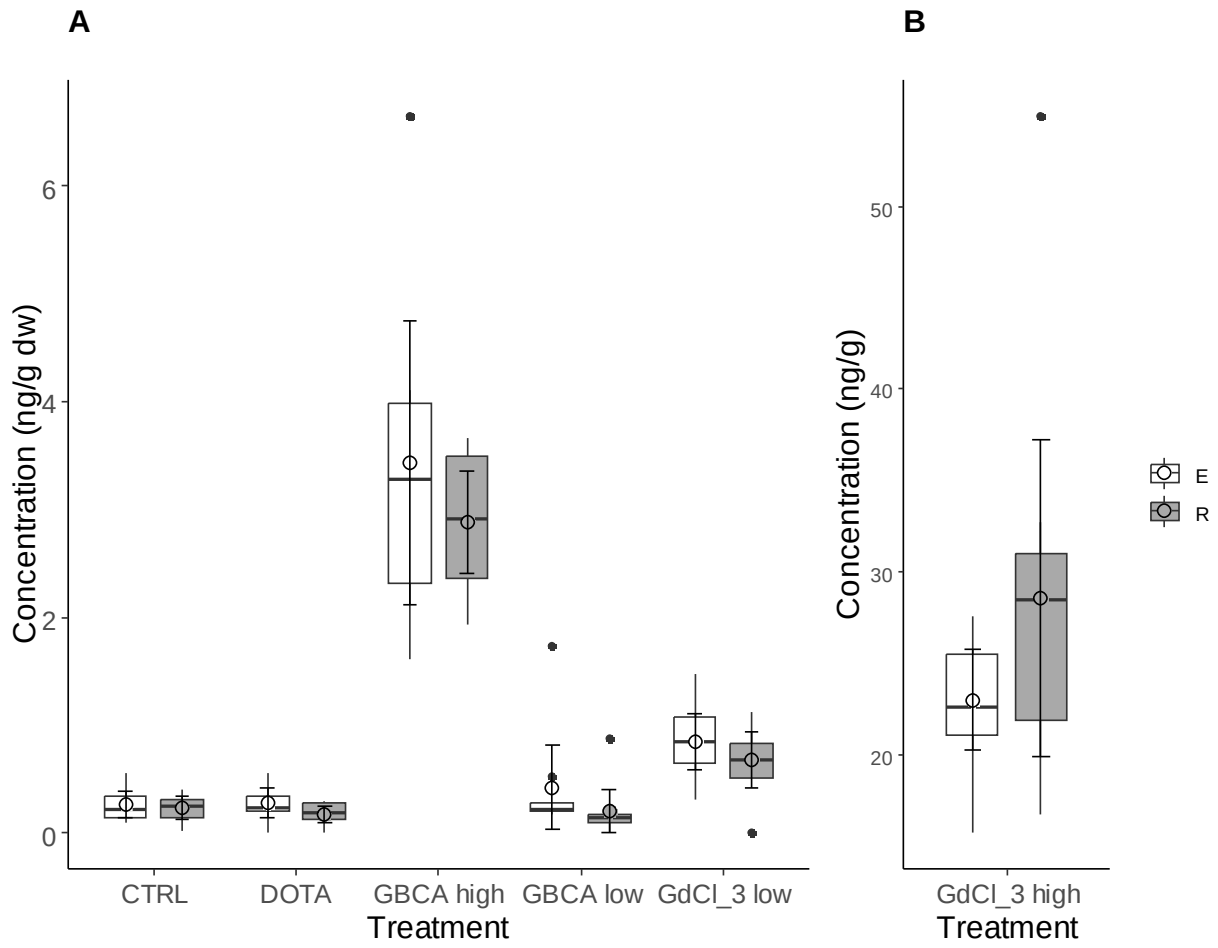


Figure 4. Gd concentrations (ng/g dw) in kidneys of CTRL, DOTA, GBCA low, GBCA high, $GdCl_3$ low (A) and $GdCl_3$ high (B) and the exposed (E, white) and recovered (R, grey) groups from each treatment. n=17 for CTRL (n=8 for exposed and n=9 for recovered groups), n=18 for all other exposure treatments (n=9 for exposed and n=9 for recovered groups). The line in each box represents the median, the circle represents the average concentrations, and the vertical whiskers with horizontal stop–lines are standard error bars, the vertical whiskers without horizontal stop–lines indicate the spread of the data points. Outliers are shown as dots.

3.7 Gd accumulation in livers

In livers, average Gd concentrations (in ng/g dw) were highest in $GdCl_3$ high>GBCA high> $GdCl_3$ low>GBCA low> CTRL. Concentrations were reduced after a period of recovery for all treatments. Gd concentrations determined in the livers of each exposure group are given in Table 2 and Figure 5.

Gd concentrations in the livers for $GdCl_3$ high exposed (E) were significantly higher compared to that in CTRL and DOTA ($p=0.0027$; $p=0.0027$, respectively), with measured concentrations being 179 times higher than CTRL. $GdCl_3$ high recovered (R) also had significantly higher concentrations compared to CTRL and DOTA ($p=0.0027$; $p=0.0027$, respectively), with Gd concentrations 125 times higher than in CTRL.

Gd concentrations in the livers for GBCA high E were significantly higher compared to DOTA and CTRL ($p=0.0054$; $p=0.0054$, respectively), with measured concentrations being 31 times higher than CTRL. A significant difference was also found for GBCA high R, where concentrations were significantly higher compared to that in CTRL and DOTA ($p=0.0027$; $p=0.0027$, respectively), and Gd concentrations were 28 times higher than in CTRL.

$GdCl_3$ high (E+R) and GBCA high (E+R) treatments both had significantly higher Gd concentrations compared to all other treatments.

$GdCl_3$ low E also had a significantly higher Gd concentration compared to CTRL ($p=0.0326$), with concentrations being 5 times higher than CTRL.

No significant differences were found between CTRL and GBCA low, or between the recovered and exposed groups for each treatment. A full overview of all p–values can be found in the appendix, section A4, Table A.4.3. An overview of calculated factors for all treatments is found in the appendix, section A5, Table A.5.1.

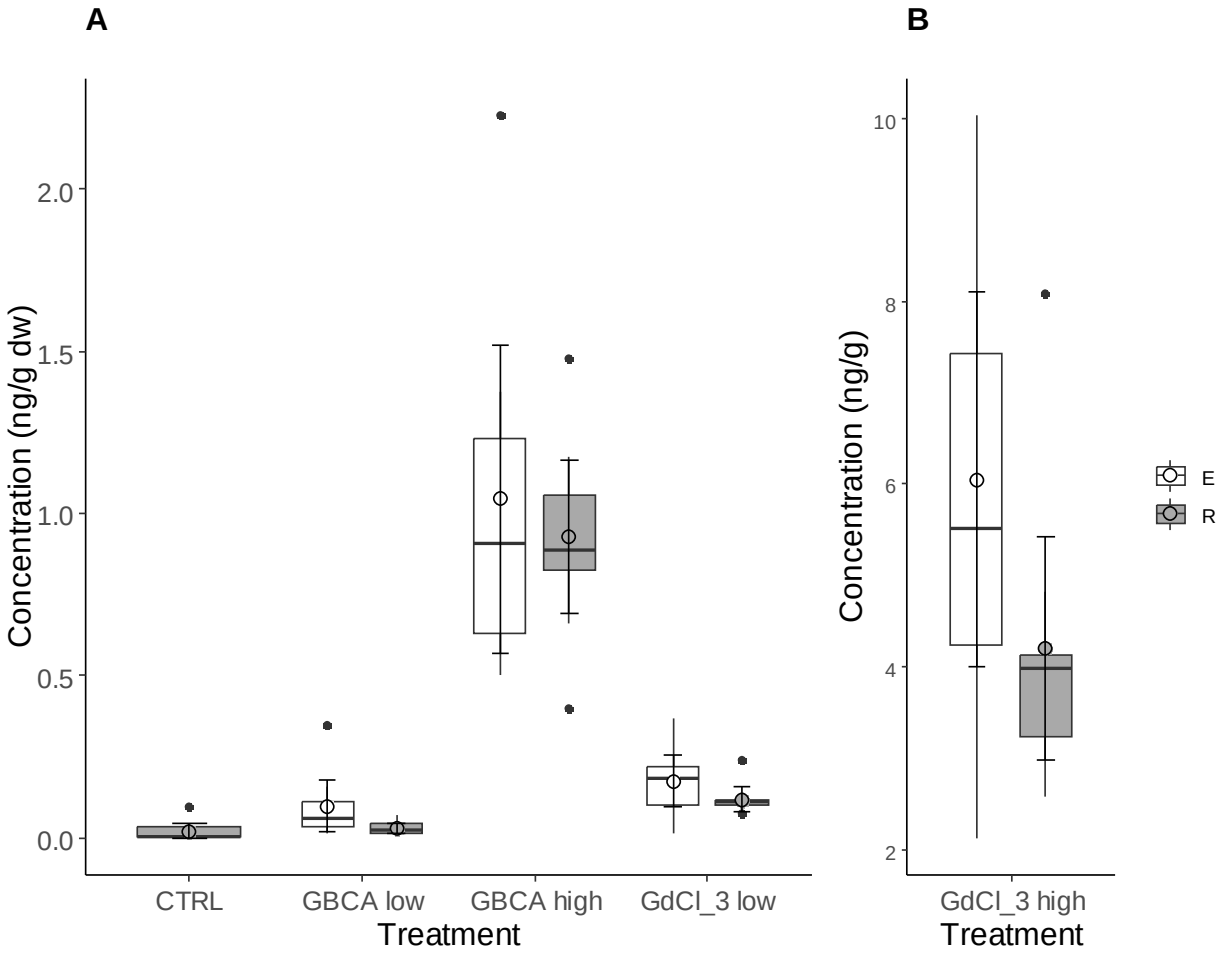


Figure 5. Gd concentrations (ng/g dw) in the livers of CTRL, GBCA low, GBCA high, $GdCl_3$ low (A) and $GdCl_3$ high (B) and the exposed (E) and recovered (R) groups from each treatment (n=9 for exposed and n=9 for recovered groups). The line in each box represents the median, the circle represents the average concentrations, and the vertical whiskers with horizontal stop–lines are standard error bars, the vertical whiskers without horizontal stop–lines indicate the spread of the data points. Outliers are shown as dots.

3.8 Gd accumulation in brains

In the brains, average Gd concentrations (in ng/g dw) were highest in $GdCl_3$ high>GBCA high>CTRL. Gd concentrations determined in the brains of each exposure group are given in Table 2 and Figure 6. Gd accumulation in $GdCl_3$ high E was 10 times higher than CTRL, and $GdCl_3$ high R was 4.3 times higher than CTRL. GBCA high was around 3 times higher than measured Gd values in CTRL, a full overview can be found in the appendix, section A5, Table A.5.1.

Fish exposed to GBCA high and $GdCl_3$ high seemed to have higher Gd concentrations in their brains after 10 days of recovery compared to exposed fish (Figure 6). However, no significant differences ($p>0.05$) were found for any of the treatments. An overview of p–values can be found in the appendix, section A4, Table A.4.4.

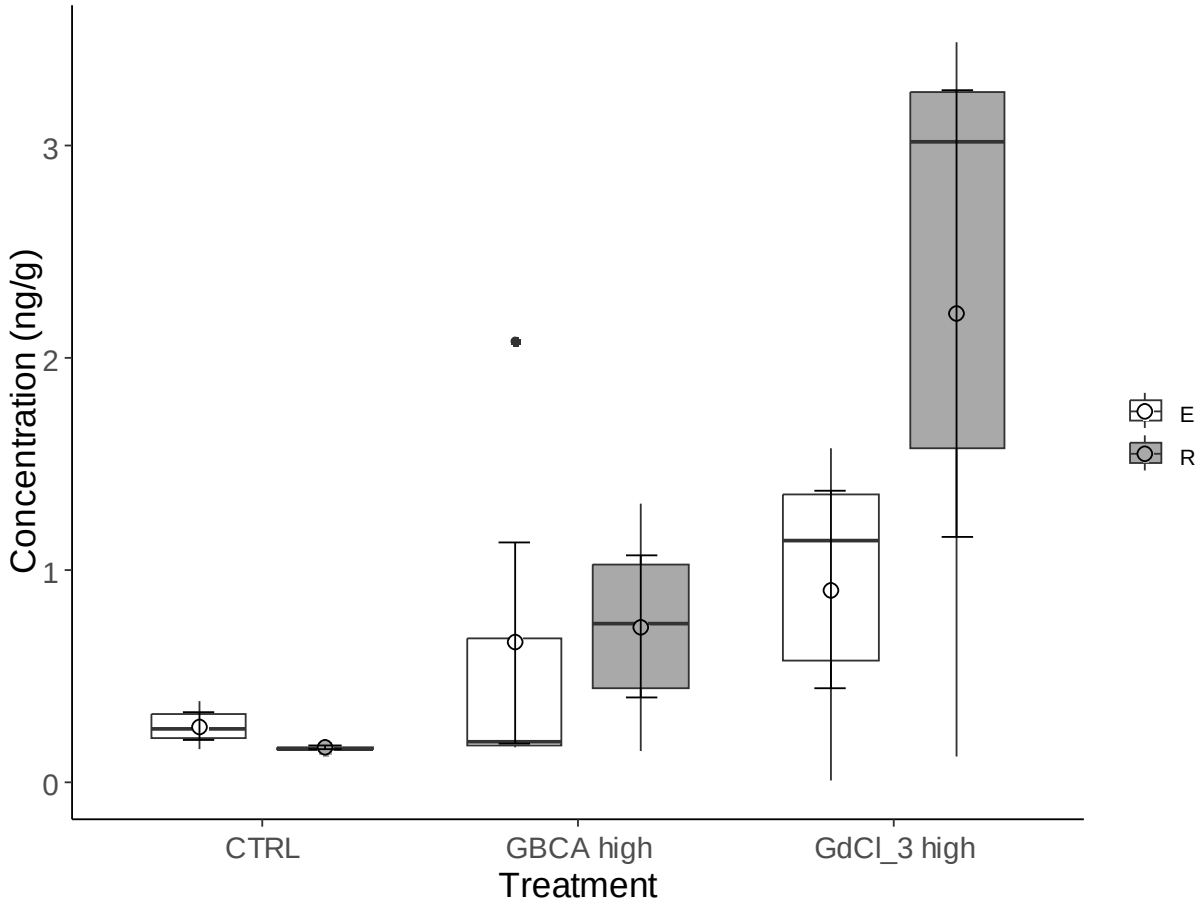


Figure 6. Gd concentrations (ng/g dw) in the brains for CTRL, GBCA high and $GdCl_3$ high and the exposed (E, white) and recovered (R, grey) groups from each treatment (n=4 for GBCA high E, for all other treatments; n=3 for recovered and n=3 for exposed). The horizontal line in each box represents the median, the circle represents the average concentration, and the vertical whiskers with horizontal stop–lines are standard error bars, the vertical whiskers without horizontal stop–lines indicate the spread of the data points. Outliers are shown as dots.

3.9 Organ–specific bioconcentration factor

Organ–specific bioconcentration factor (BCF) is highest for $GdCl_3$ high (60.8) in gills, $GdCl_3$ low (3.49) in kidneys, $GdCl_3$ low (0.71) in livers, and $GdCl_3$ high (0.04) in brains. A full overview of BCF for each organ considering each treatment is given in Table 3.

Table 3. Organ–specific BCF for each treatment during the experiment. The data is calculated based on average Gd concentrations in the different organs of lumpfish given in Table 2, and the average Gd exposure over 24 hours for each treatment, calculated from the Gd concentration at the start of the experiment (max concentration), given in the appendix, section A.2, Table A.2.1.

Organ	$GdCl_3$ high	$GdCl_3$ low	GBCA high	GBCA low
Gills	60.8	24.2	0.42	8.92
Kidneys	0.95	3.49	0.25	1.51
Livers	0.25	0.58	0.05	0.38
Brains	0.04	0.71	0.03	

3.10 Correlations

There was a slight negative correlation between fish weight and Gd concentration, where p=0.013 and Rho=-0.13, meaning a slight reduction in Gd concentration in organs with an increased weight of the fish.

A full overview of all calculated correlations and results can be found in the appendix, section A6, Table A.6.1.

4 Discussion

This study revealed a significant accumulation of Gd in organs of lumpfish (*Cyclopterus lumpus*) exposed to high concentrations of GBCA and $GdCl_3$. In contrast, no significant Gd accumulation was observed in the gills and brains of fish exposed to low concentrations of Gd (GBCA low and $GdCl_3$ low). Fish exposed to $GdCl_3$ low exhibited a significantly higher Gd concentration in kidneys and livers. Gd concentrations in organs were generally highest in the gills, followed by kidneys and livers. The smallest concentrations were found in brains (Table 2). Following a recovery period of 10 days, concentrations had decreased, indicating excretion during the recovery. An exception was found for the kidneys and brains of fish exposed to $GdCl_3$ high, where concentrations increased post–recovery.

In this study, we observed the following organotropism in lumpfish: gills>kidneys>livers>brains, both after the exposure and recovery period. Previous studies of bioaccumulation and organotropism of inorganic Gd in fish have revealed varying patterns depending on species and exposure concentrations. Lortholarie et al. (2021) measured REE accumulation in European eels (*Anguilla anguilla*), caught in the field, and found different organotropism based on life stage and gender. They observed the highest accumulation of Gd in gills (126.90±50.78 μg/kg dw) and livers (181.78±62.04 μg/kg dw for males; 203.79±111.86 μg/kg dw for females). Qiang et al. (1994) exposed carp (*Cyprinus carpio*) to 0.50 mg/L Gd dissolved in water for 45 days and found a slightly different organotropism: internal organs (48.5 μg/g, wet weight (ww)) > gills (5.33 μg/g, ww) > skeleton (2.30 μg/g, ww) > muscles (1.50 μg/g, ww). Similarly, Cardon et al. (2020) conducted a study on rainbow trout (*Oncorhynchus mykiss*) exposed to different yttrium concentrations (86, 144, 240, and 400 μg/L) through waterborne or food exposure. They reported the following organotropism: intestines (concentration not specified) > fish body (min–max: 12–243 μg Y/kg ww) > gills (44–143 μg Y/kg ww) > livers (11–61 μg Y/kg ww) > muscles (concentration not specified). However, it is important to consider that the species in the latter two studies are freshwater species, which may lead to differences in uptake pathways compared to marine species, such as lumpfish. Factors such as species, physiology, uptake and excretion pathways can contribute to these variations. For example, a study by Squadrone et al. (2020b) on Cuban lionfish (*Pterois* spp.), showed higher accumulation of waterborne ∑REEs in kidneys compared to liver, which is in agreement with our study.

The results in this study further suggest that gills are among the main tissues for Gd accumulation, with the accumulation of inorganic Gd at high concentration being significantly

higher than all other treatment groups (Figure 3), and having the highest BCF (60.8). Similar to this study, previous studies have also identified gills among the organs with the highest REE bioaccumulation (Cardon et al., 2020; Lortholarie et al., 2021; Qiang et al., 1994). This can be attributed to the fact that gills are the primary route for metal uptake and bioaccumulation in fish due to their direct contact with water. Additionally, marine fish rely on gills as the primary absorption pathway for calcium (Ca) before it reaches the intestines (Baldisserotto, 2019; Flik et al., 1995). Consequently, this can enhance the uptake of Gd, as Gd^{3+} has a similar ionic radius as Ca^{2+} and can thus bind to Ca^{2+} transporters in cell membranes, acting as an agonist (Cui et al., 2012; Figueiredo et al., 2018; Martin & Richardson, 1979; Switzer, 1978). It is also worth noting that gills also serve as an important site for Ca excretion and ionic exchange, which can contribute to the excretion of Gd during a recovery period. Furthermore, the results obtained from this study demonstrate a significantly higher concentration of Gd in the gills of lumpfish exposed to the high concentration of inorganic Gd (1468±473.3 ng/g dw) compared to European eels (*Anguilla anguilla*) (126.90±50.78 ng/g dw) (Lortholarie et al., 2020). However, it is important to consider that the study on European eels was based on environmental concentrations of Gd in an estuary. Other factors such as physiological differences between these species should also be considered. In this study, no significant differences between CTRL and groups exposed to low concentrations of organic and inorganic Gd were found in the gills. This lack of difference can likely be attributed to the relatively low Gd concentrations in these treatment groups (close to detection limits), leading to an inevitable increase in the analytical uncertainty of measurements. As a result, no significant differences were found between CTRL, DOTA, GBCA low, and $GdCl_3$ low, despite observing generally higher Gd concentrations in the gill tissues compared to all other organs.

The significant Gd uptake we detected in the kidneys, followed by livers (Table 2), highlights the importance of the livers and kidney as xenobiotic transformation and excretion sites. Both organs exhibited significant accumulation of high exposure concentrations to inorganic Gd (22.98±3.636 ng/g dw in kidneys and 6.05±2.673 ng/g dw in livers), followed by high exposure to organic Gd (6.16±8.347 ng/g dw in kidneys and 1.396±1.184 ng/g dw in livers). We expected concentrations to be higher in the liver than kidneys prior to this study, however, the literature confirms this pattern of organotropism in Indo–Pacific lionfish (*Pterois spp.*), which is also a marine fish species (Squadrone et al., 2020b). The higher concentration in the kidneys can be attributed to their higher water content (Table 1) in comparison to the livers (83%; 50.8%, respectively). Kidneys also show higher BCF compared to livers for all treatments (Table 3).

Furthermore, kidneys serve as the primary site for divalent ionic excretion, including Ca (Flik et al., 1995). Therefore, the increased accumulation of Gd in the kidneys may also result from interaction with Ca pathways. On the other hand, livers may be more efficient in xenobiotic excretion, which could explain the lower accumulation rate of Gd compared to the kidneys. Both the kidneys and liver play vital roles in metal sequestration and elimination in fish, as these organs contain high amounts of metal–binding protein. The increased concentrations of Gd in these organs align with existing literature (Pannetier et al., 2016 Kumari & Swamy, 2023; Masresha et al., 2021; Olaifa et al., 2010; Squadrone et al., 2020b).

High variability in the results from our study indicates some uncertainties regarding accumulation in the brain of lumpfish, which is related to the small sample size and thus low mass of tissue available for analyses. The relatively small size of the lumpfish brain may be an adaptation to conserve energy by minimizing unnecessary costs associated with maintaining a larger brain. Consequently, the measurement of Gd concentration in the brain is limited, as there is an increased risk of values falling below the detection limit. Despite the high standard error and lack of significant difference between treatments and groups, one cannot exclude the potential bioaccumulation of Gd in the brains, which could potentially be observed in brains with a larger mass.

Differences were observed between the exposed and recovered groups in this study, suggesting Gd excretion from certain organs over time, particularly for low concentrations of both inorganic and organic Gd. These findings correlate with our expectations prior to the study. However, in the brains and kidneys of fish exposed to $GdCl_3$ high, higher average Gd concentrations were observed in recovered groups compared to exposed groups (R=2.210±1.821 ng/g dw; E=0,905±0.805 ng/g dw in brains and R=28.54±11.32 ng/g dw; E=22.98±3.636 ng/g dw in kidneys). This change in concentration patterns might be attributed to the transport of Gd through the bloodstream, leading to the movement of Gd between organs and changes in organotropism. Previous research has shown that Gd tends to accumulate in tissues with increased concentrations of Ca^{2+}, which are typically bones, blood, and the brain, where Ca^{2+} acts as signalling molecules (Flik et al., 1995). High amounts of Gd in the brain might therefore be explained by its affinity to Ca^{2+} binding sites. Similarly, the increase of Gd concentration in kidneys over time could be attributed to the organs' filtration of blood, containing Gd transported from other organs. These patterns of accumulation require further investigation over an extended period and measurements of Gd concentrations in the blood to draw definitive conclusions.

The study also found differences in bioaccumulation of organic Gd (GBCA) and inorganic Gd ($GdCl_3$), showing a significantly higher accumulation of high concentrations of inorganic Gd compared to organic Gd for all treatments, and in all organs. The higher accumulation of inorganic Gd compared to organic Gd aligns with our hypothesis and existing literature (Hanana et al., 2017). Results suggest that the chelating molecule DOTA, coating the Gd in GBCA acts as a protective barrier, reducing the bioaccumulation of GBCA. The GBCA molecule is also a larger molecule, potentially inhibiting the deployment of GBCA over the gills. As a result, ingestion of Gd from GBCA via fish drinking the water may cause accumulation in the kidneys before the liver due to ingestion pathways. The detection of Gd following exposure to GBCA may indicate that GBCA has a certain bioavailability, despite its coating. This is further supported by the elevated Gd concentrations in fish exposed to GBCA, found even after the recovery period, suggesting incomplete Gd excretion. Alternatively, it can indicate some breakdown of the Gd chelate in tissues or Gd^{3+} displacement from the ligands by other elements, as proposed by Mann (1993).

Findings in this study are relevant considering natural exposures to anthropogenic Gd in marine environments. Previous studies have found concentrations of Gd ranging from 0.62–17.5 ng/L in the Arctic (Laptev Sea) (Laukert et al., 2017), and from 0.03–15.4 ng/L in coastal and open waters in Germany, Greece, and Spain (Bau et al., 1997; Cánovas et al., 2020; Paffrath et al., 2020). The exposure to low concentrations of GBCA (1.37±0.039 µg/L) and $GdCl_3$ (1.29±0.04 µg/L) in this study are still considerably higher than concentrations found in the mentioned studies. Although no significant accumulation was found for exposure to organic and inorganic Gd at low concentrations in the gills and brains, exposure to low concentration of inorganic Gd caused significant accumulation in livers and kidneys. Additionally, the timeframe of this study should be considered, as the exposure lasted for 10 days. Continuous exposure to Gd in the marine environment may lead to accumulation despite low concentrations.

Uptake, accumulation, and organotropism of metals such as Gd in aquatic organisms are influenced by various environmental parameters, including temperature, pH and physio–chemical characteristics, and bioavailability of Gd. Additionally, species–specific characteristics such as detoxification pathways, trophic level, feeding, absorption, gender, and life stages play significant roles (Lortholarie et al., 2020; Lortholarie et al., 2021). All these vary depending on the environmental conditions in which one studies and can change over time. Several of these factors were not accounted for during this study, highlighting the need for further investigation.

5 Future outlook

Accumulation of organic and inorganic Gd was examined for brain, liver, kidney, and gill tissues during this study. Further investigations into the kinetic processes of internalization of Gd might be beneficial to better understand the underlying mechanisms and impacts of Gd accumulation and gain deeper insights into Gd organotropism in lumpfish. It might also be beneficial to investigate the accumulation of Gd in several other tissues, especially tissues containing higher amounts of Ca^{3+}, such as bones, muscle tissue, and blood to increase the understanding and knowledge of how Gd accumulates and distributes after both exposure and recovery. Investigating Gd accumulation throughout the entire organism will also facilitate the calculation of BCF for the entire organism. Fish with a larger brain size would be beneficial to investigate, to collect data based on a higher brain mass for increased accuracy of the results. Gender differences in accumulation and accumulative patterns between organs have been investigated and found in fish, however, this was not accounted for during this experiment and might be relevant to include in future studies.

Expanding the scope of research to include other marine species, particularly those inhabiting the bottom regions, would be valuable for future investigations. As Gd tends to accumulate at the ocean floor over time, studying species in close proximity to the bottom can provide valuable insights into the impacts of anthropogenic Gd enrichment on marine ecosystems. Additionally, examining species from various trophic levels can offer indications of potential biomagnification within marine food webs.

Considering the current limited knowledge regarding the effects of Gd, it is important to adopt a comprehensive approach that investigates the potential relationships between Gd and its anthropogenic uses. This broader perspective will enhance our understanding of the ecological implications of Gd and contribute to knowledge–based decision–making regarding its usage.

6 Conclusion

In the present study, Gd was quantified in the brain, kidney, liver, and gill tissues of *Cyclopterus lumpus* after a period of exposure to different concentrations of organic Gd (GBCA) and inorganic Gd ($GdCl_3$). Exposure to high concentrations of $GdCl_3$ resulted in significantly higher Gd accumulation compared to all other treatments, for all organs. Exposure to a high concentration of GBCA also leads to significant Gd accumulation in all organs. However, the accumulation of Gd in fish exposed to a high concentration of $GdCl_3$ was still significantly higher than in fish exposed to the high concentration of GBCA. These findings were similar for all organs, indicating a certain similarity in the accumulation patterns of inorganic and organic Gd. The differences between Gd accumulation in fish exposed to $GdCl_3$ and GBCA can be explained by a high rate of Gd dissociated from $GdCl_3$ in contrast to the DOTA–coated GBCA. This dissociation process might enhance the transport of Gd^{3+} across ion channels, contributing to an increased accumulation of Gd. The highest concentrations of Gd were found in the gills, followed by kidneys, then livers, and lastly brains. High concentrations in the gills can be explained by their central role in osmoregulation and ionic transfer between the organism and the water, to which the exposure solvents were added. Gills are also the main route for Ca uptake, and the agonistic properties of Gd towards Ca may cause a higher accumulation of Gd in this organ. High concentrations in kidneys and livers are explained by their central role in metal excretion and xenobiotic transformation. Furthermore, Gd excretion was observed for all organs after a period of recovery, except in the brains and kidneys of fish exposed to the high concentration of inorganic Gd. These findings indicate changes in organotropism over time, potentially due to bloodstream transport between organs. Research into several aspects, such as potential toxicity of Gd and accumulation and excretion mechanisms, is still needed to advance our understanding of anthropogenic Gd impacts on marine ecosystems.

References

Baldisserotto, B. (2019). *Fish osmoregulation*. CRC Press.

Bau, M., Balan, S., Schmidt, K., & Koschinsky, A. (2010). Rare earth elements in mussel shells of the Mytilidae family as tracers for hidden and fossil high-temperature hydrothermal systems. *Earth and Planetary Science Letters*, *299*(3–4), 310–316. https://doi.org/10.1016/J.EPSL.2010.09.011

Bau, M., & Dulski, P. (1996). Anthropogenic origin of positive gadolinium anomalies in river waters. *Earth and Planetary Science Letters*, *143*(1–4), 245–255. https://doi.org/10.1016/0012-821X(96)00127-6

Bau, M., Knappe, A., & Dulski, P. (2006). Anthropogenic gadolinium as a micropollutant in river waters in Pennsylvania and in Lake Erie, northeastern United States. *Chemie Der Erde*, *66*(2). https://doi.org/10.1016/j.chemer.2006.01.002

Bau, M., Möller, P., & Dulski, P. (1997). Yttrium and lanthanides in eastern Mediterranean seawater and their fractionation during redox-cycling. *Marine Chemistry*, *56*(1–2), 123–131. https://doi.org/10.1016/S0304-4203(96)00091-6

Blaise, C., Gagné, F., Harwood, M., Quinn, B., & Hanana, H. (2018). Ecotoxicity responses of the freshwater cnidarian Hydra attenuata to 11 rare earth elements. *Ecotoxicology and Environmental Safety*, *163*, 486–491. https://doi.org/10.1016/J.ECOENV.2018.07.033

Brünjes, R., & Hofmann, T. (2020). Anthropogenic gadolinium in freshwater and drinking water systems. In *Water Research* (Vol. 182). https://doi.org/10.1016/j.watres.2020.115966

Cánovas, C. R., Basallote, M. D., & Macías, F. (2020). Distribution and availability of rare earth elements and trace elements in the estuarine waters of the Ría of Huelva (SW Spain). *Environmental Pollution*, *267*. https://doi.org/10.1016/j.envpol.2020.115506

Cao, X., Chen, Y., Gu, Z., & Wang, X. (2000). Determination of trace rare earth elements in plant and soil samples by inductively coupled plasma-mass spectrometry. *International Journal of Environmental Analytical Chemistry*, *76*(4). https://doi.org/10.1080/03067310008034137

Caravan, P., Ellison, J. J., McMurry, T. J., & Lauffer, R. B. (1999). Gadolinium(III) chelates as MRI contrast agents: Structure, dynamics, and applications. *Chemical Reviews*, *99*(9). https://doi.org/10.1021/cr980440x

Cardon, P.-Y., Roques, O., Caron, A., Rosabal, M., Fortin, C., & Amyot, M. (2020). Role of prey subcellular distribution on the bioaccumulation of yttrium (Y) in the rainbow trout. *Environmental Pollution*, *258*, 113804. https://doi.org/https://doi.org/10.1016/j.envpol.2019.113804

Charalampides, G., Vatalis, K. I., Apostoplos, B., & Ploutarch-Nikolas, B. (2015). Rare Earth Elements: Industrial Applications and Economic Dependency of Europe. *Procedia Economics and Finance*, *24*. https://doi.org/10.1016/s2212-5671(15)00630-9

Cui, J., Zhang, Z., Bai, W., Zhang, L., He, X., Ma, Y., Liu, Y., & Chai, Z. (2012). Effects of rare earth elements La and Yb on the morphological and functional development of zebrafish embryos. *Journal of Environmental Sciences*, *24*(2), 209–213. https://doi.org/10.1016/S1001-0742(11)60755-9

Davies, J., Siebenhandl-Wolff, P., Tranquart, F., Jones, P., & Evans, P. (2022). Gadolinium: pharmacokinetics and toxicity in humans and laboratory animals following contrast agent administration. In *Archives of Toxicology* (Vol. 96, Issue 2). https://doi.org/10.1007/s00204-021-03189-8

Dubé, M., Auclair, J., Hanana, H., Turcotte, P., Gagnon, C., & Gagné, F. (2019). Gene expression changes and toxicity of selected rare earth elements in rainbow trout juveniles. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *223*, 88–95. https://doi.org/10.1016/J.CBPC.2019.05.009

Ebrahimi, P., & Barbieri, M. (2019). Gadolinium as an emerging microcontaminant in water resources: Threats and opportunities. In *Geosciences (Switzerland)* (Vol. 9, Issue 2). https://doi.org/10.3390/geosciences9020093

EPA. (2012). Rare Earth Elements : A Review of Production , Processing , Recycling, and Associated Environmental Issues. *United States Environmental Protection Agency*, *December*.

Espejo, W., Chiang, G., Kitamura, D., Kashiwada, S., O'Driscoll, N. J., & Celis, J. E. (2023). Occurrence of rare earth elements (REEs) and trace elements (TEs) in feathers of adult and young Gentoo penguins from King George Island, Antarctica. *Marine Pollution Bulletin*, *187*, 114575. https://doi.org/https://doi.org/10.1016/j.marpolbul.2023.114575

Farkas, J., Polesel, F., Kjos, M., Carvalho, P. A., Ciesielski, T., Flores-Alsina, X., Hansen, S. F., & Booth, A. M. (2020). Monitoring and modelling of influent patterns, phase distribution and removal of 20 elements in two primary wastewater treatment plants in Norway. *Science of The Total Environment*, *725*, 138420. https://doi.org/10.1016/J.SCITOTENV.2020.138420

Figueiredo, C., Grilo, T. F., Lopes, C., Brito, P., Diniz, M., Caetano, M., Rosa, R., & Raimundo, J. (2018). Accumulation, elimination and neuro-oxidative damage under lanthanum exposure in glass eels (Anguilla anguilla). *Chemosphere*, *206*. https://doi.org/10.1016/j.chemosphere.2018.05.029

Flik, G., Verbost, P. M., & Bonga, S. E. W. (1995). 12 Calcium Transport Processes in Fishes. In C. M. Wood & T. J. Shuttleworth (Eds.), *Fish Physiology* (Vol. 14, pp. 317–342). Academic Press. https://doi.org/https://doi.org/10.1016/S1546-5098(08)60251-4

Freitas, R., Costa, S., D Cardoso, C. E., Morais, T., Moleiro, P., Matias, A. C., Pereira, A. F., Machado, J., Correia, B., Pinheiro, D., Rodrigues, A., Colónia, J., Soares, A. M. V. M., & Pereira, E. (2020). Toxicological effects of the rare earth element neodymium in Mytilus galloprovincialis. *Chemosphere*, *244*, 125457. https://doi.org/10.1016/J.CHEMOSPHERE.2019.125457

Fu, F. F., Akagi, T., Yabuki, S., Iwaki, M., & Ogura, N. (2000). Distribution of rare earth elements in seaweed: Implication of two different sources of rare earth elements and silicon in seaweed. *Journal of Phycology*, *36*(1). https://doi.org/10.1046/j.1529-8817.2000.99022.x

Garcia-Solsona, E., Jeandel, C., Labatut, M., Lacan, F., Vance, D., Chavagnac, V., & Pradoux, C. (2014). Rare earth elements and Nd isotopes tracing water mass mixing and particle-seawater interactions in the SE Atlantic. *Geochimica et Cosmochimica Acta*, *125*. https://doi.org/10.1016/j.gca.2013.10.009

Ghio, A. J., Soukup, J. M., Dailey, L. A., Richards, J., Deng, Z., & Abraham, J. L. (2011). Gadolinium exposure disrupts iron homeostasis in cultured cells. *Journal of Biological Inorganic Chemistry*, *16*(4). https://doi.org/10.1007/s00775-011-0757-z

Guimarães, D., Praamsma, M. L., & Parsons, P. J. (2016). Evaluation of a new optic-enabled portable X-ray fluorescence spectrometry instrument for measuring toxic metals/metalloids in consumer goods and cultural products. *Spectrochimica Acta - Part B Atomic Spectroscopy*, *122*. https://doi.org/10.1016/j.sab.2016.03.010

Gulani, V., Calamante, F., Shellock, F. G., Kanal, E., & Reeder, S. B. (2017). Gadolinium deposition in the brain: summary of evidence and recommendations. *The Lancet Neurology*, *16*(7), 564–570. https://doi.org/10.1016/S1474-4422(17)30158-8

Gwenzi, W., Mangori, L., Danha, C., Chaukura, N., Dunjana, N., & Sanganyado, E. (2018). Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants. In *Science of the Total Environment* (Vol. 636). https://doi.org/10.1016/j.scitotenv.2018.04.235

Hanana, H., Turcotte, P., André, C., Gagnon, C., & Gagné, F. (2017). Comparative study of the effects of gadolinium chloride and gadolinium – based magnetic resonance imaging contrast agent on freshwater mussel, Dreissena plymorpha. *Chemosphere*, *181*. https://doi.org/10.1016/j.chemosphere.2017.04.073

Hatje, V., Bruland, K. W., & Flegal, A. R. (2016). Increases in Anthropogenic Gadolinium Anomalies and Rare Earth Element Concentrations in San Francisco Bay over a 20 Year Record. *Environmental Science and Technology*, *50*(8). https://doi.org/10.1021/acs.est.5b04322

He, M. L., & Rambeck, W. A. (2000). Rare earth elements - A new generation of growth promoters for pigs? *Archives of Animal Nutrition*, *53*(4). https://doi.org/10.1080/17450390009381956

Henderson, P. (1984). Chapter 1 - General Geochemical Properties and Abundances of the Rare Earth Elements. In P. Henderson (Ed.), *Developments in Geochemistry* (Vol. 2, pp. 1–32). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-444-42148-7.50006-X

Henriques, B., Coppola, F., Monteiro, R., Pinto, J., Viana, T., Pretti, C., Soares, A., Freitas, R., & Pereira, E. (2019). Toxicological assessment of anthropogenic Gadolinium in seawater: Biochemical effects in mussels Mytilus galloprovincialis. *Science of The Total Environment*, *664*, 626–634. https://doi.org/10.1016/J.SCITOTENV.2019.01.341

Hissler, C., Stille, P., Iffly, J. F., Guignard, C., Chabaux, F., & Pfister, L. (2016). Origin and Dynamics of Rare Earth Elements during Flood Events in Contaminated River Basins: Sr-Nd-Pb Isotopic Evidence. *Environmental Science and Technology*, *50*(9). https://doi.org/10.1021/acs.est.5b03660

Keersemaker, M. (n.d.). *SPRINGER BRIEFS IN EARTH SCIENCES Suriname Revisited: Economic Potential of its Mineral Resources*. http://www.springer.com/series/8897

Klaver, G., Verheul, M., Bakker, I., Petelet-Giraud, E., & Négrel, P. (2014). Anthropogenic Rare Earth Element in rivers: Gadolinium and lanthanum. Partitioning between the dissolved and particulate phases in the Rhine River and spatial propagation through the Rhine-Meuse Delta (the Netherlands). *Applied Geochemistry*, *47*, 186–197. https://doi.org/10.1016/J.APGEOCHEM.2014.05.020

Krebs Greenwood, R. E., & Bracken, J. D. (1999). Chemical Education Today The History and Use of Our Earth's Chemical Elements: A Reference Guide. *Journal of Chemical Education*, *76*(475).

Kulaksiz, S., & Bau, M. (2007). Contrasting behaviour of anthropogenic gadolinium and natural rare earth elements in estuaries and the gadolinium input into the North Sea. *Earth and Planetary Science Letters*, *260*(1–2). https://doi.org/10.1016/j.epsl.2007.06.016

Kulaksiz, S., & Bau, M. (2013). Anthropogenic dissolved and colloid/nanoparticle-bound samarium, lanthanum and gadolinium in the Rhine River and the impending destruction of the natural rare earth element distribution in rivers. *Earth and Planetary Science Letters*, *362*. https://doi.org/10.1016/j.epsl.2012.11.033

Kulaksız, S., & Bau, M. (2011). Anthropogenic gadolinium as a microcontaminant in tap water used as drinking water in urban areas and megacities. *Applied Geochemistry*, *26*(11), 1877–1885. https://doi.org/https://doi.org/10.1016/j.apgeochem.2011.06.011

Kümmerer, K., & Helmers, E. (2000). Hospital effluents as a source of gadolinium in the aquatic environment. *Environmental Science and Technology*, *34*(4). https://doi.org/10.1021/es990633h

Laukert, G., Frank, M., Bauch, D., Hathorne, E. C., Gutjahr, M., Janout, M., & Hölemann, J. (2017). Transport and transformation of riverine neodymium isotope and rare earth element signatures in high latitude estuaries: A case study from the Laptev Sea. *Earth and Planetary Science Letters*, *477*. https://doi.org/10.1016/j.epsl.2017.08.010

Lawrence, M. G., Ort, C., & Keller, J. (2009). Detection of anthropogenic gadolinium in treated wastewater in South East Queensland, Australia. *Water Research*, *43*(14), 3534–3540. https://doi.org/10.1016/J.WATRES.2009.04.033

Lerat-Hardy, A., Coynel, A., Dutruch, L., Pereto, C., Bossy, C., Gil-Diaz, T., Capdeville, M. J., Blanc, G., & Schäfer, J. (2019). Rare Earth Element fluxes over 15 years into a major European Estuary (Garonne-Gironde, SW France): Hospital effluents as a source of increasing gadolinium anomalies. *Science of The Total Environment*, *656*, 409–420. https://doi.org/10.1016/J.SCITOTENV.2018.11.343

Li, J. X., Zhu, Z. W., Yin, X. F., Han, B., Zheng, L., Wang, J. T., & Wang, X. R. (2015). Analysis of Contents and Distribution Patterns of Rare Earth Elements in the Surface Sediments of the South Mid-Atlantic Ridge. *Chinese Journal of Analytical Chemistry*, *43*(1), 21–26. https://doi.org/10.1016/S1872-2040(15)60796-4

Liang, T., Li, K., & Wang, L. (2014). State of rare earth elements in different environmental components in mining areas of China. *Environmental Monitoring and Assessment*, *186*(3). https://doi.org/10.1007/s10661-013-3469-8

Liu, J., Cao, L., & Dou, S. (2019). Trophic transfer, biomagnification and risk assessments of four common heavy metals in the food web of Laizhou Bay, the Bohai Sea. *Science of the Total Environment*, *670*. https://doi.org/10.1016/j.scitotenv.2019.03.140

Lortholarie, M., Poirier, L., Kamari, A., Herrenknecht, C., & Zalouk-Vergnoux, A. (2021). Rare earth element organotropism in European eel (Anguilla anguilla). *Science of The Total Environment*, *766*, 142513. https://doi.org/10.1016/J.SCITOTENV.2020.142513

Lortholarie, M., Zalouk-Vergnoux, A., Couderc, M., Kamari, A., François, Y., Herrenknecht, C., & Poirier, L. (2020). Rare earth element bioaccumulation in the yellow and silver European eel (Anguilla anguilla): A case study in the Loire estuary (France). *Science of the Total Environment*, *719*. https://doi.org/10.1016/j.scitotenv.2019.134938

Lürling, M., & Tolman, Y. (2010). Effects of lanthanum and lanthanum-modified clay on growth, survival and reproduction of Daphnia magna. *Water Research*, *44*(1), 309–319. https://doi.org/10.1016/J.WATRES.2009.09.034

MacMillan, G. A., Chételat, J., Heath, J. P., Mickpegak, R., & Amyot, M. (2017). Rare earth elements in freshwater, marine, and terrestrial ecosystems in the eastern Canadian Arctic. *Environmental Science: Processes and Impacts*, *19*(10). https://doi.org/10.1039/c7em00082k

Malhotra, N., Hsu, H. S., Liang, S. T., Roldan, M. J. M., Lee, J. S., Ger, T. R., & Hsiao, C. Der. (2020). An updated review of toxicity effect of the rare earth elements (REEs) on aquatic organisms. In *Animals* (Vol. 10, Issue 9). https://doi.org/10.3390/ani10091663

Mann, J. S. (1993). Stability of gadolinium complexes in vitro and in vivo. *Journal of Computer Assisted Tomography*, *17*. https://doi.org/10.1097/00004728-199301001-00004

Martin, R. B., & Richardson, F. S. (1979). Lanthanides as probes for calcium in biological systems. *Quarterly Reviews of Biophysics*, *12*(2). https://doi.org/10.1017/S0033583500002754

Mashitah, S. M., Shazili, N. A. M., & Rashid, M. K. A. (2012). Elemental concentrations in Brown Seaweed, Padina sp. along the east coast of Peninsular Malaysia. *Aquatic Ecosystem Health and Management*, *15*(3). https://doi.org/10.1080/14634988.2012.705774

Nozaki, Y., Lerche, D., Alibo, D. S., & Tsutsumi, M. (2000). Dissolved indium and rare earth elements in three Japanese rivers and Tokyo Bay: Evidence for anthropogenic Gd and In. *Geochimica et Cosmochimica Acta*, *64*(23), 3975–3982. https://doi.org/10.1016/S0016-7037(00)00472-5

Paffrath, R., Pahnke, K., Behrens, M. K., Reckhardt, A., Ehlert, C., Schnetger, B., & Brumsack, H. J. (2020). Rare Earth Element Behavior in a Sandy Subterranean Estuary of the Southern North Sea. *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00424

Palasz, A., & Czekaj, P. (2000). Toxicological and cytophysiological aspects of lanthanides action. In *Acta Biochimica Polonica* (Vol. 47, Issue 4). https://doi.org/10.18388/abp.2000_3963

Pannetier, P., Caron, A., Campbell, P. G. C., Pierron, F., Baudrimont, M., & Couture, P. (2016). A comparison of metal concentrations in the tissues of yellow American eel (Anguilla rostrata) and European eel (Anguilla anguilla). *Science of the Total Environment*, *569–570*. https://doi.org/10.1016/j.scitotenv.2016.06.232

Parant, M., Sohm, B., Flayac, J., Perrat, E., Chuburu, F., Cadiou, C., Rosin, C., & Cossu-Leguille, C. (2019). Impact of gadolinium-based contrast agents on the growth of fish cells lines. *Ecotoxicology and Environmental Safety*, *182*. https://doi.org/10.1016/j.ecoenv.2019.109385

Pasquini, L., Napolitano, A., Visconti, E., Longo, D., Romano, A., Tomà, P., & Espagnet, M. C. R. (2018). Gadolinium-Based Contrast Agent-Related Toxicities. In *CNS Drugs* (Vol. 32, Issue 3). https://doi.org/10.1007/s40263-018-0500-1

Piarulli, S., Hansen, B. H., Ciesielski, T., Zocher, A. L., Malzahn, A., Olsvik, P. A., Sonne, C., Nordtug, T., Jenssen, B. M., Booth, A. M., & Farkas, J. (2021). Sources, distribution and effects of rare earth elements in the marine environment: Current knowledge and research gaps. In *Environmental Pollution* (Vol. 291). https://doi.org/10.1016/j.envpol.2021.118230

Ponnurangam, A., Bau, M., Brenner, M., & Koschinsky, A. (2016). Mussel shells of Mytilus edulis as bioarchives of the distribution of rare earth elements and yttrium in seawater and the potential impact of pH and temperature on their partitioning behavior. *Biogeosciences*, *13*(3). https://doi.org/10.5194/bg-13-751-2016

Qiang, T., Xiao-rong, W., Li-qing, T., & Le-mei, D. (1994). Bioaccumulation of the rare earth elements lanthanum, gadolinium and yttrium in carp (Cyprinus carpio). *Environmental Pollution*, *85*(3), 345–350. https://doi.org/10.1016/0269-7491(94)90057-4

Ramos, S. J., Dinali, G. S., Oliveira, C., Martins, G. C., Moreira, C. G., Siqueira, J. O., & Guilherme, L. R. G. (2016). Rare Earth Elements in the Soil Environment. In *Current Pollution Reports* (Vol. 2, Issue 1). https://doi.org/10.1007/s40726-016-0026-4

Reindl, A. R., Saniewska, D., Grajewska, A., Falkowska, L., & Saniewski, M. (2021). Alimentary exposure and elimination routes of rare earth elements (REE) in marine mammals from the Baltic Sea and Antarctic coast. *Science of The Total Environment*, *754*, 141947. https://doi.org/10.1016/J.SCITOTENV.2020.141947

Rogowska, J., Olkowska, E., Ratajczyk, W., & Wolska, L. (2018). Gadolinium as a new emerging contaminant of aquatic environments. In *Environmental Toxicology and Chemistry* (Vol. 37, Issue 6). https://doi.org/10.1002/etc.4116

Roskill, R. E. (2021). *Rare Earths: Outlook to 2030*. Roskill London, UK.

Services, R. I. (2013). *Vanadium: Global Industry Markets and Outlook*. Roskill Information Services Limited.

Song, H., Shin, W. J., Ryu, J. S., Shin, H. S., Chung, H., & Lee, K. S. (2017). Anthropogenic rare earth elements and their spatial distributions in the Han River, South Korea. *Chemosphere*, *172*, 155–165. https://doi.org/10.1016/J.CHEMOSPHERE.2016.12.135

Squadrone, S., Brizio, P., Battuello, M., Nurra, N., Sartor, R. M., Benedetto, A., Pessani, D., & Abete, M. C. (2017). A first report of rare earth elements in northwestern Mediterranean seaweeds. *Marine Pollution Bulletin*, *122*(1–2), 236–242. https://doi.org/10.1016/J.MARPOLBUL.2017.06.048

Squadrone, S., Brizio, P., Stella, C., Favaro, L., Da Rugna, C., Florio, D., Gridelli, S., & Abete, M. C. (2019). Feathers of Humboldt penguin are suitable bioindicators of Rare Earth Elements. *Science of The Total Environment*, *678*, 627–631. https://doi.org/10.1016/J.SCITOTENV.2019.05.032

Squadrone, S., Brizio, P., Stella, C., Mantia, M., Battuello, M., Nurra, N., Sartor, R. M., Orusa, R., Robetto, S., Brusa, F., Mogliotti, P., Garrone, A., & Abete, M. C. (2019a). Rare earth elements in marine and terrestrial matrices of Northwestern Italy: Implications for food safety and human health. *Science of the Total Environment*, *660*. https://doi.org/10.1016/j.scitotenv.2019.01.112

Squadrone, S., Brizio, P., Stella, C., Mantia, M., Battuello, M., Nurra, N., Sartor, R. M., Orusa, R., Robetto, S., Brusa, F., Mogliotti, P., Garrone, A., & Abete, M. C. (2019b). Rare earth elements in marine and terrestrial matrices of Northwestern Italy: Implications for food safety and human health. *Science of The Total Environment*, *660*, 1383–1391. https://doi.org/10.1016/J.SCITOTENV.2019.01.112

Squadrone, S., Brizio, P., Stella, C., Mantia, M., Favaro, L., Biancani, B., Gridelli, S., Da Rugna, C., & Abete, M. C. (2020a). Differential Bioaccumulation of Trace Elements and Rare Earth Elements in the Muscle, Kidneys, and Liver of the Invasive Indo-Pacific Lionfish (Pterois spp.) from Cuba. *Biological Trace Element Research*, *196*(1). https://doi.org/10.1007/s12011-019-01918-w

Squadrone, S., Brizio, P., Stella, C., Mantia, M., Favaro, L., Biancani, B., Gridelli, S., Da Rugna, C., & Abete, M. C. (2020b). Differential Bioaccumulation of Trace Elements and Rare Earth Elements in the Muscle, Kidneys, and Liver of the Invasive Indo-Pacific Lionfish (Pterois spp.) from Cuba. *Biological Trace Element Research*, *196*(1), 262–271. https://doi.org/10.1007/s12011-019-01918-w

Switzer, M. E. (1978). The lanthanide ions as probes of calcium ion binding sites in biological systems. *Science Progress*, *65*(257).

Telgmann, L., Wehe, C. A., Birka, M., Künnemeyer, J., Nowak, S., Sperling, M., & Karst, U. (2012). Speciation and isotope dilution analysis of gadolinium-based contrast agents in wastewater. *Environmental Science and Technology*, *46*(21). https://doi.org/10.1021/es301981z

Tommasi, F., Thomas, P. J., Pagano, G., Perono, G. A., Oral, R., Lyons, D. M., Toscanesi, M., & Trifuoggi, M. (2021). Review of Rare Earth Elements as Fertilizers and Feed Additives: A Knowledge Gap Analysis. In *Archives of Environmental Contamination and Toxicology* (Vol. 81, Issue 4). https://doi.org/10.1007/s00244-020-00773-4

Trapasso, G., Chiesa, S., Freitas, R., & Pereira, E. (2021). What do we know about the ecotoxicological implications of the rare earth element gadolinium in aquatic ecosystems? *Science of the Total Environment*, *781*. https://doi.org/10.1016/j.scitotenv.2021.146273

Tyler, G. (2004). Rare earth elements in soil and plant systems - A review. In *Plant and Soil* (Vol. 267, Issues 1–2). https://doi.org/10.1007/s11104-005-4888-2

van der Molen, A. J., & Bellin, M. F. (2008). Extracellular gadolinium-based contrast media: Differences in diagnostic efficacy. *European Journal of Radiology*, *66*(2). https://doi.org/10.1016/j.ejrad.2008.02.010

Wahsner, J., Gale, E. M., Rodríguez-Rodríguez, A., & Caravan, P. (2019). Chemistry of MRI contrast agents: Current challenges and new frontiers. In *Chemical Reviews* (Vol. 119, Issue 2). https://doi.org/10.1021/acs.chemrev.8b00363

Wang, Z., Yin, L., Xiang, H., Qin, X., & Wang, S. (2019). Accumulation patterns and species-specific characteristics of yttrium and rare earth elements (YREEs) in biological matrices from Maluan Bay, China: Implications for biomonitoring. *Environmental Research*, *179*. https://doi.org/10.1016/j.envres.2019.108804

Wen, B., Yuan, D. an, Shan, X. quan, Li, F. liang, & Zhang, S. zhen. (2001). The influence of rare earth element fertilizer application on the distribution and bioaccumulation of rare earth elements in plants under field conditions. *Chemical Speciation and Bioavailability*, *13*(2). https://doi.org/10.3184/095422901783726825

Yang, L., Wang, X., Nie, H., Shao, L., Wang, G., & Liu, Y. (2016). Residual levels of rare earth elements in freshwater and marine fish and their health risk assessment from Shandong, China. *Marine Pollution Bulletin*, *107*(1), 393–397. https://doi.org/10.1016/J.MARPOLBUL.2016.03.034

Zhao, Y., Liang, J., Meng, H., Yin, Y., Zhen, H., Zheng, X., Shi, H., Wu, X., Zu, Y., Wang, B., Fan, L., & Zhang, K. (2021). Rare Earth Elements Lanthanum and Praseodymium Adversely Affect Neural and Cardiovascular Development in Zebrafish (Danio rerio). *Environmental Science and Technology*, *55*(2). https://doi.org/10.1021/acs.est.0c06632

Zhou, B., Li, Z., & Chen, C. (2017). Global potential of rare earth resources and rare earth demand from clean technologies. In *Minerals* (Vol. 7, Issue 11). https://doi.org/10.3390/min7110203

7 Appendix

A1 Water parameters

Measurements were taken from each tank before the start of the experiment and during the experiment to ensure constant water parameters and increase the welfare of the fish. Preliminary testing of water–stop is shown in Table A.1.1, these values were further used to determine the length of the exposure (1 hour daily) during the exposure period to ensure minimal distress through changes in water parameters.

Measurements of water parameters were taken several times from each tank during the experiment to ensure constant parameters and increase the welfare of the fish. An overview over measurements of salinity (‰), dissolved oxygen (DO%) is given in Table A.1.2, measurements of temperature (°C) and ammonium levels (NH_4, mg/L) are shown in Table A.1.3. Average measurements of salinity, dissolved oxygen and temperature is given in Table A.1.4.

Table A.1.1. Measurements done during preliminary testing of water_stop (3 days before the experiment started), of temperature (°C), salinity (‰), NH_4 concentration (mg/L), in selected tanks at timepoints, start, after 30 minutes (30min) , 1 hour (1h), 1.5 hours (1.5h) and 2 hours (2h)) and dissolved oxygen (%) in all tanks at the same timepoints. The results in this table were further used to determine the period of water_flowthrough stop during the main experiment.

Tank#	Temp °C	Salinity (‰)	NH_4 (mg/L)					Dissolved Oxygen (%)				
			start	30min	1h	1.5h	2h	start	30min	1h	1.5h	2h
1	7.5	34.99						83.1	84.4	85.5	85.5	84.8
2	7.0	34.92	<0.05	0.10	0.10	0.10–0.20	0.10–0.20	88.9	87.5	88.4	88.4	88.3
3	7.0	34.90						86.4	86.0	85.9	85.9	86.3
4	6.5	34.90						89.9	89.1	88.8	88.8	89.0
5	7.0	34.88	0.05–0.10	0.10	0.20	0.20	0.20–0.30	87.5	86.8	87.7	87.7	87.7
6	7.0	34.88						85.5	85.8	85.9	85.9	85.2
7	7.0	34.88						88.9	88.9	88.7	88.7	88.7
8	6.9	34.89	0.05–0.10	0.05–0.10	0.10	0.10	0.10–0.20	89.6	89.1	88.8	88.8	88.5
9	7.0	34.88						90.3	90.4	90.3	90.3	90.1
10	6.9	34.88						90.7	90.9	90.6	90.6	90.7
11	7.0	34.87						90.7	90.8	90.3	90.3	90.3
12	7.0	34.88	<0.05	0.10	0.10	0.10–0.20	0.10–0.20	89.4	89.0	88.9	88.9	88.2
13	7.0	34.88						86.3	85.2	84.4	84.4	84.1
14	6.9	34.87						88.6	87.8	87.8	87.8	87.9
15	7.0	34.87	<0.05	0.05	0.05-0.10	0.05–0.10	0.10	88.4	88.2	88.2	88.2	88.0
16	6.9	34.88						89.6	89.3	88.9	88.9	88.9
17	7.0	34.88						89.5	90.2	90.0	90.0	90.2
18	7.0	34.88	0.05–0.10	0.10	0.10	0.20	0.20	89.0	89.4	89.5	89.5	89.2

Table A.1.2. Measurements of salinity (‰) and dissolved oxygen (DO%) in tanks 1–18 on different times and at different timepoints (m=morning, exp=experiment).

TANK #	Salinity (‰)					DO (%)				
Date	10.05.2022	11.05.2022	16.05.2022	19.05.2022		10.05.2022	11.05.2022	16.05.2022	19.05.2022	
Timepoint	after food m	after food m	before exp	end exp	end exp	after food m	after food m	before exp	end exp	end exp
1	34.8	35.0	34.8	34.1	34.9	86.6	83.1	85.1	86.8	87.2
2	34.8	34.9	34.8	34.9	34.9	89.3	88.9	85.0	88.2	87.7
3	34.8	34.9	34.8	34.8	34.9	87.1	86.4	83.9	85.1	85.4
4	34.8	34.9	34.8	34.8	34.9	89.1	89.9	87.5	89.0	89.2
5	34.8	34.9	34.7	35.0	34.9	87.0	87.5	86.5	88.7	87.4
6	34.8	34.9	34.8	34.8	34.8	85.5	85.5	85.5	87.5	85.2
7	34.8	34.9	34.8	35.0	34.8	89.0	88.9	87.6	90.7	88.1
8	34.8	34.9	34.8	34.8	34.8	88.2	89.6	86.9	90.3	89.1
9	34.8	34.9	34.8	34.8	34.8	89.5	90.3	87.0	91.1	89.4
10	34.8	34.9	34.8	34.8	34.8	90.2	90.7	87.9	90.6	90.3
11	34.8	34.9	34.8	34.8	34.8	90.6	90.7	87.7	92.2	90.7
12	34.8	34.9	34.9	34.8	34.9	89.7	89.4	84.4	90.4	89.4
13	34.8	34.9	34.8	34.9	34.9	84.7	86.3	86.2	87.2	88.3
14	34.8	34.9	34.9	34.8	34.9	87.8	88.6	70.4	88.2	86.5
15	34.8	34.9	34.8	34.8	34.8	89.1	88.4	86.3	88.4	86.5
16	34.8	34.9	34.8	34.8	34.8	87.3	89.6	86.9	88.7	86.1
17	34.8	34.9	34.8	34.8	34.8	88.8	89.5	86.3	89.4	87.7
18	34.8	34.9	34.8	34.8	34.8	89.0	89.0	86.8	89.5	87.3

Table A.1.3. Measurements of temperature (°C) and dissolved concentration of NH_4 (mg/L) in tanks 1–18 on different times and at different timepoints (m=morning, exp=experiment).

TANK #	Temperature °C					NH_4			
Date	10.05.2022	11.05.2022	16.05.2022	19.05.2022		10.05.2022	11.05.2022	18.05.2022	
Timepoint	after food m	after food m	Before exp	end exp	end exp	after food m	after food m	before exp	end exp
1	7.5	7.5	6.9	7.5	8.0	0.10		0.20	
2	7.0	7.0	6.9	7.3	7.6		<0.05	0.10	
3	6.9	7.0	6.9	7.3	7.5			0.20	
4	6.8	6.9	6.8	7.2	7.4			0.20	0.40
5	6.9	7.0	6.8	7.3	7.6		0.05–0.10	0.20	
6	6.8	7.0	6.8	7.1	7.4	0.10		0.10–0.20	
7	6.9	7.0	6.8	7.2	7.5	0.10		0.10	
8	6.8	6.9	6.8	7.1	7.4		0.05–0.10	0.10	
9	6.9	7.0	6.8	7.2	7.5			0.10	
10	6.8	6.9	6.8	7.2	7.4			0.10	
11	6.9	7.0	6.9	7.3	7.6	<0.05		0.05–0.10	
12	6.9	7.0	6.9	7.2	7.5		<0.05	0.10	
13	6.9	7.0	6.8	7.3	7.5			0.05-0.10	
14	6.9	6.9	6.9	7.2	7.5	<0.05		0.20–0.30	
15	6.9	7.0	6.8	7.2	7.4		<0.05	0.20	0.40
16	6.9	6.9	6.8	7.1	7.5			0.20	
17	6.9	7.0	6.8	7.1	7.5	<0.05		0.05–0.10	
18	6.9	7.0	6.8	7.2	7.4		0.05-0.10	0.20–0.30	

Table A.1.4. Average measurements of salinity (‰), dissolved oxygen (DO%) and temperature (Temp °C) in tank 1–18, based on measurements shown in Table A.1.2. and Table A.1.3.

	Average	SE
Salinity (‰)	34.83	0.09
DO%	87.80	2.61
Temp °C	7.10	0.27

A2 Exposure concentrations

Highest water exposure concentrations were found in treatments GBCA high, followed by $GdCl_3$ high, GBCA low and $GdCl_3$ low. An overview over Gd concentrations in the tanks of treatments at different timepoints during the exposure can be found in Table A.2.1.

Table A.2.1. Measured Gd exposure for the treatments (**µ**g/L), CTRL, DOTA, GBCA low, GBCA high, $GdCl_3$ low and $GdCl_3$ high based on average measurements of Gd concentration on day 2 and day 6 at the timepoints: before exposure (before), start of exposure (start), end of exposure (end), after 1.5 hours (1.5h) and after 3 hours (3h).

	Timepoint				
Treatment	Before	Start	End	1.5h	3h
CTRL	0.015	0.022	0.019	0.010	0.024
SE	0.013	0.047	1.470	0.988	1.383
DOTA	0.009	0.009	0.007	0.007	0.006
SE	0.004	0.006	0.003	0.002	0.002
GBCA low	0.011	1.370	1.350	0.784	0.476
SE	0.015	0.039	0.056	0.034	0.037
GBCA high	0.110	131.4	132.7	77.21	49.23
SE	0.020	5.360	2.420	3.280	6.520
$GdCl_3$ low	0.050	1.290	1.140	0.690	0.420
SE	0.060	0.040	0.060	0.050	0.050
$GdCl_3$ high	0.320	127.7	110.0	59.25	33.01
SE	0.190	1.770	15.00	13.55	7.340

A3 Gd concentration in wet weight of organs

Element analyses showed that the accumulation of Gd varied between different treatments and between exposed and recovered groups from each treatment. The accumulation of Gd in the organs of lumpfish (wet weight, ww) was highest in kidneys, followed by liver and lastly brain. An overview over the accumulation in the different organs and for each treatment is given in Table A.3.1. Gills are not included in this dataset as the wet weight of gills were not weighed during sampling.

Table A.3.1. Concentration of Gd (mg/kg wet weight (ww)) in kidney, liver, and brain of lumpfish (*Cyclopterus lumpus*) for CTRL, DOTA, GBCA low, GBCA high, $GdCl_3$ low and $GdCl_3$ high and exposed and recovered groups (Gr) from each treatment. Treatment groups that are not included due to high uncertainty in the measurements are marked with <LOD. n is the number of samples.

Element	Organ	Treatment	Gr.	n	Median	Average	SE	Min	Max
Gd	Kidney	CTRL	E	8	0.1487	0.2345	0.1848	0.0388	0.5833
			R	9	0.1211	0.1353	0.0813	0.0131	0.2616
		DOTA	E	9	0.2237	0.2095	0.1267	0.0096	0.3875
			R	8	0.1074	0.1203	0.0768	0.0097	0.2749
		GBCA low	E	9	0.1510	0.3100	0.4075	0.1012	1.3618
			R	6	0.0787	0.1337	0.1716	0.0037	0.4717
		GBCA high	E	8	2.0404	2.2905	1.1068	1.0032	4.1505
			R	9	1.9486	1.8696	0.4949	0.9353	2.7085
		$GdCl_3$ low	E	9	0.5092	0.6288	0.3578	0.2381	1.3119
			R	9	0.3518	0.3694	0.2038	0.0049	0.7160
		$GdCl_3$ high	E	9	15.082	15.979	3.9157	11.178	23.081
			R	8	17.516	17.840	9.4769	2.1482	30.628
	Liver	CTRL	E	9	-	-	-	-	<LOD
			R	9	0.0004	0.0034	0.0053	0.0003	0.0158
		DOTA	E	9	-	-	-	-	<LOD
			R	9	-	-	-	-	<LOD
		GBCA low	E	9	0.0087	0.0220	0.0242	0.0019	0.0780
			R	9	0.0032	0.0052	0.0044	0.0019	0.0139
		GBCA high	E	8	0.1690	0.1879	0.1020	0.0796	0.3523
			R	9	0.1538	0.1542	0.0617	0.0562	0.2439
		$GdCl_3$ low	E	8	0.0349	0.0394	0.0243	0.0159	0.0959
			R	9	0.0171	0.0191	0.0118	0.0104	0.0491
		$GdCl_3$ high	E	9	0.9839	1.3405	0.8238	0.2848	2.8351
			R	8	0.7718	0.7704	0.3433	0.3366	1.4348
	Brain	CTRL	E	3	0.0048	0.0049	0.0028	0.0022	0.0078
			R	3	2.0497	2.1078	0.2651	1.8765	2.3971
		GBCA high	E	4	2.9028	6.5712	7.4257	2.7710	17.708
			R	3	9.0910	9.1506	7.3447	1.8359	16.525
		$GdCl_3$ high	E	3	14.815	2.5285	11.391	0.1679	22.602
			R	3	40.801	34.150	29.747	1.6402	60.010

A4 Multiple group comparisons

The Kruskal–Wallis test was followed by a Wilcoxon rank–sum test (also known as Mann–Whitney U test) with Bonferroni adjustments for p–values (to control family–wise error rates) to investigate the significance of differences between treatment groups for all organs. The results for gills are given in Table A.4.1, results for kidneys are given in Table A.4.2, results for livers are given in Table A.4.3, results for brains are given in Table A.4.4.

Table A.4.1. Results from the Kruskal–Wallis test followed by Wilcoxon rank–sum test with Bonferroni adjustments for the p–value for the gills. The results are based on initial measurements of Gd concentrations in all sampled gills. Values where $p<0.05$ are marked in bold. CTRL R and DOTA R are excluded from the dataset due to high uncertainty of initial measurements of Gd concentrations.

Gills		**CTRL**	**DOTA**	**GBCA low**		**GBCA high**		**$GdCl_3$ low**		**$GdCl_3$ high**
	Group	**E**	**E**	**E**	**R**	**E**	**R**	**E**	**R**	**E**
DOTA	E	1.0000								
GBCA low	E	1.0000	1.0000							
	R	0.5131	**0.0217**	**0.0217**						
GBCA high	E	**0.0027**	**0.0054**	**0.0109**	**0.0027**					
	R	1.0000	1.0000	**0.0109**	**0.0027**	**0.0027**				
$GdCl_3$ low	E	1.0000	0.1231	**0.0205**	**0.0054**	0.0109	0.0054			
	R	1.0000	1.0000	1.0000	**0.0027**	**0.0027**	**0.0027**	**0.0027**		
$GdCl_3$ high	E	**0.0027**	**0.0054**	**0.0054**	**0.0027**	**0.0027**	**0.0027**	**0.0027**	**0.0054**	
	R	**0.0027**	**0.0054**	**0.0054**	**0.0027**	**0.0027**	**0.0027**	**0.0054**	**0.0027**	**0.0326**

Table A.4.2. Results from the Kruskal–Wallis test followed by Wilcoxon rank–sum test with Bonferroni adjustments for the p–value for the kidneys. The results are based on intial measurements of Gd concentrations in all sampled kidneys. Values where p<0.05 are marked in bold.

Kidneys		CTRL		DOTA		GBCA low		GBCA high		$GdCl_3$ low		$GdCl_3$ high
	Group	E	R	E	R	E	R	E	R	E	R	E
CTRL	R	1.0000										
DOTA	E	1.0000	1.0000									
	R	1.0000	1.0000	1.0000								
GBCA low	E	1.0000	1.0000	1.0000	1.0000							
	R	1.0000	1.0000	1.0000	1.0000	1.0000						
GBCA high	E	**0.0054**	**0.0103**	**0.0054**	**0.0054**	**0.0060**	**0.0103**					
	R	**0.0027**	**0.0054**	**0.0027**	**0.0027**	**0.0014**	**0.0054**	1.0000				
$GdCl_3$ low	E	**0.0190**	**0.0217**	**0.0190**	**0.0027**	0.1972	0.1629	**0.0054**	**0.0027**			
	R	0.5131	0.3638	0.9367	0.2633	1.0000	1.0000	**0.0054**	**0.0027**	1.0000		
$GdCl_3$ high	E	**0.0027**	**0.0054**	**0.0027**	**0.0027**	**0.0014**	**0.0054**	**0.0054**	**0.0027**	**0.0027**	**0.0027**	
	R	**0.0027**	**0.0054**	**0.0027**	**0.0027**	**0.0014**	**0.0054**	**0.0054**	**0.0027**	**0.0027**	**0.0027**	1.0000

Table A.4.3. Results from the Kruskal–Wallis test followed by Wilcoxon rank–sum test with Bonferroni adjustments for the p–value for livers. Based on intial measurements of Gd concentrations in all sampled livers. Values where p<0.05 are marked in bold. CTRL E, DOTA E and DOTA R are excluded from the dataset due to high uncertainty of initial measurements of Gd concentrations. Excluded grups are CTRL E, DOTA E and DOTA R due to high uncertainty in initial measurements of accumulated Gd.

Livers		CTRL	GBCA low		GBCA high		$GdCl_3$ low		$GdCl_3$ high
	Group	R	E	R	E	R	E	R	E
GBCA low	E	0.5131							
	R	1.0000	1.0000						
GBCA high	E	**0.0054**	**0.0054**	**0.0054**					
	R	**0.0027**	**0.0027**	**0.0054**	1.0000				
$GdCl_3$ low	E	**0.0326**	1.0000	**0.2633**	**0.0054**	**0.0027**			
	R	**0.0109**	1.0000	**0.0027**	**0.0054**	**0.0027**	1.0000		
$GdCl_3$ high	E	**0.0027**	**0.0027**	**0.0027**	**0.0109**	**0.0027**	**0.0027**	**0.0027**	
	R	**0.0027**	**0.0027**	**0.0027**	**0.0054**	**0.0027**	**0.0027**	**0.0027**	1.0000

Table A.4.4. Results from the Kruskal–Wallis test followed by Wilcoxon rank–sum test with Bonferroni adjustments for the p–value for the brains, based on initial measurements of Gd concentration in all sampled brains. Values where p<0.05 are marked in bold.

Brains		CTRL		GBCA high		$GdCl_3$ high
	Group	E	R	E	R	E
CTRL	R	1.0000				
GBCA high	E	1.0000	0.0860			
	R	1.0000	1.0000	1.0000		
$GdCl_3$ high	E	1.0000	1.0000	1.0000	1.0000	
	R	1.0000	1.0000	1.0000	1.0000	1.0000

A5 Factor between treatments

The factor was calculated between each treatment for all organs using the average concentration in CTRL as a reference. The highest difference is between $GdCl_3$ and CTRL for all organs, followed by GBCA high, $GdCl_3$ low (except factor value $GdCl_3$ low R in kidneys which is higher than GBCA), GBCA low and lastly DOTA. There are differences considering organs and treatment groups, which are given in Table A.5.1.

Table A.5.1. Calculation of factor between the different treatments: DOTA, GBCA low, GBCA high, $GdCl_3$ low and $GdCl_3$ high and the CTRL groups for gills, kidneys, livers, and brains. The factor was calculated using average concentrations from each treatment in each organ, divided by average concentration in CTRL for the same treatment and organ, to get a ratio. For gills, DOTA R, GBCA R are excluded due to high uncertainty in the initial measurements of Gd concentrations. For liver, DOTA R is excluded due to high uncertainty in the initial measurements of Gd concentrations.

Factor	DOTA		GBCA low		GBCA high		$GdCl_3$ low		$GdCl_3$ high	
	E	R	E	R	E	R	E	R	E	R
Gills	0.593		0.427	0.105	3.870	1.099	2.157	0.388	535.5	172.7
Kidney	0.699	0.699	1.630	0.902	13.66	11.48	3.408	113.7	91.57	113.7
Liver			2.893	0.833	30.99	27.61	5.177	3.490	179.8	125.0
Brain					3.089	3.451			4.258	10.40

A6 Spearman correlation

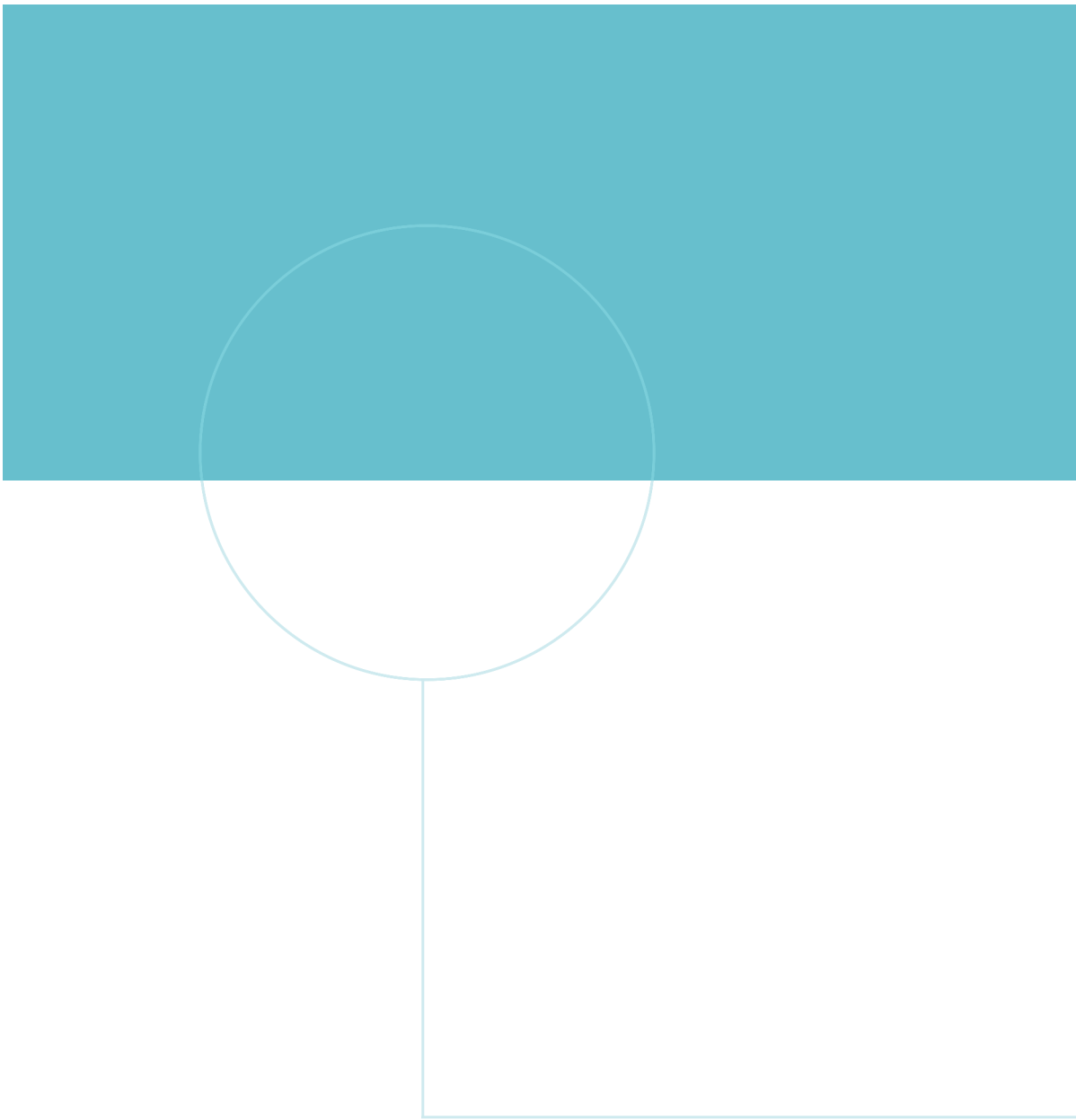
Spearman correlation test of the collected data showed a slight negative correlation between fish weight and Gd accumulation considering comparison of all the data and for the gills, meaning as the weight increases, the Gd concentration decreases. An overview over the results of the Spearman correlation test is given in Table A.6.1.

Table A.6.1. Spearman correlation test between different body parameters of the fish (length, weight, organ weight) and concentrations of Gd for all the data collected (n=342), and for the individual organs; gills (n=108), kidneys (n=107), livers (n=108) and brains (n=19) using the Spearman's rank correlation method. Results indicating correlations are marked in **bold**. n shows the total number of samples the correlation is based on, comparisons show what types of parameters were compared, for the Spearman test, both the p–values and Rho values are given. Excluded datapoints are those from CTRL R (gills), DOTA R (gills), CTRL E (liver), DOTA R (liver), DOTA E (liver) due to high uncertainty in initial measurements of Gd concentrations.

	n	Comparisons		Spearman	
				p–value	Rho
All data	342	Length	Fish weight	$< 2.2\times10^{-16}$	0.7738
		Length	Gd	0.3522	-0.0505
		Fish weight*	Gd*	**0.0129**	**-0.1343**
Gills	108	Fish weight**	Gd**	**0.0022**	**-0.2935**
		Fish length	Gd	0.0546	-0.1863
Kidney	107	Fish length	Gd	0.7847	0.0277
		Fish weight	Gd	0.4611	-0.0745
		Organ weight	Gd	0.4744	-0.0723
Liver	108	Fish length	Gd	0.8773	-0.0153
		Fish weight	Gd	0.2392	-0.1159
		Organ weight	Gd	0.4378	-0.0765
Brain	19	Fish weight	Organ Weight	0.1348	0.1469
		Fish Length	Organ Weight	0.0479	0.1935
		Organ Weight	Gd	0.2091	0.3019

*Fish Weight and total Gd accumulation: slight negative correlation. As weight increase, the Gd accumulation decreases

** Fish Weight and Gd accumulation in gills: slight negative correlation. As weight increase, the Gd accumulation decreases



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