# Risk of PFAS contamination within aquatic food species in the Netherlands

What are the ecotoxicological risks of PFAS pollution in Lake IJssel and Western Scheldt for the European eel (*Anguilla anguilla*), crayfish species (*Astacoidea spp. & Parastacoidea spp.*), and the Chinese mitten crab (*Eriocheir sinensis*)?

Project name: PFAS-risk assessment: Investigate the potential problem of PFAS in (shell)fish in the Netherlands.

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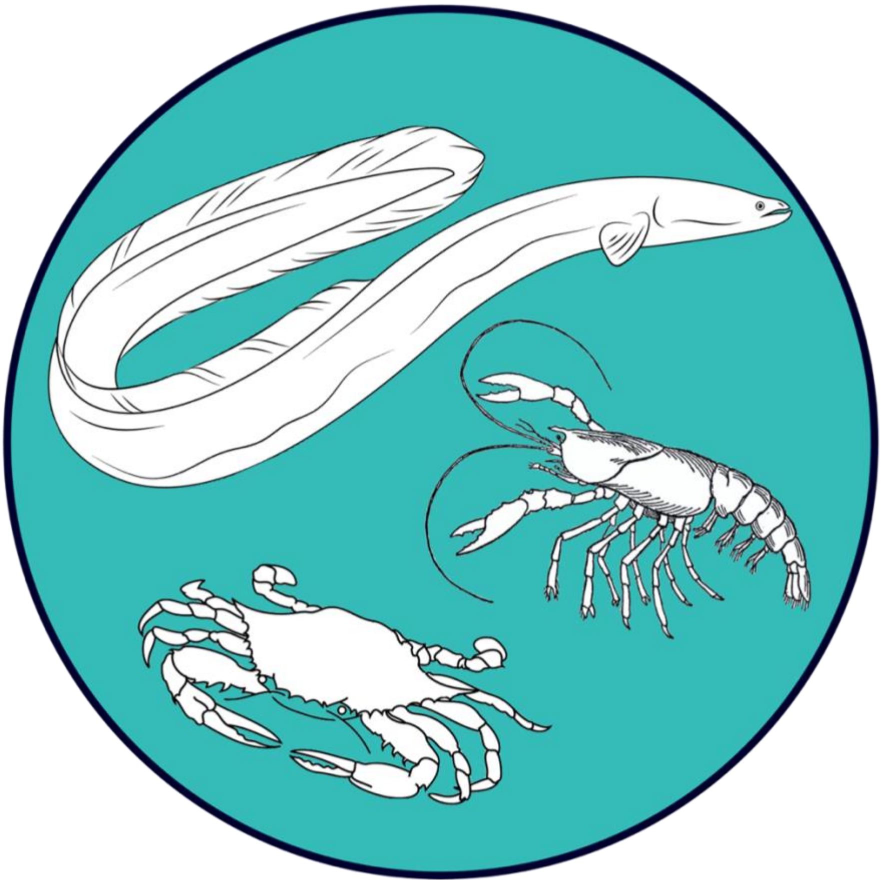
May 2022



Consultancy team: **PFISH**
Company: Good Fish
Commissioner: Tom Koppenol Msc.

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# Executive summary

Poly- and perfluoroalkyl substances (PFASs) are known for possessing desirable traits such as heat, fire, and water resistance. However, this class of organic chemicals is also known for being extremely difficult to degrade and tend to bioaccumulate in the environment.

This research investigates the effects the current levels of PFAS contamination in Dutch waters, with a special focus on Lake IJssel and the Western Scheldt, have on the wellbeing of the European Eel (*Anguilla anguilla*), invasive species of crayfish (*Astacoidea spp. & Parastacoidea spp.*) and Chinese mitten crab (*Eriocheir sinensis*) and the Dutch population who would consume them. This project was performed to aid the Good Fish Foundation in their various projects regarding the European eel, crayfish, and Chinese mitten crab. Literature research supplemented with interviews with researchers was used to assess the risks of PFAS pollution for these species.

Perfluorooctane sulfonic acid (PFOS) concentrations in the Western Scheldt were found to exceed the 0.65 ng/L European quality standard (EQS). Lake IJssel was found to be cleaner, however recent data for Lake IJssel was lacking and limited to PFOS and perfluorooctanoic acid (PFOA). Once again only PFOS was found to be exceeding the EQS.

In eels, concentrations of PFAS were found to be higher than regulatory levels set for biota by the European food safety association (EFSA) and it was found that these levels could prove detrimental to eel health. Evidence was found suggesting liver damage as a result of PFAS contamination in eels. PFAS therefore must be considered a very real threat to the European eel. However, due to the lack of data and understanding of PFAS, it might prove more effective to prioritize other concerns such as habitat accessibility and connectivity, overfishing, and other toxins in conservation exploits.

For the crayfish, no health effects are reported outside of behavioural changes. No data regarding PFAS concentrations in Dutch crayfish was available. Calculated estimates based on PFAS concentrations found in the Hudson River watershed were made which show that it is likely PFAS concentrations in Dutch crayfish will exceed the EFSA regulatory levels. However, these estimations are only preliminary and really show the need for proper measurements.

Very little information is available regarding PFAS contamination and toxicity within the Chinese mitten crab. Evidence of immunotoxicity is available, however significant health effects have not been observed yet. Data on PFAS concentrations within crabs is severely lacking with no data available anywhere in the world.

In humans, PFAS is known to be hepatotoxic and immune-toxic. Evidence exists which suggests carcinogenicity and based on lowered sperm motility and counts it is also reasonable to assume developmental toxicity. No regulations have been established which dictate maximum levels of PFAS in food. Intake levels have been established but have seen regular adjustments over the past years.

Based on current intake and PFAS concentration levels it was determined that average consumption of wild-caught European eel would entail exceedance of the intake levels and would be detrimental to human health.
A similar assessment was performed using the estimated data obtained for the crayfish. Using the PFAS concentration estimations, it was determined that consumption of one average meal of Dutch crayfish would likely mean exceeding the intake levels for PFAS. However, this assessment is heavily subject to assumptions and statistical outliers. Therefore, proper measurements are needed to assess the safety of consumption of Dutch caught crayfish.

Finally, for the Chinese mitten crab, it was impossible to determine the safety for consumers as far as PFAS contamination goes. Therefore, it is vital that PFAS concentrations within Dutch caught crabs are determined and the safety reassessed.

It is evident that PFAS will be the subject of research for years to come and will have long-lasting effects far into the future. A lot of research is being done on the effects of PFAS and this research shows that a lot more research can and will have to be done to get a full grasp of PFAS contamination within the environment, biota, and humans.

Having been met with quite some reluctance and hesitancy, it was determined that PFAS as it stands, is a hot topic with high sensitivity. Researchers were reluctant to speak with the notion that anything they said could be held against them would it turn out to be wrong or even detrimental to society. It is therefore likely that regulations will change in the future and that reassessment of the dangers of PFAS will have to be done frequently to keep the dangers of PFAS up to date in the future.

## Keywords

Risk assessment – PFAS – PFOA – PFOS – Ecotoxicology – European eel (*Anguilla anguilla*) – Crayfish (*Astacoidea spp. & Parastacoidea spp.*) – Chinese mitten crab (*Eriocheir sinensis*) – Western Scheldt – Lake IJssel

# Table of content

# Figures

# Tables

# Equations

# Appendices

# Abbreviations

| Abbreviation | Explanation |
|---|---|
| ALT | Alanine aminotransferase |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| BAF | Bioaccumulation factor |
| bw | Body weight |
| DDT | Dichlorodiphenyltrichloroethane |
| DNA | Deoxyribonucleic acid |
| DSSB | DNA single-strand breaks |
| ECHA | European Chemicals Agency |
| EFSA | European Food Safety Authority |
| EFSA CONTAM Panel | European Food Safety Authority Panel on Contaminants in the Food Chain |
| EG | Expert Group |
| EPA | United States Environmental Protection Agency |
| EQSs | Environmental Quality Standards |
| $EQS_{Biota}$ | Environmental Biota Quality Standards |
| GAO | Government Accountability Office |
| ICES | International Council for the Exploration of the Sea |
| IUCN | International Union for Conservation of Nature |
| MADEP | Massachusetts Department of Environmental Protection |
| ML | Maximum limit |
| MRL | Minimal risks levels |
| MRM | Rhône-Mediterranean Migrants Observatory<br>(Observatoire des poissons migrateurs amphihalins Rhône Méditerranée) |
| NHDES | New Hampshire Department of Environmental Services |
| NVWA | The Dutch Food and Consumer Product Safety Authority<br>(Nederlandse Voedsel en Warenautoriteit) |
| PCB | Polychlorinated biphenyls |
| PCDDs | Polychlorinated dibenzodioxins (dioxins) |
| PCDF | Polychlorinated dibenzofurans |
| PFAAs | Per- and polyfluorinated alkyl acids |
| PFAS | Per- and polyfluoroalkyl substances |
| PFCs | Perfluorinated compounds |
| PFHxS | Perfluorohexanesulfonic acid |
| PFNA | Perfluorononanoic acid |
| PFOA | Perfluorooctanoic acid |
| PFOS | Perfluorooctanesulfonic acid |
| PFTeDA | Perfluorotetradecanoic acid |
| POPs | Persistent organic pollutants |
| RIVM | National Institute for Public Health and the Environment<br>(Rijksinstituut voor Volksgezondheid en milieu) |
| ROS | Reactive oxygen species |

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|---|---|
| RWS | Directorate-General for Public Works and Water Management (Rijkswaterstaat) |
| T4 | Thyroxine |
| TDI | Tolerable daily intake |
| TWI | Tolerable weekly intake |
| WFD | Water Framework Directive |
| ww | Wet weight, the weight before water is removed from a plant or animal. For fish this is often the total body weight. |

# Definitions

| Concept | Definition |
|---|---|
| Actin cytoskeleton | A dynamic network made up of actin polymers involved in cell shape, axonal growth, cell migration, organelle transport, and phagocytosis. |
| Amphiphilic | Molecules characterized by a polar water-soluble part and an apolar lipid-soluble part. |
| Benthic | Related to the lowest part of waterbodies. |
| Bioaccumulation | Accumulation of substances in an organism |
| Bioconcentration | Accumulation of chemicals in an organism if the chemical occurs only in the water. |
| Bioindicator | (Group of) species whose function, population, or status reveals the qualitative status of the environment. |
| Biomagnification | Accumulation of chemicals in an organism through the diet of the organism. |
| Biomarker | Molecules that can be used as a first indicator for the biological condition of an organism due to their sensitivity to stressors. |
| Biota | All living organisms in an ecosystem. |
| Bioturbation | Manipulation of soils and sediments by animals or plants. |
| Catadromous | Fish species that live for a large part of their lives in freshwater and move to the sea to spawn. |
| Cephalothorax | Part of the arthropod body that contains the head and thorax fused. |
| Diadromous | Fish species that migrate between freshwater systems and marine systems. |
| Ecotoxicology | Study regarding effects of toxic compounds on ecological systems (organisms, population, community, ecosystem, biosphere). |
| Endangered species | An endangered species is a species that faces a risk of extinction due to environmental, human, or genetic threats. It is a general term that corresponds to the ‘Threatened categories’ of the Red list established by the International Union for Conservation of Nature (IUCN). ‘Endangered species’ have a high risk of extinction in the wild. ‘Critically endangered’ species face an extremely high risk of extinction in the wild. A critically endangered species is characterized by a decrease in population numbers in the last 10 years, they are predicted to become extinct in the wild within the next 10 years if no measures are taken. Critically Endangered’ species require urgent management to prevent its extinction. |
| Estuary | Partially enclosed water body containing brackish water connecting rivers to open seas. |
| Fecundity | Maximum potential reproductive output of an individual over its lifetime. |
| Hepatopancreas | Organ of the digestive tract of arthropods and molluscs with the same function as the pancreas and liver in mammals. |
| Hepatosomatic index | The ratio of liver weight to total body weight. |

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|---|---|
| **Immunoglobulin** | An antibody. It is a protein with a characteristic Y-shape used by the immune system. |
| **Immunotoxicity** | Adverse effects on the immune system caused by exposure to toxic substances. |
| **Interleukin** | Signaling molecules secreted by primarily white blood cells. |
| **Ion exchange resins** | A resin or polymer acting as a medium for ion exchange. |
| **Littoral zone** | Seashore, the part of the sea that is underwater at high tide but falls dry at low tide. |
| **Macroinvertebrates** | Animals without a backbone that are visible without using a microscope. |
| **Mutagenic** | Compounds that are able to permanently alter an organism’s genes. |
| **Neurotoxicity** | Exposure to toxic compounds that can alter normal processes of the nervous system. |
| **Oxidative stress** | Imbalance between accumulation reactive oxygen species and the ability of an organism to detoxify the reactive intermediates or to repair the resulting damage. |
| **Panmixia** | Ability of individuals in a population to interbreed without restrictions. |
| **Pharmacokinetic model** | This model describes movement of a drug to organs or tissues throughout the organism. |
| **Risk assessment** | Process to identify hazards and analyze potential effects if a hazard occurs. |
| **Semelparous** | Reproductive strategy where an organism only has one reproductive event in its lifetime. |
| **Synteny** | Physical co-localization of genetic loci on the same chromosome in an individual or species. |
| **Toxicodynamic** | Dynamic interactions of a toxic compound with a biological target (organ or tissue) and its biological effects. |
| **Toxicokinetic** | Describes the rate a toxic compound enters an organism and the metabolisation before excretion of the compound. |
| **Tributary** | Smaller river that flows from larger river to another river or lake. |
| **Trophic level** | Number of steps in the food chain from primary producer to species of interest. |

# 1. Introduction

This chapter provides an introduction to the project. It contains general information about the subject and the relevance and goal of this project. Additionally, the problem and our focus and scope in this study are defined. Lastly, the research question and sub-questions are established.

## 1.1. Project description

'Don't eat fish from the Western Scheldt, PFAS levels are too high' (Omroep Zeeland, 2021). More often headers of news articles look like this. Concerns about PFAS pollution have been growing as a reaction to the advice given by the GGD against eating self-caught fish from the Western Scheldt, as consumption of the fish exceeds the safe European limits of PFAS contamination (GGD Zeeland, 2021). Attention has been given to PFAS discharges by the 3M factory upstream of the Western Scheldt. Already 60% of blood samples of the local residents in the area of the factory have significant PFAS-levels that could lead to potential health issues in the long term (Omroep Zeeland, 2022). Also, the RIVM concluded in 2021 that the human intake of PFAS through food and drinking water is dangerously high. PFAS are man-made compounds and they were first introduced in the 1940s. They were widely applied because of their favourable characteristics. However, PFAS are very persistent and the retention time of PFAS in the environment is high (Brase et al., 2022). Humans are mostly exposed to PFAS by consuming animal products, and this poses potential threats to human health (Zafeiraki et al., 2019). In the Netherlands, increased levels of PFAS are found in fish, shellfish, and crustaceans due to bioaccumulation and biomagnification (Zafeiraki et al., 2019). The European Food Safety Authority (EFSA) reported PFAS contamination in a wide range of edible wild fish, farmed fish, molluscs, and shellfish species throughout Europe (Knutsen et al., 2018). Good Fish is concerned that consumption of these fish species will lead to an exceedance of safe levels of PFAS exposure, threatening human health.

## 1.2. Commissioner: Good fish

Our project with Good Fish is relevant since it will support them in reaching a healthy and sustainable fish industry. Good Fish aims to ensure that by 2030 only 'good fish' is being sold and consumed in the Netherlands. With 'good fish' they mean fish that is caught (or farmed) sustainably, without harming nature and ecosystems. Additionally, they are concerned with providing sustainable alternatives to fish they do not view as 'good'. They aim to reach this goal by working together with fishermen, fish farmers, processors, supermarkets, etcetera. A part of reaching their goal is informing the consumer on what 'good fish' entails. When the consumer has more information on what is sustainable, they can demand the fish industry to work more sustainably by using their money as a 'vote' (Moraes et al., 2011).

Good Fish is facing multiple challenges in achieving its goal. It might prove difficult to change the behaviour of consumers (Lubowiecki-Vikuk et al., 2021). Good Fish aims to reach consumers by providing information in the Viswijzer app, on websites, and in folders. For this to work, consumers need to actively search for this information. Consumers that do not care for the sustainability of their food will probably not look for information and thus these consumers will not be reached. Good Fish understands and sees this problem, and therefore they are moving their resources toward market parties, such as fish retailers and supermarkets. Furthermore, it may be problematic to change the view of the fish industry. As a lot of money is involved in this industry, they probably will not be keen to change to a sustainable way if this is less lucrative.

Currently, Good Fish has projects on the European eel (*Anguilla anguilla*), crayfish (*Cambarus spp.*), Mussels (*Mytilus edulis*), red mullet (*Mullus surmuletus*), grunt (*Haemulidae spp.)* and squid (*Decabrachia spp.*). This study focuses on the endangered European eel, the invasive crayfish (*Astacoidea spp. & Parastacoidea spp.),* and the Chinese mitten crab (*Eriocheir sinensis*). Investigation of PFAS contamination could add an important dimension to the advice of Good Fish for the fishing and consumption of these fish.

## 1.3. Problem definition

Since eels are bottom-dwelling animals and have a high-fat percentage, they are especially vulnerable to contamination by persistent organic pollutants (POPs). From 2011 onwards, there is a ban on commercial fisheries on European eels in the large Dutch rivers like Rhine, Meuse, and IJssel, since the areas are heavily polluted with POPs, such as dioxins, and the consumption of these eels is unsafe (Zafeiraki et al., 2019). PFAS are POPs that mainly accumulate in adipose tissue. As the eel is a long-living benthic predator, PFAS accumulates in the eel to a greater extent compared to other farmed fish (Figure 2). Compared to other fish, the level of average PFAS concentrations (consisting mostly of PFOS and PFNA) in the eel is higher (Zafeiraki et al., 2019). PFAS could also accumulate in crabs and crayfish as they are scavengers and feed near the sediments contaminated with PFAS (Ip et al., 2005). Additionally, their gills have a large surface area that filtrates large volumes of water (Yang et al., 2007).

Recently, PFAS and its impact in the Western Scheldt and other Dutch waters received extra attention. Research on the potentially harmful compounds is still ongoing. Because it is currently unclear how PFAS affects the environment no clear regulations have been implemented by the government yet. However, the Netherlands, together with Germany, Sweden, Denmark, and Norway has submitted a restriction proposal to the European Chemicals Agency (ECHA) to ban PFAS. This proposal includes 6.000 PFAS compounds in total, making this the most extensive and complex restriction (Rijksoverheid, 2021).

In this project, the aim is to investigate if and how the levels of PFAS in Lake IJssel and the Western Scheldt affect the European eel (*Anguilla anguilla*). Furthermore, we intend to broaden the investigation toward the Chinese mitten crab (*Eriocheir sinensis*) and crayfish species (*Procambarus spp.*). The PFAS contamination within these species will be assessed as a risk to human health. Finally, the aim is to highlight current knowledge gaps that need to be closed to proceed in.

## 1.4. Research question and sub-questions

Based on the problem statement and project purpose, the following research question has been formulated.

**What are the ecotoxicological risks of PFAS pollution in Lake IJssel and Western Scheldt for the European eel (*Anguilla anguilla*), crayfish species (*Astacoidea spp. & Parastacoidea spp.*), and the Chinese mitten crab (*Eriocheir sinensis*)?**

Since the main question covers a broad scope, the question has been broken down into sub-questions.

### What is the ecotoxicological risk in the area of interest, and for each targeted species?

- What are the characteristics of PFAS?
- What is the level of PFAS in sediment and water in Lake IJssel and the Western Scheldt?
- What is the contamination of the European eel in the area?
- How and where does PFAS bioaccumulate in eels?
- Is PFAS posing a threat to the European eel?
- What are the PFAS exposure levels of crayfish and the Chinese mitten crab?

### What are the risks to human health?

- What are the potential dangers of PFAS contamination for human health?
- How much PFAS enters humans due to the consumption of species of interest?
- Are the species of interest safe to consume?

# 2. Methodology

To answer our main research question and sub-questions, a risk assessment strategy was adopted. In this report, two assessments can be distinguished. The first risk assessment that was performed assessed the risks of PFAS contamination within the three target species and can be seen as an ecotoxicological risk assessment. This assessment can be classified as a classical risk assessment, consisting of the general steps shown in Figure 1. The second risk assessment assessed the risks the PFAS-contamination within the three aquatic species poses to humans who consume these species as a food source. This assessment is similar to the first assessment yet has different boundaries. The focus for hazard identification and characterization here is on humans. Exposure assessment was limited to only PFAS uptake through consumption of the three target organisms. It is important to note that we do not communicate the risks beyond our commissioner Good Fish.

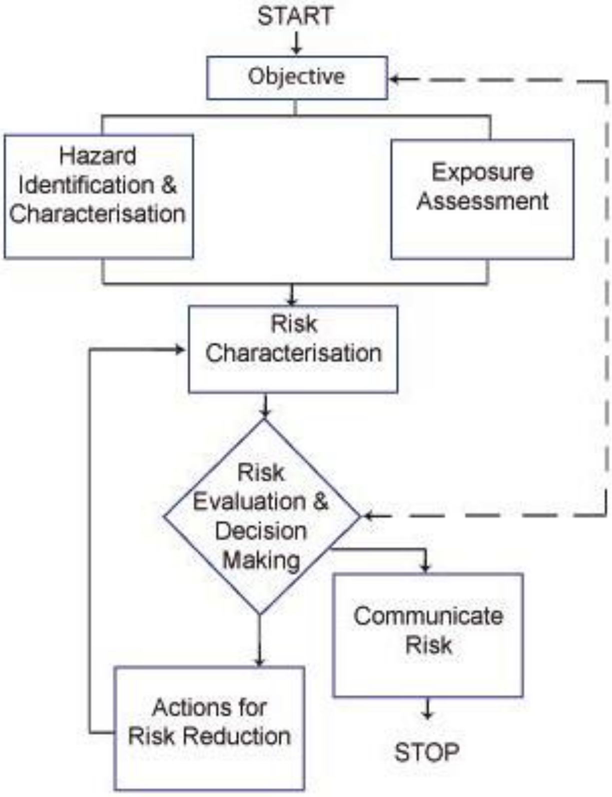


*Figure 1: General flowchart of risk assessment strategies. From Eurosafe, 2008.*

To gather information to execute the risk assessment, a literature study was performed. Information was gathered with the use of the Wageningen Library and Google (Scholar) from both scientific and relevant grey literature. Also, the ECOTOX database was used. This is a comprehensive, publicly available database providing single chemical environmental toxicity data on aquatic life, terrestrial plants, and wildlife. Scientific publications, project reports, and information from the Dutch Government (i.e. Rijkswaterstaat) are assessed and reviewed. If possible, data found outside of the target areas was extrapolated with relevant scientific calculations. However, for these extrapolations assumptions were made, and thus they only serve to give a preliminary assessment of the possible severity.

Existing knowledge gaps form a core problem within this project. Due to the limited available data and knowledge, literature research was not sufficient. Encouraged by our commissioner, the decision was made to perform interviews with researchers and other stakeholders. All the interviews were structured on forehand. Per interview, an agenda with topics and/or questions to discuss was set up. The topics and/or questions were tailored to the scientists or stakeholders' expertise. The topics and/or questions were shared with the interviewee on forehand. This was partly done to ensure that every important topic would be discussed. Additionally, this allowed the interviewee to prepare, or to choose which questions he or she would (not) like to answer. The latter was important since the topic of PFAS is sensitive. Meetings were all conducted online, using Microsoft Teams and Google Meet. With the permission of the interviewee, the interview was recorded using Microsoft Teams and subsequently transcribed. An overview of the agendas sent to each interviewee is included in an external appendix. During the interviews, minutes have been made. The transcripts of the interviews are included in the external appendix.

Dr. C. Belpaire, a researcher working at the Research Institute Nature and Forest in Flanders, was interviewed first. Mr. Belpaire works on ecological and ecotoxicological projects regarding the eel and is an advisor on eel conservation. His expertise was used as a starting point in this project, he pointed us to important literature. Additionally, P. Feitsma MSc was interviewed. Mr. Feitsma is a business analyst at the Nederlandse Voedsel en Warenautoriteit (NVWA). He provided us with insights regarding the toxicity of PFAS and the dangers of PFAS in our food. He shared his knowledge of the implementation of the regulations and he explained which parties are involved in the decision-making process. Additionally, he elaborated on what occurs when safety limits are exceeded. Lastly, an interview with M. Schiphouwer MSc took place. Mr. Schiphouwer is a project manager at RAVON, and he provided us with valuable information about the life history traits of the eel, such as their reproduction, diet, and threats they face. Additionally, Chiel Jonker provided us with data on PFAS levels in the Western Scheldt.

During the writing of our proposal, it was discovered that PFAS is a sensitive and controversial subject. To provide Good Fish with some insights into why this topic is sensitive, the aim was to ask the researchers not willing to be interviewed why they were hesitant in cooperating. When potential interviewees did not respond to our e-mails, they were called to elaborate on the motive for their hesitancy.

PFAS could potentially be polluting waters or endangering food safety. However, food safety concerns could be in direct conflict with food provision. Besides, not only animal life but also human rights should be considered. A discovery could cause a social-ethical dilemma of choosing between fishermen's jobs, food supply, and ecological health. If fish are contaminated with PFAS and this remains unknown, some humans could suffer from intoxication. Banning all fisheries is also not viable, because of the many livelihoods at stake. We took these ethics into account during this project.

# 3. Background information

This chapter reports relevant background information to form a basic understanding of the most important concepts related to this project. The chapter starts with a chemical and functional presentation of the pollutants of interest: Poly- and perfluoroalkyl substances, PFAS. Furthermore, general information is given for the European eel, crayfish, the Chinese mitten crab, and the Western Scheldt and Lake IJssel.

## 3.1. Poly- and perfluoroalkyl substances (PFAS)

### 3.1.1. *Description of PFAS*

Poly- and perfluoroalkyl substances (PFAS) are man-made organic compounds consisting of a carbon backbone substituted with fluor moieties. Most PFAS are known as amphiphilic molecules consisting of a hydrophilic functional group head and a hydrophobic fluorinated carbon tail. The fluorinated carbon tail consists of a lot of carbon-fluor (C-F) bonds, known as the strongest organic single bond (O'hagan, 2008). These bonds give the PFAS extreme stability even at high temperatures. These chemical properties cause PFAS to possess desirable characteristics, such as resistance to heat, water, and fire. Because of these properties, they are used in numerous products such as non-stick coatings in pans, water-resistant clothing, firefighting foams, paints, and food packaging. However PFAS molecules are hard to degrade. This, together with the fact that they are mobile, makes them potentially dangerous since they accumulate in the environment and organisms. Over 6,000 PFAS compounds exist of which perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) (Figure 2) are most often encountered in the environment. The omnipresence of PFOS and PFOA has resulted in regulations that came into force in 2020 (Gagliano et al., 2020). However, most other PFAS compounds remain unregulated and the production of alternative PFAS compounds is progressing.

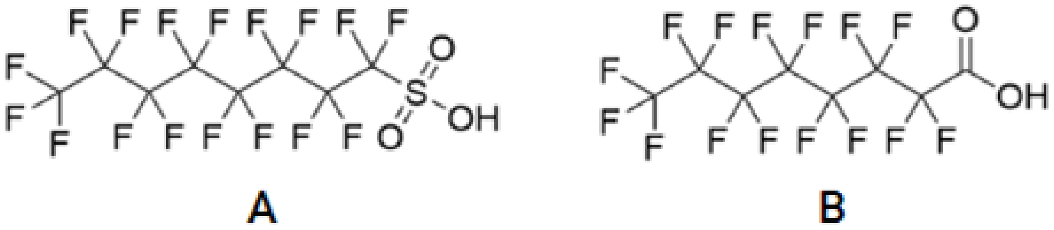
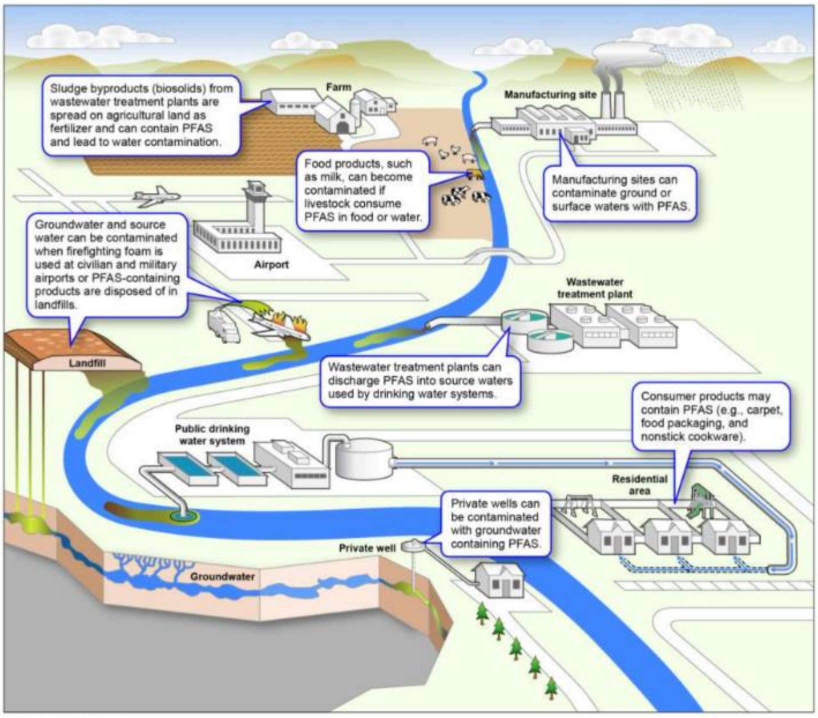


*Figure 2: Chemical structures of PFOS (A) and PFOA (B). From personal source, made with Chemdraw®, 2021.*

PFAS have been encountered worldwide in sediments, water air, and biota (Mussabek et al., 2019). They enter the environment via many pathways. In the Netherlands, PFAS often enters the environment through waste and discharge from several industries (Jans & Berbee, 2020). Industrial factories, like the 3M factory near Antwerp actively produce PFAS. The elevated levels in, for instance, the Ghent-Terneuzen Canal, which flows directly into the Western Scheldt, can be attributed to this 3M factory (Möller et al., 2010; Jans & Berbee, 2020). Another common source of PFAS is the use of the earlier mentioned firefighting foams (Mooij et al., 2011). Furthermore, paper is an often-overlooked source of

PFAS pollution. PFAS can be present in the coating of disposable paper products and paper fibres may act as vectors transporting PFAS into the aquatic environment (Langberg et al., 2021).

Wastewater treatment, while becoming increasingly effective in PFAS removal, still struggles with the complete and especially efficient removal of PFAS. Currently implemented methods such as sorption, ion-exchange resins, and filtration all suffer from various downsides. Sorption is inefficient when dealing with smaller PFAS molecules and often requires a lot of adsorbents that could interfere with other constituents. Filtration and ion-exchange resins, while more effective for large and small PFAS, are a lot more costly and result in concentrated streams of PFAS polluted water for which no proper treatments exist (Mastropietro et al., 2021). Therefore, wastewater treatment, meant to improve PFAS removal, is still an active source of PFAS pollution. Before reaching the wastewater treatment plants PFAS must enter the wastewater itself. As said before, PFAS is used in a lot of household utilities and consumables such as non-stick pans, packaging as well as the food and drinks we consume. Through excrement and discharge of these items, the compounds may reach the wastewater as well as the environment directly (Jans & Berbee, 2020). Figure 3 below shows an overview of different PFAS sources.



Sludge byproducts (biosolids) from wastewater treatment plants are spread on agricultural land as fertilizer and can contain PFAS and lead to water contamination.

Food products, such as milk, can become contaminated if livestock consume PFAS in food or water.

Manufacturing sites can contaminate ground or surface waters with PFAS.

Groundwater and source water can be contaminated when firefighting foam is used at civilian and military airports or PFAS-containing products are disposed of in landfills.

Wastewater treatment plants can discharge PFAS into source waters used by drinking water systems.

Consumer products may contain PFAS (e.g., carpet, food packaging, and nonstick cookware).

Private wells can be contaminated with groundwater containing PFAS.

*Figure 3: Schematic representation of common sources of PFAS contamination. From Gomez et al, 2021.*

### 3.1.2. *Policy, regulations, and possible fishing bans*

The major source of PFAS exposure in the human population is the consumption of seafood (Haug et al., 2010; Christensen et al., 2017; Colles et al., 2020). Limiting this exposure to PFAS through partial or seasonal fishing bans could be a valuable way to decrease PFAS contamination in humans.

In 2008, the Directorate-General for Public Works and Water Management (Rijkswaterstaat, RWS) measured PFAS for the first time in the Western Scheldt (Jonker, 2021). The measurements were then limited to PFOS and PFOA, but have been elaborated since. So, for a few years, the European Union (EU) has initiated commissions to look into the challenges regarding PFAS. The commissions aim to define regulations and restrictions on the use of PFAS compounds across the EU (ECHA, n.d.). As a result of the omnipresence of PFAS, it is impossible to address them with a few regulations. Therefore, commissions concentrate their energy on specific apparent problems caused by PFAS. Because a lot of stakeholders are involved in PFAS production and consumption, the implementation of regulations usually takes a long time. In addition, one characteristic of the European Union (EU) is that every country in the EU needs to accept the regulations, which prolongs the process.

Brennan et al. (2021) divide the challenges of the PFAS discussion in the US into five categories: political, social, economic, scientific, and practical factors. The political challenge represents the creation and implementation of new regulations following many different political levels from a municipal- to an international scale. The social challenge regards the awareness of the population, e.g. are people concerned about their health risks. The economic challenges include the costs accompanied by regulation and monitoring. The scientific challenge constitutes finding supportive toxicity data to support regulations. Reliable scientific evidence helps in supporting regulations as well. Lastly, the practical challenge concerns the technical expertise that is needed to implement regulations. In this context, it could be difficult to find a balance between the positive and negative aspects that come with the use of PFAS. There also is a high discrepancy in PFAS regulations across the globe, which can be attributed to variation in scientific and social factors among cultures. For instance, developed countries invest more in PFAS-research than less developed countries. Additionally, there is a knowledge gap in the correlation between exposure of humans to PFAS and the likelihood of health risks. Also, the scale of the risks is currently not entirely understood (Abunada et al., 2020).

Formerly, PFAS have been regulated per type. However, a trend is seen in which there is a focus on chemical subgroups of PFAS, of which the toxicological endpoints are expected to be similar, allowing for extrapolation (Kwiatkowksi et al., 2020). For instance, in some states in the US, PFAS were banned from all materials that come into contact with food (State of Maine legislature, 2019). In Australia, there is a ban on PFAS in firefighting foams (Gomez et al, 2021). The Dutch RIVM works together with Denmark, Germany, Norway, and Sweden since 2020 to prohibit the use of PFAS in Europe. In July 2021 these countries have announced the prohibition of PFAS to the European Chemicals Agency (ECHA), which marks the official start of the process to come to a European prohibition. In this announced prohibition, all 6000 PFAS will be banned (except essential PFAS) making it the most elaborate restriction so far. The plan is to submit the prohibition proposal in July 2022, and it will come into force before 2025 (Rijksoverheid: Ministerie van Infrastructuur en Waterstaat, 2021). Additionally, there is a trend in which PFAS are only used in essential products. Compared to governments, it is easier for retailers and manufacturers to make changes, and they can also make changes faster. Consumers often demand retailers sell products that contain fewer or no harmful chemicals. Retailers such as IKEA phased out PFAS use in textiles, and many other retailers agreed to stop using PFAS in their products (IKEA, 2017; Kwiatkowski et al., 2021).

For both governments and retailers, it is easier to manage the use of PFAS when a comprehensive approach is taken, by categorizing chemical subgroups in PFAS. However, it is also recognized that regulating in this way might lead to an underestimation of the overall risks of PFAS to human health (Kwiatkowski et al., 2021). With this in mind, the RIVM formulated 'no-regret' measures, which are precautionary measures based on extrapolation from data when information for a type of PFAS is missing. These measures indicate to citizens how they can limit exposure to products containing PFAS to limit the health risks. The 'no-regret' measures also address food sources, as food is the main route of exposure to PFAS in humans (Vrancken, 2021). In 2020, the European Food Safety Authority (EFSA) has established a tolerable weekly intake (TWI) for four different types of PFAS: PFOS, PFOA, PFNA, and PFHxS. The RIVM is performing research on how these TWIs can be translated toward norms for water quality in the Netherlands, considering PFAS exposure of humans via seafood (Smit, 2021). In the Netherlands, as well as in Belgium, there is stringent advice not to consume freshwater fish caught in the Western Scheldt. This advice is merely based on measures of PCBs, dioxins, and flame retardants, but currently, also PFAS is taken into account (Nederland Sportvisserij, 2022). Unfortunately, these 'no-regret' measures are hard to enforce (Vrancken, 2021).

The European Food Safety Authority (EFSA) evaluates a large number of PFAS in food in relation to human health. The EFSA established a tolerable weekly intake (TWI), a value that describes a maximum intake of PFAS before the risk of health effects becomes significant. This TWI gets published in a concept opinion. Then, a public consultation round follows all the EU member states (Steenbergen-Biesterbos, J., 14-04-2022, personal communication). The ministries of health and other authorities, such as safety authorities, review the EFSA concept opinion and after implementing all the feedback from these states, a final TWI is decided upon. The most recent EFSA CONTAM panel established TWI comes from 2020, this is a TWI of 4.4 ng/kg body weight per week for the sum of PFHxS, PFNA, PFOA, and PFOS (Schrenk et al., 2020).

Currently, dioxins and polychlorobiphenyls (PCBs) have been assessed for several fish species to ensure safe consumption. It was found that the fishing ban for eels in Dutch inland waters was more than justified. People consuming eels contaminated with dioxins and PCBs had 2.5 times higher levels of dioxin and 10 times higher levels of PCBs in their bodies (van den Dungen et al., 2016). Since 2017, there is a law in the Netherlands that forbids catching eels (or Chinese mitten crabs) when the levels of PCBs or dioxins exceed the norms for two consecutive years in a certain area (Rijksdienst voor ondernemend Nederland, 2021). In other countries, such as Australia, fishing bans were put into place as a result of too high dioxin and PCB levels in seafood as well (Manning et al., 2017). The European Water Framework Directive (WFD) monitors bioaccumulative substances in fish. On the list of priority substances, we can only find PFOS. For the other PFAS frequently detected in fish, there are no environmental quality standards yet (Rüdel et al., 2022). In 2020 the European Commission initiated setting up European standards for other PFAS and they submitted a proposal in 2021 including standards for PFOS, PFOA, PFNA, and PFHxS in food originating from animals (Vrancken, 2021).

## 3.2. Species of interest

PFAS are known to accumulate in aquatic systems close to industrial areas. However, these areas are also home to a wide array of fish species, three of these species are of interest for this project.

### *3.2.1. The European eel (Anguilla anguilla)*

Eels belong to the Anguillidae family in which currently 19 species and/ or subspecies are recognized. Of these 19 species, *A. anguilla*, *A. rostrata*, and *A. japonica* have been studied the most (Righton et al., 2021). This study focuses on *A. anguilla*, the European eel. The European eel is a fish with an elongated body, that can reach sizes between 60 and 100 cm, with a weight of up to 6 kg as an adult (Van Ginneken & Maes, 2006; Verreycken, 2011). In the wild, an eel's lifespan ranges from 3 to 20 years, with a generation length of 13 years (IUCN, 2010).

#### 3.2.1.1. Critically endangered species

Eel populations have been declining for decades now (Stone, 2003). Decreases of nearly 99% are observed, compared to population numbers in 1980 (Van Ginneken & Maes, 2006). *A. anguilla* has a place on the International Union for Conservation of Nature (IUCN) ''Red List of Threatened Species'' since 2008 (IUCN, 2010). It is since then characterized as 'critically endangered' (Figure 4). The IUCN is in charge of monitoring endangered species in the world. It is crucial to make efforts to prevent the European eel from becoming extinct.

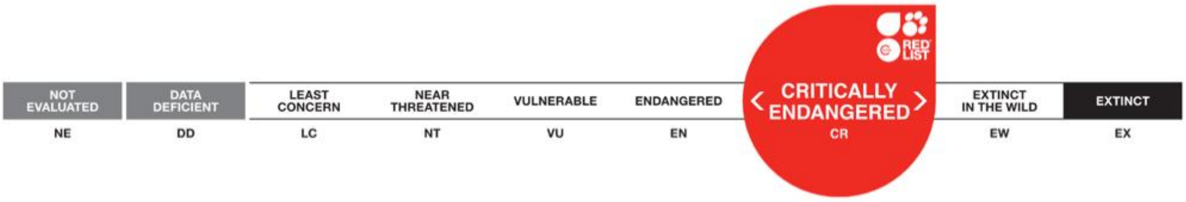


*Figure 4: The UICN Red List assessment of European eels. From Crook & Gollock, 2210.*

The European eel faces many potential threats, among which both natural and anthropogenic threats, such as climate change, overexploitation by fisheries, predation, barriers to migration, pollution, etc. (Stone, 2003; Drouineau et al., 2018). Up to date, it remains unclear what the exact cause of the decline is (Stone, 2003). It is possible that all threats synergistically affect population numbers (Jacoby et al., 2015).

The life history of the eels largely depends on ocean conditions and therefore climate change is expected to affect the European eel population. Eels depend on oceanic conditions for maturation, migration, spawning, etc. (Van Ginneken & Maes, 2006). Climate change might cause ocean currents to change, and additionally, the water temperature rises, and the food availability is altered (Righton et al., 2021). For example, it is possible that through climate change the currents in the Gulf Stream change resulting in Leptocephali larvae, which is the first life stage of eels, never reaching Europe to mature.

Eels are commercially important since especially the freshwater life stages are harvested. Juvenile glass eels are harvested to be used as stock 'seed' in aquaculture since breeding eels in captivity is not yet possible (Butts et al., 2016 as cited in Musing et al., 2018). Yellow eels and silver eels are mainly caught for human consumption (Crook & Nakamura, 2013 as cited in Musing et al., 2018).

The blockage of migration routes, for instance by hydropower dams, dykes, or sluices poses a threat to the European eel. They can cause (sub) lethal damage to the eels (Pedersen et al., 2012; Leeningen, 2020), and delay or even prevent juvenile eels from reaching their upstream habitat (Van Ginneken & Maes, 2006; Righton et al., 2021). In the Netherlands, for instance, the Afsluitdijk makes the migration of eels difficult. Currently, improving accessibility and increasing connectivity between the marine and freshwater habitats eels live in is one of the strategies of, for instance, RAVON to conserve the European eel (Schiphouwer, M., 22-04-2022, personal communication). This is already being implemented; the ‘Vismigratierivier’, an idea from the ‘Afsluitdijk Wadden Center’, connects Lake IJssel with the Wadden Sea, so many migratory fish can freely move in and out (de Afsluitdijk, 2022).

Lastly, the effects of pollutants on eels have been studied relatively extensively. There is a wide range of contaminants that have been reported in eels, often in high concentrations. Examples are PCBs, dioxins, and heavy metals (Belpaire & Goemans, 2007). It is thought that pollution contributed to the population decline of *A. anguilla*, but the effects of contaminants on, for instance, reproduction, remain elusive and need to be further investigated (Righton et al., 2021). Furthermore, more research needs to be conducted on the synergistic effects of different contaminants (Righton et al., 2021). In this study, we extensively address PFAS as a pollutant.

### 3.2.1.2. The European eel life cycle

The European eel has a semelparous reproductive strategy, which entails that they reproduce only once in their lifetime. Adult European eels are thought to breed in the Sargasso Sea. They spawn their eggs in the Sargasso Sea, and these eggs develop into so-called Leptocephali larvae. Through the tides, these larvae drift toward Europe to mature. When eels reach the adult stage they migrate back to the breeding area in the Sargasso Sea before dying there (Figure 5) (Righton et al., 2021).

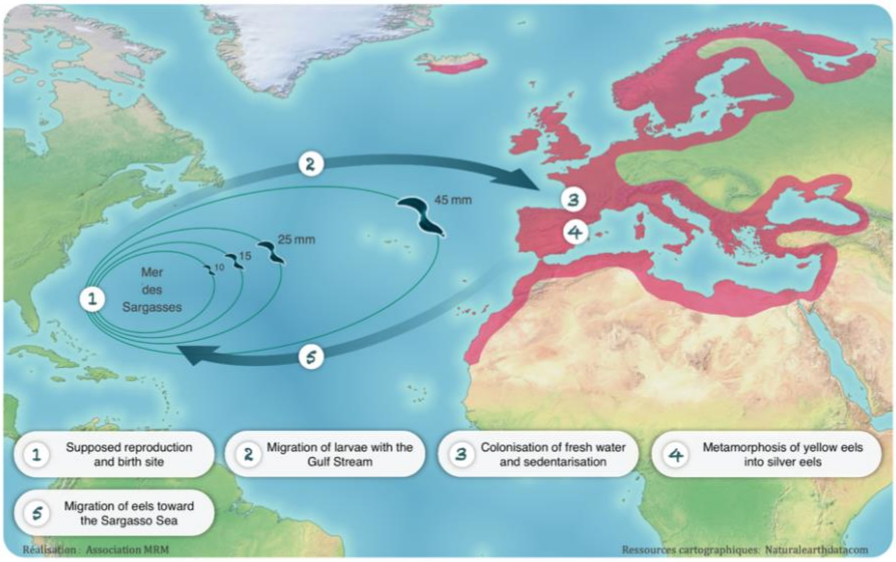


*Figure 5: Migration of European eels during the life cycle. From Observatoire MRM, 2021.*

The life of the European eel can be divided into six different phases, they mature from eggs to Leptocephali larvae, to glass eels, to elvers, to yellow eels, and ultimately to silver eels, that spawn and subsequently die (Cresci, 2020). Up to date, the life cycle of eels is not fully understood (Van Ginneken & Maes, 2006). Progress has been made due to the improvement of technology such as DNA techniques, but some parts of the life cycle remain elusive. It is for instance not well documented where breeding takes place, it is expected to happen in the Sargasso Sea, but there is little evidence (Van Ginneken & Maes, 2006). It is crucial to understand the eel’s life cycle, as this will aid in the protection and thus conservation of this species.

## Spawning and Leptocephali larva

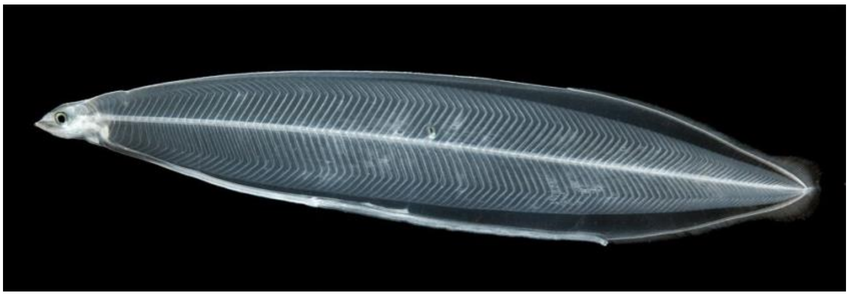


*Figure 6: Larva of European eel (Leptocephalus). From biology.duke.edu, the Johnsen Lab, n.d.*

The Leptocephali larvae that develop from spawned eggs in the Sargasso Sea are small (between 5 and 80 mm long), and they are completely transparent (Figure 6). Their appearance is so different from the appearance of adult eels that researchers first defined this stage as a different fish species named *Leptocephalus brevirostris* (Tesch et al., 2003). The European eel stays in the Leptocephalus larvae stage for a period ranging from 6 to 12 months. This duration corresponds to the time they need to migrate from the spawning area in the Sargasso Sea to the European continental shelf (Van Ginneken & Maes, 2006; Adam et al., 2008).

Most research on the eel's diet has been done for the yellow eel stage, and therefore there remains a lack of understanding of the feeding behaviour of Leptocephali larvae and glass eel stages. The lack of understanding of the feeding behaviour makes deciphering the eel's life cycle more difficult. Understanding the eel's life cycle is crucial for the conservation of the European eel (Righton et al., 2021).

It is known that adult eels spawn in a relatively productive zone in the Sargasso Sea, which ensures food security for the larvae (Van Ginneken & Maes, 2006; Riemann et al., 2010). Leptocephali larvae possess large but thin teeth (Figure 7) that aid them in the consumption of large but soft objects, in particular gelatinous pellets of marine zooplankton, and marine snow, organic matter falling from upper water to the deep ocean (Miller, 2009; Riemann et al., 2010; Ayala et al., 2018; Righton et al., 2021).

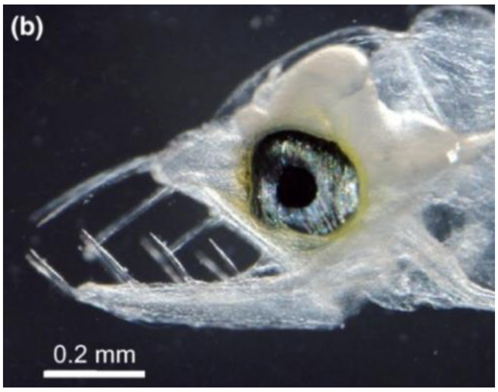


*Figure 7: Head and jaw of a Leptocephalus larva of the species* Anguilla marmorata *showing their long, thin teeth. From Miller, 2009.*

Leptocephali larvae are expected to depend on ocean currents to reach their destination on the European continent. However, it remains unclear whether the migration of Leptocephali larvae is a passive or an active process (Van Ginneken & Maes, 2006). It is known that Leptocephali larvae of more than 5 mm in size change their vertical distribution in the water column based on diurnal patterns, whereas larvae of less than 5 mm in size do not. This indicates that the developmental stage influences the active migration of the larvae (Van Ginneken & Maes, 2006). Furthermore, Naisbett-Jones and colleagues (2017) have shown that Leptocephali larvae use a magnetic map to orient themselves and find their way to the Gulf Stream that transports them toward Europe. This could explain why the migration time of larvae (6 to 12 months) is shorter than the migration time of inert particles (3 years) over the same distance (Adam et al., 2008).

### Glass eel

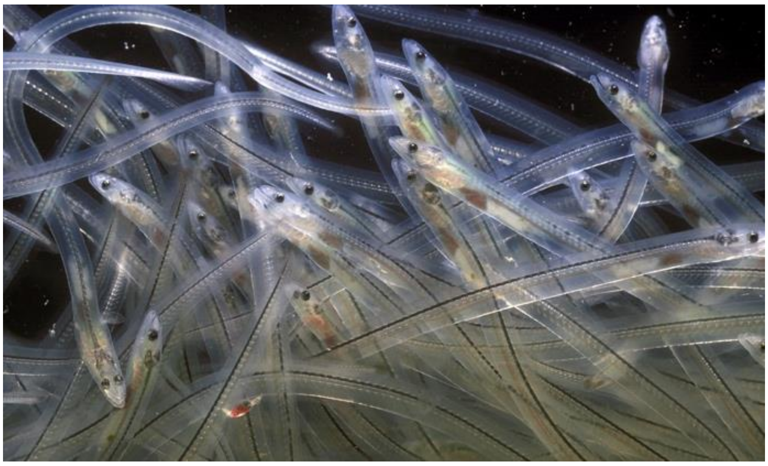


*Figure 8: Glass eels of the European eel. From Philippe Garguil – Science, n.d.*

After about 18 months, leptocephali larvae are capable of metamorphosis. They then enter the glass eels stage. In this stage the eels do not have any pigmentation; the vertebrae of the eels can be seen which gives rise to the name glass eel (Figure 8) (Tesch & Henderson, 1977; Adam et al., 2008). During this stage, eels migrate from the continental shelf to estuaries in Europe. The diet of glass eels is not extensively studied, but it is known that they do not eat much. However, glass eels of *A. japonica* are known to eat algae and shark eggs (Schiphouwer, M., 22-04-2022, personal communication). A lot of energy is used for the metamorphosis and migration. This, and the lack of food ingestion results in a reduction of the eel's weight during this phase. Glass eels are usually constant in size, around 8 cm long (Adam et al., 2008).

Glass eels move in a completely different manner compared to Leptocephali larvae. They have adopted a swimming mode called anguilliform, in which their body makes sinusoidal waves (e.g. snake-like) (Tesch et al., 2003). This allows glass eels to swim against currents to reach the European estuaries. The colonization of estuaries occurs from December to April, and this precise window may indicate that glass eels are sensitive to water temperature, and depend on it to start migrating toward the estuaries (Tesch & Henderson, 1977).

### Elver

When glass eels reach the estuaries, they start to eat again. The sunlight, salinity, and temperature together act on the pigmentation of the eels, and a brown/ red pigmentation slowly appears (Adam et al., 2008). This pigmentation change indicates the onset of the juvenile stage, in which the eels are called elvers. During this phase, the eels reach a size ranging from 8 up to 30 cm (Tesch et al., 2003). At the onset of the elver stage, the eels migrate from estuaries upstream into rivers and thus change from a marine to a brackish to a freshwater habitat (Tesch & Henderson, 1977).

## Yellow eel

*Figure 9: Yellow eel. From Adam et al., 2008.*

The yellow stage is the most dominant stage in the eel's life cycle, the biggest part of their life is spent in this stage. After the elver stage, the eels adopt a yellowish pigmentation, giving rise to the name yellow eel (Figure 9). During the yellow eel phase, eels are still not fully matured. Female eels spend between 5 and 12 years in this stage whereas male eels spend between 3 and 8 years as a yellow eel (Tesch & Henderson, 1977). The actual age of the maturation is known to differ per geographical area. For instance, in Northern Europe, females reach maturity when they are between 12 and 20 years old (Van Ginneken & Maes, 2006). Yellow eels slowly grow to reach their adult size, ranging up to 50 cm for males, and up to 100 cm for females (Tesch & Henderson, 1977).

During the yellow eel phase, the migration is ceased (Adam et al., 2008). The feeding behaviour of yellow eels has been studied relatively well, yellow eels improve their hunting skills and can be viewed as predatory fish (Tesch et al., 2003). There are two different phenotypes within the yellow eel population; broad-heads and narrow-heads. The expression of these phenotypes is dependent on the diet of the eels. De Meyer and colleagues (2018) found that eels eating harder food, develop a broader head than eels that feed on softer substances.

## Silver eel

*Figure 10: External features of yellow (top) and silver (bottom) eels. From Smithman, 2015.*

After spending about 8 to 15 years in the yellow eel phase, the eels undergo their last metamorphosis to the silver eel stage (Figure 10). This last metamorphosis usually occurs in spring (Adam et al., 2008). The morphology changes to prepare the adult eels for their migratory trip that brings them back to the spawning areas in the Sargasso Sea. Eels exhibit diadromous migratory behaviour, which means that they migrate between fresh and saltwater. Eels can be further defined as facultatively catadromous, which means that they spawn in marine waters, migrate to freshwaters where they mature, and migrate back to marine waters to reproduce (Adam et al., 2008). The migration consists of a long journey of over 6000 km and the destination is the spawning site in the Sargasso Sea (Van Ginneken & Maes, 2006).

The body of the silver eels has to adapt to going from freshwater conditions to saltwater conditions and thus the eel changes behaviour, physiology, anatomy, and morphology (Adam et al., 2008). Apart from a change in colouration, the eyes of the eels enlarge in this phase, as well as the pectoral fins (Tesch & Henderson, 1977). Additionally, silver eels are no longer capable of eating, because their digestive tract degenerates (Aoyama & Miller, 2003). They live on reserves of fat, proteins, and carbohydrates that they accumulated during the yellow eel stage (Van Ginneken & Maes, 2006). The migration back to the Sargasso Sea usually starts in autumn (Adam et al., 2008), but some studies have shown that when the silver eels are not able to migrate in autumn, they will become inactive in the winter, to migrate in the next spring (Tesch & Henderson, 1977).

When the adult silver eels have migrated back to the Sargasso Sea, they are thought to breed and spawn there. There is some uncertainty about the exact position of breeding and spawning areas of the European eel. Additionally, it remains unclear whether all European eels belong to the same population. According to the generally accepted panmixia theory, all eels reproduce in one single population in the Sargasso Sea. However, some theories state that there are several spawning populations, based on molecular studies using genetic markers (Van Ginneken & Maes, 2006). The debate on this topic is currently ongoing.

The peak in the spawning of the European eel is known to occur in April (Schmidt 1925, as cited in Van Ginneken & Maes, 2006; Tesch & Henderson, 1977). Spawning may be triggered by pheromones (Van Ginneken & Maes, 2006), the lunar cycle, and/ or temperature (Adam et al., 2008). After breeding and spawning, eels are thought to die (Figure 11). More experiments need to be conducted to understand the reproductive behaviour of eels (Adam et al., 2008).

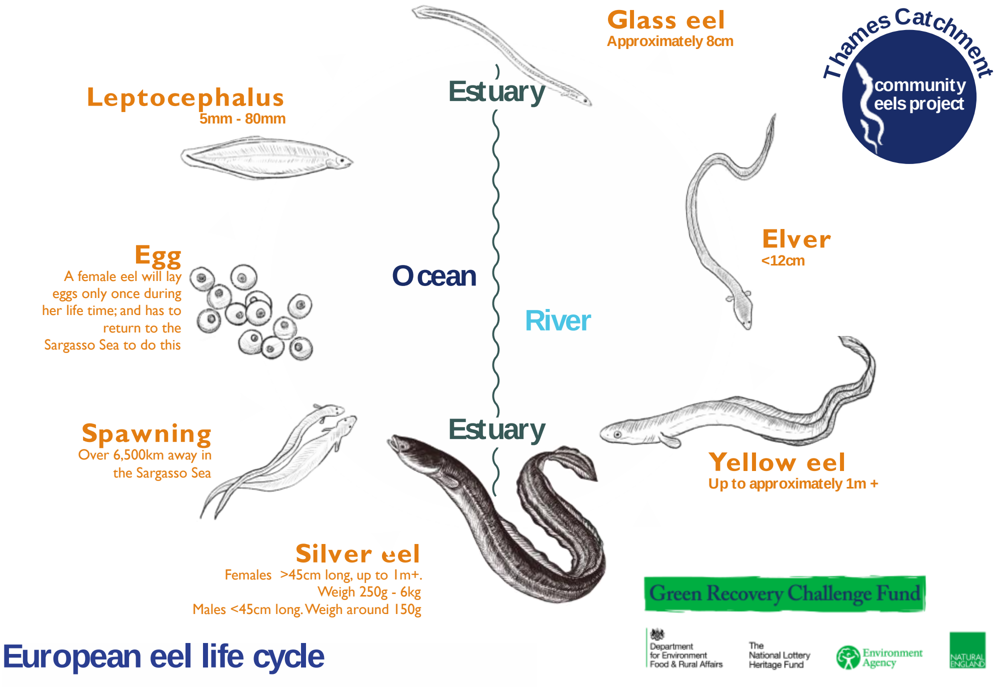


*Figure 11: Life cycle of the European eel. From Thames River trust, 2021.*

### 3.2.2. Crayfish species (*Astacoidea spp. & Parastacoidea spp.*)

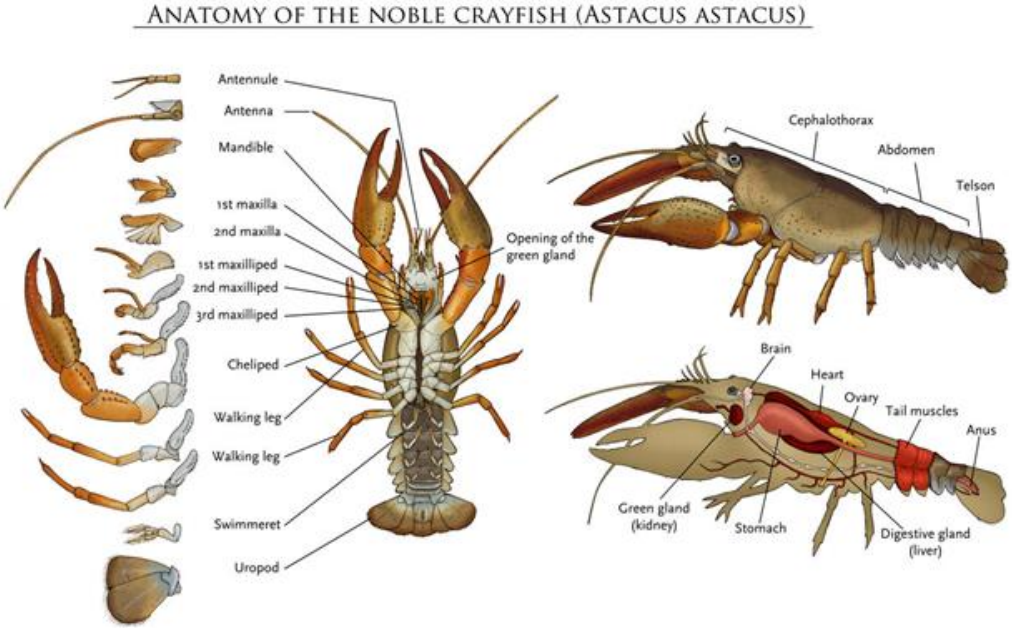


*Figure 12: Simplified anatomy of the Noble/European Crayfish. From Karala, 2018.*

Crayfish (*Astacoidea spp. & Parastacoidea spp.)* are crustaceans that are predominantly found in freshwater. While they closely resemble lobsters (*Nephropidae spp.)*, they are generally smaller in size. Most crayfish reach average lengths of 10 cm and weigh around 50 g. Some species of lobster have no pincers while crayfish are exclusively found with pincers (chelae). Apart from their pincers, crayfish are characterized by their joined head and thorax, called a cephalothorax, followed by a segmented abdomen (Figure 12). Their cephalothorax is lined with 5 pairs of legs, four of which are used for walking, and the front pair is equipped with pincers (Holdich, 2002). Along the abdomen, they have 5 pairs of smaller appendages called swimmerets which are mostly used for swimming (Seichter et al., 2014). Discharge is released from the green gland which functions as a kidney, together with the digestive gland (or hepatopancreas), cleansing the body of contaminants (Figure 12) (Jewell et al., 1997).

Crayfish are inhabitants of the littoral zone, the shallow zone characterized by penetration of sunlight down into the sediment level allowing the growth of aquatic plants. The crayfish play an integral role in ecosystems both through their diet and their tendencies to engage in bioturbation while creating burrows (Dorn & Wojdak, 2004). While preferentially carnivorous (Momot, 1995), crayfish are classified as polytrophic omnivores. Their diet includes algae, aquatic macrophytes, detritus, invertebrates, fish eggs, and carcasses (Hobbs, 1993). This varied feeding behaviour causes crayfish to transcend the conventional trophic levels, they occupy a flexible position in the food web. Crayfish act as ecosystem engineers. By consuming algae and macrophytes, their burrowing behaviour, and their active search for food within the sediment, they shape the structure of the habitat (Creed & Reed, 2004; Dorn & Wojdak, 2004).

While their feeding behaviour may imply that crayfish can be found at the top of the food chain, they are not safe from predators. Young crayfish fall prey to any kind of fish of a suitable size. The larger adult crayfish are only realistically threatened by larger predatory fish species such as pike (*Esox lucius*), perch (*Perca fluviatilis*), and catfish (*Silurus glanis*) (Hobbs, 1993; Soes & Koese, 2010). The European eel also predates upon the crayfish, it actively hunts both young and adult crayfish. Other predators include predatory birds such as herons *(Ardeidae spp.)* and loons *(Gavia spp.)*, as well as mammals, such as minks *(Mustelinae spp.),* otters *(Lutra spp.)* and raccoons *(Procyon spp.*) (Soes & Koese, 2010). Furthermore, humans are also avid consumers of crayfish.

## Invasion and lethal disease

As of 2010, 10 distinct species, 9 of which are invasive, have been spotted in the Netherlands. Six of the sighted species, the Spiny cheek crayfish (*Faxonius limosus*), narrow-clawed crayfish (*Pontastacus leptodactylus*), red swamp crayfish (*Procambarus clarkii*), signal crayfish (*Pacifastacus leniusculus*), virile crayfish (*Faxonius virilis*) and the white river crayfish (*Procambarus acutus*) have established themselves in the Netherlands (Soes & Koese, 2010; van Kuijk et al., 2021). Only the noble crayfish, more commonly known as the European crayfish (*Astacus astacus*) is native to the Netherlands. Figures 13a and 13b show the distribution of the crayfish. The release of crayfish by humans, a lack of natural predators, and optimal habitats being common, have caused the crayfish to flourish in a larger niche than what is naturally observed (Soes & Koese, 2010). In Europe, crayfish have one of the highest degrees of successful invasion amongst introduced aquatic species. Due to their robustness, high degree of individual and population growth, and high fecundity crayfish have been able to achieve remarkable success in invading the European continent. Especially the species with high fecundity and short life cycles are well suited to habitats that have been heavily disturbed by humans (Lindqvist & Huner, 2017). Native species on the other hand have longer life cycles and lower fecundity leaving them vulnerable to competitive species.

Additionally, some of the invasive species, especially the Signal crayfish (*Pacifasficus leniusculus*), carry what is commonly known as the Crayfish plague (*Aphanomyces astaci*) which is a water mold that infects crayfish. This mold possibly is an even more invasive species, and a bigger threat than the crayfish themselves (Kozubíková et al., 2009). The invasive crayfish species and the mold have been associated for centuries. Crayfish have become relatively resistant to the mold, however, in the native species the mold causes a lethal plague. Populations of various invasive Northern American species are contaminated with mold in the Netherlands (Tilmans et al., 2014). As of the most recent assessment by the IUCN, the Noble crayfish, the native species in the Netherlands, has been assessed as vulnerable, or even close to extinction (Edsman et al., 2010). Therefore, the invasion of non-native crayfish species can be considered to be a real problem in the Netherlands. Active promotion of consumption and fishing of invasive crayfish species could potentially be a method to combat the further spreading of these invasive species. It is therefore vital that contaminants are assessed to prevent contamination of humans with potentially dangerous chemicals.

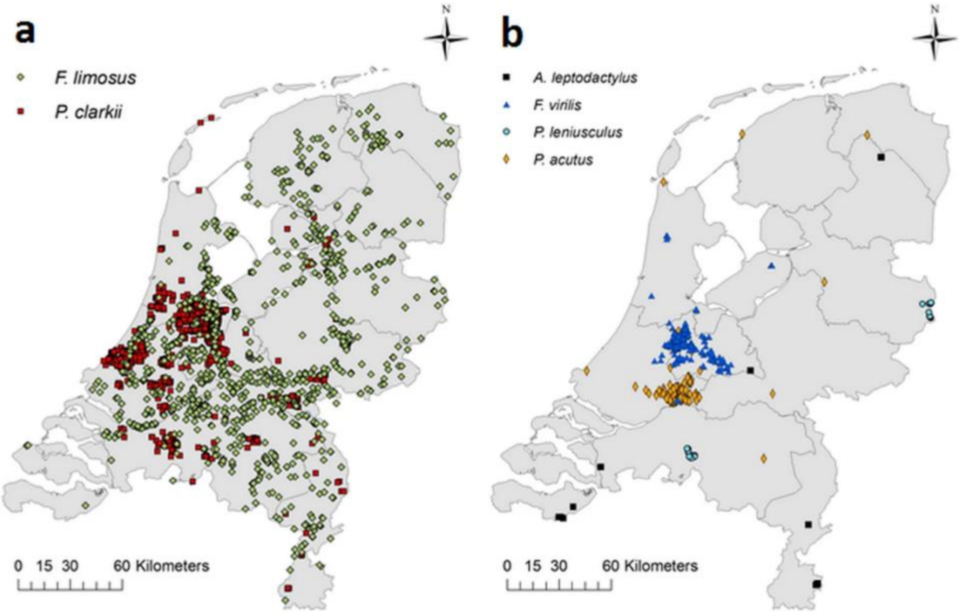


*Figure 13: Distribution of invasive crayfish species in the Netherlands. From van Kuijk et al., 2021.*

### 3.2.3. Chinese mitten crab (*Eriocheir sinensis*)

An invasive freshwater and brackish crab is the Chinese mitten crab (*Eriocheir sinensis*) (Figure 14). It has a life span ranging up to five years, depending on the salinity and temperature levels of its habitat. The Chinese mitten crab is a semelparous organism, both male and female crabs only mate once in their lifetime (Panning, 1939). The crabs require marine- or brackish water for spawning but spent the rest of their life in freshwater. Either four or five stages can be distinguished in the lifecycle of the Chinese mitten crab, depending on the temperature of the location where they occur.

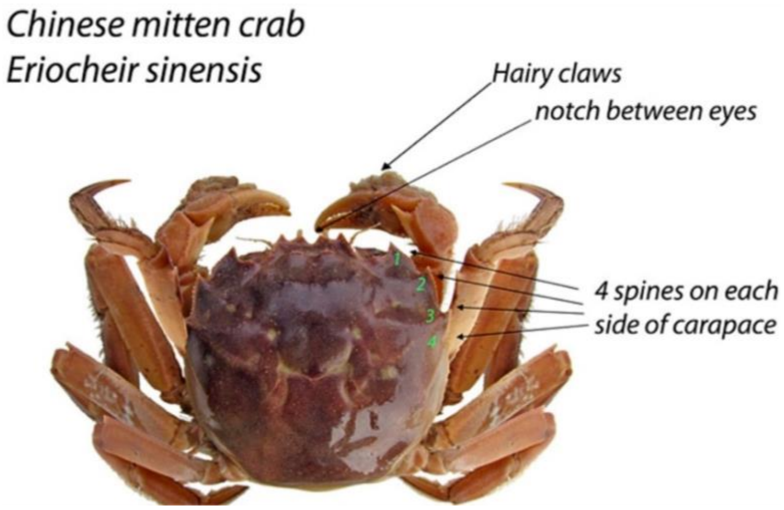


*Figure 14: A Chinese mitten crab with important features indicated. From Wildlife, 2020.*

**Invasion and nutritional value**

The Chinese mitten crab originates from Korea and China and ended up in German waters around the year 1900 (Bouma & Soes, 2010). They are considered omnivorous, consuming both plant and animal materials (Bouma & Soes, 2010). The crab has spread to many North-Western European waters (Dittel & Epifiano, 2009). The population in these waters grew extremely fast, causing them to compete with native species. The crabs are an aggressive and large species, and their bottom-dwelling and burrowing activities are a major threat to stream banks and dikes (Eline et al, 2013). The crabs cause erosion and release pollutants and phosphates from the sediment, thereby decreasing the water quality and weakening constructions. Chinese mitten crabs have been a point of attention for over a longer time, because of their potentially big economically and ecological impact. However, it turns out to be difficult to stop the invasion due to the existing high abundance of crabs in streams and rivers in the Netherlands (Soes et al, 2017). Besides, the crabs have a high rate of reproduction, a high physical tolerance range, and many possible pathways to spread (possibly even via land). Because of their tolerance, the population of Chinese mitten crab has been established in different waters in almost all provinces of the Netherlands (Bouma & Soes, 2010).

The Chinese mitten crab was seen as a plague by fishermen since they were repressing commercial fish species and destroying fishing nets. Nowadays, the crab is a delicacy in the Asian community and holds high commercial value (Van Leeuwen, 2013). The nutrient value is high, the crab’s meat is a source of iron, zinc, and other minerals. The specific amino acid composition also makes the crab an excellent protein source and it is categorized as a polyunsaturated fatty acid-rich food (Chen et al, 2007).

## 3.3. Locations

Two areas providing habitat to the eel in the Netherlands will be discussed in this research, the Western Scheldt and Lake IJssel. For the population stability and health of the eel, it is essential to understand the impact of these pollutants in these areas.

### 3.3.1. *The Western Scheldt*

The Western Scheldt, or Westerschelde in Dutch, is a water body in the south of the Netherlands. The estuary covers an area from the Belgium City Antwerp to the Dutch city Vlissingen. The Western Scheldt is part of a 360 km long river Scheldt. This river has its origin in the French city Saint-Quentin and mainly flows through Belgium. The mouth of the river flows into the North Sea, which can be seen in figure 15. The salinity level at this mouth is approximately 17,900 mg/L. The salinity declines in correlation with distance to the sea, in the middle of the Western Scheldt it is around 12,000 mg/L while it is decreased to 900 mg/L close to Antwerp (Rijkswaterstaat, 2022).

The Western Scheldt is around 55 km long and has a varying width ranging from 2,000 to 8,000 m. Since the estuary is connected to the sea, there is an average tidal amplitude of 4.5 m with a 120 $m^3/s$ average discharge (Wang et al, 2002). The physical characteristics of the Western Scheldt estuary play a significant role in the orientation and migration of the European eel. The tides and waves make the distinguishment between flooding and ebbing, and bio-efficient selective tidal stream transport is possible for the eels (Verhelst et al., 2018). Glass eels enter the Western Scheldt via the North Sea, where the salinity concentration is high. Subsequently, they follow the Western Scheldt inland and mature further into silver eels in the freshwater area.

The Western Scheldt used to be an important area for fisheries, but this has changed because the water quality declined. Nowadays, the few professional fisheries on the Western Scheldt catch mainly shrimp (*Crangon spp.*), sole (*Solea solea*), and eel (Scheldemonitor, n.d.). Upstream of the Western Scheldt, a 3M factory is located that produces chemical products. Their activities contribute to a substantial part of the estuary's PFAS contamination. Recently, part of the processes of the 3M factory was shut down temporarily, since the exposure risks for residents must be decreased.

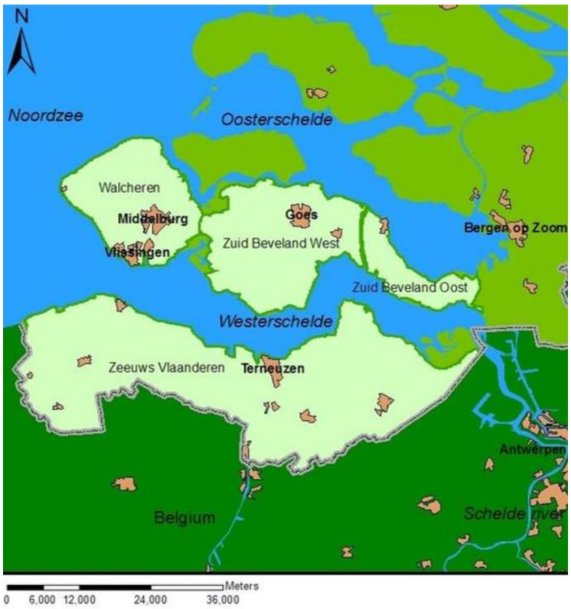


*Figure 15: Overview of the Western Scheldt and surrounding water and land in the Netherlands. From De Bruin, 2008.*

### 3.3.2. *Lake IJssel*

Lake IJssel, or IJsselmeer in Dutch, is a large lake that is situated in the middle of the Netherlands. Lake IJssel is surrounded by land and enclosed by dikes. Before 1932, the waterbody was called Zuider Zee (Southern Sea). It was an estuary around 3,650 km$^2$ and directly connected with the Wadden Sea and the North Sea. The estuary changed gradually into a freshwater lake after the Afsluitdijk (1932) was built. During the following years, the Lake IJssel decreased further in size due to the building of polders, where agriculture and cities grew. Currently, the eutrophic water is divided into two main parts separated by the Houtribdijk, the Lake IJssel, and the Markermeer. This study focuses on the Lake IJssel which is approximately 1,820 km$^2$ (Dekker, 2004) (Figure 16). The river IJssel, being a tributary of the Rhine, supplies the Lake IJssel with a continuous influx of water. The salinity level has been stable for years and fluctuates around 88 mg/L (Rijkswaterstaat, 2022).

*Figure 16: Overview of the IJsselmeer and surrounding water and land in the Netherlands. From D.W. Dekker, 1999.*

Since the construction of the Afsluitdijk, freshwater fisheries have been intensively developed, mainly for perch (*Perca fluviatilis*) and eel (Dekker, 2004). Glass eels, among other fish species, enter the Lake IJssel through sluices in the dykes to develop into yellow and then silver eels.

Increased PFAS concentrations have been found in many locations in the Netherlands (Figure 17). From 2011 onwards, there is a ban on commercial fisheries on European eels in the large Dutch rivers like Rhine, Meuse, or IJssel, since the area is heavily polluted with POPs like dioxins and the eels are not safe to eat anymore (Zafeiraki et al., 2019). In Lake IJssel, the levels of POPs (PCDD/F and PCB) are not exceeding the current regulatory levels (Zafeiraki et al., 2019). Therefore, the eel stocks in Lake IJssel are heavily exploited (Mous, 2000).

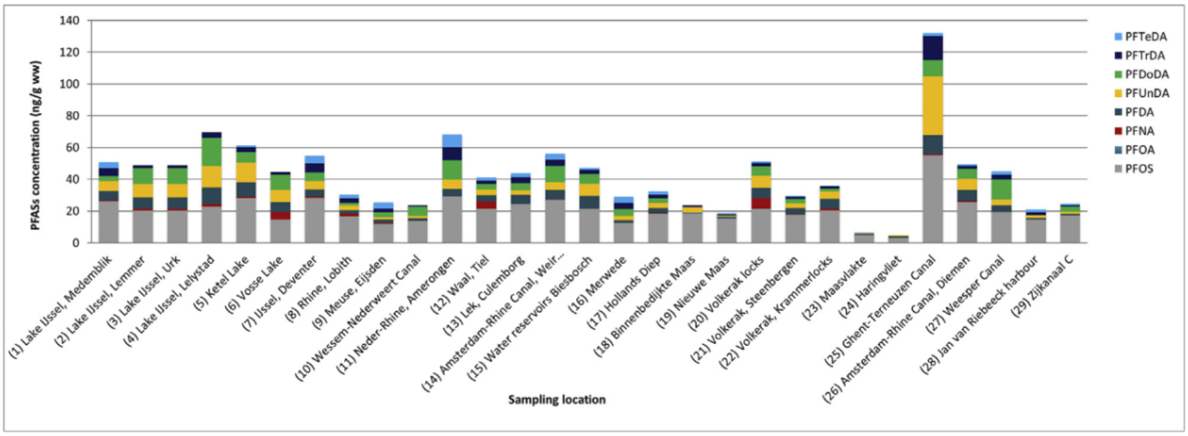


Figure 17: PFAS concentrations (ng/g ww) in eel in locations in the Netherlands. The locations Lake IJssel (at Medemblik, Lemmer, Urk and Lelystad; 1, 2, 3, 4) and 'Ghent- Terneuzen Canal' (upstream of the Western Scheldt; 25) are included. From Zafeiraki et al., 2019.

# Summary Chapter 3

## *PFAS*

Poly- and perfluoroalkyl substances (PFAS) are man-made organic compounds consisting of a carbon backbone substituted with fluor moieties. They have a strong C-F bond which results in a high resilience. Therefore, PFAS is extremely hard to degrade and accumulates in the environment. PFAS brings a political challenge of implementing regulations, a societal challenge of creating awareness, a scientific challenge of obtaining reliable evidence, and an economic challenge of distributing the monitoring and regulation costs. From a regulatory point of view, a trend is seen in which there is a focus on chemical subgroups of PFAS, of which the toxicological endpoints are expected to be similar, allowing for extrapolation. Of all PFAS substances, only PFOS is being monitored by the European Water Framework Directive, but some European countries including the Netherlands, are working together with the European Chemicals Agency (ECHA) to establish a European prohibition and ban the use of PFAS before 2025. Still, the implementation of regulations takes a long time due to many stakeholders and countries that are involved.

## *The European eel* (*Anguilla anguilla*)

The European eel (*Anguilla anguilla*) is characterized as endangered species by the IUCN. For the eel, there are three most important threats identified: the blocking of migration routes, the pollution of their habitat, and the overexploitation by fisheries. This is mainly why the population keeps rapidly declining. The life of the European eel can be divided into six different phases, they mature from eggs to Leptocephali larvae, to glass eels, to elvers, to yellow eels, and ultimately to silver eels, that semelparous spawn in the Sargasso Sea. Yellow and silver eels are caught for human consumption, glass eels for aquaculture. The habitat of the eels is freshwater for the longest part of their life, the development. The body of the silver eels has to adapt to going from freshwater conditions to saltwater conditions, and vice versa, and thus the eel changes behavior, physiology, anatomy, and morphology.

### ***Crayfish*** *(Astacoidea spp. & Parastacoidea spp.)* ***and Chinese mitten crab*** *(Eriocheir sinensis)*

The invasive Crayfish (*Astacoidea spp. & Parastacoidea spp.)* are inhabitants of the littoral zone. The invasion of non-native crayfish species is a problem in the Netherlands. Crayfish show a high population growth and robustness, and they are polytrophic omnivores giving them a flexible position in the food web. Birds, perch, and other fishes like the European eel are predators of crayfish. Humans also often consume the crayfish, but most of the time exclusively the tail is being eaten.

The invasive Chinese mitten crab (*Eriocheir sinensis*) requires marine- or brackish water for spawning but spent the rest of its life in freshwater. They destroy constructions like dikes and decrease water quality with their bottom-dwelling activities. It is hard to stop the invasion due to their abundance, high tolerance range, omnivorous diet, and high production range. The crab is being exploited and has a high commercial value since it has valuable nutrients and is popular in the Asian community.

For both the invasive species, a risk assessment must be performed concerning contaminants since they are heavily promoted and consumed.

### *The Western Scheldt*

The Western Scheldt is an estuary in the south of the Netherlands. Glass eels enter the Western Scheldt via the North Sea, where the salinity concentration is high. Subsequently, they follow the Western Scheldt inland and mature further into silver eels in the freshwater area. Due to declining water quality, there are almost no (eel) fisheries in the Western Scheldt anymore.

### *Lake IJssel*

Lake IJssel is a large Dutch freshwater lake. Glass eels, among other fish species, enter the Lake IJssel through sluices in the dykes to develop into yellow and then silver eels. The levels of POPs (PCDD/F and PCB) are not exceeding the current regulatory levels in Lake IJssel, so the eel stocks in Lake IJssel are heavily exploited at this moment.

# 4. Ecotoxicological risk assessment

This chapter includes a risk assessment of the European Eel in the Western Scheldt and Lake IJssel. First, European norms for PFAS in surface water are mentioned, then location-specific concentrations of PFAS are established. Thereafter, the PFAS accumulation in eels is investigated to determine if the known PFAS occurrence in the Western Scheldt and Lake IJssel could end up in the eel. If the eel has taken up the PFAS, then it is interesting to know where this PFAS is stored and how it could cause short- and long-term health effects for the eel. Lastly, long-term health effects and their effect on the population numbers of the European eel are discussed.

## 4.1. PFAS levels in sediment and water of The Western Scheldt and of Lake IJssel

Currently, there are concentration norms for surface water for three types of PFAS. They regard the maximum allowed year averages in surface water. For PFOS a differentiation is made between fresh and marine water; the PFOS concentration in the fresh surface water is allowed to be 0.65 ng/ L, whereas in marine surface water concentrations are only allowed to be 0.13 ng/ L. For PFOA in surface water, the norm is 48 ng/ L, and for GenX-substances the norm in surface water amounts to 118 ng/ L (Jonker, 2021). According to Jonker (2021), it is worrying that norms have only been established for three of the PFAS types.

### *4.1.1. The Western Scheldt*

In 2010, the sum of PFAS concentrations was 95.3 ng/L (Möller et al., 2010). To our knowledge, there is no available data on the sum of PFAS concentrations for the entire Western Scheldt after 2012. Jonker (2021) did not take into account the total PFAS concentration. He instead measured the fourteen most common PFAS types, among which PFOS and PFOA, in locations throughout the Netherlands. He found that for all sample locations, the norm for PFOS was exceeded. Furthermore, the norms for PFOA and GenX were not exceeded in any of the sample locations (Figure 18). If we consider these fourteen most common PFAS types as a total, Jonker (2021) found that the total PFAS concentration in the Netherlands ranges between 7.96 ng/L and 326.8 ng/L.

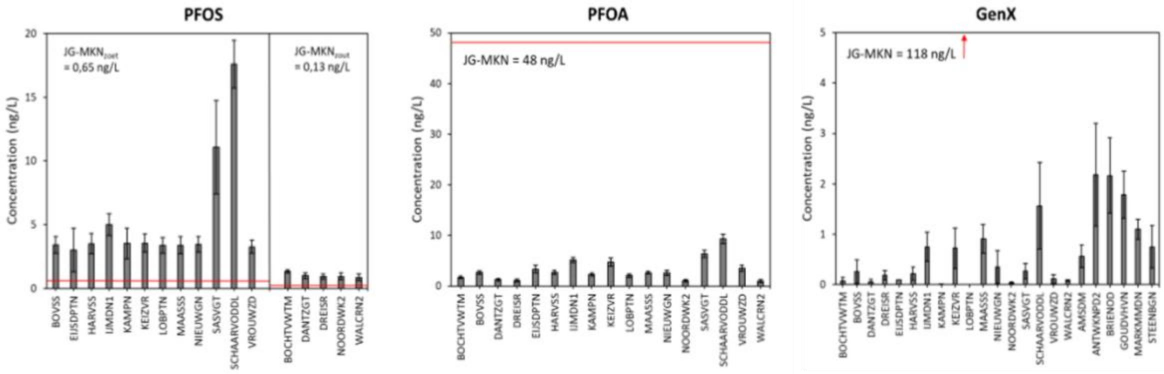


*Figure 18: Concentrations of PFOS (left), PFOA (middle), and GenX (right) in 17 (marine and freshwater) locations in the Netherlands. The norms for PFOS and PFOA are depicted by the red lines, indicating levels of 0.65 ng/L in freshwater and 0.13 ng/L in marine water for PFOS and levels of 48 ng/L for PFOA. The norms for GenX fall outside of the graph but are established at 118 ng/L. From Jonker, 2021.*

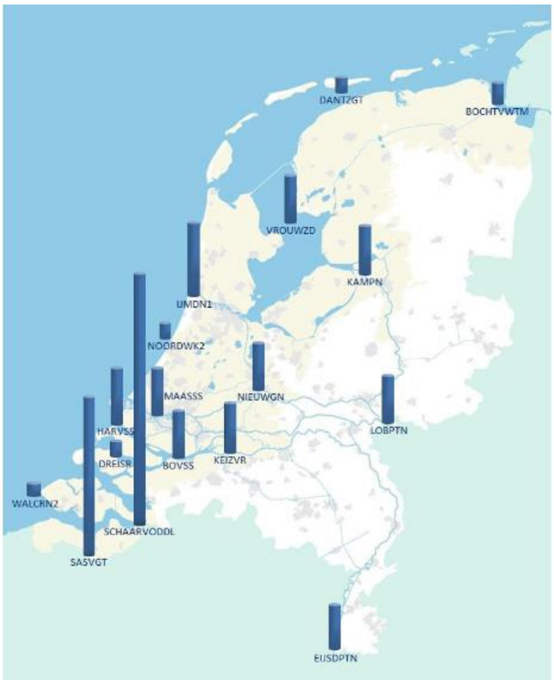


*Figure 19: Average PFOS concentrations in waters in 2020. From Jonker, 2021.*

Total PFAS concentrations of two locations near the Western Scheldt: (1) Schaar van Ouden Doel (Western Scheldt) and (2) Sas van Gent (Ghent-Terneuzen Canal, flowing into the Western Scheldt) range from minimally 67.9 ng/L to maximally 326.2 ng/L (Jonker, 2021). Eschauzier et al. (2014) found total PFAS concentrations in Schaar van Ouden Doel ranging from 389 ng/ L in 2011 and 568 ng/ L in 2012. In both locations, concentrations in the range of 11-18 ng/ L for PFOS were found. Concentrations of PFOS thus exceeded the norm in the Western Scheldt. Both locations are marked as 'hot spots'. This could be indicative of a source nearby, being the 3M factory in Zwijndrecht, Belgium (Figure 19). At these locations, concentrations in the range of 6.4-9.3 ng/ L for PFOA were found, which are not exceeding the norm of 48 ng/ L for PFOA, see Figure 18 where the height of each bar indicates the concentration at the specific location (Jonker, 2021).

The PFOS and PFOA concentrations in the river Scheldt, have decreased by a factor of three from 2012 until 2017 (Figure 20). However, the concentrations of, for instance, PFOS still exceed the norm of 0.65 ng/L in freshwater and 0.13 ng/L in marine waters, sometimes even with a factor of 27 (Jonker, 2021). Moreover, Jonker found that concentrations of PFOS in fish (European flounder) in the Western Scheldt are very high compared to other European lakes, with 140 µg/ kg wet weight.

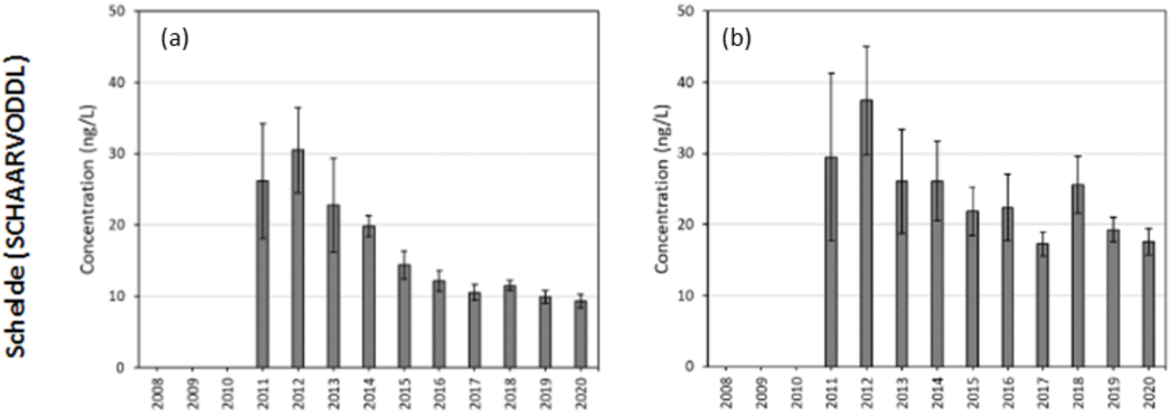


*Figure 20: Concentrations of PFOA (a) and PFOS (b) between 2008 and 2020 in the Scheldt (Location: Schaar van Ouden Doel). From Jonker, 2021.*

### 4.1.2. Lake IJssel

In 2010, Möller and colleagues found total PFAS concentrations of 91.1 ng/L in Lake IJssel (Möller et al., 2010). Eschauzier et al., (2014) found total PFAS values ranging from 93 ng/L in 2010, to 42 ng/L in 2012 in the location Vrouwezand (Lake IJssel). To our knowledge, no total PFAS concentrations for Lake IJssel after 2012 are available. However, according to de Leeuw & Van Donk (2020), Lake IJssel is viewed as relatively free of contaminants (compared to other surface waters); none of the substances that are currently monitored in Lake IJssel have increased in concentration. Significant decreases were even found for heavy metals, dioxins, and also for PFAS in Lake IJssel but this does not mean that PFAS and the other contaminants are not present in the sediment or the biota (de Leeuw & Van Donk, 2020). No exact concentrations were presented, however.

In summary, PFOS exceeds the European norm (0.65 ng/ L) at every measured location in the Netherlands, according to Jonker (2021). PFOA does not exceed the norm (48 ng/ L) in any of these measure locations in the Netherlands.

## 4.2. Bioaccumulation of PFAS in the European eel

Possible consequences of bioaccumulation of toxins in eels are physiological disturbances, lowered resistance, impaired reproduction, and increased mortality (De Meyer et al., 2018). One exposure route of PFAS resulting in bioaccumulation in the eel is bioconcentration, where the accumulation of PFAS results from exposure to PFAS concentration in the water. PFAS concentrations in the water often fluctuate, they are influenced by the solubility of the PFAS, but also by how much is absorbed by the sediment (Jonker, 2021). It is known that concentrations in the water fluctuate more than those in the sediment. After a PFAS spill in waters surrounding Amsterdam, Kwadijk et al. (2014) found that concentrations in the water went back to normal faster than the concentrations in sediment and the biota do. Fluctuations in the PFAS concentration in the sediment occur due to bioturbation, dispersal, or release of PFAS from the sediment (Kwadijk et al., 2014). Kwadijk and colleagues (2010) found that PFOS is the dominant PFAS type found in the European eel samples at different locations in the Netherlands, including locations in the Western Scheldt and Lake IJssel. Other PFAS types, such as PFCs and PFHxS were detected in eels at a ten times lower level. PFBS was detected in eels, but not in water samples, indicating that PFAS is more persistent in the biota than in water (Kwadijk et al., 2010).

The European eel is a benthic species, meaning it resides near the sediment and therefore it is meaningful to take concentrations of PFAS in the sediment into account. The absorption of PFAS to the sediment depends on the length of the perfluorinated chains (Teunen et al., 2021). There is a negative correlation between the chain length and the solubility of PFAS (Jonker, 2021) and a positive correlation between the length of the chains and the sorption of PFAS into the sediment. The hydrophobicity of PFAS increases with increasing chain length, and therefore, PFOS shows the highest accumulation in both biota and sediment (Kwadijk et al., 2014; Mussabek et al., 2019; Jonker, 2021). PFAS tends to bind to particulate organic matter present in the sediment (Mussabek et al., 2019). Therefore, the eel is not only exposed to more soluble PFAS compounds, but also the compound with longer chains.

Generally, the PFAS compounds found most frequently in eels are PFAS with long (eight or more carbon atoms) perfluorinated chains (Zafeiraki et al., 2019). Short-chain PFAS was only scarcely detected, due to the low potential to bioaccumulate (Kwadijk et al., 2010). Kwadijk et al. (2010) discovered that the shorter the fluorinated alkyl chain, the lower the potential of the PFAS to bioaccumulate.

Bioaccumulation can be quantified using bioaccumulation factors (BAFs). BAFs are calculated according to Equation 1. Values greater than 1 can be interpreted as follows: accumulation within the biota is higher than in the surrounding environment. In aquatic ecosystems, it is most common to divide the concentration of contaminant within the organism by the concentration of contaminant found within the water. When sampling over multiple locations pooled BAFs are used (Equation 2). Where BAF is the bioaccumulation factor and $n_n$ is a weighted value to correct for sample size per location.

*Equation 1:*

$$BAF = \frac{[contaminant]in\ organism}{[contaminant]in\ water}$$

*Equation 2:*

$$BAF_{pooled}\frac{(BAF_1n_1) + (BAF_2n_2) + (BAF_3n_3) + (BAF_nn_n)}{n_1n_2n_3n_n}$$

Fish can absorb PFAS through their gills and skin, where it is rapidly taken up, but also depleted, resulting in PFAS levels quickly coming into equilibrium with the aquatic environment (De Meyer et al., 2018; Zafeiraki et al., 2019). Presumably, size (and therefore age) does not influence the bioaccumulation of PFAS in the fillet of the eel (Zafeiraki et al., 2019). On the other hand, Teunen and colleagues (2021) stated that accumulated concentration increase with size and age. A lot of contradicting information exists on the correlation between bioaccumulation and body size. Therefore, it is currently unclear how the body size of the European eel influences the bioaccumulation of PFAS. In the future, bioaccumulation experiments should be conducted to get more precise information.

As the eel is primarily a secondary carnivore (carnivore that feeds on both herbi-detritivores and primary carnivores), another cause of accumulation of PFAS in the eel is biomagnification. Biomagnification is an accumulation of compounds, like PFAS, in the tissues of organisms through the diet (De Meyer et al., 2018). When foraging higher up the food chain, biomagnification can have a larger effect on the organism (De Vos et al, 2008). The major uptake of POPs in this case not through their gills and skin, but their consumption (De Meyer et al, 2018). Therefore, it is not sufficient to solely monitor water and sediment concentrations to estimate the risk of exposure to PFAS in the aquatic environment (Teunen et al., 2021). PFOS has a high affinity for proteins and is not particularly hydrophobic, causing biomagnification through the food chain (Teunen et al., 2021). As a result, PFOS concentrations in top predators (e.g. salmon and tuna) reach high levels, which potentially are harmful not only to the predator but also to humans if they then consume these top predators.

According to De Vos et al. (2008), the European eel has a trophic level of 3.5 based on Veltman et al. (2005), where the third and fourth trophic level is composed of, among others, carnivorous fish. Eels have a high position in the aquatic food chain (Teunen et al., 2021). Using the stable isotope Nitrogen ($\delta15N$) to identify the trophic position, the trophic position of the eel is between 10.2 and 13.0 (De Meyer et al, 2018). Expected is that the diet of the larger eels consists of more animals of a higher trophic level and therefore results in more uptake of PFAS (Belpaire, C., 06-04-2022, personal communication). Thus, the higher the trophic level the higher the PFAS accumulation in the animal, based on biomagnification.

Due to different types of morphology, the diet within the eel species differs. Yellow eels occur in two morphs, eels with a broad head and eels with a narrow head, showing a trophic divergence (De Meyer et al., 2018). Broad-headed eels consume proportionally more and their prey items are from a higher trophic level than narrow-headed eels (De Meyer et al., 2018). The higher the lipophilicity of a POP, the higher the potential for biomagnification in the food chain, resulting in potentially a higher POP concentration in broad-headed eels (De Meyer et al., 2018). POPs have mostly a lipophilic nature. Since most PFAS are not very lipophilic POPs, the accumulation of PFAS can deviate from most other POPs.

In 2006, the fillet of the European eel was sampled and assessed for the occurrence of PFOS/PFOA. The samples were gathered at three locations near Terneuzen in the Western Scheldt. Accumulation of PFOS was shown in the simplified food chain of the Western Scheldt (Van den Heuvel-Greve et al., 2006).

From 44 locations in Belgium, eels were sampled for their PFOS concentration. 58% of the locations were exceeding the European Environmental Biota Quality Standards ($EQS_{biota}$) of WFD, based on a human health threshold of 9.1 µg PFOS/kg ww (Beuthe et al., 2016; Teunen et al., 2021). This indicates there might be potential risks to food webs and consumers (especially top predators) of fish. However, a threshold of 33 µg PFOS/kg ww is determined for the protection of top predators against secondary poisoning, resulting in the exceedance of only 7% of sampling locations for eel (Teunen et al., 2021).

Different PFAS types have been found to bio magnify in eels in Lake IJssel and the Western Scheldt (Zafeiraki et al., 2019). In general, the most frequently detected compounds were PFOS, PFNA, and PFTeDA. The Ghent-Terneuzen Canal (upstream of the Western Scheldt) has the highest levels of concentration of PFOS and the sum of PFAS based on samples collected between 2010 and 2016 (Zafeiraki et al., 2019). The average PFOS concentration found in the Western Scheldt is 55.2 ng/g ww. Samples are taken from 5 different locations in Lake IJssel: Medemblik, Lemmer, Urk, Lelystad and the Ketelbrug. When combining the data from the 5 locations in Lake IJssel, both PFOS and sum of PFAS results in average concentrations respectively 25.5, and 55.86 ng/g ww (Table 2).

*Table 1: Average PFOS and sum of PFAS concentrations in eel in the Western Scheldt and Lake IJssel. From Zafeiraki et al., 2019.*

| PFAS type | Western Scheldt | Lake IJssel | | | | |
|---|---|---|---|---|---|---|
| | Ghent-Terneuzen | Medemblik | Lemmer | Urk | Lelystad | Ketelbrug |
| PFOS (1) | 55.2 | 26.2 | 30.2 | 20.2 | 22.9 | 28.1 |
| PFOA (1) | 0.0 | 0.0 | 0.0 | <0.3 | <0.3 | 0.0 |
| ΣPFAS (1) | 133 | 50.9 | 47.3 | 48.8 | 69.5 | 62.8 |

(1) Concentration in ng/g wet weight.

PFOA was under the detection norm in both Lake IJssel and the Western Scheldt (Zafeiraki et al., 2019). Compared to Lake IJssel, the PFOS and the sum of PFAS concentrations in eel found in the Western Scheldt are 2.6 and 2.4 times higher. Overall, the PFOS concentration in eel in the Dutch rivers was consistent compared to other studies taking place in the Netherlands and surrounding (Kwadijk et al.; 2010, Hölzer et al., 2011; Couderc et al., 2015). Besides, PFOS makes up around half of the total sum of PFAS which is roughly in line with PFAS concentration found in eels in Belgium by Teunen et al. (2021). Closer to the sea, the PFAS concentrations found in eels declined steadily, as a result of the strong dilution due to sea currents and tides (Zafeiraki et al., 2019).

## 4.3. Contamination of the European eels

### 4.3.1. *PFAS ingestion and storage in the European eels*

Contaminants in the environment of the eel can enter the eel via two pathways: (1) an active pathway through the diet of the eel and (2) a passive pathway through water filtration in the gills or absorption through the skin. Regarding PFOS, the uptake pathway is predicted to be similar to slightly hydrophobic compounds (De Vos et al., 2008). From slightly hydrophobic compounds, like short- or medium-chain fatty acids, it is known that they cross cell membranes by diffusion (Kamp & Hamilton, 2006), and thus it is speculated that PFOS will enter the blood circulation of eels in the same manner (De Vos et al., 2008). Since PFOA and PFOS have largely the same structure and characteristics it can be reasonably assumed that PFOA also diffuses across membranes as an uptake pathway. Once in the blood circulation of the eels, PFOS can be stored in different tissues.

A study performed by Giari and colleagues in 2015 assessed PFOS concentrations in different tissues in eels taken from two locations (Comacchio Lagoon and the Po River). They found that the PFOS concentration is the highest in the blood at a level of 3.12 ± 1.20 ng/g ww and muscles had the lowest value of 0.89± 0.58 ng/g ww, liver tissue had a moderate value of 1.75 ± 1.17 ng/g ww (Giari et al., 2015). The values found for PFOA were higher: blood concentrations reached an average of 13.90 ± 23.79 ng/g ww the lowest PFOA concentrations were found in the muscle at 2.11 ± 5.11 ng/g ww, liver tissue had a moderate value of 7.27 ± 15 ng/g ww. The two-fold difference in mean values for PFOA and PFOS concentrations can be explained by large outliers in the dataset since the median values for both compounds support the finding that PFOS is more prevalent in the biota than PFOA. The PFOS median equals on average 1.78 ng/g ww in contrast to an average median PFOA concentration of 0.27 ng/g ww. However, the study also mentioned that when both types of PFAS are present in one individual, PFOA accumulates at higher concentrations than PFOS (Giari et al., 2015). The underlying mechanism, however, is unclear and more research is needed to explain this.

Regarding the life stages of the European Eel, it is speculated that most PFAS accumulation, or biochemical accumulation in general, takes place in the yellow eel phase. The reason for this is that firstly, the yellow eel phase is the longest life stage (spanning over 3 to 15 years) and secondly, in this life stage the eel grows considerably and thereby accumulates large energy reserves (Santillo et al., 2006).

### 4.3.2. *Short- and long-term effects of PFAS in the European eel*

Not much specific information is known regarding the dangers of PFAS-contamination within the European eel. Observational studies have shown high concentrations of PFOS and PFOA within eel caught in Europe (Guhl et al., 2014; Couderc et al., 2015; Zafeiraki, 2019), however, none have been linked directly to increased mortality rates. While no acute toxicity has been documented, deviations in biomarkers (molecules that can be used as an indicator for biological conditions of organisms) and protein markers have been observed (Hoff et al., 2005; Roland et al 2013). Increased serum Alanine Aminotransferase (ALT) concentrations were measured which is a known indicator of liver damage (Hoff et al., 2005). Therefore, it is highly likely that contamination of the liver with PFAS causes liver damage. While the liver damage itself may not be lethal, it does leave the eel in the long-term more vulnerable to other toxins.

Additionally, altered protein expression was observed as a response to PFOS exposure. These altered protein patterns show the effect PFOS exposure has on the eel's stress response, metabolism, and cell signalling but also on the actin cytoskeleton (Roland et al., 2013). The actin cytoskeleton is a dynamic network involved in cell structural support, axonal growth, cell migration, organelle transport, and phagocytosis. Finally, as far as adverse health effects observed within eels go, a significant negative correlation between lipid content and PFAS contamination was observed for eels caught in one location along the Loire in France (Couderc et al., 2015).

In other fish species, PFAA (per and polyfluorinated alkyl acids; PFOA among others) have been shown to induce many types of disruptive behaviour at the molecular level. Through activation of nuclear receptors, induction of reactive oxygen species, hormone disruption or membrane interaction a host of different responses may occur resulting in different types of toxicity. Metabolic, reproductive, and developmental toxicity along with oxidative stress and thyroid disruption are among the different long-term effects observed in various aquatic species (Lee et al., 2020). In zebrafish (*Danio rerio*), a transgenerational study was performed and it was found that PFOS exposure could lead to changes in sex ratio over time shifting to a more female dominated population. Also, a decrease in larval survival and sperm density was found (Wang et al., 2011). Regarding developmental toxicity, PFAS exposure caused growth inhibition and malformation (e.g.: spinal curvature and pericardial oedema) in zebrafish larvae (Zhang et al., 2018). Oxidative stress in zebrafish may result in increased expression of genes involved in uptake of cysteine. Cysteine is an amino acid involved GSH synthesis (Sant et al., 2018). GSH functions as a powerful antioxidant and detoxifier making oxidative stress a self-propagating process that can ultimately lead to cell damage (Hoseinifar, 2020). Lastly, thyroid disruption in zebrafish resulted in alterations at the tissue-level (e.g. pore formation in epithelial cell junctions or vacuole formation in the mitochondria) and thus thyroid functioning resulting in lower whole-body thyroxine hormone (T4) contents (Chen et al., 2018).

Comparison of eel and zebrafish genomes showed a high amount of synteny (conservation of gene location on chromosomes) among bony fish suggesting that the effects contaminants can have on gene expression in both species can be compared as well (Kai et al., 2014). Furthermore, PFAS have been observed to act as agonists or antagonists to other environmental pollutants resulting in various combinatorial toxicological effects.

### 4.3.3. *Health issues related to PFAS contamination in the European eels*

Currently, little is known about health issues in eels related to PFAS contamination. The dose-response relationships are unknown for the European eel. However, studies have been performed on related species like the common carp (*Cyprinus carpio*) and model species like the zebrafish (*Danio rerio*). Common carp is chosen as a related species since both eel and common carp are oily fish (lipid content of around 30% in their fillets). These studies focused on PFOA and PFOS as these two types of PFAS are the most ubiquitous in aquatic environments.

#### Common carp (*Cyprinus carpio*)

One study performed on common carp looked at the toxicological effects of PFOA and PFOS on five biomarkers (DNA single-strand breaks, vitellogenin concentration, activities of 7-ethoxyresorufin-O-deethylase, acetylcholinesterase, and catalase) (Riemann et al., 2010). It is important to look at biomarkers in these types of studies as biomarkers are molecules that are very sensitive to environmental polluters and respond quickly to the absorption of pollutants (Peakall & Walker, 1994). The researchers found that after an exposure period of four days biomarker responses could already be identified. PFOA concentrations of 5,000 µg/L and 10,000 µg/L markedly increased vitellogenin and catalase levels. Vitellogenin is a protein precursor of egg yolk, and it functions as a protein and lipid transporter. It transports protein and lipids from the liver through the blood to the growing oocytes, where it becomes part of the egg yolk (Robinson, 2008). Catalase acts as a protective enzyme to protect cells from oxidative damage by reactive oxygen species (ROS). The enzyme catalyses the decomposition of hydrogen peroxide into water and oxygen. The exposure to PFOS at concentrations of 5,000 µg/L and 10,000 µg/L resulted in a significant increase in DNA-single strand breaks (DSSB). These breaks can result in the loss of a single nucleotide and damage to the 5’- and/or 3’ terminal sites (Caldecott, 2008). Fortunately, these breaks can often be repaired without large consequences (Hedges & Belancio, 2011). However, if DSSB cannot be repaired quickly or correctly it can affect the genetic stability of the cell and thus the cell survival (Caldecott, 2008).

A second study performed on common carp focused on the effects of PFOS on liver tissue (Hagenaars et al., 2008). After fourteen days of exposure to PFOS at concentration levels of 0.1, 0.5, and 1 mg/L effect was seen on gene expression of genes involved in energy metabolism, reproduction, and stress response. Hagenaars and colleagues also found that after these two weeks of exposure the hepatosomatic index, a relevant factor to assess the liver condition, as well as glycogen reserves showed a significant decrease in value. They argue that PFOS contamination in common carp can lead to energy depletion possibly because of lower feed uptake and increased energy usage because of increased detoxification activity in liver tissue. A possible trade-off in energy expenditure is proposed between detoxification activity and processes vital to survival (Hagenaars et al., 2008). As both eel and carp are oily fish, uptake and storing of toxins could follow the same pathways in both species but more research is needed to confirm this.

**Zebrafish (*Danio rerio*)**

An experiment performed on zebrafish embryos found that in the first 72 hours after fertilization the exposure to PFOA at levels ranging from 4.14 mg/L to 133 mg/L resulted in decreased methylation, thus decreased expression, of the *vasa* gene and increased methylation, thus increased expression, of the *vtg1* gene (Bouwmeester et al., 2016). The *vasa* gene is involved in germ cell determination and function. The *vtg1* gene is involved in producing the major egg yolk protein vitellogenin. This protein has the same function in zebrafish as in common carp (Polzonetti-Magni et al., 2004). Blanc and colleagues (2019) looked at the effects of PFOS contamination in zebrafish embryos. Their research found that after 96 hours of exposure to PFOS at a concentration of 17.5 mg/L the expression of the *dnmt3ab* gene is slightly but significantly decreased. The *dnmt3ab* gene provides instructions to make DNA methyltransferase enzymes involved in DNA methylation (Blanc et al., 2019). DNA methylation at one location in the genome can affect the activity of that DNA part. So, through altered DNA methylation resulting from toxic contamination, gene expression can become inhibited causing problems in embryonic development.

### 4.3.4. *Effect of PFAS exposure on the population stability of the European eels*

No data is available on the effects PFAS has on the population stability of eels. However, studies were conducted on other species (Sinclair et al., 2004). This study examined several fish species that live in waters near Michigan and New York, and it was found that eight of these fish species produce eggs that were contaminated with PFOS. The amount of PFOS present ranged between 7.7 - 381 ng/g ww (Sinclair et al., 2004). When eggs of fish are already contaminated with PFOS, this could pose risks to the development of the embryos, as was previously mentioned. For eels, however, more research needs to be done to confirm this.

Other contaminants like PCBs, DDT, or dioxins are expected to pose larger risks to the population stability of eels as these occur at a 20 to 100 times higher concentration (Belpaire, C., 06-04-2022, personal communication). Research has been done on PCBs and DDT and it was found that these contaminants negatively affect lipid contents in eels (Geeraerts et al., 2007, Geeraerts et al., 2010). Decreases in lipid contents can indirectly decrease egg production through impaired gonad maturation

(Henderson and Tocher, 1987). Decreased egg production can then consecutively influence population stability. Dioxin-like contaminants are known to possibly damage eel reproductive organs and affect embryogenesis when they are re-metabolised during migration. As dioxins are lipophilic, they are readily stored in the lipid reserves. During migration to the Sargasso Sea, the lipid reserves are metabolised together with these toxic compounds (Larsson et al., 1990). Through metabolization, the dioxins enter the bloodstream again and are distributed through the body causing potential damage to the reproductive organs. Thereby possibly indirectly affecting population stability in this way. Not much is known about the pathway PFAS takes when they are re-metabolised.

## 4.4. Invasive species exposure

In addition to the risk assessment of the European eel, the project has been extended to other marine and freshwater species. These species are the crayfish and Chinese mitten crab, which are both invasive species and belong to the crustaceans. The risk assessment carried out in this chapter is less extensive than the risk assessment of the European eel, but still covers the most important aspects regarding PFAS exposure to the crayfish and Chinese mitten crab. The uptake of PFAS is discussed, where the PFAS is stored and if any health threats regarding PFAS exposure are known. Besides, location-specific information is reported whenever possible and applicable.

### *4.4.1. Crayfish exposure*

Due to their amphiphilic nature, PFAS are most often encountered within blood, livers, and organs with similar functions (Hoff et al., 2005; Sunderland et al., 2018). In crayfish, filtration and neutralization of chemicals, toxins, and other contaminants are performed by the hepatopancreas before being discharged through the green gland. Analysis of hepatopancreases of crayfish found in Lake Vättern, Sweden, revealed a sum of PFAS concentrations ranging from 18.9 ng/g to 59 ng/g hepatopancreas (Ericson Jogsten & Hyötyläinen, 2020). Another study investigating the PFAS contamination within benthic macroinvertebrates such as crayfish in the Hudson River watershed found PFAS values within the whole crayfish body ranging from 0.50 ng/g ww to 3.53 ng/g ww for PFOS and ranging from 0.17 ng/g ww to 2.38 ng/g ww for PFOA (Brase et al., 2022).

General toxicological effects have yet to be established regarding PFAS contamination within crayfish, however, this present research has shown that PFAS is actively accumulating within crayfish. While acute and chronic health effects in crayfish have not been studied, observational research has shown changes in crayfish behaviour because of PFAS contamination of aquatic ecosystems. At low concentrations, decreased foraging behaviour and increased antipredator sheltering were observed. This change in behaviour at low concentrations makes crayfish potentially a suitable bioindicator for PFAS contamination (Steele et al., 2020). These behavioural changes may not seem that impactful but considering that crayfish are ecosystem engineers they might be more impactful than they seem. Ecosystem engineers are species that modify their surrounding ecosystems in a significant way resulting in the creation of new habitats or the modification of existing ones to suit their needs (Jones et al., 1994). Therefore, changes in their behaviour will significantly affect their surrounding ecosystems. However, further research is required to draw proper conclusions regarding these effects.

Due to the limited data that is available regarding PFAS contamination within crayfish. PFAS within crayfish in Dutch waters has yet to be assessed let alone quantified. It is therefore impossible to make definite statements regarding the PFAS accumulation in crayfish caught in the Netherlands. However, using Eq. 1 & 2 and previously found data, a calculated estimate can be made on the amounts of PFOA and PFOS in Dutch crayfish. Pooled BAFs for PFOA and PFOS were determined in crayfish in the Hudson River watershed (Brase et al., 2022). Together with PFAS concentration in water found in different locations in the Netherlands (Jonker, 2021) Equation 1 can be used to make an estimation. Using these data does require some assumptions, however. While the crayfish species encountered in the Hudson River watershed have become established in the Netherlands and would be a preferred target for consumption it is uncertain whether feeding, bioturbation, antipredator and migratory behaviour are similar. Abiotic factors such as climate, water currents, water depth, and sediment types have also been assumed to be similar. Furthermore, the most recent available data on water contamination stems from 2020 so one could argue it is not completely up to date. Personal correspondence with Dr. Ir. Jonker revealed that more recent data, measured in 2021, will be available in the future. Additionally, he shared the PFOS and PFOA concentrations averaged over the past years (Jonker, 2021). Finally, these calculations assume a linear correlation between environmental PFAS concentrations and bioaccumulation which is highly unlikely (Brase et al., 2022). However, this extrapolation gives the best possible characterization of PFAS contamination in crayfish in the Netherlands based on currently available data.

Estimations for each of the locations assessed by Jonker (2021) are shown in Appendix 3. It is important to note, however, that not all locations actively harbour crayfish. As Figures 13A&B from paragraph 3.2.2. show, crayfish are found almost exclusively inland and mostly in fresh and sometimes brackish water. Table 2 below shows the relevant values for freshwater locations where crayfish are found.

*Table 2: Relevant estimates of PFOA and PFOS concentrations in Dutch crayfish. Appendix 3 contains full data for all locations. From Jonker, 2021.*

| Location | PFOA in water (ng/L) | PFOA in crayfish (ng/g ww) | PFOS in water (ng/L) | PFOS in crayfish (ng/g ww) |
|---|---|---|---|---|
| Nieuwegein | 2.61 | 0.57 ± 0.27 | 3.45 | 2.96 ± 2.89 |
| Vrouwezand | 3.52 | 0.77 ± 0.37 | 3.27 | 2.80 ± 2.74 |
| Kampen | 2.31 | 0.51 ± 0.24 | 3.53 | 3.03 ± 2.96 |
| Eijsden Ponton | 3.36 | 0.74 ± 0.35 | 3.02 | 2.59 ± 2.53 |
| Lobith Ponton | 2.07 | 0.45 ± 0.22 | 3.38 | 2.90 ± 2.83 |
| Keizersveer | 4.75 | 1.04 ± 0.50 | 3.56 | 3.05 ± 2.98 |
| Maassluis | 2.66 | 0.58 ± 0.28 | 3.4 | 2.91 ± 2.85 |

It is tough to conclude from these data regarding the effects on crayfish as no dose-response data is available. However, this data shows that it is likely that the EQS for the sum of PFAS in biota will likely be exceeded. Furthermore, this data can be used to give a preliminary view of the current state of PFOA and PFOS contamination in Dutch crayfish as a food source. This is further assessed in chapter 5.3.2.

### *4.4.2. Chinese mitten crab exposure*

Chemical pollutants can pose potential dangers to the health of the Chinese mitten crab as well. Crustaceans, that crabs belong to, take up contaminants mainly through their food. The hepatopancreas is responsible for the absorption of nutrients. Therefore, it makes sense that a large part of PFAS is accumulated in this organ, which corresponds to results found in research (Hoogenboom et al., 2015; Brust et al., 2016).

The hepatopancreas is part of the brown meat component of the crab, just as the gonads and the body. The appendages (claws), legs, and other parts of the body are part of the white meat component. White meat is predominantly muscle meat, so it is high in protein and low in fat. The brown meat contains a high fat percentage. Several studies showed that levels of contaminants are higher in brown meat than in white meat (BuRO, 2019). These investigators looked at dioxins, PCB, and PCDD/F levels in waters in the Netherlands and UK. The reason that most pollutants are stored in brown meat remains unclear. Possible reasons are the high vascularity in the brown meat or the fat content. It is known that contaminants stored in adipose tissue get released during catabolism, for instance during spawning, or parental care periods (Van den Brink et al., 1998).

It has been observed that high PFAS concentrations can lead to immunotoxicity in the Chinese mitten crab (Zhang et al., 2015). This could impair the high survival rate of this crab. To be able to better map the PFAS toxicity and perform a solid risk assessment for several fish species, some biomarkers can be used. For the Chinese mitten crab, some recommended biomarkers are phenoloxidase, acid phosphatase, and hemocyte counts (Zhang et al., 2015).

# Summary Chapter 4

### ***The European eel*** (*Anguilla anguilla*)

The major exposure route to PFAS for eels is biomagnification, meaning that uptake of these contaminants goes through their diet rather than via passive absorption through skin or gills, which would be called bioconcentration. Recent values of surface water in the Western Scheldt exceeded EQS norms of 0.65 ng/L water, no recent data for the sum of PFAS concentrations is found on Lake IJssel, the most recent data found was from 2012 and that was below the EQS norm. It has been indicated that PFAS is more persistent in the biota than in water. A sampling of eels in Western Scheldt and Lake IJssel showed that PFOS was the most dominant PFAS type found in the European eel samples at different locations in the Netherlands. Since the water levels of the Western Scheldt exceeded EQS norms and PFAS are more persistent in biota than in the water, it is safe to say that the total concentration of PFOS in biota in the Western Scheldt also exceeds the European norm of 9.1 µg/ kg body weight set out for all biota. Since a strict PFOS norm specifically for eels does not yet exist, but eel is regarded as being part of biota, it can be assumed that the EQS norm is also exceeded for eels in Lake IJssel. Concentrations of PFAS in fish in the Western Scheldt are very high compared to other European lakes but the concentrations found in fish in Lake IJssel are comparable to those of other European lakes.

Most PFAS accumulation takes place in the yellow eel life stage. If PFAS is taken up by the eel, then the PFAS is stored in many types of cells, muscle cells, blood cells, and liver cells. However, the concentration differs between all these types of cells, the highest concentration was found in blood cells and the lowest in muscle cells. The storage of PFAS in eel could potentially bring some health dangers to the eel. A potential health effect of PFAS exposure in eels is liver damage. This was observed in a study regarding eels, Common Carp, and zebrafish. No direct evidence has been found for other health effects in eels based on published studies that used eels as target species. However, in related fish species, it has been shown that PFOS influences embryonic development. In eels, there has not been any research on the influence of PFAS on population stability.

### ***Crayfish and Chinese mitten crab*** *(Astacoidea spp. & Parastacoidea spp. and Eriocheir sinensis)*

The hepatopancreas is an important organ in both the crayfish and Chinese mitten crab and it is responsible for filtration, digestion, and neutralization of both chemicals and food. A large part of PFAS is accumulated in this organ in both species. For the Chinese mitten crab, immunotoxicity has been observed as a health effect related to PFAS exposure, which could potentially cause a threat to the population stability of this crab. In contrast with the Chinese mitten crab, no studies have been published on the general toxicological, acute, and chronic health effects regarding PFAS contamination in crayfish. However, observational research has shown changes in crayfish behaviour because of PFAS contamination in aquatic ecosystems. No definitive conclusion can be drawn about the impact of PFAS on crayfish and mitten crab in the Netherlands due to a lack of dose-response data. However, a rough estimation has been made of PFOA and PFOS concentrations in Dutch crayfish by extrapolation.

# 5. Human risk assessment

Eel, crayfish, and crab all get consumed by the general population. If a high concentration of PFAS is present in these marine species, this could also elevate PFAS concentrations in humans if they consume these fish species. Therefore, this chapter must first be investigated if PFAS contamination poses a health threat to humans by searching for the potential short- and long-term dangers of PFAS. Especially the health effects that are already observed in either model animal species or epidemiological studies can give clear indications for potential health effects. Then, the next step is to identify at what PFAS exposure level these health effects could occur. This is also known as the dose-response part of a risk assessment. Finally, it has been assessed for the eel, crayfish, and Chinese mitten crab how often humans could consume these species without exceeding their tolerable intake.

## 5.1. Potential dangers of PFAS contamination for human health

PFAS could be hazardous to human health. Just as for other chemical substances, two important aspects need to be considered when estimating the potential dangers of PFAS contamination to humans. The first aspect includes the severity of the effects on human health. From a toxicological point of view, there are acute health effects and chronic health effects, which are based on lifelong exposure. Examples of health effects with a significantly large impact are mortality, fertility, and immobilization. The second aspect includes health effects that are supported by science the most and are therefore the most reliable. The severity of these two aspects determines the level of danger PFAS have to humans.

There has been an ongoing investigation by diverse institutions into the risk of PFAS on human health for several years. Thorough measurements of blood and semen samples of people in developed countries revealed that PFAS is present in measurable concentrations in the general population, at levels that could potentially cause severe health effects (Stubleski et al., 2016; Cakmak et al., 2022). The list of potentially harmful effects keeps increasing every year and more evidence is appearing in the literature to support the already reported effects. A summary of the potential dangers of PFAS contamination in humans is illustrated in Figure 21 (Fenton et al., 2021).

Not all the different PFAS substances have been undergoing testing for toxicological and health effects. The ones that have been tested are mainly tested in animals, but there are large differences in toxicodynamic and toxicokinetic factors between animals and humans. This results in large uncertainties in the interpretation of the effects of low PFAS concentrations on human health. This knowledge gap is slowly being tackled by an increase in epidemiological research. In this type of research, human populations are monitored for several years in areas where PFAS is a known problem and the occurred health effects are compared to national population data. From the existing knowledge, it is discovered that the toxicities of PFAS in humans potentially include neurotoxicity, developmental toxicity, carcinogenicity, and immunotoxicity (Sunderland et al., 2018). A few of the most concerning health effects are further discussed in detail below.

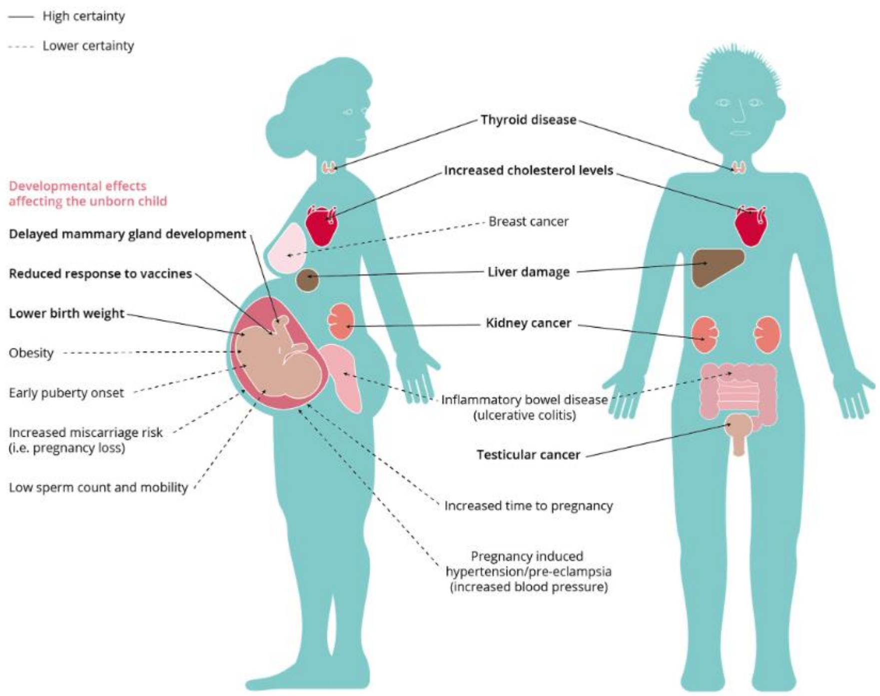


*Figure 21: Potential PFAS effects on human health. Reprinted from Fenton et al., 2021.*

### Cancer

A frequently reported health effect of PFAS that could have a potentially great impact on human health is cancer. PFAS exposure could increase the risk of kidney, liver, and testicular cancer (Massoud & Charlton, 2018; Stanifer et al., 2018; Program, 2020). There is an ongoing debate among several institutes on whether PFAS exposure can be linked to the occurrence of cancer in humans. A study revealed that DNA damage can be induced by PFAS exposure, but only at concentrations that are relatively high compared to the average exposure of PFAS in humans (Schrenk et al., 2020). Due to this high level of PFAS dose that is needed before DNA damage occurs, PFAS is unlikely to cause cancer in the general population, hence, the EFSA states that PFAS is not of mutagenic concern at this point (Schrenk et al., 2020). However, a study focused on the population from Veneto in Italy showed preliminary suggestions of increased kidney cancer mortality due to exposure to PFAS (Mastrantonio et al., 2018). A higher risk of several types of cancer such as thyroid cancer was also found in the population of Merrimack in the United States (Messmer et al., 2022). Furthermore, several researchers concluded that there is scientific evidence for a link between PFOA and kidney and testicular cancer (Zodrow et al., 2022). Thus, there is an indication that PFAS results in a higher risk of cancer in humans at certain concentrations, but there is still no definitive agreement on the carcinogenic risk for the general population due to uncertainty in the level of exposure.

### Fertility, embryonic development, and pregnancy

Different PFAS concentrations have been measured in the blood serum of men and women belonging to the same community (Daly et al., 2018). This is because PFAS is being eliminated through the blood during menstruation as a supplementary way of excretion, which can result in higher or lower excretion levels, depending on the use of birth control-related medicine. PFAS has also been measured in the blood serum of children and infants, sometimes even at higher elevated levels than adults, for which the reason could be the way of intake and body mass (Graber et al., 2019). Concentrations were detected in the plasma samples, but also breast milk and the blood of the umbilical cord. This means that PFAS is transferred during the pre-and postnatal period, during either breastfeeding or transplacental transfer (Emmett et al., 2006; Blake et al., 2020). Some studies of PFAS related to reproductive toxicity have been conducted in mice, where for example high doses decreased the fecundity (Fei et al., 2009).

Pregnancy issues have been mainly based on reduced birth weight measurements in human populations. However, the inferences that can be made from these studies on the association between PFAS exposure and developmental and pregnancy issues are often weak. This is because the glomerular filtration rate and plasma blood volume expansion differ greatly and have a large influence on birth weight during pregnancy (Zodrow et al., 2022). Other problems during pregnancy, such as birth defects, miscarriages, and preterm births, have not been researched much and are only able to provide small indications of PFAS dangers. There has only been some epidemiological evidence found on pregnancy-induced hypertension (Zodrow et al., 2022). Research has been done on semen samples of men to investigate the potential dangers of PFAS on semen quality. These studies gave reasonable indications that PFAS potentially lowers sperm concentration and motility, thereby decreasing the semen overall quality (Joensen et al., 2009; Song et al., 2018; Pan et al., 2019).

### Immunotoxicity and cholesterol

Other health effects that are of actual concern for the general population, but with a less direct impact on human life are elevated cholesterol levels and an impaired immune system. Longitudinal and cross-sectional studies of several populations indicated increased low-density lipoprotein levels and serum total cholesterol levels associated with PFAS exposure, both in children and adults (Steenland et al., 2009; He et al., 2018; Dong et al., 2019; Lin et al., 2019). Even a low-PFAS dose has been recorded to increase the risk of high cholesterol levels in conducted risk assessments (Gleason et al., 2017; Li et al., 2020). According to a pharmacokinetic model, a rise of more than 20% in serum cholesterol concentrations is to be expected in about half of the PFAS-exposed population (Chou & Lin, 2020). Overall, there is strong evidence for a statistically significant association between high cholesterol levels and PFAS exposure, which was also concluded by a group of experts (Zodrow et al., 2022).

Impairment of the immune system is indicated as the most sensitive effect of PFAS dangers in humans, according to several institutes and researchers (MADEP, 2019; NHDES, 2019). The effect is regarded as sensitive since impairment occurs at very low doses already and in sensitive populations, the effect is also relevant for (young) children (Den Braver, M., 14-04-2022, personal communication). The health effects on the immune system can be measured directly through parameters like responses of antibodies to T-cell-dependent antigens or with more indirect parameters like the total immunoglobin levels, interleukins expression levels, or changes in the weights of lymphoid organs (Schrenk et al., 2020). A decrease in the functionality of the immune system can reduce resistance to tumors, infectious diseases, and infectious agents in vaccines (World Health Organization, 2019; Looker et al., 2014; National Toxicology Program, 2016). Thus, a relatively large amount of research shows that PFAS has immunosuppressive potential. It must also be mentioned that a decreased response to vaccinations is not regarded as a disease, but as a risk for a disease, which decreases the direct effect of PFAS contamination on human health.

## 5.2. Dose assessment for human health

PFAS contamination brings some potential dangers to humans, as discussed before. The consequences of the PFAS-exposure depend on the duration, the route, and the magnitude of the exposure. The probable health effects depend on the sex, age, health status, genetics, and ethnicity of the exposed individual (Schrenk et al., 2020). That is why it is difficult, but important, to determine when exposure levels of PFAS are likely to result in health effects for the general population. The European reference laboratory is debating with the European Commission on maximum limit (ML) values for PFAS in food. This is difficult, if the ML is set too low, the current analytical methods cannot measure all food samples because the methods are not sensitive enough (Den Braver, M., 14-04-2022, personal communication). The PFAS concentrations cannot be measured at a sufficient level with a low ML and all investigations will report a conclusion lower than the detection limit, which is not feasible for a risk assessment. On the other hand, if the ML is set too high, there is the danger of disregarding significant health risks.

For humans, extrapolation of dose-response relationships from, for instance, mice often occurs (Andersen et al., 2021). Dose-response relationships describe how an organism responds to a certain stimulus or stressor. These relationships are usually established in a laboratory setting. Dose-response curves have been established for multiple PFAS substances (Canova et al., 2020; Goodrum et al., 2021). If several components have a similar dose-response curve, relative potency factors could be potentially derived. Relative potency factors are correction factors, they predict the risk resulting from exposure to a mixture of compounds by concentration addition data (Den Braver, M., 14-04-2022, personal communication). There have been efforts to derive relative potency factors for the different PFAS (Zeilmaker et al., 2018; Bil et al., 2021). However, a debate is going on whether it is appropriate to apply relative potency factors to PFAS substances since it is uncertain if there is sufficient research available to apply them. Sensitive effects, disease endpoints, and target organ potencies could differ between PFAS (Goodrum et al., 2021). Without the use of relative potency factors, due to some missing data, it is assumed that all PFAS are equally potent for the critical effect which also seems unlikely.

The EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel) has evaluated 27 PFAS in food and investigated the relation to human health. Some established health effects of PFAS exposure, like immune system impairments, were used to determine a tolerable weekly intake (TWI) (Schrenk et al., 2020). They decided in 2018 that the TWI for PFOS was 13 ng/kg body weight (bw) per week and for PFOA 6 ng/kg bw per week (Knutsen et al., 2018). These numbers were reviewed in 2020 again by the EFSA CONTAM Panel and were significantly decreased based on more recently published research.

The accumulation and long half-lives of the PFAS were given greater weight in the determination of a low-risk intake level. The inclusion of the half-life of PFAS was only done recently because a half-life determination of these substances appears to be difficult to research. The half-life of PFAS in model animal species like mice has a range of weeks maximally. In humans, the half-life for most PFAS is in the range of years (Zhang et al., 2013; Dourson et al., 2019; ATSDR, 2021). Therefore, the determination of an exposure level at which PFAS causes adverse health effects in humans and the establishment of a half-life for all PFAS compounds in humans is still an ongoing challenge (Li et al., 2018). Nevertheless, the EFSA CONTAM Panel decided on a TWI of 4.4 ng/kg bw per week for the sum of PFHxS, PFNA, PFOA and PFOS (Schrenk et al., 2020). This number was based on an estimated daily intake of 0.63 ng/kg bw per day, following a study where serum levels of mothers were measured, and a model was created for infants that received breastfeeding for approximately 12 months. Thus, the TWI was based on the maximum toleration of infants. Since infants are considered the most sensitive and vulnerable group of the population, no additional uncertainty factors were required to investigate. This makes the TWI a straightforward measure to use because differences between individuals in toxicodynamic and toxicokinetic factors do not need to be considered. The EFSA CONTAM Panel concluded that this TWI is efficient for all the critical points that were regarded as important in 2018 (Knutsen et al., 2018). These points were: reduced birth weight increased cholesterol and liver enzyme (ALT) blood serum levels. Additionally, this TWI prevents the serum levels of the mother reach a concentration that would result in decreased immune efficacy in infants.

The average lower bound exposure of several adolescents and the elderly have been investigated by the EFSA. A weekly average of 3 to 22 ng/kg bw PFAS exposure was observed. For children and infants, the mean exposure average was two times higher (Schrenk et al., 2020). The highest weekly mean average of adults, 22 ng/kg bw, is five times higher than the determined TWI. This is a concerning discovery because adverse health effects on humans can occur if the TWI is exceeded for a longer period.

## 5.3. Consumption of species of interest

### *5.3.1. Safe consumption levels of the European eel*

PFAS is being taken up by humans through several polluted sources in their environment. The exposure to PFAS differs for the sub-populations of society, for example, factory workers or fishermen are expected to have elevated PFAS exposure levels concerning the general population. The most acknowledged and relevant exposure routes of PFAS for humans are dust and air inhalation, dermal absorption, and ingestion of drinking water and food (De Silva et al., 2021). For the general population, the ingestion of drinking water and food is seen as the major pathway of contamination, the dust, and dermal adsorption intake pathways are more concerning for specific sub-populations (Kaiser et al., 2010; Post et al., 2012; Franko et al., 2012; Augustsson et al., 2021). When PFAS enters the human body, it travels via the blood and then follows several distributions and elimination routes. For example, it gets absorbed by transport proteins that have a high affinity for these substances. Then, it gets stored in either the kidney, the liver, or the blood plasma covalently bound to albumin (Taylor et al., 2017; Schrenk et al., 2020). Since PFAS is mostly stored in these places, most well-known health effects are related to these organs. PFAS can be eliminated again through the urine, menstrual blood, or bile. However, the overall absorption of PFAS is higher than the elimination because of a high occurrence of renal and biliary reabsorption (Schrenk et al., 2020).

**Eel landings**

To assess the human exposure to PFAS through the diet, specifically through eel consumption, it is important to have numbers on how much eel is caught. In the Netherlands in 2018, 461 tonnes of European eel were caught. Preliminary data for 2019 states that 484 tonnes of eel were caught (Bryhn et al., 2021). Additionally, Van der Hammen et al. (2021) show the average yearly catches in tonnes (Table 3). It is shown that after the European eel was characterized as critically endangered in 2008 (Crook & Gollock, 2020), the catches plummeted from 1,005 tonnes from 2006 to 2008, to 485 tonnes in the next three years.

*Table 3: Average yearly freshwater catches in tonnes for both Yellow-, and Silver eels, as well as for recreational and commercial fisheries. From Van der Hammen, 2021.*

| | Commercial | | | Recreational | Total |
|---|---|---|---|---|---|
| Period | Silver eel | Yellow eel | Total | Yellow eel | Commercial + Recreational |
| 2006-2008 | 280 | 525 | 805 | 200 | 1,005 |
| 2009-2011 | 174 | 234 | 410 | 75 | 485 |
| 2012-2014 | 140 | 187 | 327 | 36 | 363 |
| 2015-2017 | 143 | 191 | 334 | 10 | 344 |
| 2018-2020 | 201 | 268 | 469 | 10 | 479 |

### Consumption

Currently, there are no maximum levels for PFAS in food (Zafeiraki et al., 2019), but there are Environmental Quality Standards (EQSs) in the WFD. For only one PFAS type there is a norm for biota living in the surface water, which also partly considers human safety. For instance, for PFOS the EQS is 9.1 µg/ kg wet weight in biota, and this value is based on an outdated Tolerable Daily Intake (TDI) set by the EFSA, which was 150 ng/kg body weight per day, for a person of 70 kg eating 115 g eel per day (Jonker, 2021). According to Zafeiraki and colleagues (2019), 93% of the eel samples exceed this EQS for PFOS, especially eels sampled near the Western Scheldt, reaching approximately seven times the EQS.

According to the research of the Working Group on Eel of ICES, contamination levels of many contaminants, among which PFOS, exceed the human consumption standards in many countries (Geeraerts & Belpaire, 2010). In Flanders, there is a catch-and-release obligation in contaminated regions, issued because the concentrations of PCBs in eels were exceeding the TWI (Goemans & Belpaire, 2004). Bilau et al. (2007) studied the intake of PCBs via the consumption of eels by Flemish recreational fishermen that caught eels for consumption. They also compared this intake to the intake of the rest of the Flemish population. They modelled the intake of eel consumption by combining the distribution of eel consumption and the distribution of PCB contamination in Flanders in a probabilistic model. They observed that 7.2% of recreational fishermen consumed their caught eel and that 11.2% of the Flemish population consume eel. The fishermen taking their caught eel home, eat 498 g of eel per week, maximally.

A similar assessment was done by Teunen et al. (2021) for PFOS and PFOA. The mean consumption in Flemish recreational anglers was 18 g of eel (ANB-VF/2015/4, 2016). When using the TWI EFSA advises, the maximum amount of eel consumed without health risk was exceeded. Additionally, when viewing a worst-case scenario, with the highest found concentrations for PFOS and PFOA combined, the maximum edible amount of eel was 2.85 g/day. Flemish fishermen are thus likely to experience negative effects from accumulating PFAS in their bodies. It should be considered that mean consumption levels were calculated, and that there are thus fishermen that consume even more, and thus have an even higher risk. We are not aware of similar probabilistic models for eel consumption in the Dutch population.

It also must be considered that PFAS enters the human body via many routes, as stated, for instance, via drinking water or plastic packaging (Curtzwiler, 2021). This means that the uptake of PFAS is possibly underestimated when only the intake through fish species is taken into account. Even if the PFAS concentration in eel would not exceed the Minimal Risk Level (MRL), the general human population can still have an intake of PFAS above the tolerable weekly intake. This could result in a misinterpretation and underestimation of the dangers for humans in terms of health effects.

In eels growing up in the Lake IJssel near Urk, the PFOS concentration was found to be 19 ng/ gram body weight, which equals 19 µg/ kg body weight, and near Medemblik it was 24 ng/g body weight which equals 24 µg/ kg body weight (Leenders et al., 2020). Comparing these values to the TWI of 4.4 ng/kg bw set by the EFSA we find that a person of 70 kg eating 115 g of eel will exceed the TWI set by over seven times when consuming eel caught near Urk and by over nine times for eel caught near Medemblik when only considering the levels of PFOS. Currently, we cannot say how much of the sum of PFAS concentration measured by Zafeiraki et al (2019) is due to PFNA and PFHxS so it is tough to make sound conclusions using the data in Table 1. However, as the sum of PFAS concentrations found for the different locations are composed of much more than only PFOS it is likely that current and future TWI's set for PFAS will be exceeded by even more considerable margins.

### 5.3.2. *Safe consumption levels of crayfish*

Currently, fishing on crayfish in the Netherlands is mostly localized to the provinces of Utrecht, South Holland, Friesland, and the North of Flevoland. Yearly, approximately 30 to 40 tons of crayfish are caught and sold at markets, wholesalers, and restaurants. Figure 22 shows the distribution of crayfish fisheries and sellers in the Netherlands. Consumers can also purchase crayfish at supermarkets. However, these crayfish are farmed and imported from China and are not recognized as a sustainable food source.

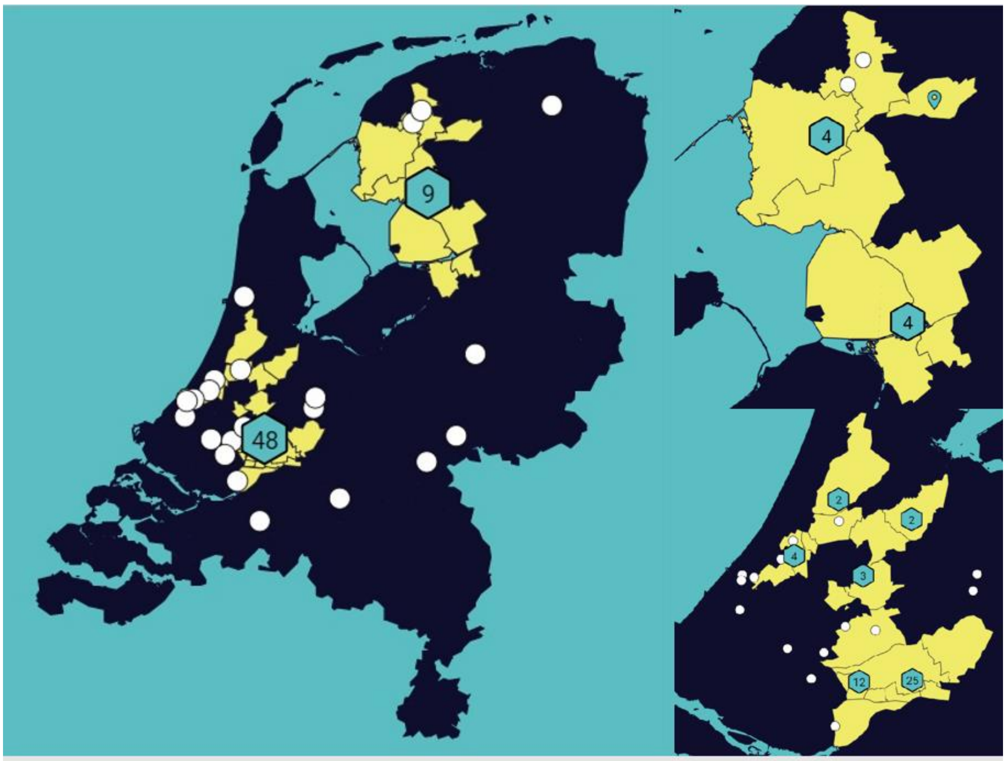


*Figure 22: Number (turquoise) and location (yellow) of crayfish fisheries and location of crayfish sellers (white dots) in the Netherlands. From Good fish, n.d.*

Based on this data there are three locations assessed in Jonker (2021) that are relevant for caught and consumed crayfish. Therefore, the earlier estimated PFOA and PFOS concentrations in crayfish for Kampen, Vrouwezand, and Nieuwegein (Table 4 & Appendix 3) were used in the following assessment. Calculations were made based on an average crayfish meal size of 0.1 kg for an average person of 75 kg using TWI's of 6 ng/kg bw for PFOA and 13 ng/kg bw for PFOS. In the calculation, a distinction between best-case, worst-case and average scenarios is made corresponding to the range in each of the estimated concentrations. The results of these calculations are shown in Table 4. To better illustrate these calculations an example calculation is written below for the PFOA concentration estimated for Nieuwegein.

For crayfish caught in Nieuwegein, a PFOA concentration of 0.57 ± 0.27 ng/g ww was estimated. In this case, the best-case scenario would then be 0.30 ng/g ww as a starting point and the worst-case scenario corresponds to 0.84 ng/g ww. The following calculation was then performed:

*Equation 3:*

$$\frac{(Estimated\ concentration\ (ng \cdot kg^{-1}) \cdot 0.1\ kg)}{75\ kg} = \text{Intake based on average crayfish consumption}$$

For PFOA in Nieuwegein, this intake corresponds to 0.4 ng/kg bw in the best-case scenario, 0.76 ng/kg in the average-case scenario, and 1.12 ng/kg bw in the worst-case scenario. This data can then be compared to the TWI's to get a rough estimate of how often one could consume crayfish from this area.

*Table 4: Intake values based on the calculated estimates for PFOA and PFOS concentrations in crayfish in areas with active crayfish fishery. Calculations were based on the assumption of the average consumption of 100 g of crayfish by an average person (75 kg). From Jonker (2021).*

| **Location** | **PFOA (ng/kg bw)** | | | **PFOS (ng/kg bw)** | | |
|---|---|---|---|---|---|---|
| | Worst-case | Average-case | Best-case | Worst-case | Average-case | Best-case |
| Nieuwegein | 1.12 | 0.76 | 0.40 | 7.80 | 3.95 | 0.09 |
| Vrouwezand | 1.52 | 1.03 | 0.53 | 7.39 | 3.73 | 0.09 |
| Kampen | 1 | 0.68 | 0.36 | 7.99 | 4.04 | 0.09 |
| Average | 1.21 | 0.82 | 0.43 | 7.72 | 3.91 | 0.09 |

Comparing these data to the 2018 TWI's set for PFOA (6 ng/kg bw) and PFOS (13 ng/kg bw) it is not likely that consumption of one average crayfish meal will result in an exceedance of these TWI's. However, the most recent EFSA guidelines have set the TWI for the sum of PFHxS, PFOA, PFOS, and PFNA at 4.4 ng/kg bw. This TWI would be exceeded with the average case already (0.82 + 3.91 = 4.73). It is once again important to note that these calculations are heavily subject to assumptions discussed previously. Additionally, this final calculation is only based on an average meal which has been set at 100 g of crayfish. Higher accounts of intake are known in Louisiana, United States, where crayfish boils are a delicacy, with intakes reported reaching 300 to 500 g (LA crawfish, 2022). Furthermore, it is very important to note that the pooled BAF used in this calculation has a very large standard deviation resulting in an inaccurate estimation. This pooled BAF is based on three data points with one stark outlier resulting in a high pooled BAF and a large standard deviation (Brase et al., 2022). A quick calculation excluding the outlier resulted in values for PFOS ranging from 0.8 ng/kg in the best case to 2.0 ng/kg bw in the worst case. This calculation together with the outlier only further emphasizes the need for actual measurements of PFAS concentrations within Dutch crayfish. Therefore, as of now, it is impossible to conclude that the concentration of PFAS in crayfish will be too high for human consumption. However, based on these calculations the concentrations are expected to lie somewhere around the set TWI's. Inclusion of the other two PFAS molecules PFNA and PFHxS in this assessment will most likely result in exceedance of the TWI, however, these conclusions can only be drawn when more detailed data is available.

### *5.3.3. Safe consumption levels of mitten crab*

The fishing season of the Chinese mitten crab in the Netherlands spans from September to November. An average mitten crab meal consists of four to five crabs, and the average consumer eats Chinese mitten crab once every two weeks. Only a low amount (around 18%) of crab consumers stated that they also consume the crab outside of its catching season (BuRO, 2019). For toxic equivalency calculations, it has been assumed that the brown meat of crabs is not regularly consumed. For example, for the Dutch regulation (EG) nr. 1881/2006, only maximum consumable levels of white meat for the Chinese mitten crab have been established, not for the brown meat (BuRO, 2019). However, for the Chinese mitten crab, the assumption that brown meat is not eaten is incorrect because the body of the crab contains both white and brown meat. No studies were found regarding accumulation values of PFAS in crabs, making a more elaborate risk assessment infeasible.

## Summary Chapter 5

Not all the diverse PFAS substances have been undergoing testing for human toxicological and health effects, most research has only been focused on the most commonly present compounds instead of the sum of all PFAS. Besides, the hazards regarding PFAS contamination concerning the dangers of for example dioxins have not been researched yet, making it impossible to estimate the total health effects of several contaminants groups accumulating in humans.

What is known, is that PFAS in humans gets stored in either the kidney, the liver, or the blood plasma. Therefore, much exposure research investigated potential diseases related to these organs. There is strong evidence for a statistically significant association between high cholesterol and liver enzyme levels and PFAS exposure. Furthermore, a relatively large amount of research shows that PFAS has immunosuppressive potential. Impairments of the immune system are indicated as the most sensitive effect of PFAS dangers in humans. These health effects are found to be interspecific. Liver damage was found both in eels and humans, and immunotoxicity was encountered in both Chinese mitten crab and humans. Cancer has only been researched as a potential danger in humans, but it is thought to also be a potential risk for other species that are exposed to PFAS. There is a strong indication that PFAS results in a higher risk of cancer in humans at certain concentrations, but there is still no definitive agreement on the carcinogenic risk for the general population due to uncertainty in the level of exposure. A health effect that has been solely focused on humans is fertility. Studies gave reasonable indications that PFAS potentially lowers sperm concentration and motility, thereby decreasing the semen quality. But the association that can be made between developmental and pregnancy issues and PFAS exposure is too weak to base any conclusions on.

When focusing on the consumption risk for humans it was found that there are no maximum levels for PFAS in food established yet. What has been decided upon is a tolerable weekly intake of 4.4 ng/kg body weight per week for the sum of PFHxS, PFNA, PFOA, and PFOS by the EFSA CONTAM panel. Concerningly, it has been observed that the highest average weekly PFAS exposure for adults is five times higher than the determined TWI. When using the EFSA TWI, the maximum amount of PFAS uptake was exceeded for eel consumption. A preliminary assessment of Dutch crayfish showed that consumption of one average crayfish meal will be very close to the TWI, but it is impossible to make solid conclusions due to a lack of data. The same applies to the Chinese mitten crab, no solid risk assessment could be performed since no data regarding contamination levels was available.

# 6. Conclusion

The goal of this report was to investigate whether the levels of PFAS contamination within Lake IJssel and the Western Scheldt pose risks for three aquatic species; the European eel and the invasive species crayfish and Chinese mitten crab. Furthermore, the risks associated with the consumption of these contaminated species were assessed regarding human health.

First, the current state of PFAS pollution in the Western Scheldt and Lake IJssel was assessed. The sum of PFAS data was only available for the Western Scheldt and it was found to exceed the EQS norm of 0.65 ng/L water. For Lake IJssel, data was unavailable after 2012 other than PFOS and PFOA data. In 2012 the PFAS levels did not exceed the EQS norm. While PFOA did not exceed the norms anywhere in the Netherlands PFOS was found to exceed the norms all over the Netherlands.

Next, the risks for the European eel were assessed. No specific literature was found regarding health effects due to PFAS contamination within the European eel. Available data on PFAS contamination showed that PFAS readily accumulates within the eels during the yellow eel stage and is stored in various types of cells such as muscle cells, blood cells, and liver cells but was found to be most prominent in the blood. While no acute health effects are known, liver damage was suggested based on encountered biomarkers. Available PFAS levels within the eel and the PFAS levels within the locations suggest that the eels in Lake IJssel and the Western Scheldt will most likely exceed the European norm of 9.1 μg/kg bw set out for biota. Based on our literature research and interviews with researchers it is most likely that PFAS is not the main contributor to the endangered state of the eel. While it should never be forgotten and can play a part in the endangerment of the eel, it would be best to focus the preservation attention on other more pressing issues such as habitat accessibility and connectivity, overfishing, and other POPs such as dioxins and PCBs.

The assessment of the crayfish was not as straightforward. To make a proper risk assessment a lot of new data is needed. PFAS was found to be stored mostly within the blood and the hepatopancreas but based on available data and research no direct health effects are known. Observation of behavioural changes in response to PFAS pollution suggests PFAS could affect the crayfish but apart from the consequential environmental effects, no conclusive evidence is available. Using available BAF data from the Hudson River watershed, estimates were made for the PFOA and PFOS concentrations within Dutch crayfish which were found to be lower than the European norms set for biota. To make conclusions about the effects on the crayfish, more data and research is needed.

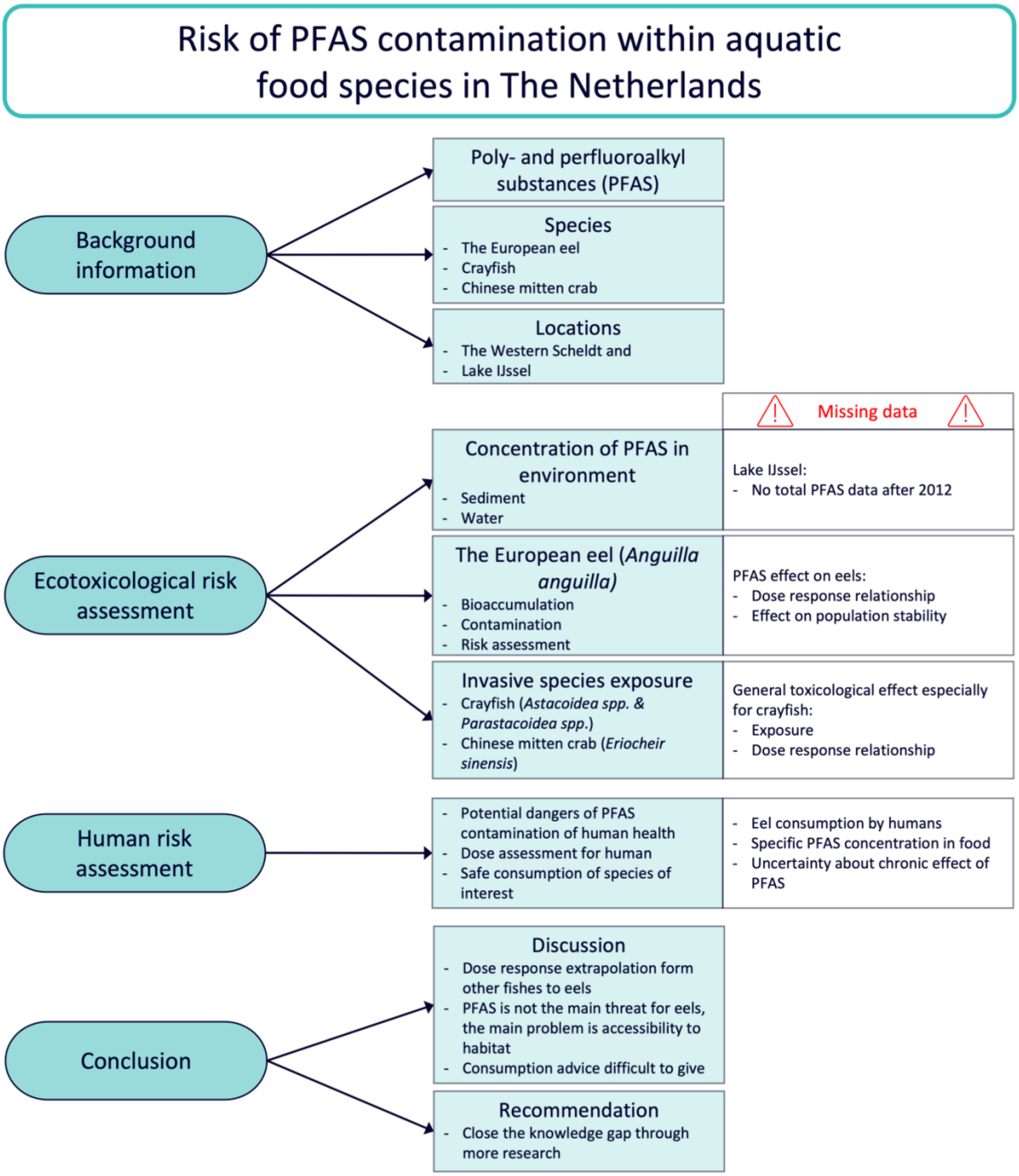
Doing a risk assessment for the Chinese mitten crab was practically inexecutable. It was found that PFAS accumulates in similar locations to those found in crayfish with the blood and hepatopancreas being the most prominent locations for PFAS accumulation. Immuno-toxicological effects were observed within Chinese mitten crab as a result of PFAS contamination which could be a potential health and population risk for these crabs. No data regarding contamination levels are currently available so the further assessment was not possible at this point.

Information on PFAS contamination and toxicology in humans is more readily available. However, most data available was focused on the most common PFAS molecules rather than cumulative PFAS toxicology. PFAS accumulates in human kidneys, livers, and blood plasma and has been linked to high cholesterol and liver enzyme expression levels. Immunotoxicity and hepatotoxicity are the most common types of toxicity observed because of PFAS contamination in humans. Additionally, studies suggest that PFAS may also exhibit carcinogenicity, however, further research is needed to confirm this suspicion. Furthermore, studies gave reasonable indications that PFAS may affect sperm concentration and motility.

Exposure to PFAS via consumption of the assessed species was assessed. Based on the set TWI of 4.4 ng/kg bw for the sum of PFHxS, PFNA, PFOA, and PFOS by the EFSA CONTAM panel it was found that at average consumption of Dutch wild eel the TWI would be exceeded. Therefore, the PFAS contamination in eels could serve as an additional argument when advising against the consumption of eels during preservation exploits. For the crayfish, a preliminary assessment was made based on estimated PFOA and PFOS concentrations. Concise conclusions cannot be made based on currently available data, however, based on the estimated data it was expected that the TWI for the sum of PFAS would either be reached or exceeded by PFOA and PFOS alone. It can therefore be expected that the inclusion of other PFAS would lead to an exceedance of the TWI. PFAS exposure through Chinese mitten crab consumption could not be assessed as no data was available regarding PFAS concentrations within these crabs.

PFAS contamination in these aquatic species requires more data to be fully assessed. However, based on currently available data and interviews the potential dangers were assessed. Overall, PFAS does not seem to be the most pressing concern for these aquatic species, however, when viewing these species as possible food sources for humans, concerns should have and have arisen. Further research is needed to properly assess the full scope of this problem and conversations with researchers have revealed that this is ongoing and will continue in the future.

### 6.1. Visual summary



## Risk of PFAS contamination within aquatic food species in The Netherlands

**Background information**

Poly- and perfluoroalkyl substances (PFAS)

Species
- The European eel
- Crayfish
- Chinese mitten crab

Locations
- The Western Scheldt and
- Lake IJssel

⚠ Missing data ⚠

**Ecotoxicological risk assessment**

| | Missing data |
|---|---|
| Concentration of PFAS in environment<br>- Sediment<br>- Water | Lake IJssel:<br>- No total PFAS data after 2012 |
| The European eel (*Anguilla anguilla)*<br>- Bioaccumulation<br>- Contamination<br>- Risk assessment | PFAS effect on eels:<br>- Dose response relationship<br>- Effect on population stability |
| Invasive species exposure<br>- Crayfish (*Astacoidea spp. & Parastacoidea spp.*)<br>- Chinese mitten crab (*Eriocheir sinensis*) | General toxicological effect especially for crayfish:<br>- Exposure<br>- Dose response relationship |

**Human risk assessment**

| | Missing data |
|---|---|
| - Potential dangers of PFAS contamination of human health<br>- Dose assessment for human<br>- Safe consumption of species of interest | - Eel consumption by humans<br>- Specific PFAS concentration in food<br>- Uncertainty about chronic effect of PFAS |

**Conclusion**

Discussion
- Dose response extrapolation form other fishes to eels
- PFAS is not the main threat for eels, the main problem is accessibility to habitat
- Consumption advice difficult to give

Recommendation
- Close the knowledge gap through more research

*Figure 23: Visual summary. From personal source, 2022.*

### 6.2. Limitations

In this paragraph, the limitations of the methods of this study are discussed. This study largely consisted of a literature review. Literature reviews are often the foundation for several types of research. Based on literature reviews, guidelines can be set up, and literature reviews often spark new ideas for future research. However, it sometimes proves difficult to conduct such a literature review with great accuracy. We believe that, by setting up our research questions carefully and taking a risk assessment approach, we have performed this literature review with decent accuracy. Nevertheless, without performing experiments, there were only limited options to validate and integrate research results.

This study covers a broad scope; multiple species and multiple locations were considered. This broad scope is interesting, and it provides Good Fish with information on multiple facets of the PFAS problem. However, taking a broad scope also inevitably meant that the information provided is of a less detailed level. This broad scope is, for instance, also the reason that the risk assessment for the crayfish species and Chinese mitten crab is less extensive than for the European eel and humans.

Additionally, this study had to be performed within a short time scale of eight weeks. This had consequences for the amount of literature that could be reviewed and taken into account. Therefore, we turned to grey literature to complement scientific literature. To collect both types of literature we started off with primary literature and used cited references to find more relevant sources, this is also known as the ‘snowball effect’.

Initially, the aim was to approach many interviewees to add to information gathered from both scientific and relevant grey literature. But due to time constraints and the sensitivity of the topic, this number was limited to three interviewees. When we discovered that this topic was sensitive, we came up with the idea to incorporate a chapter about why this topic is sensitive. We planned to first approach interviewees and ask for their cooperation. If they were not open to cooperation, we asked them for their reasoning. However, many researchers or experts we reached out to, were not open to cooperation or did not reply at all. Therefore, we did not gather as much information as we would have preferred.

## 6.3. Discussion

As stated before, in this study, we have taken a risk assessment approach. This approach ensured that all essential information was considered to assess the risk for the European eel, the crayfish, the Chinese mitten crab, and finally humans. It has become clear that not all information is available to perform a thorough risk assessment. Some crucial information is missing, and we pose this missing information below as knowledge gaps that need to be filled to obtain a better overview of the risks PFAS poses to the European eel, Crayfish species, and the Chinese mitten crab, and eventually humans. Knowledge gaps were found on a general scale and especially in the study area: Lake IJssel and the Western Scheldt. This chapter highlights these current knowledge gaps for the most important topics in the risk assessment.

### 6.3.1. Is PFAS posing a threat to the European eels in our targeted areas?

For the targeted locations (the Western Scheldt and Lake IJssel), some accumulation concentrations in the eel were found, but it is currently unknown what concentration of PFAS in the eel's body poses a threat to its health. This is because dose-response relationships have not been established yet, which often form the base for policies and regulations. Establishing dose-response relationships is possibly hampered since eels do not yet breed in captivity, because they have a relatively long and complex life cycle, and their (sexual) maturation in captivity depends on the administration of exogenous hormones (Herranz-Jusdado et al., 2019). Furthermore, the eel is critically endangered which makes it difficult and unethical to capture them from the wild and use them in experiments.

Dose-response relationships from other fish, such as zebrafish or carp, possibly can be extrapolated toward the European eel. Such comparisons are often done by applying scaling factors to correct for interspecies differences in metabolism, physiology, genetics, and biochemistry (Calabrese, 2017). The greater the similarities between the species, the more certainty can be obtained by extrapolation. However, a high similarity is not required, which for example can be seen by the fact that using zebrafish as a model species for human diseases is rapidly gaining in popularity (Bailone et al.,2020) For eels, it might be possible to establish dose-response relationships using farmed specimens as well.

Other animals in the food chain of the European eel might also be affected by PFAS. To our knowledge, no studies are researching the indirect effects of PFAS on the food sources of the European eel. Additionally, it is known that, depending on the diet, eels can accumulate more or less POPs. It is, for instance, thought that narrow-headed eels, which have a higher fat content, are less likely to accumulate POPs in their bodies than broader-headed eels (De Meyer et al., 2018). This is assumed because narrow-headed eels eat smaller organisms, which are lower in the food chain, therefore, having lower levels of biomagnification. Regarding the body size of the European eel, it is currently unclear how this influences the bioaccumulation of PFAS due to contradicting research. Humans are not the only top predators consuming the European eel, species such as the waterbirds, for example, herons (Ardeidae) also consume eels, and they may therefore also suffer from bioaccumulation and/ or biomagnification which is also still under investigation (Santillo et al., 2006).

Nevertheless, it needs to be taken into consideration that PFAS might not be the contaminant that poses the biggest threat to the eel. The measured environmental PFAS concentrations are relatively low and based on the found literature it can be likely assumed that short-term and acute effects of PFAS are of little concern for the eel at this moment. For instance, no evidence has been found of rapidly declining eel populations due to PFAS, but research on which eels died after admitting a certain dose has not been performed yet. Long-term effects are less studied and therefore we cannot exclude that those are occurring. It is probably more relevant to look at the synergistic effects of multiple contaminants, which could affect the European eel to a larger extent than PFAS do.

It is also likely that contaminants are not even the biggest threat, nor will they ever be, for eel. Bad connectivity between habitats is now for example regarded as the biggest threat to the survival of the (Schiphouwer, M., 22-04-2022, personal communication).

### 6.3.2. *Invasive species: Crayfish and Chinese mitten crab exposure in the target area, are crayfish and Chinese mitten crab in the area in danger?*

For crayfish, no dose-response relationships are established, and thus it is difficult to assess the possible health risks of PFAS crayfish. Extrapolations from dose-response relationships could be made, but we are not aware of dose-response relationships for related species. To determine PFAS concentrations in crayfish, calculations were made based on data from the Hudson River watershed and estimated PFAS levels in Lake IJssel. For these calculations, several large assumptions are considered, making the results less reliable. Research needs to be conducted to determine PFAS concentrations in crayfish in Lake IJssel. These measured data points can then be used to reassess the TWI's as is performed in this report.

For the Chinese mitten crab, no dose-response relationships are established. Extrapolations from dose-response relationships could be made, but we are not aware of dose-response relationships for related species. In contrast to crayfish, already some acute and chronic health effects have been established for the Chinese mitten crab.

### 6.3.3. *Are humans in danger due to consumption?*

For human risk assessment, some crucial information also seems to be missing. For instance, there is little knowledge on the consumption of specific species, like the European eel, crayfish, and the Chinese mitten crab. With up-to-date consumption levels, probabilistic models for the risk of the entire Dutch population could be established, as was done in Flanders by Bilau et al. (2010) and Teunen et al. (2021). This data could be obtained from doing surveys among consumers or looking at the quantity of the species of interest sold by fish retailers and/ or supermarkets. Additionally, there are no norms considering PFAS concentrations in food (Zafeiraki et al., 2019), hampering the risk evaluation. Definitive conclusions of human health effects regarding PFAS exposure are very hard to draw since there is much contradictory evidence. When an effect has been researched for example PFOS, it is hard to extrapolate to other PFAS because the adverse outcome pathway must be known to compare substances, but that field is still very much in development (Feitsma, P., Den Braver, M., 14-04-2022, personal communication).

The most eel that is sold is farmed eel. Since farmed eel is most of its life exposed to clean water, PFAS accumulation in these organisms is greatly reduced. The biggest concern for humans is when they consume a lot of wild eels. The GGD advises not to eat self-caught fish from the Western Scheldt because then consumers might be exposed to high PFAS concentrations (GGD Zeeland, 2021). Since the difference between the PFAS concentration in wild eels and farmed eels is expected to be big, there is not a specific amount of eel that is certainly safe to consume. Besides, the amount of consumption of the species significantly differs between people. Therefore, it is too complex to give one-sided advice on eel consumption when it comes to health risks caused by PFAS. However, based on the available TWI's and concentrations it is likely that consumption of wild eel will be detrimental to human health.

Lastly, for humans, it needs to be considered that PFAS enters the body with drinking water and the consumption of other food. Assessing the risks for humans when eating only eels, crayfish or Chinese mitten crab thus gives an underestimation of the potential risk for humans.

### 6.3.4. *What makes the establishment of regulations difficult?*

Currently, environmental norms have only been established for three types of PFAS, and for norms regarding PFAS levels in biota, only one type of PFAS has been assessed (Jonker, 2021). When norms are established, it will simplify risk assessments, and then more regulations can be put in place. For other contaminants such as dioxins, maximum limit values have been set and areas have been shut down based on risk assessments. The big difference between PFAS and other contaminants is that PFAS is in almost every water in the Netherlands because it is very soluble and mobile. PFAS spreads easily, making it difficult to close off areas, which for example can be way more easily implemented for dioxins that are bound to soil and create hotspots of contaminated areas.

More research needs to be conducted on current total PFAS levels. Many studies are focussing on a few PFAS types, without considering the total PFAS accumulation, there are no total PFAS concentrations present for Lake IJssel after 2010 and for the Western Scheldt after 2012. Since there is much data missing and no solid scientific evidence related to the impact of PFAS, there is a disagreement between some institutions and ministries on regulations regarding the maximum limit values, and the use and monitoring of PFAS. This is also why there are no maximum levels for PFAS in food established yet.

### 6.3.5. *Why is there some resistance and prudence to sharing knowledge and data?*

Many people, industries, and countries depend on the economy generated by PFAS. PFAS is a hot-topic but also sensitive and controversial. With the increase of scientific data and research on the negative effects of PFAS on human health and the environment, PFAS took a growing place in discussions at the international and national levels. During our research, we faced some reticence from researchers to express their opinion or share expertise about PFAS. We came across multiple reasons why several researchers were hesitant to cooperate with us. Since it is Good Fish's goal to advise consumers on their food, it is important to know why researchers cannot be transparent about their findings regarding PFAS pollution in eels.

The first reason we heard back from the researchers was that they felt they could not share sufficient information with us. Many researchers who are very knowledgeable about eels are not very familiar with PFAS and the other way around. Especially since experiments with eels are forbidden since the eel is an endangered species, and no dose-response values are available (Belpaire, C., 06-04-2022, personal communication). Therefore, some researchers feel not comfortable making statements when there are too many uncertainties. Studies about the impact of PFAS exposure on aquatic species are currently still in process, therefore some studies are not yet finished nor published. This is one of the reasons why researchers are not yet able to communicate results. The problem is not necessarily coming from reluctance to share data, but sometimes it is just not allowed to communicate about research due to confidentiality or uncertainty before publication. One of the researchers who is working on PFAS, but was yet unwilling to be interviewed, informed us of one of his motives which had to do with agreements with the government. Many research institutes have an agreement with the RIVM that they are not allowed to advise others. Research institutes only provide data to the RIVM, but it is the RIVM who makes the final advice on what is and what is not safe.

Another reason for the reluctance of researchers to make statements has to do with eel. According to one of the researchers, the eel has been a sensitive topic in the Netherlands for quite some time since the eel fisheries is a traditional sector. Fishing on eel is heavily debated because eel has been an endangered species since 2008. Making statements that are in favour of banning eel fisheries, would not only hit the sector financially but also emotionally. Not only fishermen are financially dependent on the eel fisheries, but the eel is also important for the fish retailers and shops. Because of the financial importance, the government is not keen on implementing harsh regulations and stopping the eel fisheries, even though ICES advised banning fisheries on the glass and yellow eel for a year to save the eel populations (Schiphouwer, M., 22-04-2022, personal communication). Since fisheries and the PFAS industry represent a big part of the national economy, the Dutch government and the European Union must implement regulations that will not disturb the economy too much. Therefore, they prefer to create regulations or bans only if the risk is certain, proven, and problematic for human health. It is one extra reason why there is a lot of controversy behind the PFAS subject.

PFAS are known as dangerous to humans, but since the exact concentration remains uncertain, the priority is given to the economic perspective and not the health perspective. The objective is to slowly reduce the PFAS concentration in food on a long-term scale, but regulations should first preserve the economy. The European Commission aims for a maximum limit value at which 95% of the fish is still available on the market (Feitsma, P., 14-04-2022, personal communication). This is a feasible way of regulating, since otherwise if health-based guidance values for PFAS would be used, the majority of the tested food would realistically be rejected. In addition, there is also some debate inside the scientific community about methodologies used to analyze the effects of PFAS. There are many stakeholders involved who are limited in sharing information, therefore, it is difficult for governments to define an official method. They usually go for the safest option while mainly taking the economy into account.

### 6.4. Recommendations for future research

Based on the required information and the identified knowledge gaps, some recommendations for future research are given.

Most of the eel populations are not mapped and exact population numbers are still missing. Therefore, it is hard to make a statement about the status of the eel population in Lake IJssel and the Western Scheldt. It is recommended to ask Rijkswaterstaat for population data of the European eel in, for instance, the Western Scheldt (Schiphouwer, M., 22-04-2022, personal communication). Further research could also focus on better mapping the eel populations. This will also aid in investigating the long-term effects of PFAS on the eel, such as effects on reproduction and population dynamics.

Besides long-term effects, indirect effects of PFAS are also far from well-studied and understood. For instance, the effects of PFAS on the food sources of the European eel are unknown. Additionally, we are not aware of information on the effects of PFAS on predators of the eel. Due to bioaccumulation, the consumption of eel might not only pose a threat to humans but also animals higher up in the food chain.

Further investigation of the risks and effects of PFAS exposure on aquatic animals requires a dose-response relationship and assessment. However, for many aquatic organisms, no dose-response relationships for PFAS have been established. Possibly, data can be extrapolated from animals of which a dose-response relationship is available. To do this properly more statistical modelling data and scaling factors are necessary.

Another facet of research that needs to be elaborated upon is the molecular pathways of different compounds for a better understanding of the health effects in several species. Besides more research, the interactions between contaminants should be receiving more attention and there is a need for the development of more sensitive analytical methods.

Regarding the safety of eel consumption, the RIVM can be contacted for their advice. They can also elaborate on why they withhold researchers who are working for them to give advice. Employees of the RIVM that are mentioned by researchers are Dr. Ir. Polly Boon and Dr. Marcel Mengelers. To infer what part of the Dutch population is exposed to health risks by consumption of eels, more data on eel consumption is required. When this data is collected, it can be used in probabilistic models to infer the risk for the entire Dutch population. Furthermore, minimal risk levels (MRL) need to be established to be able to advise people on safe eel consumption. Only when MRLs are set, statements can be made about whether eel can be consumed without being at risk of adverse non-cancer health effects.

Previously mentioned reasons are in the way of researchers to speak freely about the subject. For Good Fish, it is important to keep this in mind since it has an impact on the provided information about this sensitive topic.

# Advice

To Good Fish,

*Regarding the European Eel (Anguilla anguilla)*

Based on currently available data PFAS cannot be deemed a very pressing concern for the short-term health of the eels. No statement can be made yet regarding the long-term health effects of PFAS exposure on the European eel. Due to the lack of data and the ongoing research into PFAS, it is best to focus conservation exploits on better studied and understood concerns such as habitat accessibility and connectivity, overfishing, and other POPs such as dioxins, PCBs and PAHs.

The PFAS levels within wild-caught eels pose a health risk to humans and consumption should therefore not be promoted. The current state of PFAS contamination within wild eels can be used as a valid argument when discouraging the fishing and consumption of wild eels.

*Regarding the invasive crayfish (Astacoidea spp. & Parastacoidea spp.)*

Research into the health effects is needed as it is unclear whether crayfish suffer as a consequence of PFAS contamination. Furthermore, PFAS levels within Dutch crayfish need to be assessed to give a better overview of the state of PFAS contamination within these species.

Measured PFAS levels in crayfish must be compared with set TWI's before crayfish consumption can be promoted as it is likely that the levels in crayfish exceed current EFSA approved levels.

*Regarding the invasive Chinese mitten crab (Eriocheir sinensis)*

Outside of the immunotoxicity evidence, more elaborate health effects for the crab need to be assessed to gain a full scope of the contamination within the Chinese mitten crab. However, PFAS levels within the crabs in the Netherlands need to be measured before conclusions can be drawn regarding the risks of consumption.

*Yours sincerely,*

*PFISH Consultancy team*

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# Appendices

*Appendix 1: Stakeholder analysis. From personal source, 2022.*

Since the eel is already critically endangered (Crook & Gollock, 2020) and the impact of added chemical stress by increasing PFAS levels is unknown, Good Fish wants us to close this knowledge gap to enable better conservation of the eel. Figure 24 shows a Power-Interest matrix for relevant stakeholders regarding a statement on this knowledge gap: Fish is only sold/consumed when not contaminated with PFAS. Good Fish is the most important stakeholder in this project. They have an interest in this project since the information gathered will aid them in reaching their goal of healthy and sustainably caught fish by 2030. Good Fish, as an NGO, does not have much power, but with the gathered information, Good Fish can go to other, (e)NGOs and strive for collective action.

Good Fish has formerly focused on consumers, they aimed to provide information to the consumer, so the consumer could steer market parties using their money. Consumers have an interest, since contaminated fish may result in health risks. Closing the knowledge gap on PFAS will lead to consumers consuming more healthy fish, or at least them being more aware, and they will thus be positively affected.

Good Fish is moving away from consumers, toward fish retailers and supermarkets. Fish retailers and supermarkets are commercially involved and can decide what consumers can buy or not buy, and hereby push for sustainability. How they are affected by this project depends on the outcomes, since fishing bans may result in rising prices of affected fish. On the other hand, fishing bans will ensure that only safe fish is being sold.

Fishermen have an interest in this project since they will be the targets if regulations are applied because of this project. Fishermen do not have much power as of now, but a trend is developing in which fishermen are being more empowered, to create a certain notice of 'ownership', so they can push for sustainability.

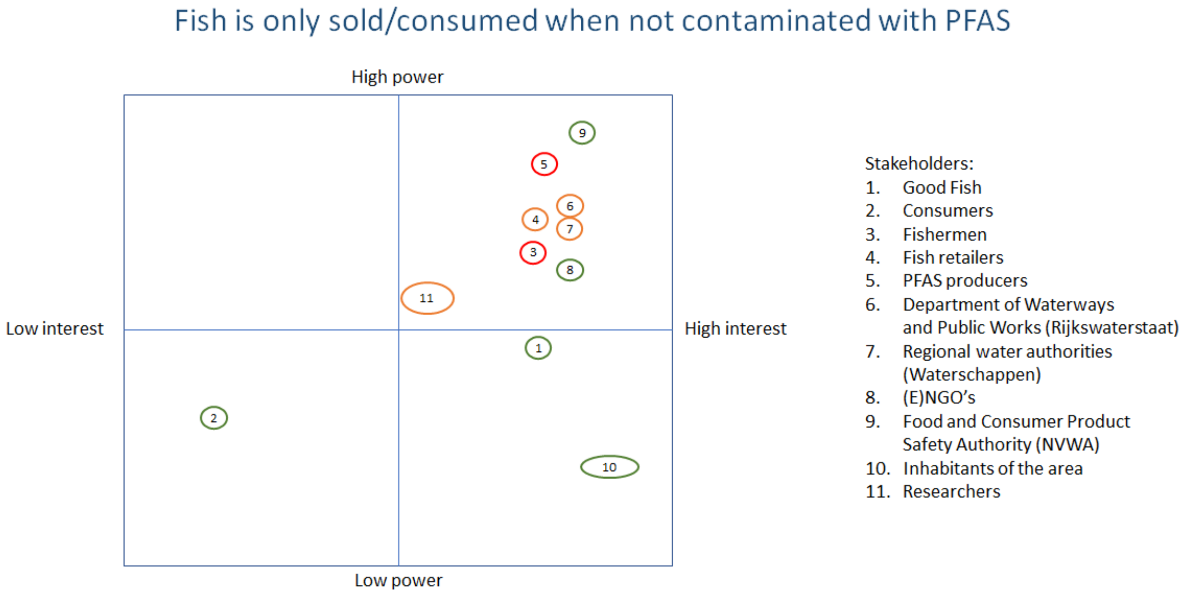

*Figure 24: Power-interest matrix of stakeholders involved in this project, with interest indicated on the x-axis, and power on the y-axis. The numbers (1- 11) indicate different stakeholders, mentioned in the legend on the right of the diagram. Colours indicate whether these stakeholders will be affected in a positive (green), negative (red), or neutral (orange) way. From personal source, 2022.*

*Appendix 2: Stakeholder long-list. From personal source, 2022.*

### Good Fish

Good Fish is our commissioner, so they will play a significant role in this project. They have an interest in this project since it could provide them with information that will aid them in reaching their long-term goal: only sustainable and healthy fish in the Netherlands by 2030. Good Fish does not have a lot of knowledge on this issue yet, which is why we were commissioned to close this knowledge gap. Good Fish is not a large NGO, but they have a strong network and plan on taking the findings of this project to other NGOs, and together they might be able to push for fishing bans or other solutions that may or may not be necessary.

### Researchers

Researchers in this specific field usually have a high level of interest since these projects lie within their field of expertise and could potentially have an influence on their future research. Newly gathered knowledge provides researchers with new research opportunities and job possibilities. They have some power since knowledge can create awareness, influence more powerful stakeholders and hereby steer decision making.

### Consumers

Consumers in this project consume fish caught and sold in the Netherlands. Consumers have been the focus of Good Fish formerly. Good Fish tried to inform the consumer so the consumer would be able to make decisions about the consumption of certain fish. Hereby, the consumer can use its power (voting with money) and push market parties toward a healthier and more sustainable industry. Good Fish now understands that it is difficult for layman consumer to use their Viswijzer, since advanced information of for example fishing gear is needed to apply the Viswijzer in a correct way. Consumers also include people that consume and/or use PFAS. Probably many people contribute to PFAS pollution without being aware since it is omnipresent.

### Fishermen

Fishermen professionally catch fish in Dutch aquatic ecosystems (e.g., marine, brackish or freshwater). Fishermen depend on fisheries for their livelihood. In this project, hobby fishing is not taken into account. Fishermen are usually the targets of regulations. They have an interest in this project, since the results of our project may result in Good Fish and other NGOs starting to demand fishing bans. However, the empowerment of fishermen is currently a relevant topic in marine resource management in which governance is shifting toward a more bottom-up, instead of the usual top-down approach. It is thought that giving fishermen more authority, might create a feeling of ownership, which could result in fishermen caring more about, for instance, sustainability (Hart, 2021).

### Fish retailers and supermarkets

The fish retailers and supermarkets have an interest because they are commercially involved. This project may result in fishing bans, which would affect the products they can sell. Good Fish is currently moving away from the consumer, toward the fish retailers and supermarkets, to push for sustainability. They have high power since they can decide what is sold and what is not. It depends on the outcome of our project whether they will be affected positively or negatively. When we find that fish is contaminated with PFAS, they possibly cannot sell that product anymore, resulting in less profit. However, the food industry also wants to sell healthy products that do not endanger their consumers.

### PFAS producers

PFAS producers have both high power and high-interest rates. Since they produce products that contain PFAS they are to a high degree responsible for the pollution of aquatic ecosystems and thus have high power in influencing the water quality. Their high-interest rate comes from the fact that they might have to change their way of production if the government decides that their manner of production is deemed too polluting.

### Department of Waterways and Public Works (Rijkswaterstaat, for Lake IJssel and Western Scheldt) / water boards

The Department of Waterways and Public Works/ water boards oversees that the water quality of seas, lakes, and rivers are within safe limits. They have high power because they decide when the water is safe for consumption, fisheries, agriculture, etc. Their high level of interest is because of their goal to ensure safe water for drinking, fisheries, agriculture, etc. (Ministerie van Infrastructuur en Waterstaat, 2021).

### Environmental (non-governmental) organisations (data collection, communication network)

Good Fish is an environmental NGO that works together with other, larger NGOs to push for more sustainability regarding fish, for our project, and nature conservation in general. They do not have a high level of power since they mainly forward information to the European Committee in the hope that they adjust their policy based on their advice.

### Food and Consumer Product Safety Authority (NVWA)

The Food and Consumer Product Safety Authority has an interest in this project since this project regards the consumption of fish that might be contaminated with PFAS. This could lead to a health risk for consumers. The NVWA has power since it aims to ensure that food is healthy and that laws and regulations regarding food safety are met (NVWA, 2019).

### Inhabitants surrounding polluted waters

People that inhabit areas that suffer from high pollution by PFAS have an interest since their health is possibly affected. They have no power, apart from informing others about their feelings and life experience, and hereby creating awareness of a certain problem.

*Appendix 3: PFOA & PFOS Concentrations in Dutch water and estimated concentrations within Dutch crayfish Table. From Jonker, 2021.*

| Area | Location | Salinity | PFOA in water (ng/L) | PFOA in crayfish (ng/g ww) | PFOS in water (ng/L) | PFOS in crayfish (ng/g ww) |
|---|---|---|---|---|---|---|
| Western Scheldt | Sas van Gent | Brackish | 6,37 | 1.40 ± 0.67 | 11,07 | 9.49 ± 9.28 |
| | Schaar van Ouden Doel | | 9,32 | 2.04 ± 0.98 | 17,6 | 15.08 ± 14.75 |
| Northern Sealand | Haringvlietsluis | Brackish | 2,73 | 0.60 ± 0.29 | 3,52 | 3.02 ± 2.95 |
| | Bovensluis | | 2,67 | 0.58 ± 0.28 | 3,42 | 2.93 ± 2.87 |
| | Dreischor | | 1,1 | 0.24 ± 0.12 | 0,94 | 0.81 ± 0.79 |
| North Sea | Walcheren | Marine | 0,98 | 0.21 ± 0.10 | 0,86 | 0.74 ± 0.72 |
| | Noordwijk | | 1,05 | 0.23 ± 0.11 | 0,95 | 0.81 ± 0.80 |
| | Ijmuiden | | 5,13 | 1.12 ± 0.54 | 5,02 | 4.30 ± 4.21 |
| Waddensea | Dantziggat | Marine | 1,25 | 0.27 ± 0.13 | 1 | 0.86 ± 0.84 |
| | Bocht van Watum | | 1,74 | 0.38 ± 0.18 | 1,33 | 1.14 ± 1.11 |
| Utrecht | Nieuwegein | Fresh | 2,61 | 0.57 ± 0.27 | 3,45 | 2.96 ± 2.89 |
| Lake IJssel | Vrouwezand | Fresh | 3,52 | 0.77 ± 0.37 | 3,27 | 2.80 ± 2.74 |
| Flevoland | Kampen | Fresh | 2,31 | 0.51 ± 0.24 | 3,53 | 3.03 ± 2.96 |
| Ponton | Eijsden Ponton | Fresh | 3,36 | 0.74 ± 0.35 | 3,02 | 2.59 ± 2.53 |
| | Lobith Ponton | | 2,07 | 0.45 ± 0.22 | 3,38 | 2.90 ± 2.83 |
| North Brabant | Keizersveer | Fresh | 4,75 | 1.04 ± 0.50 | 3,56 | 3.05 ± 2.98 |
| South Holland | Maassluis | Fresh | 2,66 | 0.58 ± 0.28 | 3,4 | 2.91 ± 2.85 |