Fish die-off in the Oder River, August 2022

Status report as of 30 September 2022

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German expert group on the fish die-off in the Oder River headed up by the German Environment Agency (UBA)

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List of abbreviations

BfG	Bundesanstalt für Gewässerkunde (Federal Institute of Hydrology)
BMUV	Bundesministerium für Umwelt, Naturschutz, nukleare Sicherheit und Verbraucherschutz (Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection)
IKSO	Internationale Kommission zum Schutz der Oder (International Commission for the Protection of the Odra River)
LALLF	Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern (Mecklenburg-Western Pomerania State Office for Agriculture, Food Safety and Fisheries)
LfU	Landesamt für Umwelt Brandenburg (Brandenburg State Office for the Environment)
LLBB	Landeslabor Berlin-Brandenburg (Berlin-Brandenburg State Laboratory)
LUNG	Landesamt für Umwelt, Naturschutz und Geologie Mecklenburg-Vorpommern (Mecklenburg-Western Pomerania State Office for the Environment, Nature Conservation and Geology)
MLUK	Ministerium für Landwirtschaft, Umwelt und Klimaschutz des Landes Brandenburg (Brandenburg Ministry of Agriculture, Environment and Climate Protection)
OGewV	Oberflächengewässerverordnung (German Surface Waters Ordinance)
SETAC	Society of Environmental Toxicology and Chemistry
THW	Bundesanstalt Technisches Hilfswerk (Federal Agency for Technical Relief)
UBA	Umweltbundesamt (German Environment Agency)
WHO	World Health Organization
WSA	Wasserstraßen- und Schifffahrtsamt (Office for Waterways and Shipping)

Summary and overall conclusion

In August 2022, massive numbers of fish died in the Oder River. The first dead fish were reported on the German side of the river on 09.08.2022 close to the city of Frankfurt (Oder). Aquatic organisms other than fish also died including snails and mussels.

The actual extent of the environmental damage and the long-term impacts on the ecosystem cannot yet be quantified at this time.

On 16.08.2022, an official inner-German expert group was formed to clarify what caused the fish die-off.

The time period of the events can be narrowed down for the German part of the Oder to the period from 01.08.2022 to 22.08.2022. The conductivity, which is a measure of the ions dissolved in the water, began to increase in Frankfurt (Oder) on 01.08.2022, and further downstream in Hohenwutzen starting on 03.08.2022. The salt discharged into the Oder was mainly sodium chloride.

The following results were reported in the period of maximum measured conductivities (≥ 2,000 µS/cm) between 07.08.2022 and 14.08.2022 in Frankfurt (Oder):

- A sudden increase in oxygen concentrations, pH value and chlorophyll-a concentrations as well as a decrease in the nitrate concentration. These changes in the parameters point to a massive algal bloom. This algal bloom was verified by high-resolution satellite images.
- The biotesting with *Daphnia* (water fleas) indicated a high toxicity level of the substances in the water of the Oder.
- In the phytoplankton (community of algae floating freely in the water) of the Oder, the brackish water alga *Prymnesium parvum*, which is predominantly found in salt water, was identified by conventional (microscopic) and molecularbiological methods. The measured cell counts per litre are comparable to data in scientific publications describing fish die-off caused by *Prymnesium parvum*.
- The brackish water alga can produce toxins. These toxins were identified by non-target screening of water samples from the Oder. The concentrations of the toxins cannot be quantified, and no evaluation criteria exist for the toxins. It cannot therefore be conclusively determined whether the toxins were present in a concentration that would trigger a massive fish die-off.
- The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and its technical by-products 2,6-dichlorophenoxyacetic acid (2,6-D) and 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) were detected at concentrations above the existing evaluation criteria. However, acute toxic effects on aquatic fauna are unlikely at the concentrations measured.
- Extensive chemical analyses including non-target screening for 1,200 known substances and additional unknown substances did not identify any probable cause of acute fish toxicity other than the toxins produced by *Prymnesium parvum*.

The changes in the parameters described above, which were initially only recorded at the monitoring site in Frankfurt (Oder) on 07.08.2022, reached the downstream monitoring site in Hohenwutzen on 08.08.2022. Due to inflows of other tributaries, especially the Warta River, and the effects of mixing, the changes in Hohenwutzen were less distinct. Water samples from Hohenwutzen showed *Daphnia* toxicity. In addition, there were also signs of the effects of a strong algal bloom with the presence of *Prymnesium parvum* and the toxin prymnesin.

No dead fish or abnormal measurements were recorded in the Kleines Haff (German part of the Stettiner Haff (Szczecin Lagoon), inland coastal waters at the mouth of the Oder) before 9 September 2022.

The expert group tested a number of hypotheses. The most plausible hypothesis is that the fish die-off was caused by high salt concentrations, which enabled massive proliferation of *Prymnesium parvum* and its associated toxins.

The conditions necessary for an algal bloom are generally present in the Oder in the summer: light and temperature conditions, increased nutrient concentrations, low water and low discharge levels and hydromorphological changes. However, the salt concentration is assumed to be the primary trigger of the observed *Prymnesium* bloom. The sources of the salts, other elements and chemicals are unclear. The primary habitats of *Prymnesium parvum* in the Oder are also unknown. According to the available data, this alga only occurs in Germany in salty transitional and coastal waters with very low cell counts.

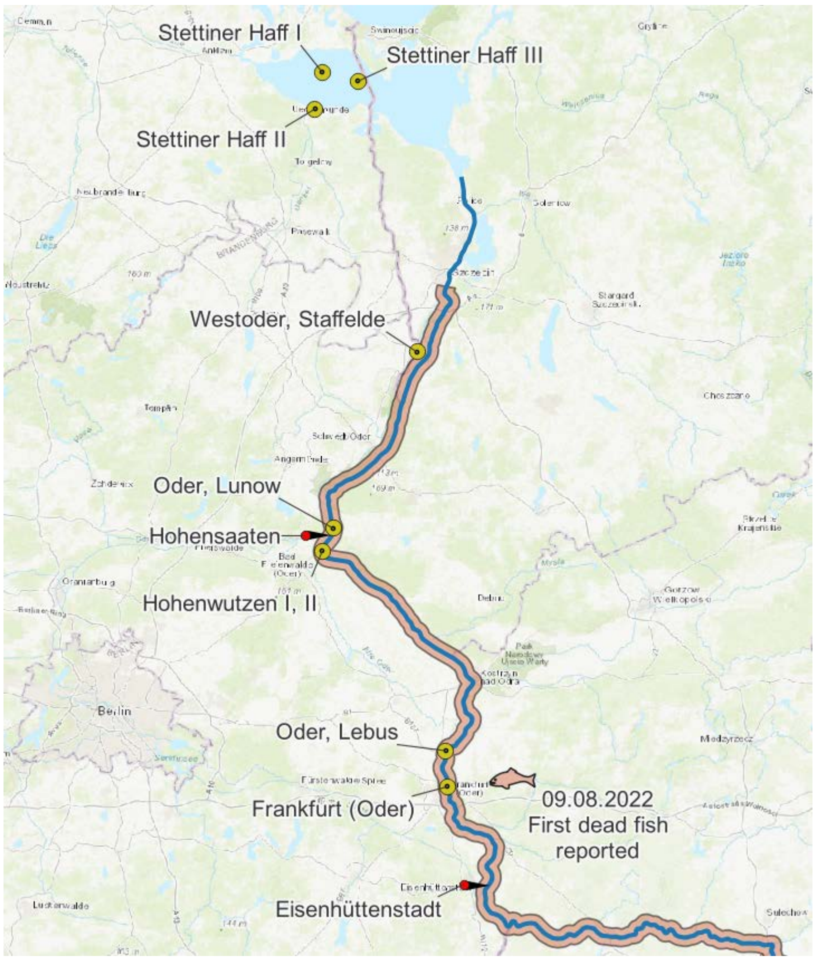
The damage to the environment in the Oder in August 2022 poses new challenges for science, water management and policy. The findings to date suggest multi-causal relationships. In the future, preventative and monitoring measures must be taken to prevent fish die-off – in the Oder and other water bodies. These measures include extensive monitoring using innovative methods, adapting warning and alert plans, reviewing discharge into water bodies and discharge permits, research into the spread of toxic algae in water bodies and strengthening the resilience of aquatic ecosystems.

Water bodies and their functionality can only be preserved in the long term if pressures, such as pollution, river engineering and straightening and excessive uses, such as the discharge of substances or water withdrawals, are reduced to a tolerable level for the flowing water ecosystem.

1 Introduction

In August 2022, the Oder suffered severe environmental damage over a length of approx. 500 km, with fish, mussels, crayfish and snails dying on an unprecedented scale. Figure 1 shows the geographical scope of the area of the Lower Oder affected by the death of fish and other aquatic species on the German side.

Figure 1 Geographical scope of the area of the Oder affected by the death of fish and other aquatic species



The fish die-off was first discovered on the German side by a ship operator and reported to the Berlin-Brandenburg State Laboratory (LLBB) on 09.08.2022. The exact volume of dead fish removed from the Oder and disposed of cannot be verified. There were mentions of several hundred tonnes in the media.

Water quality measurements at the automatic water quality monitoring station in Frankfurt (Oder) (Figure 2) recorded a sharp increase in conductivity starting on 05.08.2022; this was followed by an increase in the parameters oxygen content, pH value and chlorophyll-a starting on 07.08.2022. These parameters are indications of a critical situation in the water body that can be expected under the given conditions (low water level, high water temperature) as a result of an algal bloom. However, the nitrate concentration surprisingly ran counter to previous experience.

On 12.08.2022, the Berlin-Brandenburg State Laboratory and the Brandenburg State Office for the Environment (LfU) officially requested administrative assistance from the German Environment Agency (UBA) through the Federal Ministry for the Environment, as well as from the Federal Institute of Hydrology (BfG). At the same time, a group of experts from the German authorities in the federal states of Brandenburg and Mecklenburg-Western Pomerania and the German federal government was formed to investigate possible causes of the fish die-off on the basis of information and monitoring data.

The experts had at their disposal data from three automatic monitoring stations on the Oder in Frankfurt and in Hohenwutzen, the results of analyses using daily composite samples from these monitoring stations on the days with maximum concentrations as well as measurements from a daily pick-up water sample after the fish die-off (Figure 2, Table 1).

Figure 2 Overview of the monitoring sites along the German part of the Oder

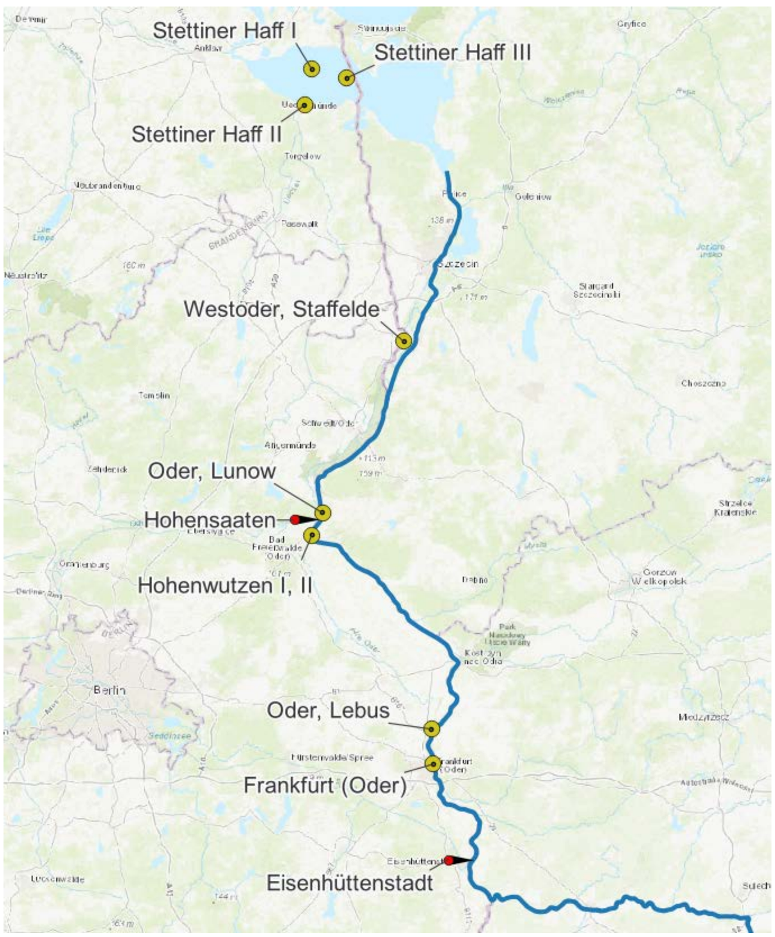


Table 1 Monitoring and sampling sites along the Oder

Monitoring sites	Details
Stettiner Haff I, II and III	Three monitoring sites in total (central area I, Uecker River mouth II, national border III)

Monitoring sites	**Details**
	Measurements, analyses • Physicochemical parameters • Elements (including salt components) • Organic substances • Phytoplankton (algae) • Fish and mussels[1]
Westoder River, Staffelde	Sampling site to record phytoplankton (algae) Measurements, analyses • Physicochemical parameters • Algae: taxonomy and abundances • Analysis of algae toxins
Oder, Lunow	Sampling site to record phytoplankton (algae) Measurements, analyses • Physicochemical parameters • Algae: taxonomy and abundances • Analysis of algae toxins
Hohensaaten	Discharge gauge
Hohenwutzen I	Automatic water quality monitoring station of the federal state of Brandenburg Automated measurements (10-minute measurement interval) • Physicochemical parameters • Chlorophyll-a • *Daphnia* toxicity test (water fleas) Additional measurements of daily composite samples • Physicochemical parameters • Elements (including salt components) • Organic substances • Other substances by means of non-target screening • *Daphnia* toxicity test (water fleas) • Algal toxins
Hohenwutzen II	Automatic monitoring station of the Federal Institute of Hydrology[2] Measurements of daily composite samples • Elements (including salt components) • Known (approx. 1,200) organic pollutants/substances and unknown substances by means of non-target screening • *Daphnia* toxicity test (water fleas) • Algal toxins • Oxidising agents
Oder, Lebus	Sampling site to record phytoplankton (algae) Measurements, analyses • Physicochemical parameters • Algae: taxonomy and abundances • Analysis of algae toxins

[1] The measurements were taken at monitoring sites other than those mentioned above. Mussels (*Dreissena* sp.) and fish (*Rutilus rutilus*) were analysed.
[2] Part of the monitoring network for environmental radioactivity in federal waterways (Section 161 Radiation Protection Act (StrlSchG))

Monitoring sites	Details
Frankfurt (Oder)	Automatic water quality monitoring station of the federal state of Brandenburg Automated measurements (10-minute measurement interval) • Physicochemical parameters • Chlorophyll-a Additional measurements of daily composite samples • Physicochemical parameters • Elements (including salt components) • Known (approx. 1,200) organic pollutants/substances and unknown substances by means of non-target screening • *Daphnia* toxicity test (water fleas) • Algal toxins • Oxidising agents
Eisenhüttenstadt	Discharge gauge

This report summarises the information, data and findings that were collected and evaluated by the authorities during the period of the fish die-off in August 2022. The following sections of this report present the findings to date, analyse possible hypotheses linked to the fish die-off, assess causal relationships and identify the need for clarification and action to prevent events of this kind in the future.

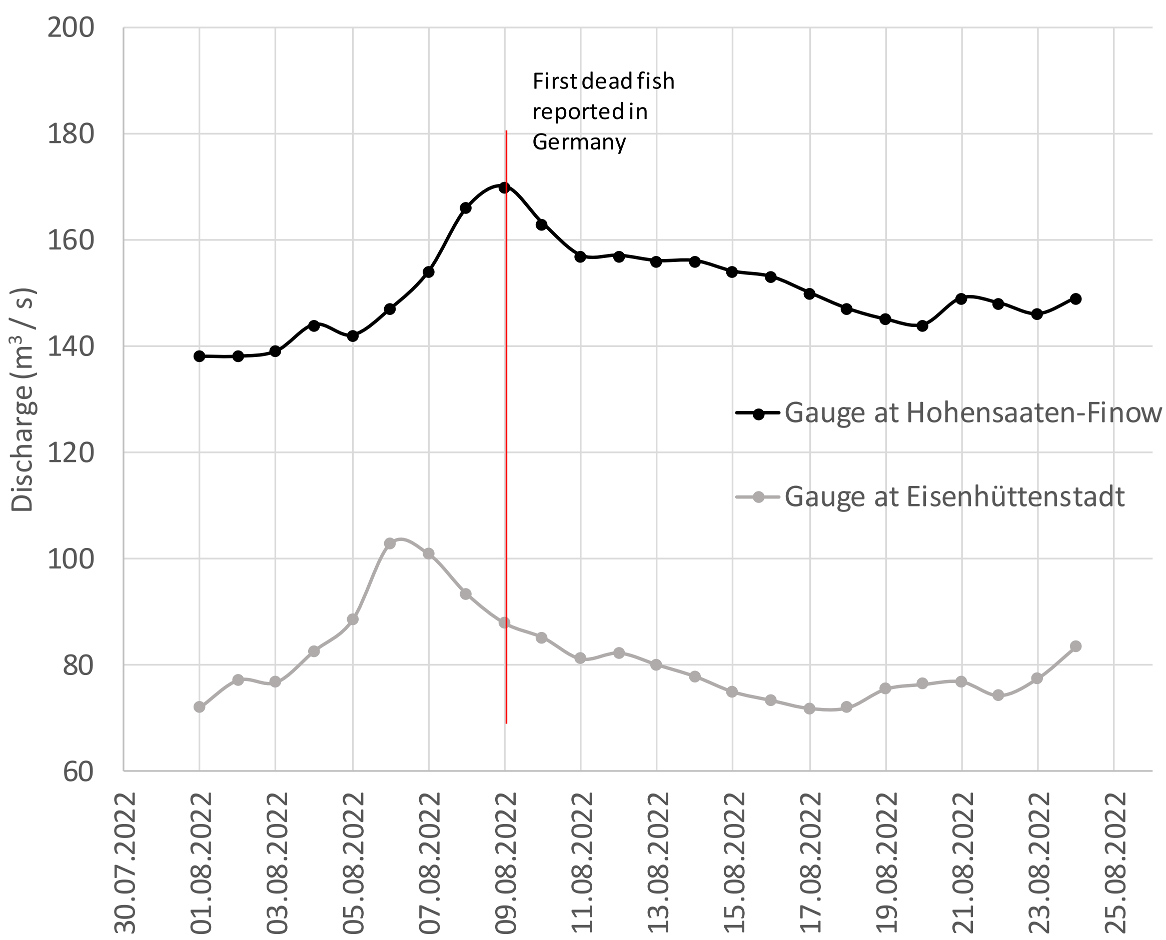
2 Results

2.1 Hydrological parameters

Figure 3 shows the hydrological conditions before, during and after the period of the fish die-off using discharge data from the Eisenhüttenstadt and Hohensaaten-Finow gauges.

At the Eisenhüttenstadt gauge there was an increase in discharge in the Oder from 04.08.2022 to 11.08.2022. The maximum value was 103 cubic metres per second (m^3/s) on 06.08.2022. The peak discharge with a maximum value of 170 m^3/s was measured after three days at the Hohensaaten-Finow gauge. It is not possible to retroactively quantify to what extent the increase in discharge is due to precipitation in the catchment area.

Figure 3: Discharges at the Eisenhüttenstadt and Hohensaaten-Finow gauge stations from 01.08.2022 to 24.08.2022 (Federal Waterways and Shipping Administration)



Compared to the mean discharges at the two gauges in the period 1951 to 2015, the mean discharge in 2022 was much lower than these values (German-Polish Border Water Commission, 2021). Overall, the discharge in the Oder was significantly lower in the monitoring period than in the previous years.

2.2 Physicochemical parameters

The physicochemical parameters analysed include water temperature, oxygen content, pH, chlorophyll-a, nutrient ratios and conductivity as a measure of salt content. These were continuously measured at the Frankfurt (Oder) and Hohenwutzen I monitoring stations as well as in daily composite and scoop samples.

Table 2 shows the evaluation criteria applicable for Germany for the physicochemical parameters according to the German Surface Waters Ordinance (Oberflächengewässerverordnung (OGewV)).

Table 2 Overview of the evaluation criteria for the physicochemical parameters for the type-specific assessment of ecological status/potential

Parameter	Unit	Evaluation criteria	Source
Water temperature	°C	21.5 to 28 (summer Apr.-Nov.) type-specific 10 (winter Dec.-Mar.) (max) type-specific	Annex 7 no. 2 OGewV
Oxygen content (dissolved)	mg/l	7 (minimum)	Annex 7 no. 2 OGewV
pH value		7.0 to 8.5 (min./max.)	Annex 7 no. 2 OGewV
Ammonium-N	mg/l	0.1 (type 9, 9.2) (annual average) 0.2 (type 15, 17, 20) (annual average)	Annex 7 no. 2 OGewV
Nitrite-N	mg/l	0.03 (type 9) (annual average) 0.05 (type 9.2, 15, 17, 20) (annual average)	Annex 7 no. 2 OGewV
Nitrate-N	mg/l	11 (average value) (converted from 50 for nitrate)	Annex 8 OGewV
Total phosphorous	mg/l	0.1 (annual average)	Annex 7 no. 2 OGewV
Orthophosphate (as P)	mg/l	0.07 (annual average)	Annex 7 no. 2 OGewV
Chloride	mg/l	200 (annual average)	Annex 7 no. 2 OGewV

2.2.1 Temperature, oxygen and pH value

The following findings relate to the Frankfurt (Oder) monitoring station:

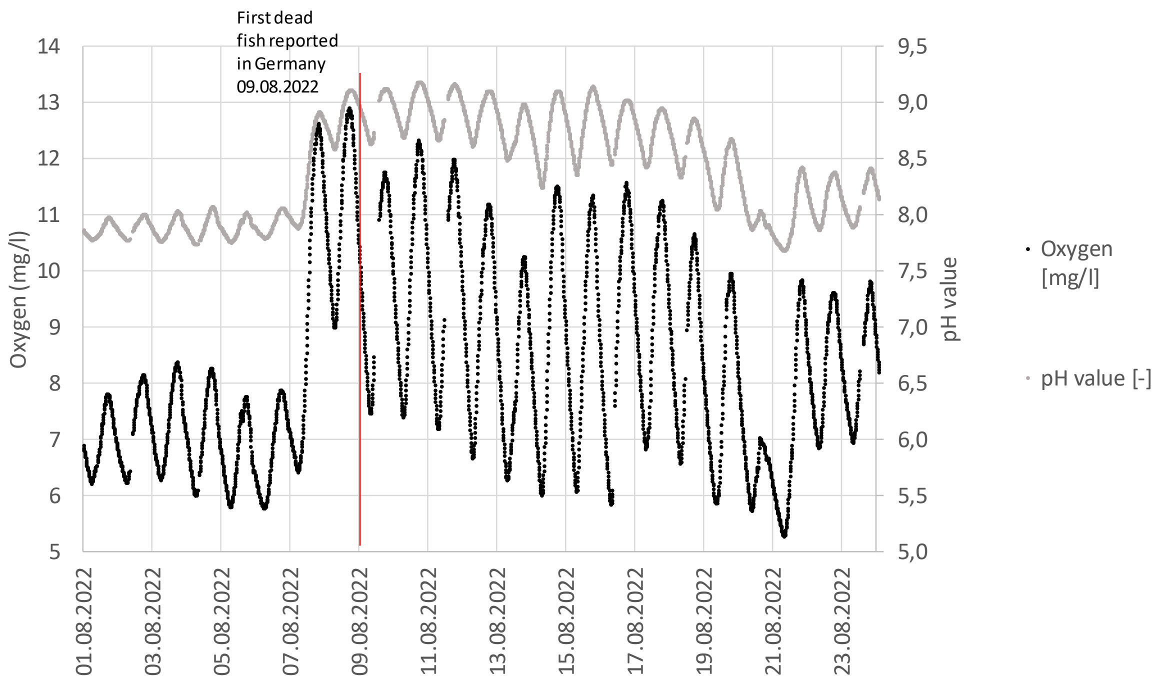
The **water temperature** was within the permissible range (OGewV) at 22-27°C during the monitoring period, and air temperatures ranging from 14-36°C were measured.

Changes in oxygen content and pH value at the Frankfurt (Oder) monitoring station from 31.07.2022 to 25.08.2022 are shown in Figure 4.

In the monitoring period, there was a sharp increase in the **oxygen content** from 6-8 mg/I to 9-13 mg/l (Figure 4) from 07.08.2022 to 12.08.2022. Furthermore, significant day-night fluctuations in oxygen content, averaging 5 mg/l, are clearly evident during this period.

The **pH value** also increased considerably at the Frankfurt (Oder) sampling site on 07.08.2022 from approx. 8 to > 9. Peaks as high as pH 9.2 were reached on subsequent days. Similar to the oxygen concentrations, the pH value showed clear day-night fluctuations of 0.5 on average. The values decreased again to between 7.8 and 8.4 starting on 20.08.2022.

Figure 4 **Changes in oxygen content and pH value at the Frankfurt (Oder) monitoring station in the period from 01.08.2022 to 24.08.2022.**



2.2.2 Nutrients

The concentrations of ammonium, nitrite and nitrate measured in the daily composite samples from Hohenwutzen I, II and Frankfurt (Oder) were either below the limits of quantification or exceeded them only very slightly.

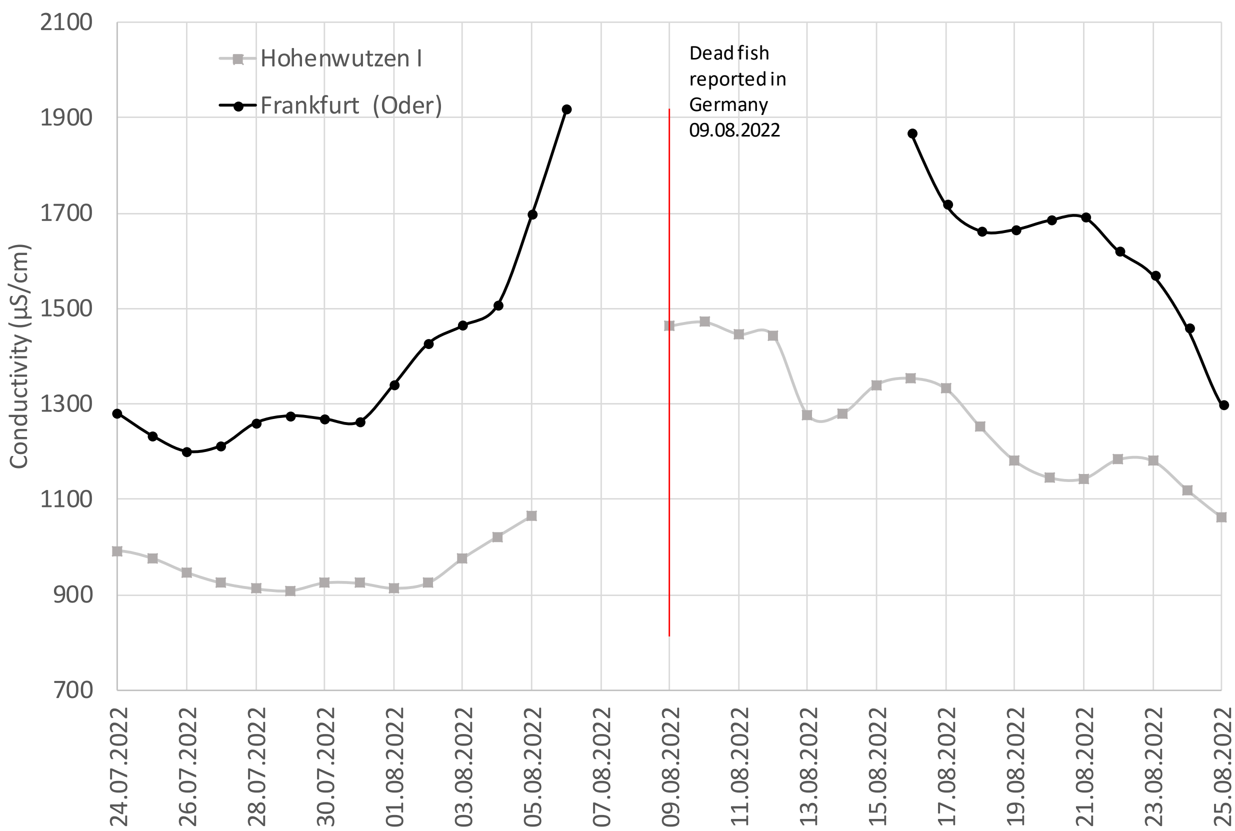
A noticeable development was a reduction in nitrate concentrations from the previous level of 1.5 mg/l to 0.6 mg/l on 07.08.2022 (measured with a measurement probe at the Frankfurt (Oder) monitoring station).

The values measured for total phosphorus and orthophosphate phosphorus during the monitoring period were in the polytrophic range, as has been usual for the Oder for many years, i.e. they were relatively high as in the previous years (Table 3).

2.2.3 Salt content

Conductivity in water is a measure of the salts dissolved in the water. The **conductivity** at the Frankfurt (Oder) monitoring station initially rose on 01.08.2022 and 02.08.2022 from approximately 1,200 µS/cm to around 1,500 µS/cm. Then, on 04.08.2022, there was another unusually sharp increase, reaching values above 1,900 µS/cm on 06.08.2022. A decrease in these values to below 1,900 µS/cm was only recorded starting on 16.08.2022. At the Hohenwutzen I monitoring station, conductivity increased from 900 µS/cm to nearly 1,500 µS/cm after one day compared to Frankfurt (Oder) (Figure 5).

Figure 5 **Conductivity at the two monitoring stations Frankfurt (Oder) and Hohenwutzen I from 31.07.2022 to 25.08.2022[3] (daily averages)**



[3] At both the Frankfurt (Oder) and Hohenwutzen I monitoring stations, some data is not available because the measuring range was exceeded or due to a power failure. The peak maximum was reached in Frankfurt (Oder) between 07.08.2022 and 12.08.2022.

In addition, filtered daily composite samples from the Hohenwutzen II monitoring site were analysed from 25.07.2022 to 15.08.2022 to determine the composition of the salts that led to the increase in conductivity.

Chloride and sodium were found to be the main components (Figure 6). In the period from 05.08.2022 to 15.08.2022, an additional 23,500 tonnes of sodium chloride were transported in the Oder in Hohenwutzen compared to 04.08.2022. The calcium (Ca) concentration decreased by around 25% during the period of high salt concentrations, possibly due to the process of biogenic decalcification[4] in the river.

Together with sodium and chloride, concentration increases of approx. 50-100% compared to the average value of the last week of July (25.07.2022 to 31.07.2022) are also evident for the elements bromine (Br), lithium (Li), molybdenum (Mo), caesium (Cs) and thallium (Tl) during this period (Figure 7). These elements were thus present in the water of the Oder in higher concentration ranges than are typically measured in German rivers.

In contrast, concentrations of other elements such as magnesium (Mg), potassium (K) and sulphur (S) did not show corresponding changes (Figure 6).

[4] Biogenic decalcification is defined as the formation of calcium carbonate minerals influenced by photosynthetically active aquatic organisms (e.g. algae). When these organisms bind carbon dioxide from hydrogen carbonate dissolved in water, OH- ions are released, which increases pH and in turn causes precipitation of calcium carbonate minerals due to a shift in solubility equilibria. This leads to the recorded decrease in calcium concentration in the water.

Figure 6 **Changes in the concentrations of chloride (Cl), sodium (Na), calcium (Ca), sulphur (S), magnesium (Mg) and potassium (K) at the Hohenwutzen II monitoring site from 25.07.2022 to 15.08.2022**

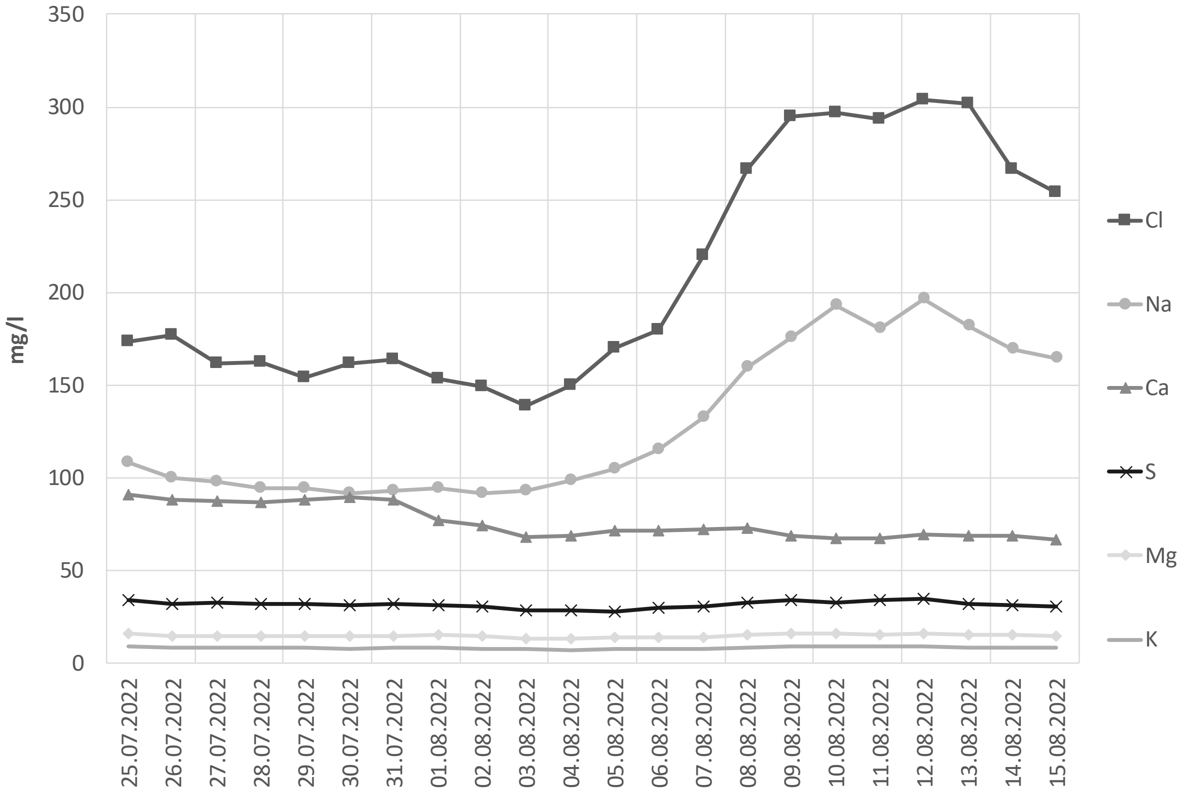
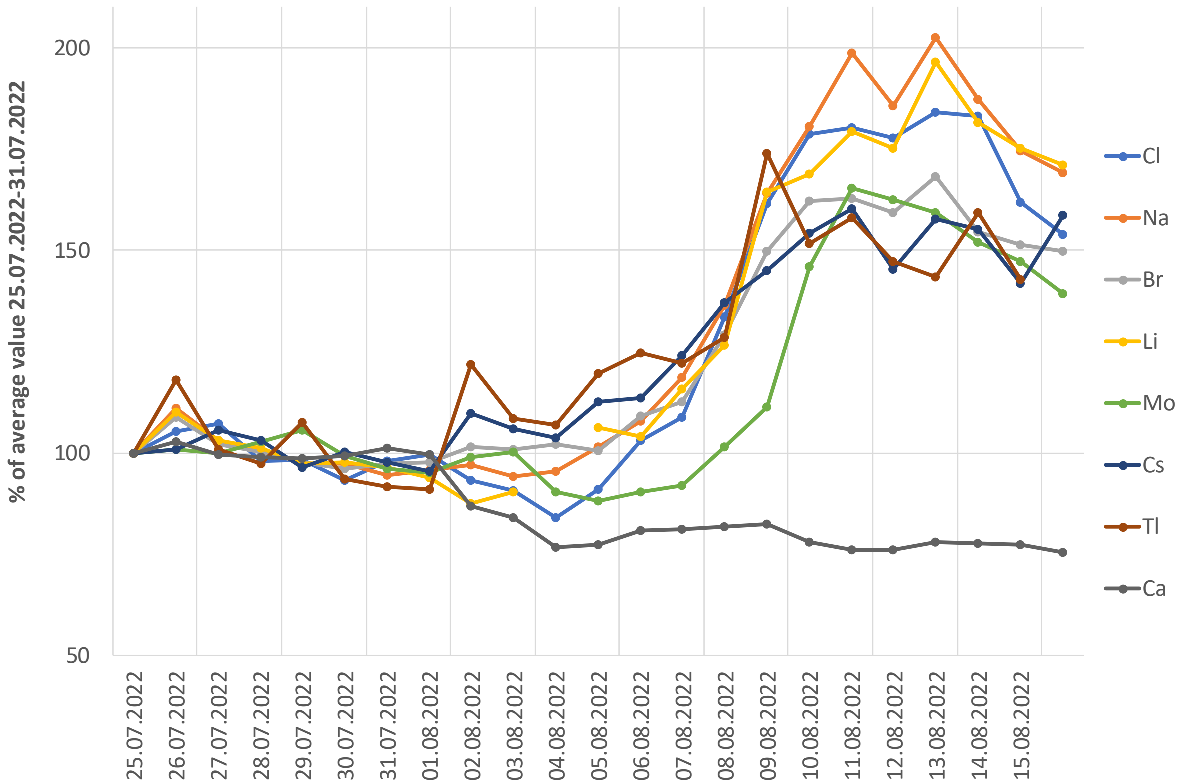


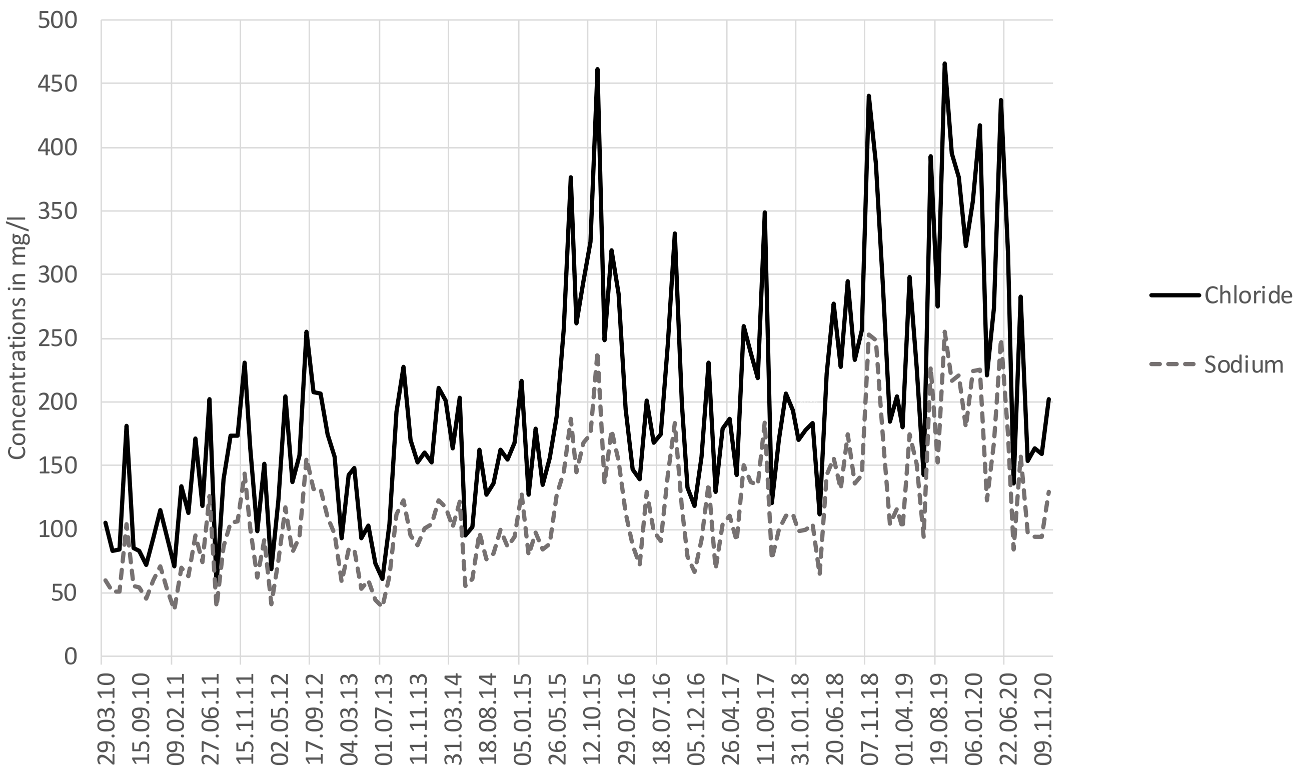
Figure 7 **Relative changes in the concentration of selected elements (dissolved) at the Hohenwutzen II monitoring site from 25.07.2022 to 15.08.2022** [5]



For many years, elevated chloride concentrations have been observed in the Middle Oder, exceeding the value of 200 mg/l specified in the Surface Water Ordinance (OGewV) . Long-term data from the Oder show a significant increase in mean annual average values for chloride and sodium over the past 10 years, while other parameters have remained unchanged (e.g. Mg and Ca) or their concentrations decreased (e.g. Pb and Fe) (Table 3). The figure showing changes in the most important salt ions over time (Figure 8) points to an increase in salt peaks over the past five years.

[5] The values shown are each normalised to the average value of the last week of July (25.07.2022-31.07.2022) as 100%. The measurements were carried out by the BfG

Figure 8 **Concentrations of sodium and chloride at the Frankfurt (Oder) monitoring site in the years 2010 to 2020**



Source: German Environment Agency using information from the Brandenburg State Office for the Environment, August 2022

Table 3 shows the annual average values of the physico-chemical parameters in the period 2011 to 2020 at the Frankfurt (Oder) monitoring site, as well as the elements and substances that are explained in more detail in the sections that follow.

Table 3 **Annual average values of various physicochemical parameters at the Frankfurt (Oder) monitoring site; source: German Environment Agency according to information from the Brandenburg State Office for the Environment, August 2022**

Parameter	Unit	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Water temperature	° C	12.3	13.4		13.9	13.3	12.7	13.2	13.6	13.8	13.5
pH value		7.99	8.1		8.04	8.17	8.02	8.25	8.28	8.25	7.92
Electrical conductivity	mS/m	88	93	84	94	124	108	105	125	135	127
Total nitrogen	mg/l	2.68	2.5	3.03	2.63	2.21	2.86	2.98	2.43	2.4	2.58
Nitrate-nitrogen	mg/l	1.13	1.03	1.55	1.55	1.0	1.67	1.66	1.59	1.32	2.21
Nitrite-nitrogen	mg/l	< 0.01	< 0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.01
Ammonium-nitrogen	mg/l	0.088	0.101	0.1	0.064	0.085	0.084	0.102	0.096	0.075	0.063
Total phosphorous	mg/l	0.114	0.124	0.136	0.109	0.13	0.13	0.131	0.12	0.121	0.105
Orthophosphate-phosphorus	mg/l	0.022	0.031	0.019	0.027	0.015	0.024	0.024	0.023	0.028	0.044
Oxygen	mg/l	11.2	11.1	10.4	10.4	10.6	10.7	11.3	11.1	11.5	10.1

Parameter	Unit	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Oxygen saturation index	%					102	99	107	105	110	96
Sulphate	mg/l	101	90	87	88	106	98	93	102	100	92
Chloride	mg/l	141	162	132	157	249	204	199	244	289	260
Filterable substances	mg/l	12.3	15.1	13.1	11.4	16.3	12.1	19.3	16.1	15.4	9.4
Total organic carbon	mg/l	7.08	7.3	8.22	5.98	6.4	6.45	7.82	7.55	7.08	6.42
Biochemical oxygen demand	mg/l	2.18	2.58	2.35	1.5	1.95	1.72	2.92	2.72	2.46	1.49
Sodium	mg/l	85	99	77	94	139	116	115	143	167	152
Potassium	mg/l	7.06	7.24	6.99	6.93	7.83	7.19	7.43	8.11	8.98	8.32
Magnesium	mg/l	14.2	14.0	14.0	13.7	15.3	12.8	13.3	14.9	15.4	14.2
Calcium	mg/l	68.9	67.7	65.6	69.9	76.0	71.6	72.6	81.2	75.7	71.1
Manganese	µg/l		148	125	98	127	137	155	132	175	119
Iron	µg/l	958	831	790	536	622	688	648	507	575	447
Arsenic	µg/l	2.48	2.52	2.31	2.41	2.13	2.48	2.49	2.12	2.32	2.54
Lead	µg/l	1.62	1.57	1.46	1.1	1.18	1.67	1.42	1.05	1.43	1.06
Cadmium	µg/l	0.13	0.104	0.091	0.079	0.11	0.071	0.084	0.062	0.072	0.048
Chromium	µg/l	1.22	1.19	0.89	0.77	0.78	1.14	0.73	1.19	0.71	0.445
Copper	µg/l	4.51	5.47	4.21	3.5	3.22	3.33	3.55	3.15	3.58	3.49
Nickel	µg/l	3.79	3.26	3.22	2.81	2.92	2.97	3.48	2.87	3.22	3.03
Mercury	µg/l	0.03	0.02	0.016	0.012	0.014	0.015	0.011	< 0.01	0.01	< 0.01
Zinc	µg/l	22	16	15	11	13	13	13	10	12	9.5

In the **Kleines Haff**, individual measurements of the physicochemical parameters were carried out at the three monitoring sites (Figure 2) and, subsequently, at four further monitoring sites close to the shore.

The findings showed that the recorded values were not unusual in the Kleines Haff. The values for conductivity, oxygen and temperature did not increase significantly. The pH value was slightly elevated compared to the annual average, but fell within the ranges of typical summer months during high biomass production.

Conclusion: Both the oxygen content values with the clearly pronounced day-night fluctuations and the increase in the pH value with the corresponding day-night fluctuations indicate high algae production in the Oder from 07.08.2022 onwards. The oxygen minima did not show a concentration that was harmful to fish at any time (OGewV).

The pH value exceeded a maximum of 9 on several days and thus the levels stipulated in the OGewV. The measured ammonium-nitrogen concentrations were almost all the time below the limit of quantification. Therefore, it is unlikely that fish were affected by this due to a shift in the degree of dissociation of ammonium to fish-toxic ammonia.

Similarly, the salt concentrations measured during the fish die-off and the resulting high conductivity cannot be classified as a direct cause of the fish die-off. The values of all relevant salt ions showed concentrations that are not directly toxic to freshwater fish.

2.3 Pollutants

2.3.1 Elements

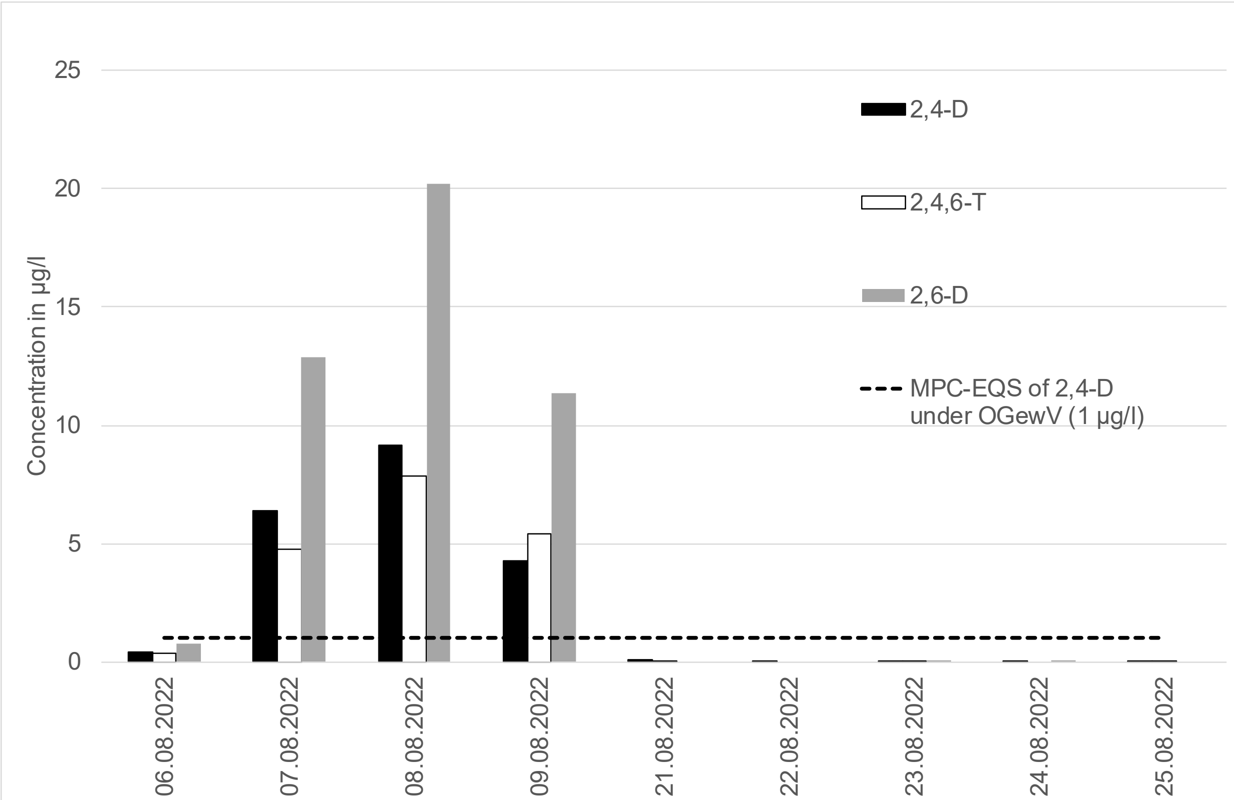
Elements in addition to those in Section 2.2.3 were analysed at the Hohenwutzen II and Frankfurt (Oder) monitoring sites. Individual samples showed elevated concentrations of manganese. Concentration of the metal rhenium was higher than in other rivers over the entire monitoring period. The values for all other inorganic compounds including mercury were unremarkable.

2.3.2 Organic compounds

Daily composite samples from the Frankfurt (Oder) and Hohenwutzen I monitoring stations on 7, 8 and 9 August 2022 showed elevated concentrations of the organic compounds 2,4-Dichlorophenoxyacetic acid (2,4-D), 2,6-Dichlorophenoxyacetic acid (2,6-D) and 2,4,6-Trichlorophenoxyacetic acid (2,4,6-T) (Figure 9). The environmental quality standard for the herbicide 2,4-D of 1 µg/l set out in the German Surface Waters Ordinance was exceeded ninefold. Exceedances related to these compounds also occurred from 2011 to 2020 in the Oder during winter, with maximum values of 2,4-D to 10.4 µg/l, 2,6-D to 10.8 µg/l and 2,4,6-T to 2.5 µg/l.

All three of these organic substances have an auxine-like mode of action that promotes uncontrolled growth in dicotyledonous plants. Aquatic dicots are also sensitive to 2,4-D, 2,6-D and 2,4,6-T (Efsa 2014), while green algae seem to have various reactions (Boyle 1980, Wong 2000, Sura 2012). In the standard aquatic toxicity tests, acute toxic effects for fish and invertebrates first occur in the mg/l range (Efsa 2014), such that the elevated concentrations can be ruled out as a cause of the fish die-off.

Figure 9 **Overview of the measured concentrations of 2,4-D, 2,4,6-T and 2,6-D at the Frankfurt (Oder) monitoring station over time**



At Frankfurt (Oder), concentration of the herbicide bentazon was measured in two times higher concentrations compared to previous peak values on 06.08.2022. A comparable concentration was found on 08.08.2022. Both values were below the environmental quality standard for the annual average value of 0.1 µg/l.

Analysis of water samples taken at Frankfurt (Oder) and Hohenwutzen I for the parameter groups of volatile halogenated hydrocarbons (e.g. mesitylene), polyflouroalkyl substances (PFAS) and organic compounds showed no abnormalities. Concentrations above the limit of quantification were found for some substances, however, these were comparable with the concentrations in other German surface water bodies (the pesticide dichlorprop, metolachlor and terbuthylazine, the pharmaceutical ingredients gabapentin, oxipurinol and the pharmaceutical metabolite valsartan acid and the solution 1,4-Dioxane). Mesitylene (1,3,5-Trimethylbenzol) and cyanide (free and total) were not present in concentrations above the limits of quantification, 0.1 µg/l and 3 µg/l respectively, in any samples.

At the three monitoring sites in the Kleines Haff, no abnormalities were found in analyses and chemical results.

2.3.3 Results of non-target screening (NTS)

Non-target screening is a new analysis method that allows observation of a large number (>1,000) of known and unknown substances based on measurement of their high resolution mass. It is especially suited to identify unknown substances. The identified substances can subsequently be quantified (e.g. concentrations) if relevant reference standards are available.

NTS of daily composite samples taken at Hohenwutzen II from 01.07.2022 to 15.08.2022 provided evidence of 70 known organic substances by comparing with a spectrum database including 1,200 substances (Jewell et al., 2020). Half of the identified substances were quantified following screening analysis. No other abnormally high concentrations of substances were found apart from the above-mentioned substances, 2,4-D, 2,6-D and 2,4,6-T as well as bentazon.

An additional suspect screening for known substances toxic to fish (90 substances with EC50 < 1 mg/l (Finckh et al., 2022) also did not reveal any unusual findings.

Analysis of two composite samples of suspended solids from the Hohenwutzen II monitoring station during the periods from 01.07.2022 to 31.07.2022 and 01.08.2022 to 15.08.2022 found around 60 substances, with seven compounds detected solely in August. Among these seven compounds were, for example, the biocide didecyldimethylammonium, the industrial chemical methyltriphenylphosphonium, the plasticiser benzyl butyl phthalate and the cyanobacteria toxins microcystin LR and microcystin YR (Table 4).

NTS detected additional, initially unknown substances. Using various statistical processes, 69 compounds have been prioritised to date. Specific potential substances have already been proposed for five of these prioritised compounds based on the statistical processes (Table 4), among these the substance hexamethoxymethylmelamine, which is used in coatings (Alhelou et al., 2019). Two additional compounds are likely dichloro- and trichlorophenolsulfonic acids, which, based on a good correlation with the intensity curve of 2,4-D, could be related to the discharge of 2,4-D, like the previously identified 2,6-D and 2,4,6-T.

Due to the high oxygen contents, the water samples were also analysed for residues of oxidising agents (called oxohalogenides and halogen acids). The results showed no abnormalities except for perchlorate. At the Hohenwutzen site, the concentrations of perchlorate increased by 2-fold between 05.08.2022 and 10.08.2022, analogous to the increase in conductivity and salt concentration.

Perchlorate is an oxidising agent used in fireworks, rockets, ammunition, flares and airbags (Trumpolt et al., 2005). In addition, elevated concentrations of perchlorate are also found in some fertilisers. Small amounts can be formed during disinfection processes. Perchlorate is, however, also formed in the atmosphere and is deposited as a salt, such that the elevated concentration is probably attributable to the increased salt inputs.

Table 4 Selection of substances detected via NTS that exhibited abnormalities during the time period of the event, source: BfG

Substance	Use/source	Detected in	Classified via
Didecyldimethylammonium	Biocide	Suspended solids	BfG spectrum database
2-Oxaspiro[4.5]decan-3-one	Unknown	Suspended solids	BfG spectrum database
Methyltriphenylphosphonium	Industrial chemical	Suspended solids	BfG spectrum database

Substance	Use/source	Detected in	Classified via
Benzyl butyl phthalate	Plasticiser	Suspended solids	BfG spectrum database
Tryptamine	Natural metabolite, basic material of chem. synthesis	Suspended solids	BfG spectrum database
Microcystin LR and YR	Cyanobacteria toxins	Suspended solids	BfG spectrum database
o,o´-Diethylthiophosphate*	Synthesis by-product and decomposition product of certain insecticides	Water	Online database
Hexamethoxymethylmelamine*	Manufacture of coatings	Water	Online database
Dichlorophenolsulfonic acid*	Unknown	Water	Manual data interpretation
Trichlorphenolsulfonic acid*	Unknown	Water	Manual data interpretation

***** *Potential substances that still require conclusive verification.*

2.3.4 Residues in aquatic organisms

Fish, snails and mussels from Brandenburg were examined for over 500 substances, substance groups and additionally screened for over 1,000 substances in the laboratory of Mecklenburg-Western Pomerania's State Office for Agriculture, Food Safety and Fisheries (LALLF). In fish, levels higher than the limit of quantification were detected only for mercury, which also exceeded the environmental quality standard of 20 µg/kg. Exceeding values were recorded between 2016 and 2019 in the Oder and many other river basins and were the main reason for failing to achieve the good chemical status in accordance with the EC Water Framework Directive for all surface water bodies in Germany. It was possible to rule out acute mercury toxicity in the period of investigation for fish fauna and other aquatic organisms.

Conclusion: Based on the analysis of more than 1,200 known organic substances and elements, no substance causing acute fish toxicity was found to date. The substances found, generally come from effluents from industrial or municipal wastewater treatment plants. Direct toxic impacts on fish fauna and invertebrates from 2,4-D, 2,4,6-T, 2,6-D and dichloro- and trichlorophenolsulfonic acids are also fairly unlikely.

The extent to which the combination of these chlorinated compounds can have ecotoxicological impacts on certain aquatic organisms remains to be clarified. Synthetic auxins such as 2,4-D can increase the growth of algae (Wong 2000, Sura 2012). Initial experiments tests with a water sample containing *Prymnesium parvum* did not produce any other indications to this effect.

Bentazon as the cause of acute fish toxicity is ruled out on the basis of the environmental quality standard of 0,1 µg/l for the annual average value.

The concentrations of the oxidising agent perchlorate were found at levels significantly below those required for acute toxicity to fish (Theodorakis et al., 2006). Overall, the results provide no indications that the elevated oxygen contents were caused by an increased discharge of oxidising agents.

Tests for pollutants in biota to date have not provided indications related to the cause of the fish die-off.

2.4 Ecotoxicological tests

Biological testing methods were used to identify pollutants and existing toxicity in water samples from the Oder. Biotesting measures the cumulative effects of all bioavailable substances with potential toxicity and therefore, provides information that complements chemical analysis. These tests used daphnia (water fleas) as well as green algae and luminescent bacteria.

Ecotoxicological testing with daphnia (*Daphnia magna*) is a fixed component of biomonitoring at the Hohenwutzen I monitoring station. The testing records various vital parameters of the daphnia by automatically evaluating images. The alarm index TI_{ges} is used to assess the potential effects. It is calculated using the swimming parameters recorded in the images. Daphnia toxicity of 10 indicates elevated toxic potential in the water samples. This criterion of 10 was exceeded from 12.08.2022 to 24.08.2022, reaching a maximum of 33 on 21.08.2022 (Figure 10).

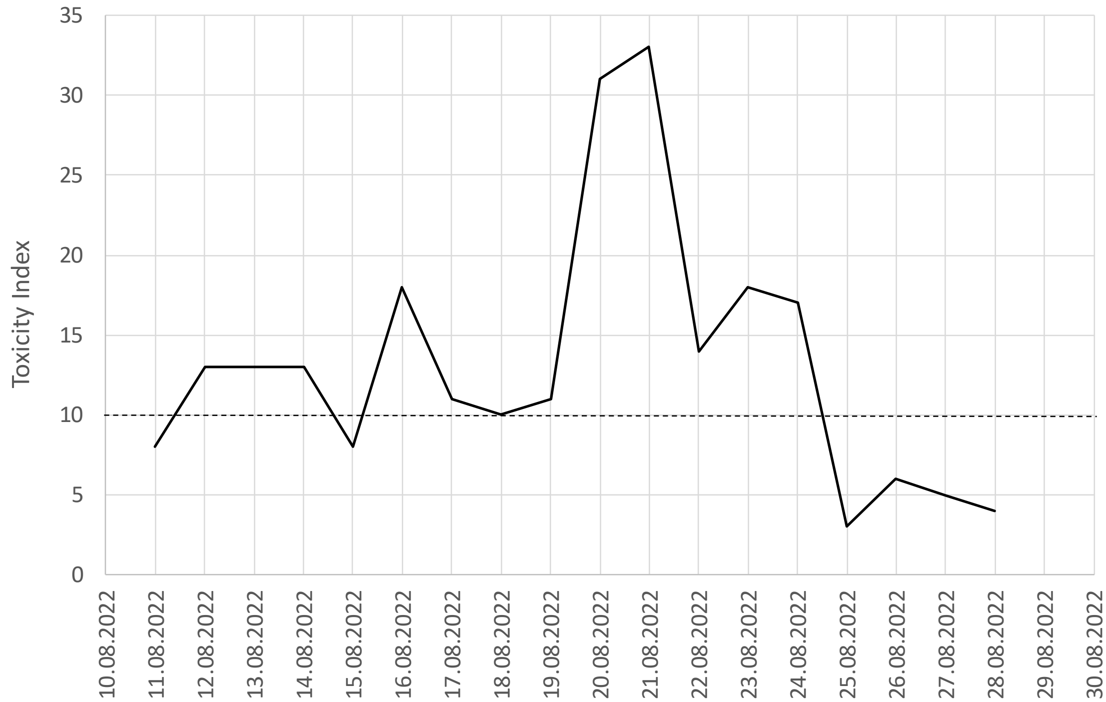
The BfG laboratory analysed a daily composite sample from the Frankfurt (Oder) monitoring site from 09.08.2022/10.08.2022 using test DIN 38412-30. The results showed daphnia toxicity of G_D 16 (toxicity up to a dilution factor of 16). The sample from 11.08.2022/12.08.2022 showed daphnia toxicity with a G_D value of 32.

A sample from the Oder-Spree canal on 06.09.2022 containing high abundances of the brackish water alga *Prymnesium parvum* showed daphnia toxicity with a G_D value of 8. Reduced toxicity with a G_D value of 4 was measured in the filtered sample. This could indicate that some of the toxic substances were present and associated with particulates, i.e. either associated with suspended solids or the biomass in the samples (including algae).

In associated parameters that were recorded in parallel (pH value, oxygen content, conductivity/salinity, ammonium concentration), no abnormalities were found that could be seen as causes for the fish die-off, except for the high conductivity of 2.100 µS/cm.

In all of the water samples analysed using green algae and luminescent bacteria testing, no inhibitory effects were found.

Figure 10 **Daphnia toxicity in the daphnia toximeter at the Hohenwutzen I monitoring station from 05.08.2022 to 30.08.2022. The alarm threshold of the toxicity index $TI_{max\ (Toxic\ Index)}$ is 10.**



Conclusion: The biotesting used only recorded adverse to lethal effects in relation to daphnia; these were found both in the daphnia monitor and in analyses using standardised biotesting. There were no inhibitory effects for luminescent bacteria and green algae. The toxic substances appear to be present in dissolved form and associated with suspended solids/biomass. It is possible to rule out the measured associated parameters as a cause. The evident impacts on daphnia (in water samples from Frankfurt (Oder) and in the daphnia monitor at Hohenwutzen I) were complementary to the fish die-off, but lasted longer.

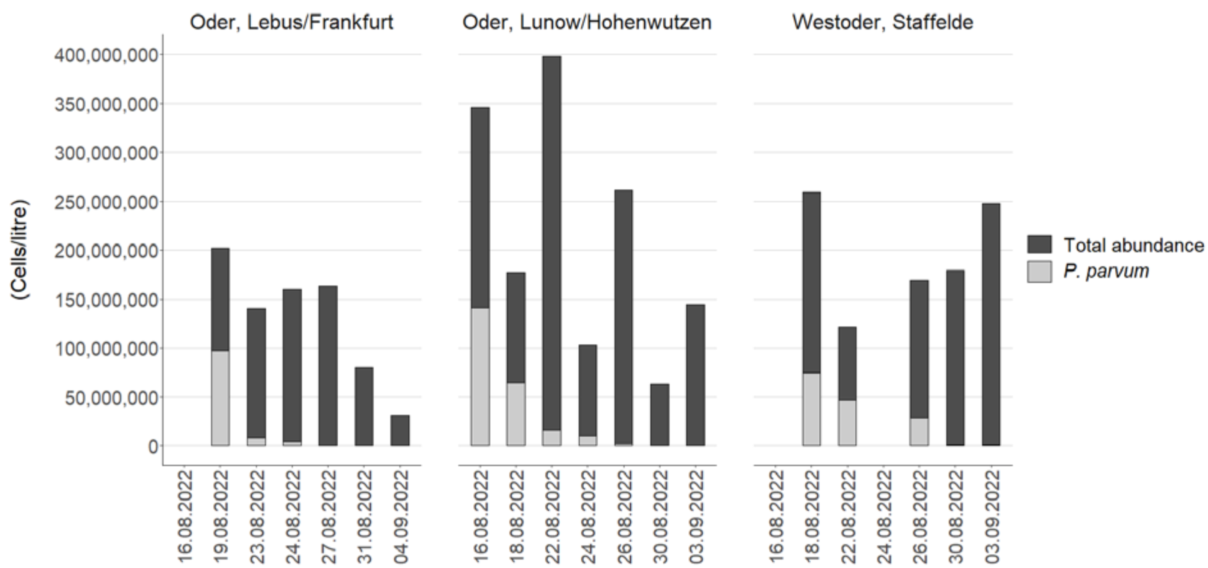
2.5 Phytoplankton

2.5.1 Abundance and distribution of phytoplankton and *Prymnesium parvum*

The composition and abundance (frequency) of phytoplankton (algae that floats freely in water) in the Oder was systematically recorded at three sampling sites from 16.08.2022 to 04.09.2022: Frankfurt near Lebus, Lunow and on the West Oder near Staffelde. In addition, samples were taken at the Hohenwutzen II monitoring station (near Lunow).

The results show that the abundance of phytoplankton at the Frankfurt (Oder) sampling site reached a maximum on 19.08.2022 at 203 million cells per litre. Of that number, 97 million cells per litre were *Prymnesium parvum* (Figure 11). At Hohenwutzen on 16.08.2022, a maximum cell count of *Prymnesium parvum* of 141 million cells per litre was recorded. For the three sampling sites during the period above, this was the maximum measured value at the peak of the algal bloom. After 19.08.2022, the abundance of *Prymnesium parvum* at all three sampling sites declined continuously.

Figure 11 Overall abundance of phytoplankton and abundance of *Prymnesium parvum* in the Oder



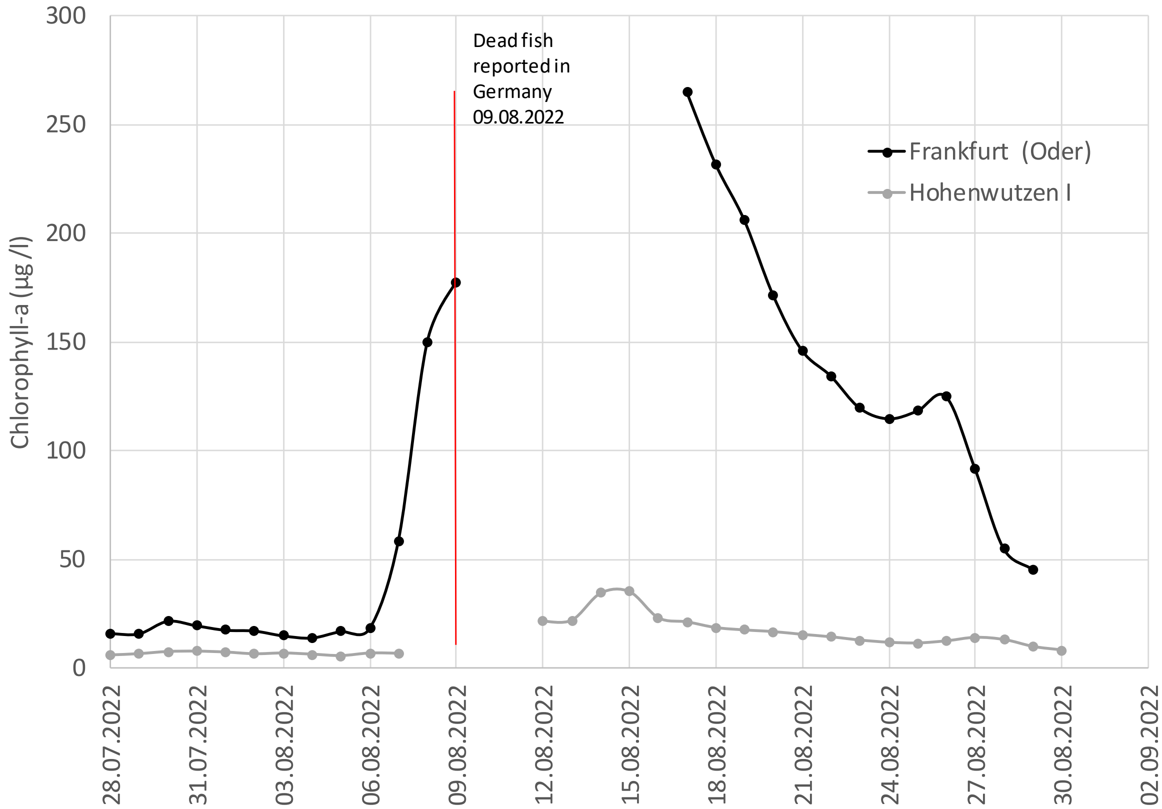
Prymnesium cells also made their way into side waters of the Oder via locks and outlets and to some extent have remained there due to lack of current. Thus, over a time period from 27.08.2022 to 04.09.2022, cell counts of over 50 million cells per litre were found in the Oder-Spree canal in Eisenhüttenstadt. The *Prymnesium* bloom presumably reached the Wriezener Alte Oder in the Oder Marshes, via the Reitwein channel, the Förstersee lake and the Quappendorf canal, where fish die-off was also reported.

A dedicated survey of the algae composition and biomasses before 16.08.2022 is not available. Chlorophyll-a is one indicator of the presence of algae. This was continuously measured at the Frankfurt (Oder) and Hohenwutzen I monitoring stations (Figure 12).

The Frankfurt (Oder) monitoring station recorded a significant increase in the chlorophyll-a concentration beginning on 07.08.2022, from 70 µg/l to more than 300 µg/l. During the monitoring period, concentrations did not return to the baseline prior to 07.08.2022.

There was also an increase in chlorophyll-a concentration at the Hohenwutzen I monitoring station over the same time period, however, it was much less significant in scale. Dilution of the chlorophyll-a in the Oder water by the upstream mouth of the Warta River could be partially responsible for this.

Figure 12 **Chlorophyll-a concentrations at the Frankfurt (Oder) and Hohenwutzen I monitoring stations from 28.07.2022 to 30.08.2022**



High-resolution satellite images show the geographic extent of algal blooms along the watercourse over time. Satellite images also make it possible to trace algal distribution in the Oder retrospectively. This data is currently being evaluated and is not part of this report.

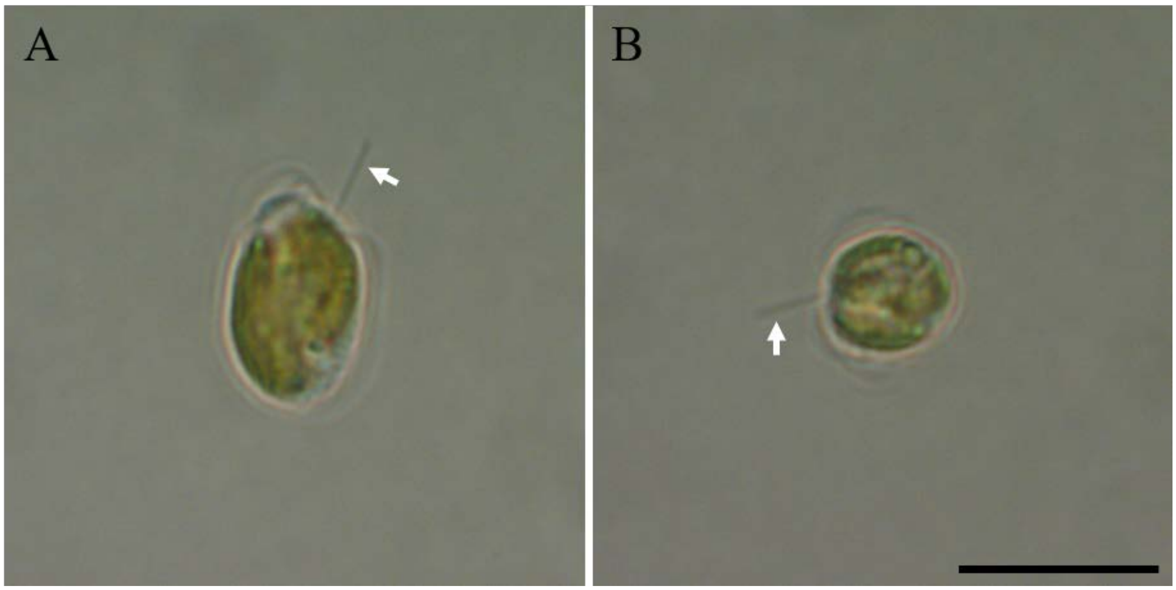
2.5.2 The brackish water alga *Prymnesium parvum*

It was possible to detect individuals of the species *Prymnesium parvum* in the taxonomic composition of the phytoplankton. This species was identified using optical microscopy in Germany by three independent institutions. Its presence was later unambiguously identified by the BfG using molecular biological testing, which included taking individual organisms from enrichment cultures and extracting their DNA. The subsequent sequencing of the DNA using Sanger sequencing provided clear molecular biological evidence. This means that methods are available that allow the detection of *Prymnesium parvum* in environmental samples within 24 hours, also without microscopic detection.

Prymnesium parvum is a planktonic, halophilic, single-celled, ellipsoid flagellate (Figure 13) that is particularly associated with brackish water conditions but can also proliferate in inland waters under appropriate conditions (high salt content, in particular). Some of the chemicals produced by *Prymnesium parvum* are poisonous to fish and other aquatic animals such as amphibians, snails, etc.

In fish, prymnesins destroy the function of the gill tissue. Blooms of this alga have already destroyed local fish and crustacean populations worldwide (e.g. Roelke et al. 2016).

Figure 13 **Optical microscope images of two cells of *Prymnesium parvum*. Both cells show the flagella (around the cell body) and the stiletto-like outstretched haptonema characteristic of *Haptophyceae*. A = oval form B = round form. Scale bar = 10 µm Photo: D. Mora, BfG.**



According to the monitoring data for the implementation of the EG Water Framework Directive, *Prymnesium parvum* was found in Germany in Schleswig-Holstein in the area of two coastal lagoons (1977 and 2004), in Saxony-Anhalt in a lake in a former opencast mine (2020) and in Mecklenburg-Western Pomerania (2009 and 2021). *Prymnesium parvum* was detected in the Baltic Sea coastal waters of Mecklenburg-Western Pomerania from 2008 to 2015. Its presence in these locations did not play a role in the algal bloom and did not trigger any fish die-off.

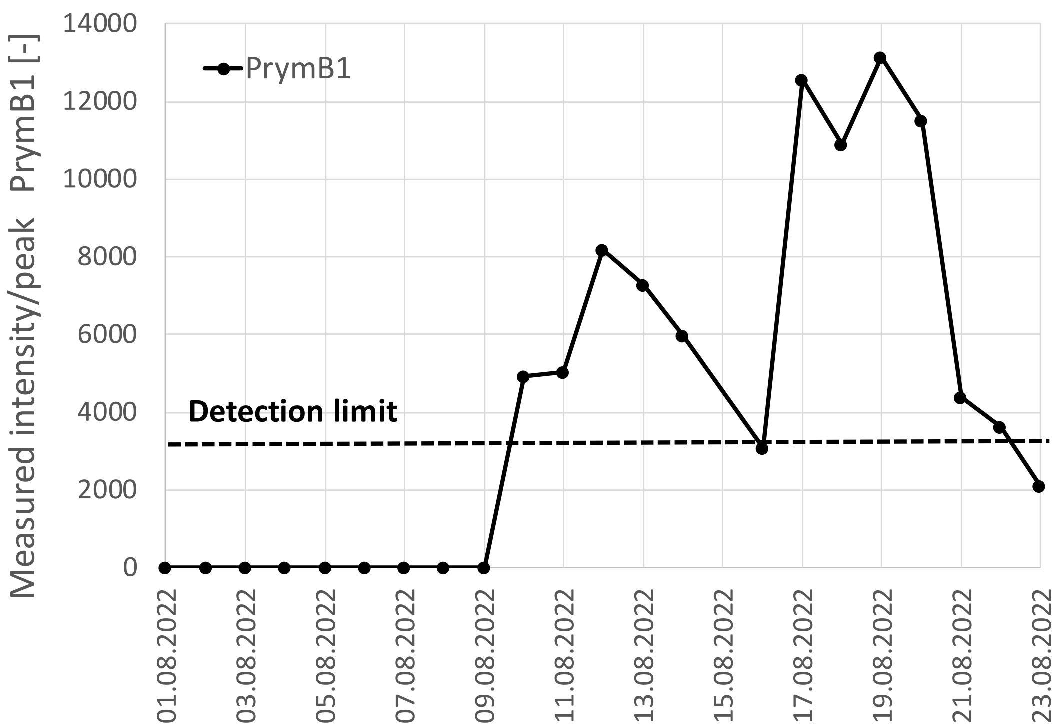
2.5.3 Algal toxins

The analyses of algae composition in the Oder samples show a comparatively high abundance of the brackish water alga *Prymnesium parvum* (see Section 2.5.2). The toxicity of *Prymnesium parvum* to fish, but also to various other organisms, is attributed to a group of chemical compounds known as prymnesins (Svenssen et al., 2019). The ecotoxicological effect of the individual toxins on different organisms has not been precisely defined to date. Toxin production can be strongly influenced by the growth phases of the algae and environmental conditions (Binzer et al., 2019; Svenssen et al., 2019). There are no known evaluation criteria for the prymnesin toxins.

While no dissolved prymnesins were detectable in the filtered water samples, during the event time period, two B-type prymnesins as well as microcystins were unambiguously detected in tested filter residues. There are no reference standards available for prymnesins. Because of this, it was only possible to determine measured intensities (peaks) of the detected prymnesins, ruling out conclusions about the absolute concentrations, but allowing instead comparisons of the relative differences in the prymnesin concentrations. Figure 13 shows the measured intensities of the detected prymnesins (prymnesin B1) in the algal biomass from the daily composite samples from the Hohenwutzen II monitoring station from 01.08.2022 to 23.08.2022. It is notable that first alga of this kind was positively identified starting on 10.08.2022 and the intensity curve shows similarities to the trend in *Daphnia* toxicity (Figure 10).

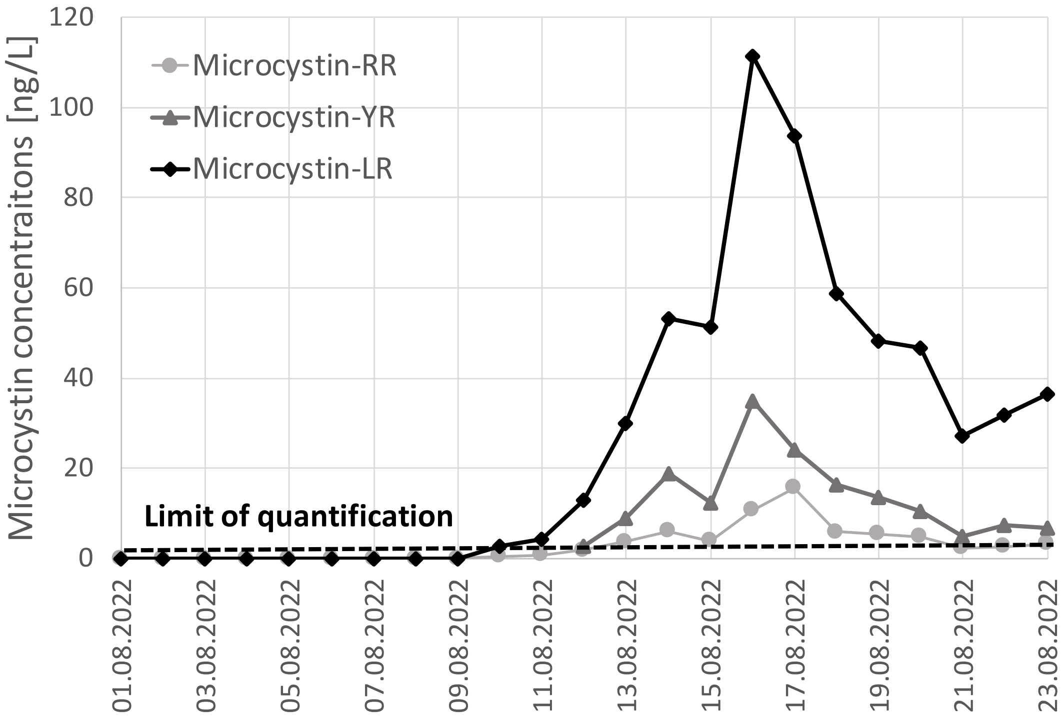
The analysis of algal toxins in Binzer et al., (2019) includes microcystins, which can be produced by blue-green algae (cyanobacteria), as well as prymnesins. Microcystins were also detected from 10.08.2022 (Figure 14). Maximum concentration was reached around 16.08.2022.

Figure 13 **Measured intensity of the algal toxin (B-type prymnesin) produced by *Prymnesium parvum* at the Hohenwutzen II monitoring station over time**[6]



[6]Because reference standards were not available, the measured intensity is listed as a peak without units.

Figure 14 **Concentrations in nanograms per litre (ng/l) of microcystin produced by cyanobacteria at the Hohenwutzen II monitoring station over time**



Conclusion: The detection of prymnesin-B1 in filter residues with algal biomass is evidence of the occurrence of algal toxins in the Oder.

The detected microcystin concentrations (Figure 14) were too low (ng/l) to have a toxic impact on fish (e.g. Malbrouck & Kestemont (2006)). These were also below the limit value for microcystin in drinking water of 1 µg/l.

3 Hypotheses and implications

3.1 Results as related to the various hypotheses explaining the fish die-off

Various assumptions have been made in the scientific community and in the media about the causes of the fish die-off. Based on the facts and data reviewed above, we can draw the following conclusions about the main hypotheses:

- **Prymnesin poisoning due to an algal bloom**
  The currently most likely hypothesis is that the fish die-off was caused by algal toxins (prymesins) from the brackish water alga *Prymnesium parvum*. This alga proliferated massively in the Oder, and the cell count was in a range that has been described in the literature as causing this type of fish die-off (> 20 million cells/l). In addition, between 10.08.2022 and 23.08.2022, prymnesins were in evidence in the algae of all tested samples taken from the Oder at Hohenwutzen.

- **High osmotic pressure in the fish due to increased conductivity**
  The elevated conductivity of more than 1,900 µS/cm at the Frankfurt (Oder) monitoring station caused by discharged salts is not sufficient to cause a fish mortality of this magnitude. However, it can be assumed that the elevated conductivity caused the fish considerable stress.

- **Discharges of currently unknown organic substances**
  Non-target screening (NTS) detected unknown substances that showed abnormal intensities during the time period of the event. Potential substances were identified for five of the total 69 prioritised compounds, among them the substances o,o´-Diethylthiophosphate (intermediate product in the manufacture of insecticides) and hexamethoxymethylmelamine (used in manufacturing coatings) as well as dichloro- and trichlorophenolsulfonic acids, which could be associated with the manufacture of 2,4-D. No conclusions can currently be drawn regarding the extent to which these substances contributed to the fish die-off. Further investigation is needed.

- **Discharges of 2,4-D, 2,6-D and 2,4,6-T**
  The herbicide 2,4-D as well as 2,6-D and 2,4,6-T were recorded in the Oder in considerable quantities at the beginning of August. It is highly likely that these are associated with industrial discharges, as 2,6-D and 2,4,6-T are known as technical by-products of manufacturing 2,4-D. Acute fish toxicity cannot be attributed to these substances based on the data available in the literature.

- **Discharges of oxidising agents**
  The monitoring results provided no indications that the increased oxygen contents in the Oder are attributable to the input of oxidising agents used for disinfection or bleaching. The only abnormality was a slight elevation in the concentrations of perchlorate coinciding with the increase in conductivity and/or salt concentrations. However, the concentrations of perchlorate were orders of magnitude below those associated with acute fish toxicity. The algal bloom, like the increased pH values, can also explain the increased oxygen contents and their daily periodic fluctuations.

- **Discharge of known substances toxic to fish such as nerve toxins/warfare agents**
  The suspect screening of non-target data for (approximately 90) substances toxic to fish did not produce any indications of their presence. This hypothesis cannot be supported by the measured data. Nonetheless, it cannot be completely ruled out that other, perhaps currently unknown substances toxic to fish were discharged. This could be due to missing analytical information.
- **Elevated ammonia concentrations**
  The concentrations of ammonium, nitrite and nitrate measured in samples from the beginning of August from Hohenwutzen I and Frankfurt (Oder) were either below or slightly above the limit of quantification. Despite the simultaneously elevated pH values, it is therefore highly unlikely that elevated ammonia concentrations triggered the fish die-off.
- **Elevated mercury or heavy metal discharges in the Oder**
  The samples from Hohenwutzen I, II and Frankfurt (Oder) showed no evidence of elevated levels of mercury concentrations or other metals. All measured mercury concentrations were within or slightly above the maximum allowable concentration in the OGewV and can therefore be ruled out as a direct cause of the fish die-off. The mercury concentrations found in fish also rule out acute mercury toxicity.
- **Discharge of caustic soda and persulfates**
  The increase in sodium concentrations correlated with the chloride concentration. This suggests discharge of sodium chloride rather than caustic soda, which, for example, is produced by paper factories. In comparison to July, no elevated sulphate concentrations were observed. The increase in pH values was attributable to algae growth and not to discharge of caustic soda, as demonstrated by the considerable day-night fluctuations.
- **Increased discharges of mesitylene or cyanide**
  Neither mesitylene nor cyanide were detected in concentrations above the limit of quantification. Therefore, any role in the fish die-off is very unlikely.

3.2 Implications

The Oder fish die-off was not the typical fish die-off that happens in many water bodies in the late summer due to oxygen deficits, also in combination with algal proliferation. The findings in this report indicate that the Oder fish die-off was triggered by multi-causal mechanisms. The high salt concentrations in the Oder encouraged the rapid growth of the brackish water alga *Prymnesium parvum* to a very high density of individuals. As a result of this growth, the Oder temporarily experienced an unusually high oxygen content, increased pH values and high chlorophyll-a concentrations. The identified alga species produces toxic substances that were detected in the Oder.

Climate conditions such as high light intensity, high temperatures, evaporation and low levels of precipitation, that are also anticipated in the future, will continue to lead to increasing concentrations of substances dissolved in water. Technical solutions to store and manage water resources can, at best, compensate in the short term. To reduce pollution with highly concentrated substances to a level that is compatible with the ecosystem, the following questions and steps should be addressed:

- What are the growth-promoting environmental conditions for the brackish water alga *Prymnesium parvum*, and how can mass growth with toxin formation be prevented?
- How can the existing warning and alert plans at national and international level be adapted to ensure early information and warnings, also across borders, to prevent or minimise damage?
- Where should monitoring infrastructure be expanded and which additional tests could ensure identification of early signs of hazards to the ecosystem and appropriate countermeasures?
- Existing permits for discharging substances into water bodies should be reviewed with regard to necessary restrictions to address the factors that led to the fish die-off.

In addition to discharges, other changes to and uses of the Oder, particularly hydromorphological changes, require re-evaluation in order to restore and permanently ensure the resilience of the ecosystem and its biota.

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