PRESENTATION OF THE PRELIMINARY REPORT SITUATION ON THE ODER RIVER

With the participation of the representatives of:

- General Directorate for Environmental Protection
- Chief Inspectorate for Environmental Protection
- Chief Veterinary Inspectorate
- Institute of Meteorology and Water Management - National Research Institute
- S. Sakowicz Institute of Inland Fisheries in Olsztyn - National Research Institute
- Maritime Institute of Fisheries - National Research Institute
- National Veterinary Research Institute - National Research Institute
- Warsaw University of Technology
- Wrocław University of Technology
- University of Gdańsk
- University of Warmia and Mazury in Olsztyn
- University of Life Sciences in Wrocław
- University of Life Sciences in Lublin
- University of Technology of West Pomerania in Szczecin

Edited by: Institute of Environmental Protection – National Research Institute

IOŚ-PIB

Narodowy Fundusz Ochrony Środowiska i Gospodarki Wodnej

Summary in numbers

14 research centres and institutions involved in the preparation of the preliminary report

A team of 49 scientists working on the preliminary report.
A team of people working in the field and the laboratories (as many as 2, 435 employees were involved on behalf of the Veterinary Inspection alone).

Over 2,700 samples tested

10 thematic issues discussed in the report

Almost 200 items of professional literature analyzed

Topics covered in the preliminary report

- Observation of fish die-off
- Toxicological and anatomopathological examination of fish
- Hydrometeorological situation in the period preceding fish die-off
- The quality of the Oder River waters in the period of fish die-off
- Analysis of satelite images
- Factual background for the formulation and verification of the hypothesis on the relationship between fish die-off and the activity of the algae in the Oder River
- Identification of the presence and bloom of *Prymnesium parvum*
- Identification of the presence of *PKS* genes
- Determination of primnesines produced by *Prymnesium parvum*
- Algae blooms in the light of research to-date

Observation of fish die-off

Observation of fish die-off

- Observations for the presence of fish die-off in the waters of the Oder River were carried out in 5 voivodeships: śląskie, opolskie, dolnośląskie, lubuskie i zachodniopomorskie.

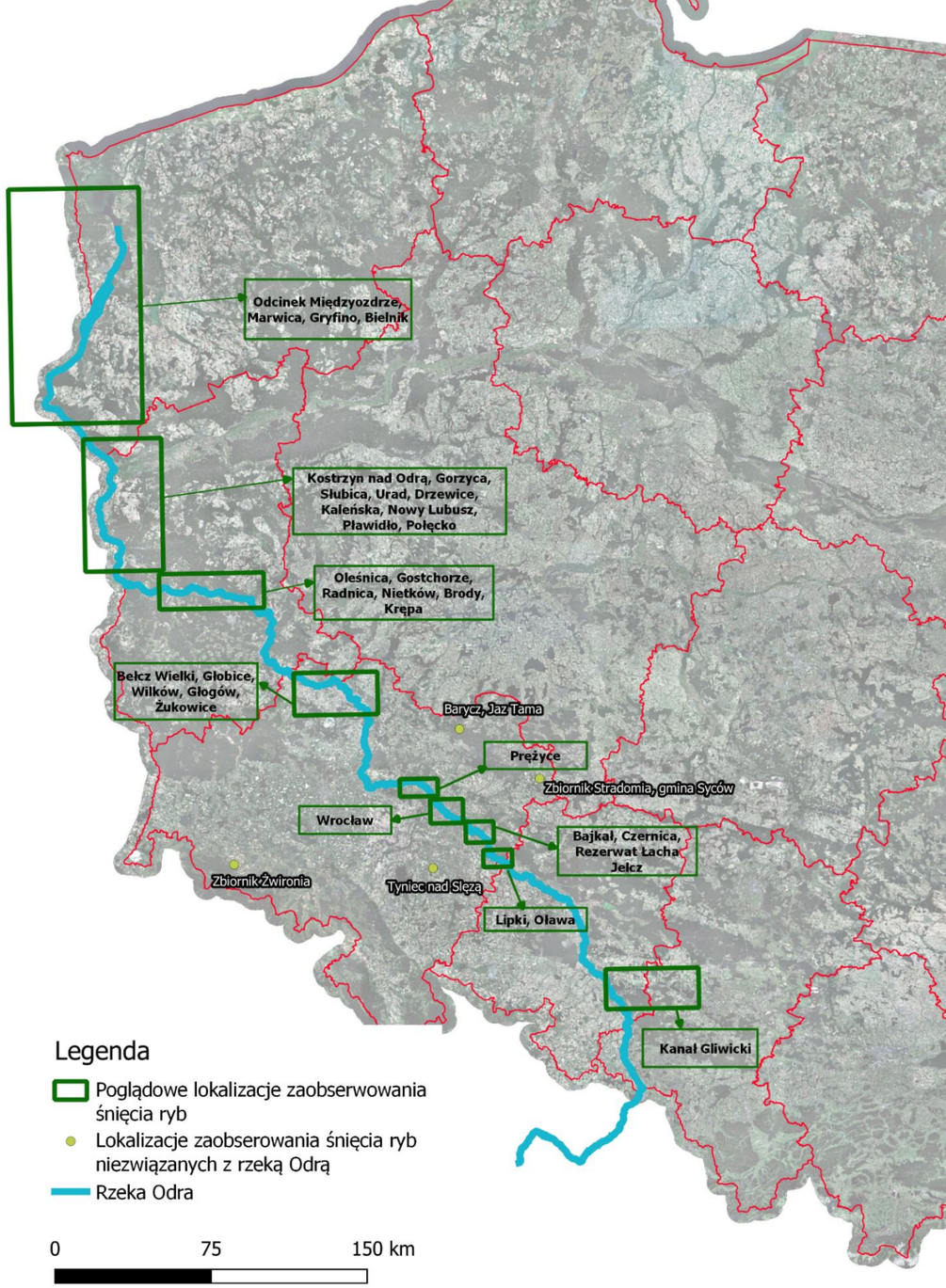
- Cyclical fish die-off was observed from the end of July 2022, but it was not a continuous phenomenon and it occurred in varous sections of the Oder and reservoirs at different time intervals..

Observtion of fish die-off

From the end of July 2022 to 12th of September 2022, a total of over 249 tonnes of dead fish were observed.

voivodeship	Amount of fish die-off [ton]
śląskie (Kanał Gliwicki)	7,3 t
opolskie (Kanał Gliwicki)	
dolnośląskie	26,1 t
lubuskie	46,42 t
zachodniopomorskie	169 t
Total:	about 249 ton

Due to diversity of the methodology of observations, the complexity of the processes and the dynamics of the situation, the data are estimates.

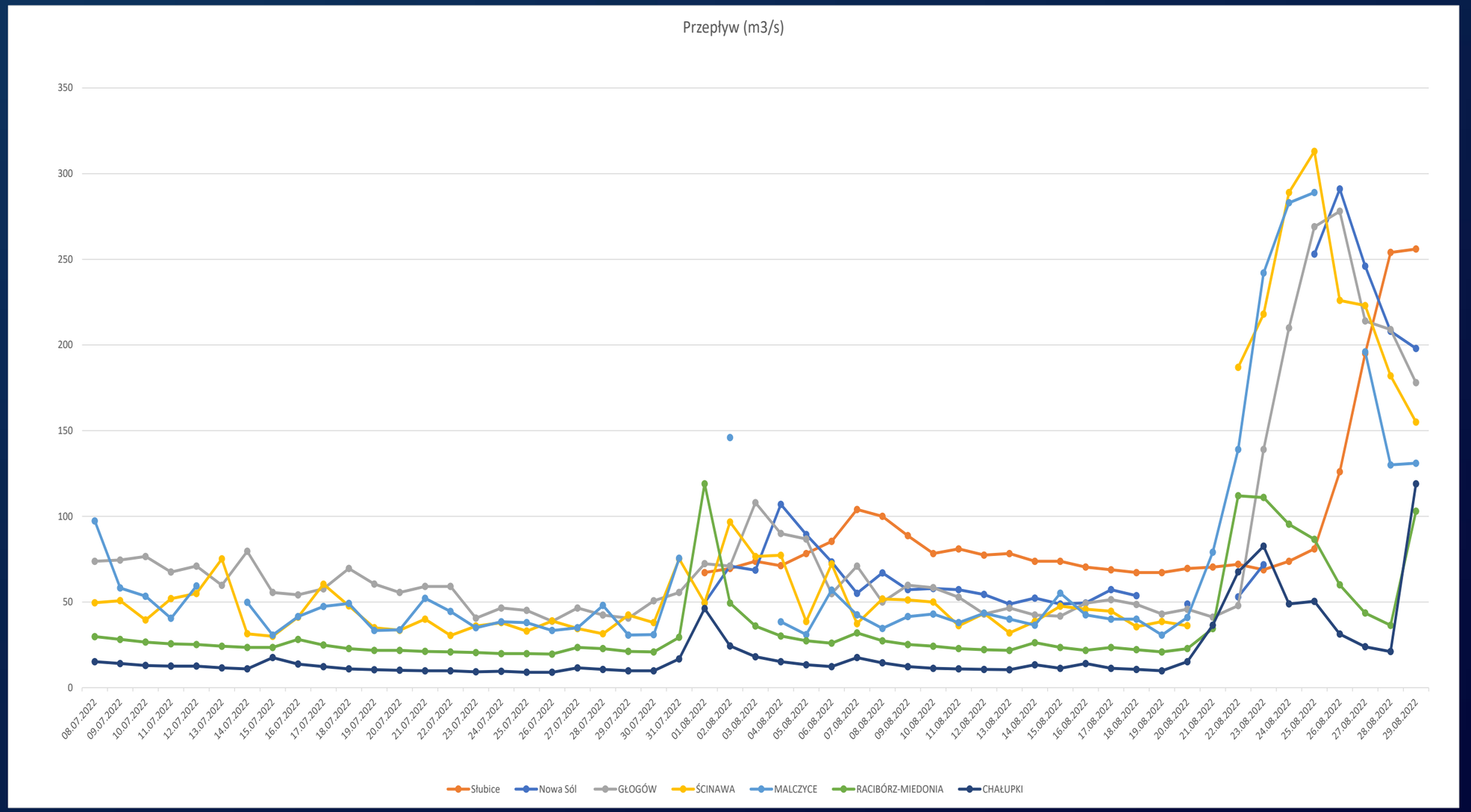


Hydrological situation of the Oder River in the preriod preceding the disaster

Hydrological situations and its impact on the conditions in the Oder River

- Almost throuhout the analyzed period (1st June 2022 – 20th August 2022), hydrographs were much below the average value of multi-year flows (SSQ), at the level closer to the value of the average low multi-year flow (SNQ).
- For more than two months, hydrographs presented in the low water zone, gradually approaching the lowest observed water levels.
- Downward trend in water levels was observed and, generally, the course was even. Higher fluctuations and increases in water levels were caused by intense rainfall.
- At the turn of July and August, exceptionally high water temperature was recorded in the Oder River (up to 27°C).
- From 21st of August, as a result of intense rainfall in the south of Poland, water levels increased along almost entire length of the Oder River, transitioning to the zone of medium levels. In the canalized section of the river and the estuary to the Szczecin Lagoon, the change trend was less noticeable. After the surge had passed, the stations experienced drops, while until the end of August the water levels were usually above the low levels.

The Oder River hydrological situation in 2022



The Oder River hydrological situation in 2022

- Hydrological situation – the condtion and corresponding flow rate – have direct impact on the water quality in the river – physical, chemical and biological conditions
  - A given load of pollutants entering the river results in the higher concentration of certain substances, the lower level of water is present in the river.
  - At lower flow, the river is more suspectible to rapied changes in the physical and chemical conditions
  - At a lower water level, the river heats up more rapidly and the temparature rise reaches deeper. Higher water temperature is usually connected with lower oxygen content.

The quality of the Oder River waters

Quality of the waters

From the begining of the year, samples are being taken in 17 control points, covering 5 voivodships: śląskie, opolskie, dolnośląskie, lubuskie and zachodniopomorskie;
The method of taking samples and the scope of the indicators are compliant with the Ordinance of the Minister of Infrastructure on the forms and methods of monitoring… (Journal of Laws 2021, item. 1576).

Results of the inspection of the status of waterbodies established on the Oder for the period 2014-2019 (source: State Env. Monitoring, CIEP).

Kod jcwp	Nazwa jcwp	Rok ostatnich badań	Klasa elementów biologicznych	Obserwacje hydromorfologiczne	Klasa elementów fizykochemicznych (grupa 3.1 - 3.5)	Klasa elementów fizykochemicznych (grupa 3.6)	Klasyfikacja stanu / potencjału ekologicznego	Klasyfikacja stanu chemicznego	Stan wód
PLRW6000191139	Odra od granicy państwa w Chałupkach do Olzy	2019	5	1	>2	2	zły	poniżej dobrego	ZŁY
PLRW600019117159	Odra od wypływu ze zb. Polder Buków do Kanału Gliwickiego	2017	4	>1	>2	2	słaby	poniżej dobrego	ZŁY
PLRW60001911759	Odra od Kanału Gliwickiego do Osobłogi	2017	5	>1	>2	>2	zły	poniżej dobrego	ZŁY
PLRW60002111799	Odra od Osobłogi do Małej Panwi	2017	5	>1	>2	>2	zły	poniżej dobrego	ZŁY
PLRW60002113337	Odra od Małej Panwi do granic Wrocławia	2019	4	1	>2	2	słaby	poniżej dobrego	ZŁY
PLRW6000211511	Odra od Wałów Śląskich do Kanału Wschodniego	2019	4	>1	>2	2	słaby	poniżej dobrego	ZŁY
PLRW60002113399	Odra w granicach Wrocławia	2017	4	>1	>2	2	słaby	poniżej dobrego	ZŁY
PLRW60002115379	Odra od Kanału Wschodniego do Czarnej Strugi	2017	4	1	>2	>2	słaby	poniżej dobrego	ZŁY
PLRW6000211739	Odra od Czarnej Strugi do Nysy Łużyckiej	2019	4	>1	>2	2	słaby	poniżej dobrego	ZŁY
PLRW60002117999	Odra od Nysy Łużyckiej do Warty	2019	4	1	>2	2	słaby	poniżej dobrego	ZŁY
PLRW60002119199	Odra od Warty do Odry Zachodniej	2019	4	4	>2	2	słaby	poniżej dobrego	ZŁY
PLRW6000211971	Odra od Odry Zachodniej do Parnicy	2019	4	>1	>2	2	słaby	poniżej dobrego	ZŁY
PLRW6000211999	Odra od Parnicy do ujścia	2019	5	>1	>2	2	zły	poniżej dobrego	ZŁY
Kanały									
PLRW6000011513	Odra od Olzy do wypływu z polderu Buków		brak klasyfikacji	brak klasyfikacji	brak klasyfikacji	brak klasyfikacji	brak możliwości klasyfikacji	poniżej dobrego	ZŁY
PLRW6000011659	Kanał Gliwicki z Kłodnicą od Kozłówki do Dramy	2019	4	>1	1	2	słaby	poniżej dobrego	ZŁY
PLRW60000117169	Kanał Gliwicki		brak klasyfikacji	2	1	brak klasyfikacji	brak możliwości klasyfikacji	poniżej dobrego	ZŁY

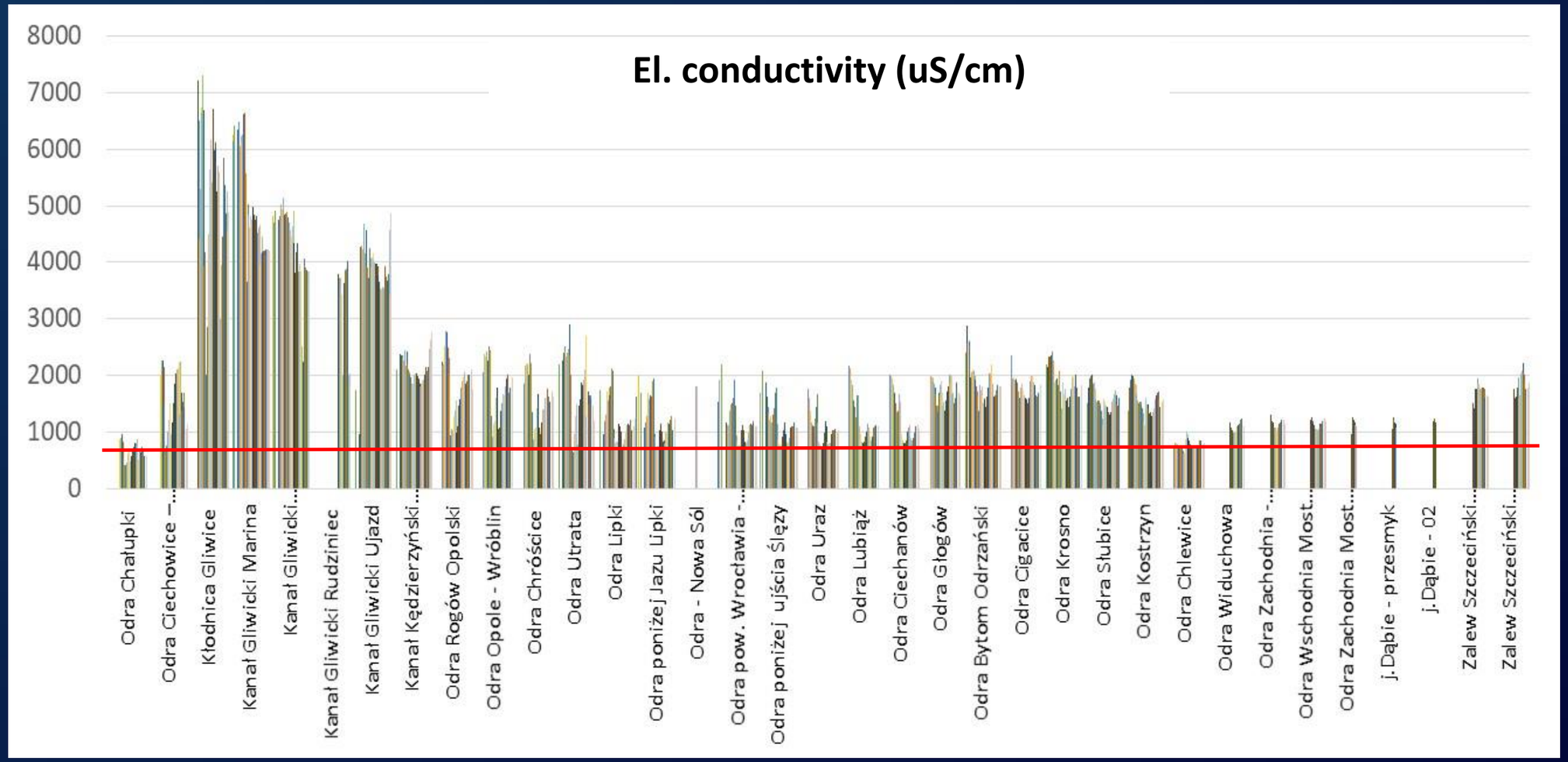
Quality of the waters

Aside from the routine water quality monitoring as part of the SEM, from 28th of July the Central Research Laboratory of CIEP, started daily sampling at additional points on the river. The numer of those points, from the middle of August, ranged between 34 and 37.

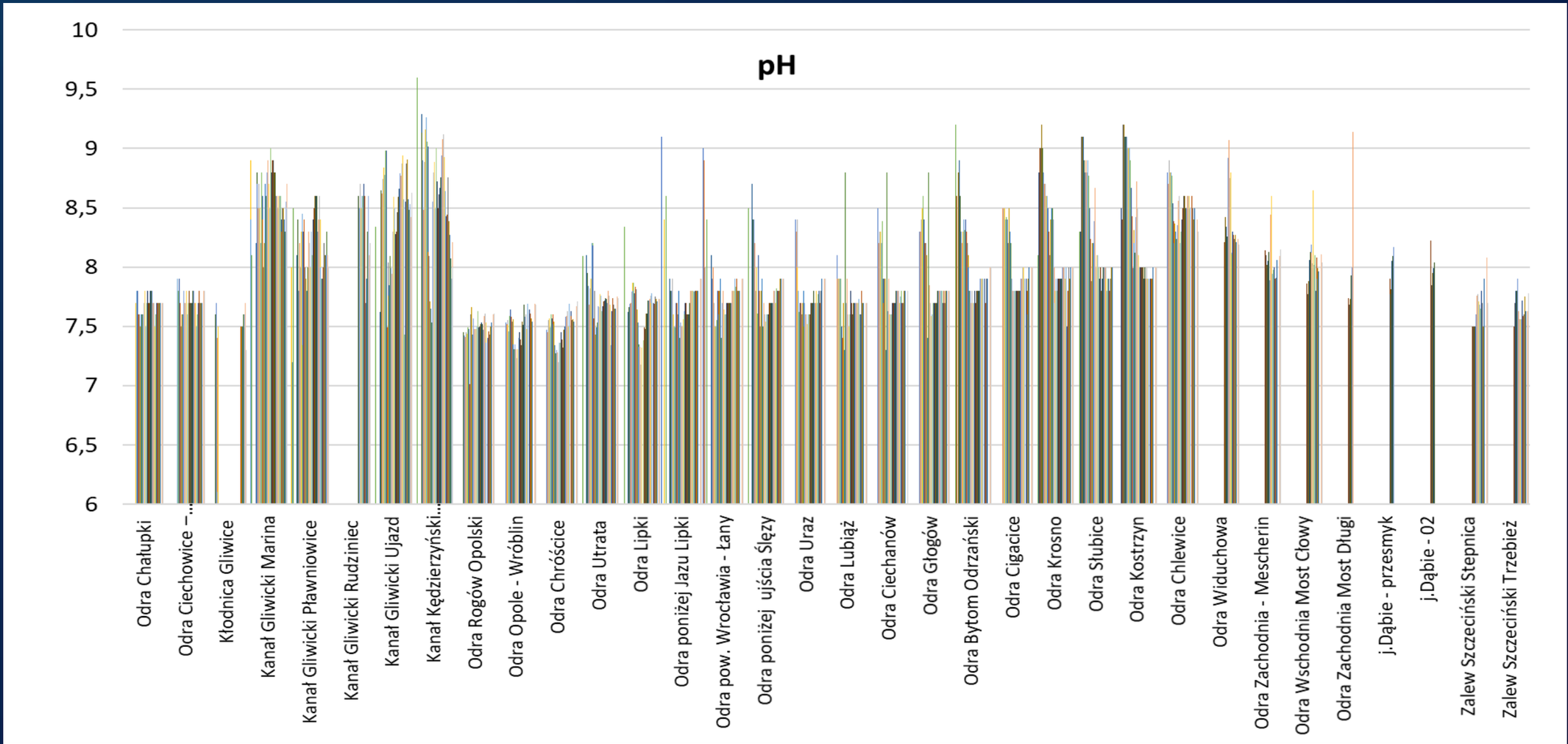
Until 20th September over 20,000 physicochemical determinations were carried out, in the fields of:

- Thermal and oxygen conditions: water temperature, dissolved oxygen, oxygen saturation, ChZT-Mn, total organic carbon, total suspended solids,
- Salinity condtions: electrolytic conductivity, sulfates, chlorides, sodium, potassium, hardness,
- Acidification conditions: pH,
- Biogenic conditions: Kjeldahl nitrogen, nitrate nitrogen, nitrite nitrogen, ammonium nitrogen, total nitrogen, total phosphorus, phosphate
- Specific pollutants: cyanides, phenol index, petroleum derivates,
- metals: chlorine, mercury, cadmium, lead, nickel,
- Elements: Li, Be, B, Al., Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Tl, Pb.

Quality of the waters: salinity indicators

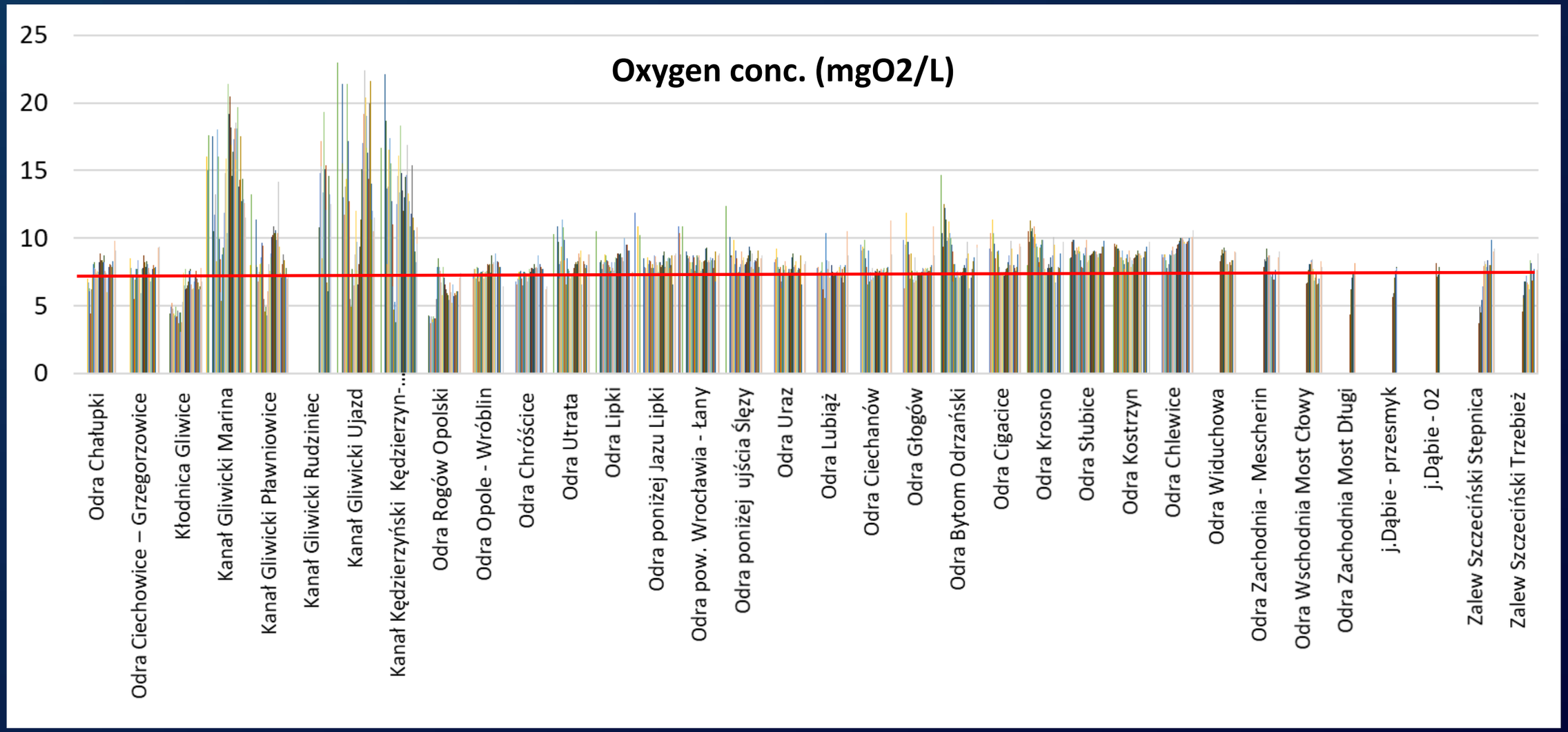


Quality of the waters – acidification indicators



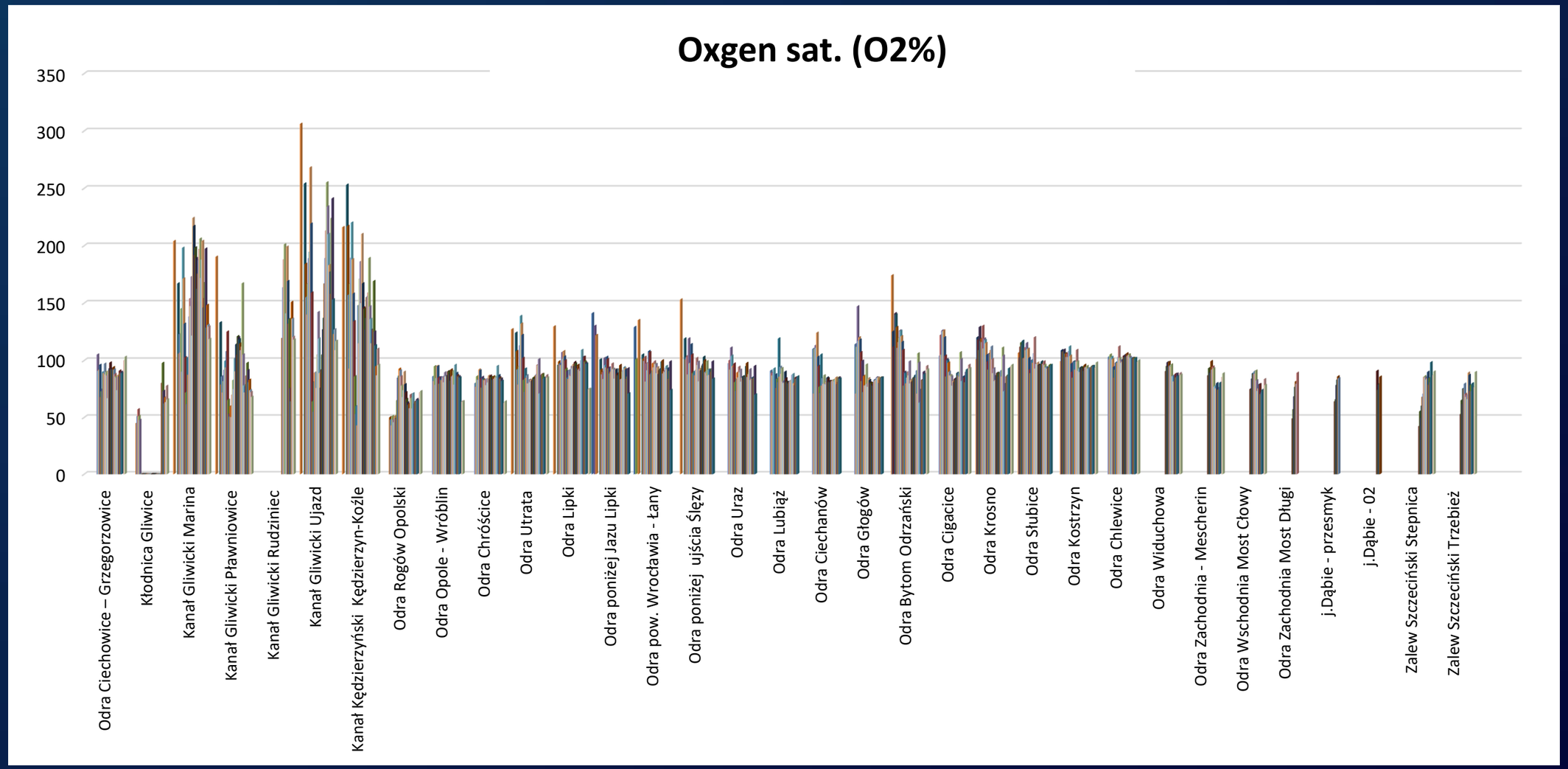
pH of surface waters is extremely variable and its periodic fluctuations are most often caused by biological processes (dependence on the intensity of assimilation or respiration). Monitored, unclassified parameter

Quality of the waters – oxygenation indicators

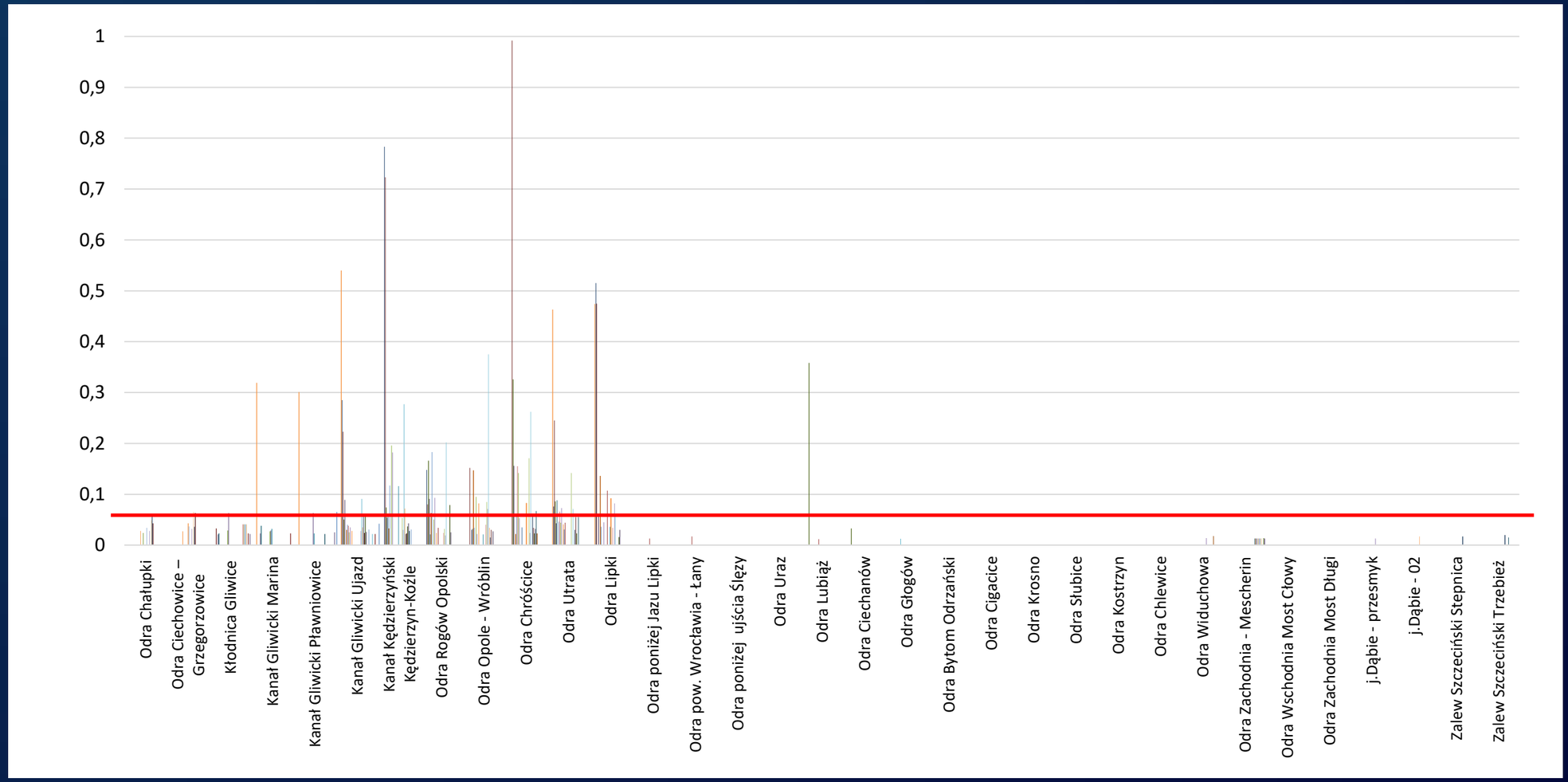


Class Limits ≥8,2 ≥7,5

Quality of the waters – oxygenation indicators



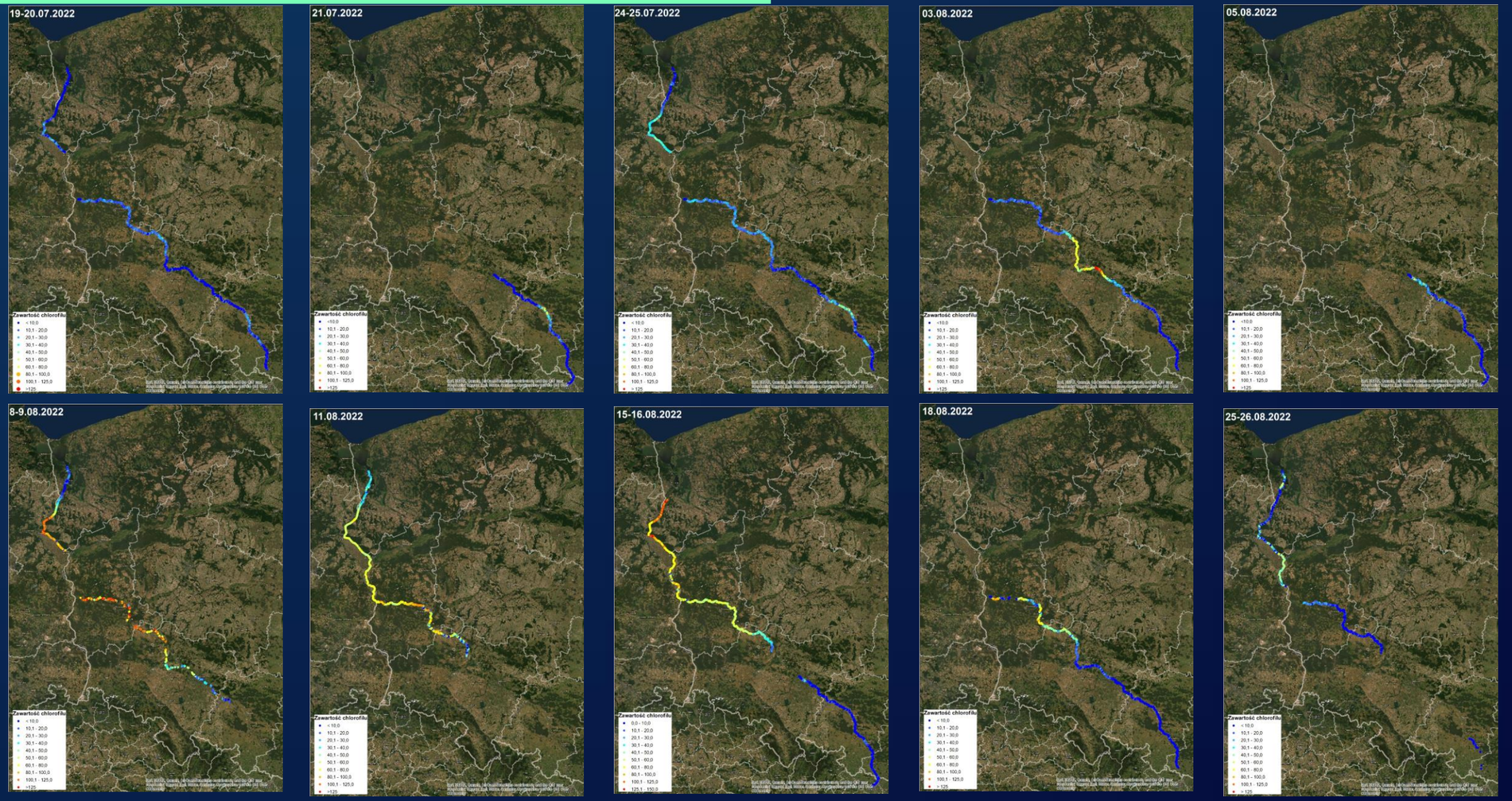
Quality of the waters - mercury



Limit ≤0,07; presented only results >GO

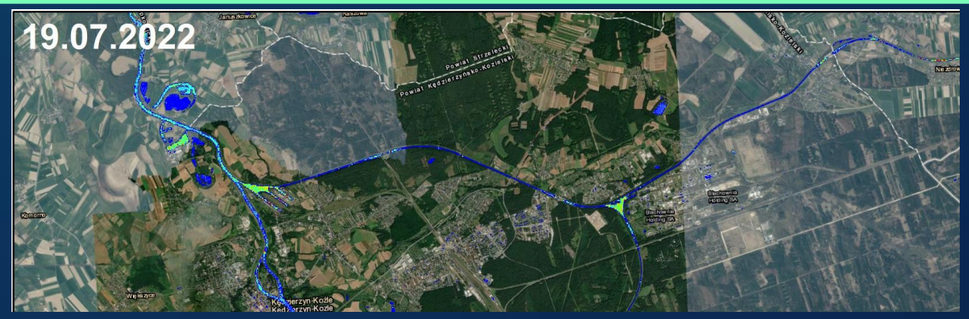
Sentinel-2 satelite data analysis

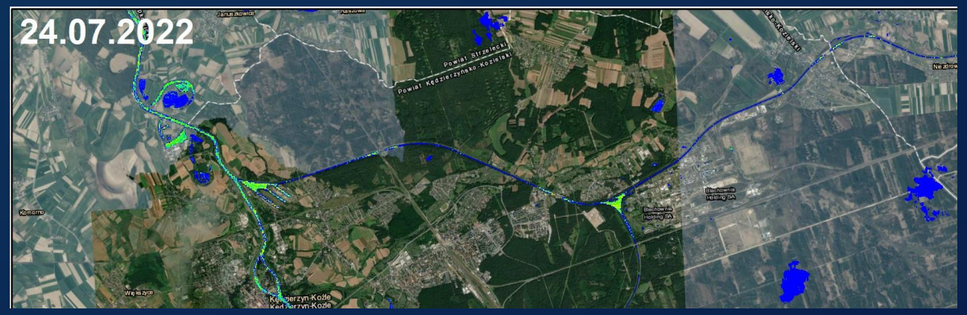
Development of the situation on the Oder in Summer 2022

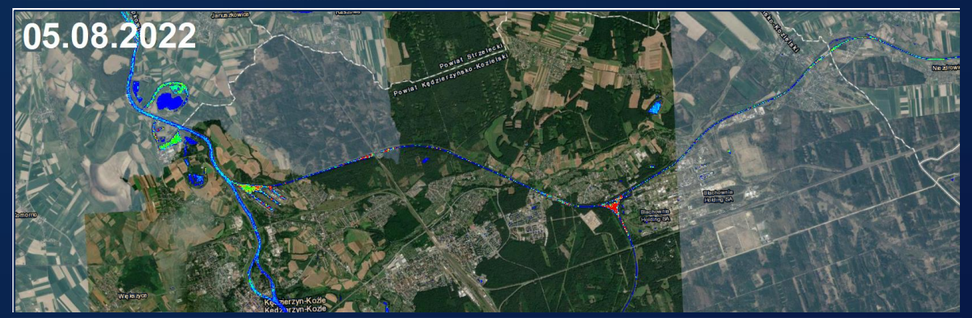


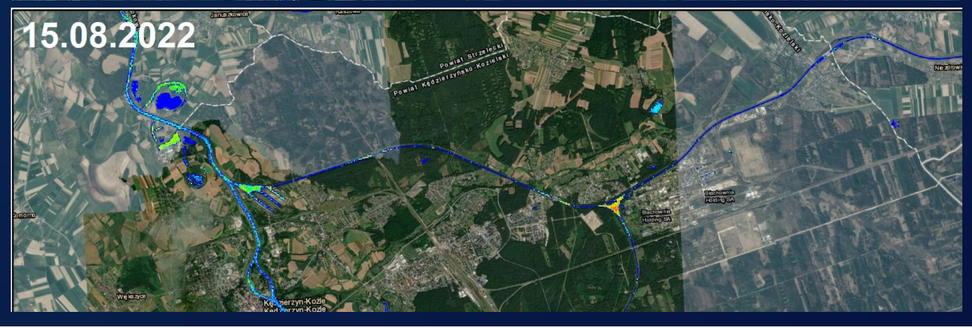
- 21-24.07.2022: there is clear, gradual increase in chlorphyll concentration from Groszkowice lock to the estuary of the Nysa Kłodzka to the Oder, as well as in the vicinity of Gliwice Canal inlet to the Oder
- 03.08.2022: high concentration of chlorphyll (over 125 mg/m3) were already recorded in the central part of the Oder
- 8-15.08.2022: high concentration of chlorphyll were already recorded in the lower part of the river
- 18.08.2022: the situation is normalising in the upper section of the Oderation is normalizing in the upper section of the Odra River.

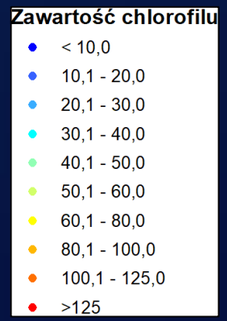
Development of the situation on the Oder in Summer 2022.





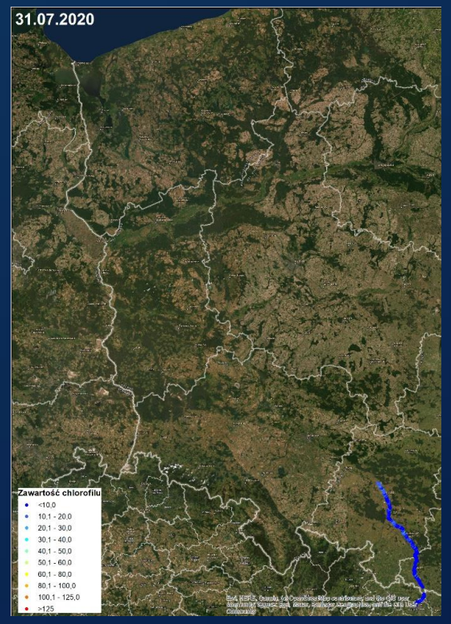


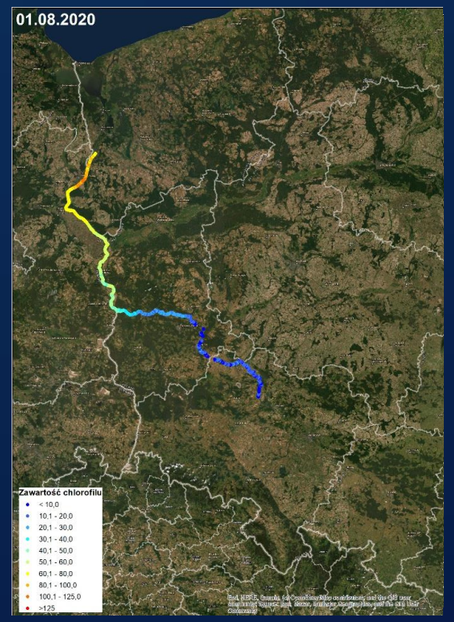


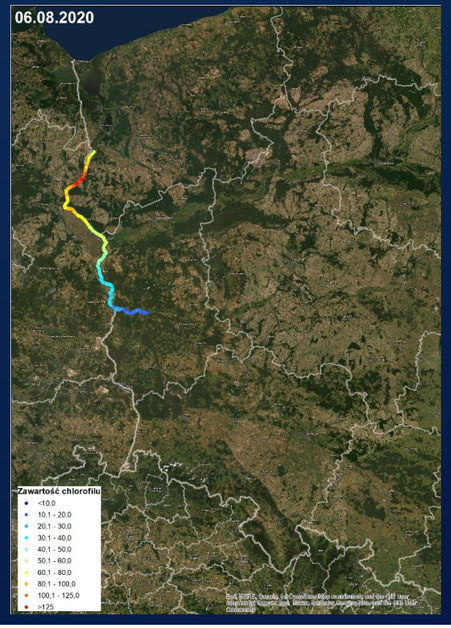


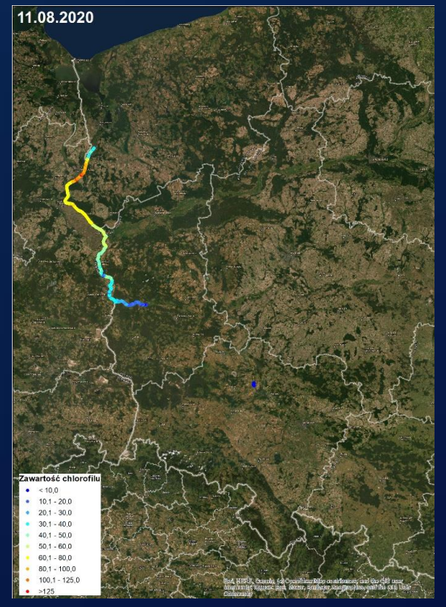
- Already on 19th of July 2022, in the inflow of the Gliwice Canal to the Oder and the Kędzierzyn Canal to the Gliwice Canal, significantly higher concentration of chrophyll was recorded compared to the upper sector of the river.
- The highest concentration of chlorophyll in the vicinity of the Gliwice Canal inlet to the Oder occurred on 24th July 2022.
- From 18th August 2022 the situation on the Oder returned to the state from before 20th of July.

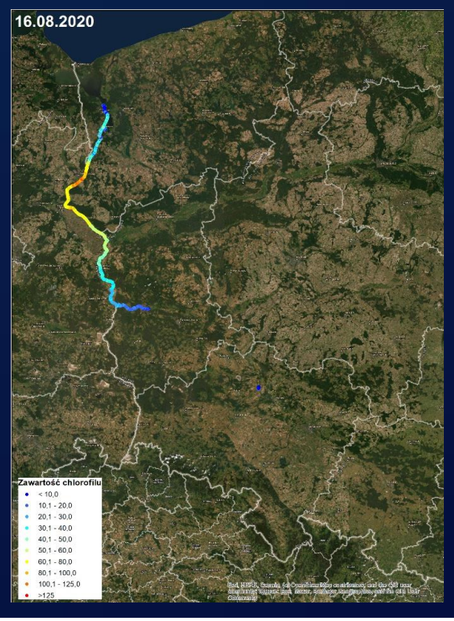
Level on the Oder in 2020

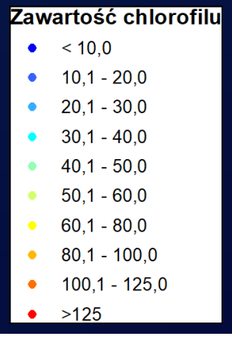












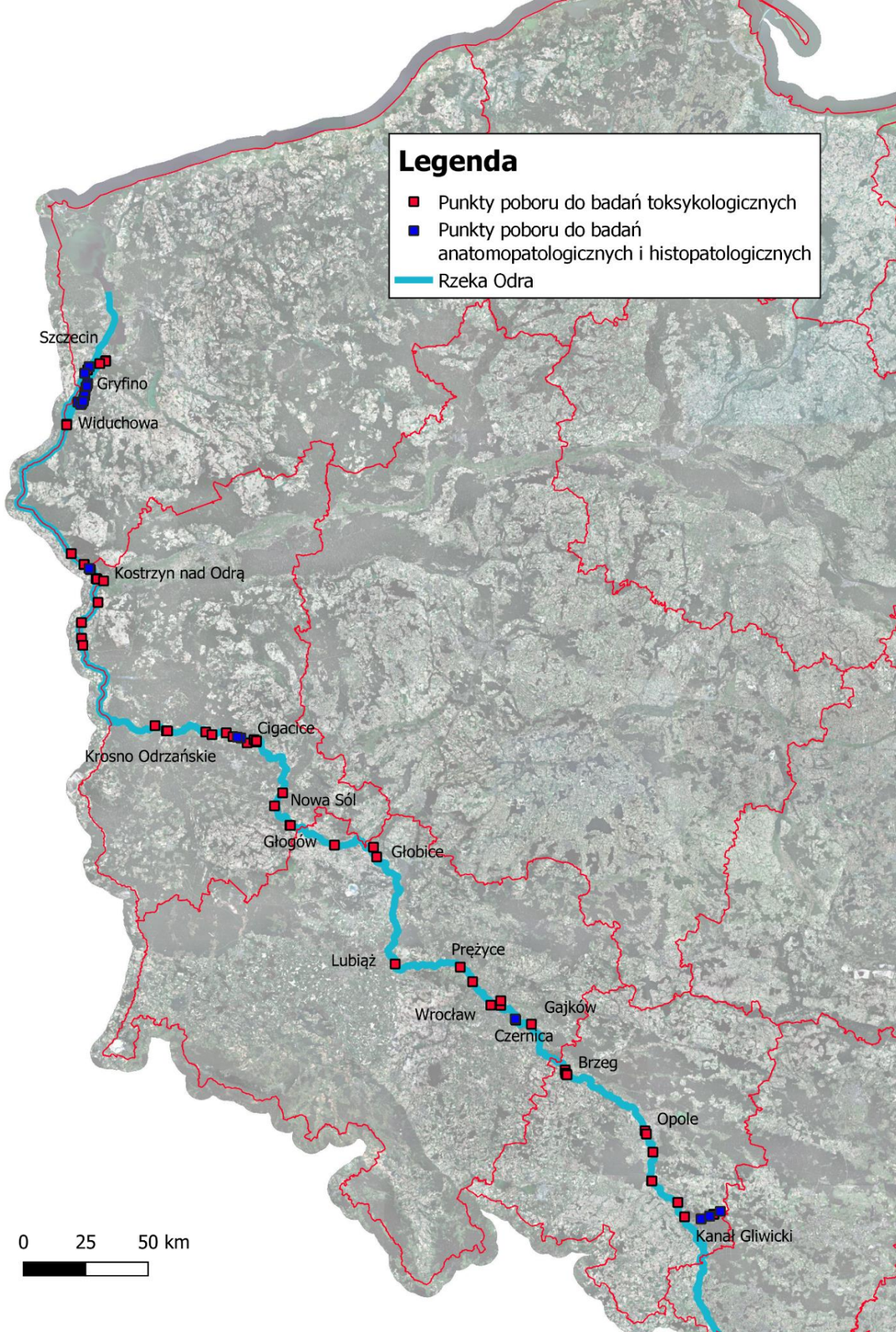
- In July there was no increased concentration of chlorophyll in upper and middle sectors of the river.
- Increased chlorophyll content was recorded in lower course of the Oder on 6-16.08.2022. From the town of Kostrzyn on the Oder- values above 50 mg/m^3 with a maximum at the level of Widuchowa (depending on a date from 80-100 mg/m^3, to even above 125 mg/m^3 on 6.08.2020).
- The situation in water reservoirs on the tributaries of the Oder, such as Lake Turawskie Duże, Lake Nyskie, Lake Otmuchowskie and Paczowski Lagoon similar to the one in 2022. Increased chlorophyll content was observed in some of tchem, indicating the appearance of algae bloom.
- In the inflow of the Gliwice Canal to the Oder and the Kędzierzyn Canal to the Gliwice Canal higher chlorophyll values were also noticable in relations to the upper course of the river.

Toxological and anatomopathological examination of fish

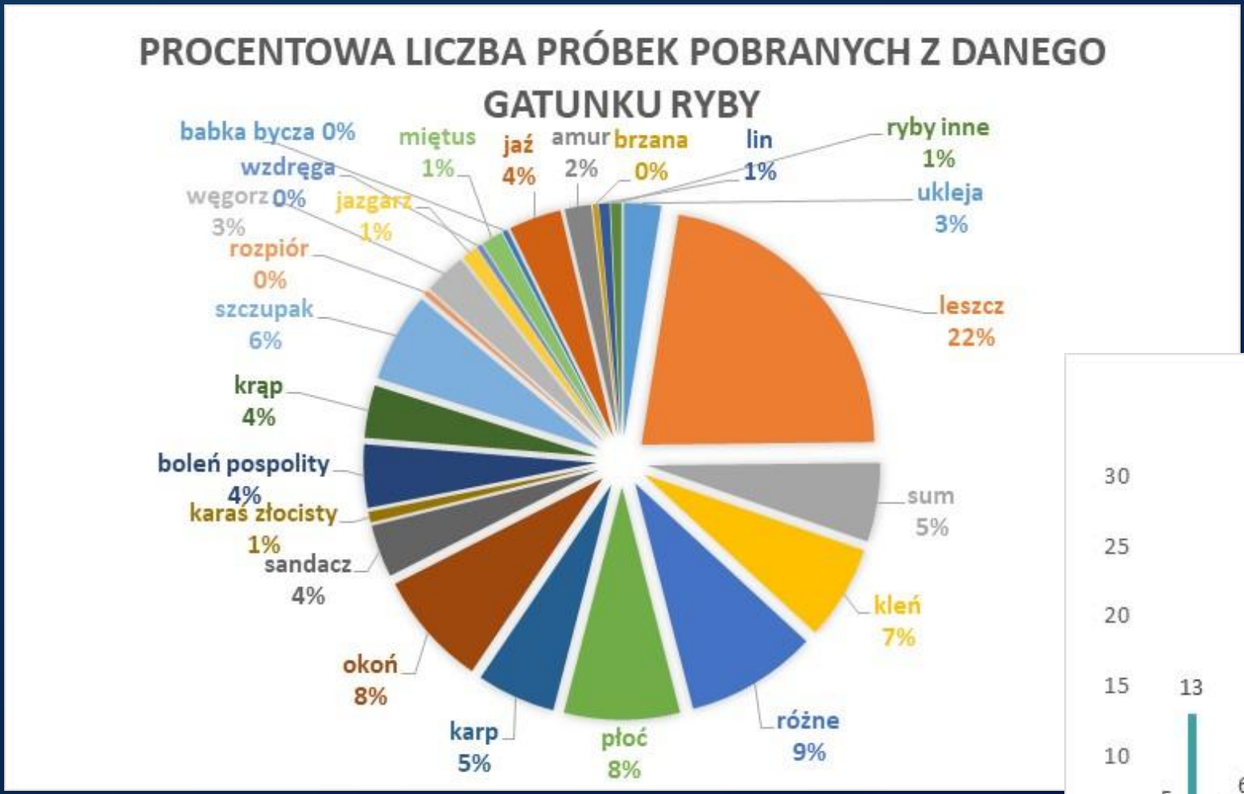
Examination of fish

Due to the situation on the Oder, the Veterinary Inspection, betweend 2nd August and 5th September, collected 334 samples for laboratory tests, that included:

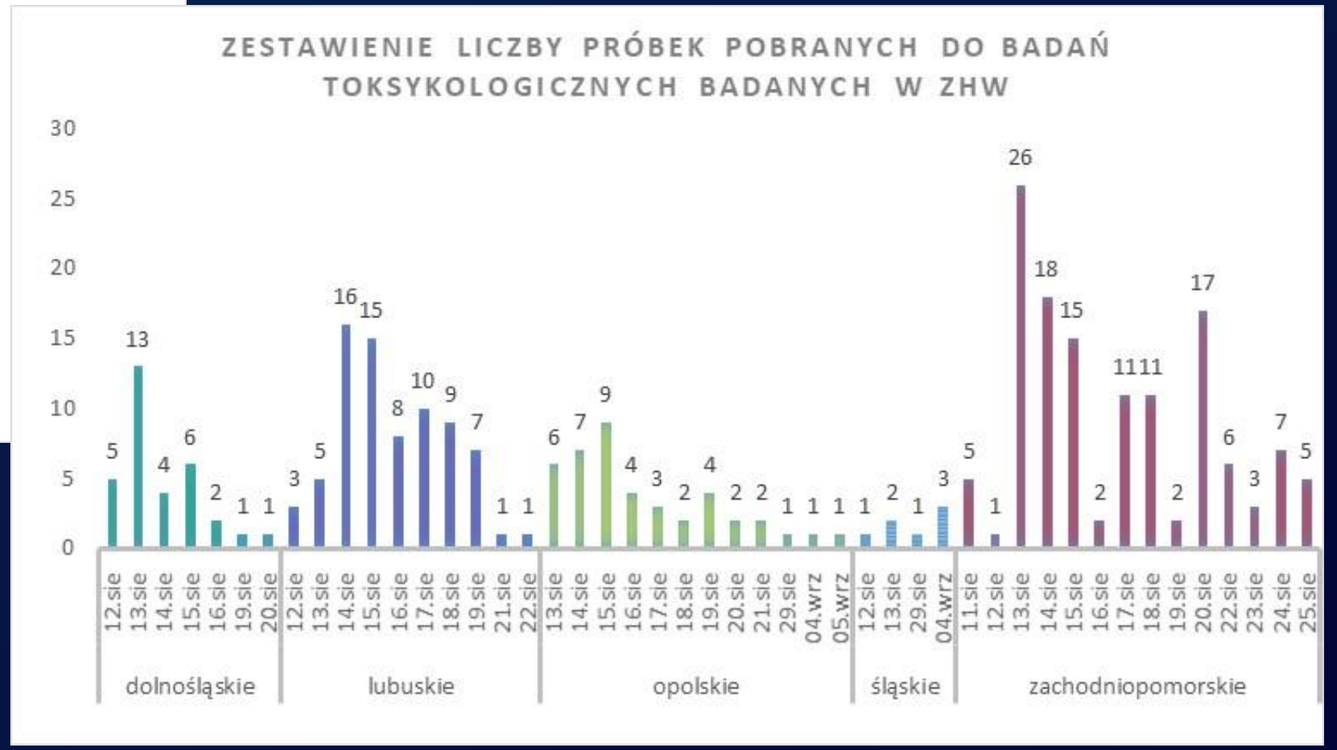
- 278 fish samples for toxicological tests
- 56 fish samples for anatomopathological and histopathological tests



Examination of fish



In total 153 samples were received by Veterinary Hygiene Institutes where 690 tests were performed.



Toxicological examination

Toxicological examination by the Veterinary Inspection

- 148 tests for lead, cadmium, mercury and arsenium.
- 98 analyzes of organochlorine pesticides and poilychlorinated biphenyls (PCB).

Toxicological examination by the National Veterinary Institute – National Research Institute

- 109 samples in the direction of toxic elements, pesticides, mold toxins and other toxic compounds.
- 6 samples in the direction of permanent organic pollutants and radioactive contamination.

In total, the samples were tested for the presence of over 300 substances.

Toxicological examination

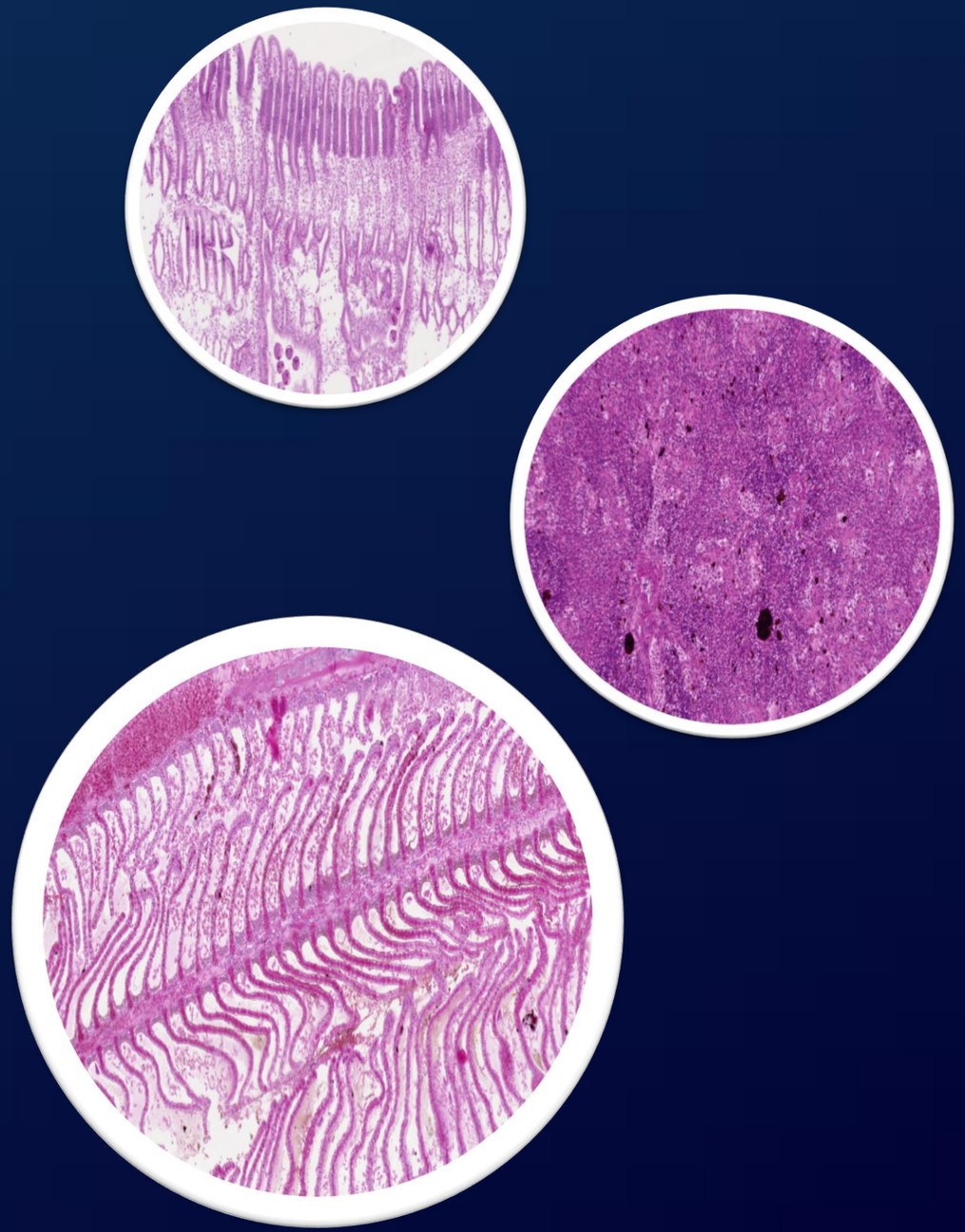
Toxicological examination carried out (total of over 300 chemical substances and trace elements) indicated that the concentration of the substances in the provided samples do not differ from the levels characteristic of environmental contamination in rivers in Poland.

Based on the current toxicological knowledge, it can be excluded that the above mentioned compounds were the cause of poisoning and the fish die-off.

Anatomopathological examination

Anatomopathological examination by IRŚ-NRI

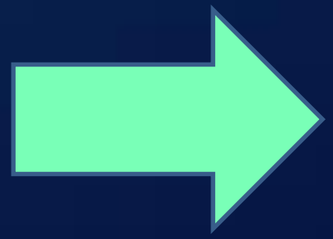
- 116 samples of fish and 7 of mules were examined.
- Clinical, anatomopathological, parasitological, bacteriological, mycological, histopathological examination
- The majority of the examined animals was healthy and showed no disease symptoms. In some studied animals, non-invasive ecto- and endoparasites were found.
- Despite the lack of clinical changes, the histopathological image of all examined animals indicated acute damage to the organs with the highest blood supply (gills, spleen, kidneys).
- Disturbances in hematopoetic processes and damage to the gills are most likely related to the action of haemolytic toxins, which incude, among others, primnesines secreted by *Prymnesium parvum*.



„Golden algae” hypothesis

The rationale for the formulation of the "golden algae hypothesis"

- Oxygen oversaturation of waters
- Spike of pH
- Significant decrease in concentration of nitrates
- Image of tissue damage in fish
- Analysis of the literature reports on ichthyotoxic blooms



ALGAE BLOOM

Identification and Quantity of *Prymnesium parvum*

Material and methods

1. In total, 211 water samples were collected and tested for phytoplankton, coming from various sections of the Oder River, and water reservoirs, canals and rivers closely related to the Oder. Sampling: August 8-12, 2022
2. Control water samples for analyzes were also collected in the following days until 21st September in order to monitor the current state.

Conclusions

In 165 (78% of the total samples) out of 211 analyzed water samples, the presence of *Prymnesium parvum* was recorded and its number was calculated.

According to the literature, fish deaths were the most common with the numbers above 50-100 million cells/L (Aquatic Invasive Species Control Plan Division of Environmental Services, Golden Alga 2021).

In the analyzed samples, the number of> 50 million *Prymnesium parvum* cells in 1 liter of water was found in approx. 35% of the samples, of which> 100 million cells/L in almost half of these samples, i.e. 22% of the entire sample pool.

Genetic analyzes – identification of the *PKS* genes

Genetic analyzes

- **PCR analyzes of the presence of genes encoding primnesin synthesis enzymes in samples**

CONCLUSION: The results indicate that the studied biological material may contain genes coding for enzymes that catalyze the production of primnesines.

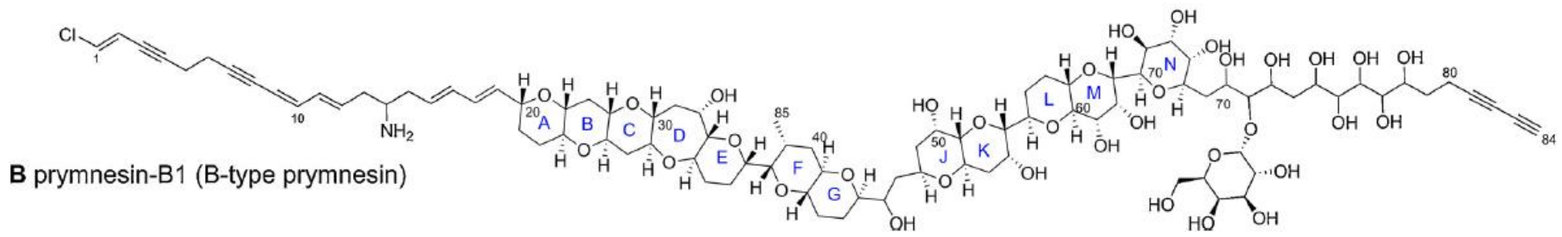
- **Gene expression examination by quantitive real-time PCR with reverse transcrptase (RT-qPCR)**

CONCLUSION: RT-qPCR analyzes indicated the possible expression of genes encoding enzymes involved in the production of primesines in the examined samples. These results show the activity of the testedgenes, which allows for inference about the possibility of primesin production by organisms in the tested samples.

- **Genomic analysis**

CONCLUSION: The obtained results indicate the presence of genes encoding enzymes / modules involved in the synthesis of pimnesines in the studies material. These modules are gatrhered in one contig, which enhances the functionality of this fragment of the genome of the *P. parvum* strain present in the tested samples.

Determination of primnesines using LC-MS / MS method



B prymnesin-B1 (B-type prymnesin)

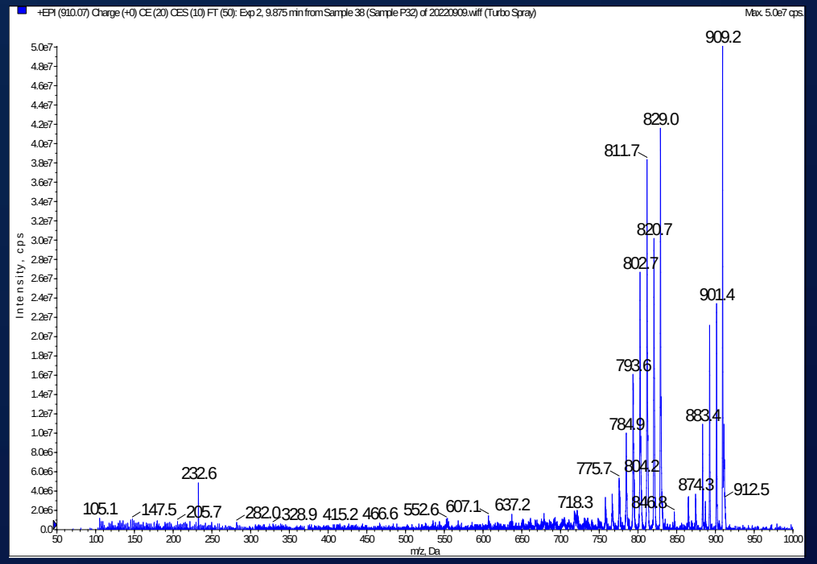
Binzer i in. Harmful Algae 2019

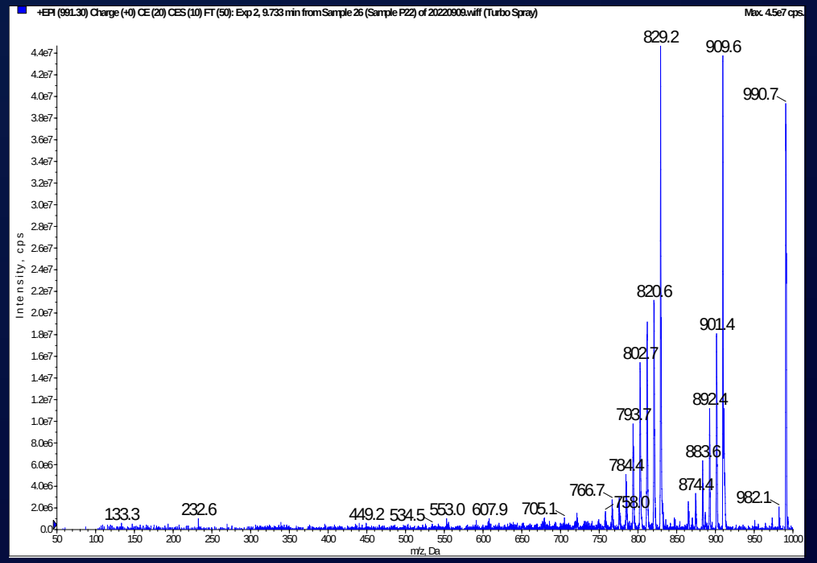
Analysed material

- Extraction and analysis of LC-MS/MS*
  - 230 filters with the material collected between 17.08-07.09.2022 (samples provided by Inland Fisheries Insitute)
  - 1 mule-based lyopilisate
  - Extraction and expedition to the University of Vienna
  - 28 filters

*LC-MS/MS – liquid chromatography coupled with tandem mass spectometry
HRMS/MS – high-resolution mass spectometry

MS/MS spectra of primesisn PRM B (Cl+1 hexose) (A) and PRM B1 (Cl+2 hexose) (B) present in the sample from the Oder. Compounds detected as doubly charged $[M+2H]^{+2}$.
The apparent loss of 81 fragments indicated the presence of hexose in the molecule.





Conclusions

- In the analyzed material, there were three compounds from the group of B primnesins.
- The presence of primesines was demonstarated on the basis of fragmentation spectrum analysis and acurate mass measurement.
- The lack of a certified standard made it impossible to carrry out a quantitive analysis.
- The results are presented in the form of the relative amount of primesins in the tested material, expressed as the ratio of the chromatographic peak of the compound to the volume of the filtered sample.

Bloom of *P. parvum* in light of research to-date

Golden algae

- The information on first blooms related to *P. parvum* as published by Liebert and Deerns in 1920
- Detailed descrption of P. parvum on the basis of specimen from a salty pond in Bembridge on the Isle of Wight was published by Carter in 1937

Blooms of golden algae were noticed, among others, in:

- The Netherlands
- Denamark
- Israel
- The USA
- Norway
- Great Britain
- Australia
- Finland
- China

Global research on golden algae

- Worldwide studies of golden algae blooms indicate that the possibility of blooms, but also the intensity of toxin production by these organisms, and finally the intensity of the toxic effec of these compound on aquactic organisms, are dtermined by a numer of factors.
- According to research, the risk of golden algae bloom increases with conductivity above 1500 uS/cm.
- Toxicity of *P. parvum* appears to be increased at a water pH greater than 7.0 and under nutrient-restricted conditions.
- According to the literaturę, toxicity is also influenced by the phase of development of the golden algae population (low in the first period, with an intensive increase in the population size, it grows after reaching the phase of population stabilization).
- The increase in toxicity can potentially be induced by changes in the environment casused by algae bloom itself – an increase in water pH, depletion of nutrients including nitrogen and change in ratio.

Physical and chemical properties of water that contains *P. parvum* in China

Physical and chemical properties of water that contains *P. parvum* in China. ChZT = chemical demand for Oxygen (according to.He, 1989).

temperature of water	**2-30°C**
water transparency	20-70 cm (dysk Secchiego)
pH	7,2-9,3
salinity	2,2-20 ‰
basicity	5-17,22 meq L-1
Cl^-	339-10800 mg L-1
SO_4^-	375-7590 mg L-1
HCO_3^-	0-68,5 mg L-1
Ca^{++}	45,9-547,6 mg L-1
Mg^{++}	187,5-905,9 mg L-1
Na^+ i K^+	75,8-3054,9 mg L-1
ChZT	23,4 -42,2 mg L-1

The golden algae bloom in the Oder waters

The intensive golden algae bloom in the Oder waters had multi-factor character.

At the turn of July and August, the waters of the Oder had favourable conditions for the development of *P. parvum* and the development of toxicity, such as significantly increased conductivity, high content of chlorides and sulphates, increased water temperature, and significant fluctuations in water parameters over time.

The hydromorphology of the Oder's waters is also significant here – the presence of many water reservoirs, as well as slowing down the flow in front of weirs, canals, i.e. places favourable to blooms.

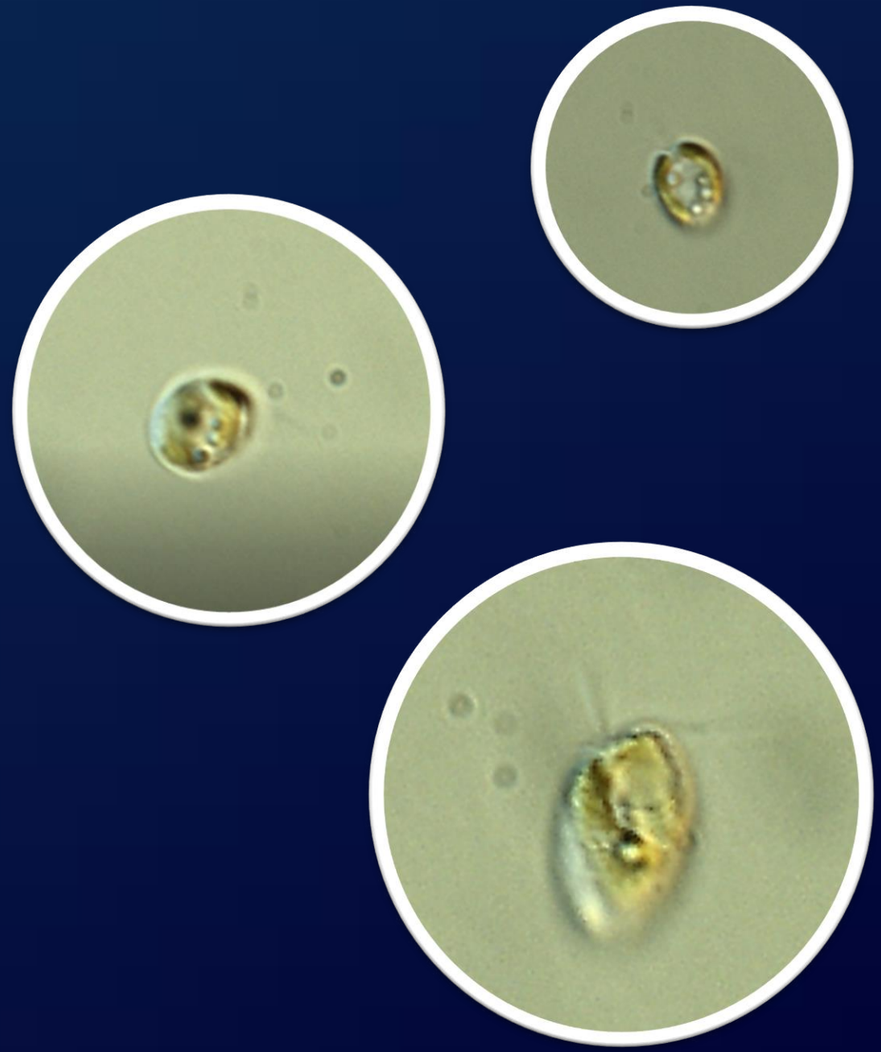
Mass blooms of golden algae in the waters of the Oder and other rivers and water reservoirs may repeat in the following years, as they did in other countries.

Tests of water samples taken from the Vistula did not reveal the presence of *Prymnesium parvum.*

Combating the algae

There are methods of suprssing golden algae blooms and reducing its toxicity by using selected chemicals, minerals, manipulating nutrients, flushing catchments, and can be used in smalller water reservoirs.

However, the literaturę does not indictae the possibility of supressing blooms in case of larger reservoirs and rivers similar in size to the Oder.



Recommendations of the Team

- Creation of a system of continuous water quality measurement in terms of selected parameters, with access to online data for all stakeholders. That shall include monitoring of patrameters related to blooms, and monitoring of golden algae itself. The smart catchment management system should use bith terrestial and satelite data. Monitoring should first of all cover water bodies in which water parameters favouring blooms are recorded. Ensuring organizational and financial conditions for the permanent functioning of the system.
- Continuation of inspections of entities conducting the discharge of polluted waters to the Oder and its tributaries, with the aim of, inter, alia, establishing the entities responsible for the quality of the Oder waters.
- Review and verification of the exisisting permits for the discharge of sewage into the waters of the Oder basin, dependence of the parameters and intensitity of the discharge on the current results of water tests, introduction of an obligation to temporarily suspend or limit discharges in an emergency.
- Improving the flow information, implementing an early warning and response system, streamlining crisis management procedures.
- Gradual, based on the best knowledge of experts, restoration of the fish population and other groups of organisms affected by the disaster.

Thank you for your attention!

IOŚ-PIB

Narodowy Fundusz Ochrony Środowiska i Gospodarki Wodnej