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A High-Density Bloom of *Prymnesium parvum* with No Apparent Toxicity in the Oder After the Ecological Disaster of 2022

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Abstract

In the summer of 2022, a massive toxic algal bloom of the haptophyte *Prymnesium parvum* devastated the Oder River in Poland and Germany. Between the end of July and the beginning of September, an estimated hundred thousand fish, mussels, and aquatic snails died. Since then, *Prymnesium parvum* has been established in the river network, as confirmed by monthly sampling, and there is the potential that such a catastrophe could repeat itself. Here, we report the occurrence of an apparently non-toxic bloom of the 'golden algae' *Prymnesium parvum* in May and June 2024 at comparable abundances to the ones observed during 2022, with a maximum abundance of 120,000 cell equivalents (CE)/mL in the main stem river without any associated fish deaths. Interestingly, in July 2024, local blooms in the West Oder even exceeded these abundances (160,000 CE/mL). The main-stem abundances decreased to 850–4250 CE/mL in July and 5–500 CE/mL in August. During the bloom, no mass mortalities of aquatic organisms were reported despite high abundances. Nutrients (total nitrogen TN, total phosphorus TP, N:P ratio) were inconspicuous and comparable to the disaster in 2022. To our knowledge, this is the first record of a massive *Prymnesium* bloom that did not result in mass fish deaths.

Keywords: fish kill; toxic algal bloom; non-toxic; golden algae; *Prymnesium parvum*; prymnesin

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1. Introduction

Harmful algal blooms (HABs) are massive proliferations of certain photosynthetic organisms that cause mass deaths of aquatic organisms, associated with the accumulation of toxins in the food web (toxin-driven events) or the development of hypoxia in the environment (hypoxia-driven events). There is considerable concern that HABs are increasing, both in size and frequency, due to anthropogenic drivers such as eutrophication and climate change [1,2]. Such blooms may last from days to months, depending on species and habitat. In freshwater, the HABs of greatest concern, receiving the most research attention, are toxic cyanobacteria and the haptophyte *Prymnesium parvum* Carter, 1937. The 'golden algae' *Prymnesium parvum* has expanded its distribution through inland waters, including reservoirs and rivers [3–5]. Despite its coastal origin [6] as an euryhaline species, it has led to catastrophic episodes at salinities between 5 and 35‰ [7,8].

From the end of July until the beginning of September 2022, extreme climatic conditions combined with anthropogenic disturbances, including river engineering, eutrophication, and salt emissions, led to a massive and long-term bloom of *P. parvum*, resulting in an environmental disaster in the Oder River. In August 2022, Central Europe experienced one of the driest and hottest summers in recent memory, with very high air temperatures and low water levels in the Oder basin. The extreme heat increased evaporation and reduced river flow. At high water temperatures around 25 °C, after incubation in highly saline tributaries of the Upper Oder, this bloom developed densities of over 100,000 cells/mL in the chronically salt- and nutrient-polluted waters [9]. The toxins synthesized by this mixotrophic algae impacted the ecosystem over approximately 500 river kilometres [10]. The bloom resulted in an estimated 1000 t of dead fish [11], a decline of more than 60% in mussel populations, and tens of thousands of dead gill-breathing snails due to the mass proliferation of mixotrophic, toxin-producing *Prymnesium parvum* [12].

Changes in the toxicity of *Prymnesium parvum* have been reported before, often attributed to clade-specific toxins named prymnesin A, B, and C [13]. Their classification is based on molecular backbone differences, and each algal strain typically produces one dominant prymnesin type [13,14]. Phylogenetic analyses have shown that prymnesin type is closely tied to genetic clade [12], suggesting a heritable component to toxin variation, possibly driving evolutionary diversification within the species complex.

These toxins are primarily delivered at close range, and variation in toxicity may promote different prey capture and phagotrophy [15]. However, the ongoing heritable variation in toxicity suggests that populations may rapidly evolve over the course of a single or multiple bloom seasons. In fact, *Prymnesium* is capable of ingesting organic matter derived from bacteria, other algae, protists, and even from larger aquatic animals, and highly toxic strains are more effective predators [15]. Combining autotrophy and heterotrophy, lytic toxins primarily function in predation.

Field and lab studies show that bloom density and toxicity are not strongly correlated, so a dense bloom does not automatically imply high toxicity. There are documented non-toxic strains of *P. parvum*, and even toxic strains may express little or no toxicity under certain environmental conditions (e.g., different nutrients, salinity, temperature, pH) [16,17].

Since the environmental causes for the mass development of *P. parvum* in the Oder River persist, it is anticipated that such a catastrophe could repeat itself. As such, a monitoring strategy was developed in order to assess the prevalence of *P. parvum* in different sections of the Oder River as an early-warning system to prepare countermeasures in the case of a renewed toxic bloom. In general, *P. parvum* can be identified through cell counts under a microscope. Assessments of the abundance, therefore, are extremely labour-intensive. For this study we applied a QPCR-based method, quantifying the number of ITS2 copies and correlating ITS2 copy numbers to cell equivalents, as previously reported [18,19]. Harvesting the algae by centrifugation, this protocol allows rapid throughput and provides high accuracy levels. The monitoring was carried out with different intensities and at different sites along the Oder River. We monitored the 20 sampling sites monthly and, at increasing densities, sampled at 2–3 d intervals at three selected sites. In this way, we were able to monitor the dynamics of a Prymnesium bloom.

Here, we report the dynamics of a non-toxic *Prymnesium* bloom in the German part of the Oder River system in spring 2024, illustrating the dynamics from spring 2024 to spring 2025. Through this, we describe the blooming dynamics of two successive and apparently non-toxic Prymnesium blooms in the Oder basin. We hypothesize that higher temperature, reduced flow, and higher salinities during the 2022 disaster resulted in an accelerated toxin synthesis that was not observed in 2024, and discuss potential factors influencing toxicity.

2. Materials and Methods

2.1. Sampling

Water samples were obtained with a monthly frequency (first week of each month) at 19 sampling sites (Figure 1, [20]) from bridges or accessible riverbanks (groynes) using a 5 L bucket attached to a rope. For *Prymnesium parvum* quantification, subsamples were taken after gentle but thorough stirring, then poured into 50 mL vials and fixed with 50 µL of acidic Lugol's iodine solution [100 g KI in 1 L distilled water, 50 g crystalline iodine, 100 g glacial distilled water], as described by Galuzzi et al. [18]. Subsamples for nutrient analyses were collected and prepared in the field.

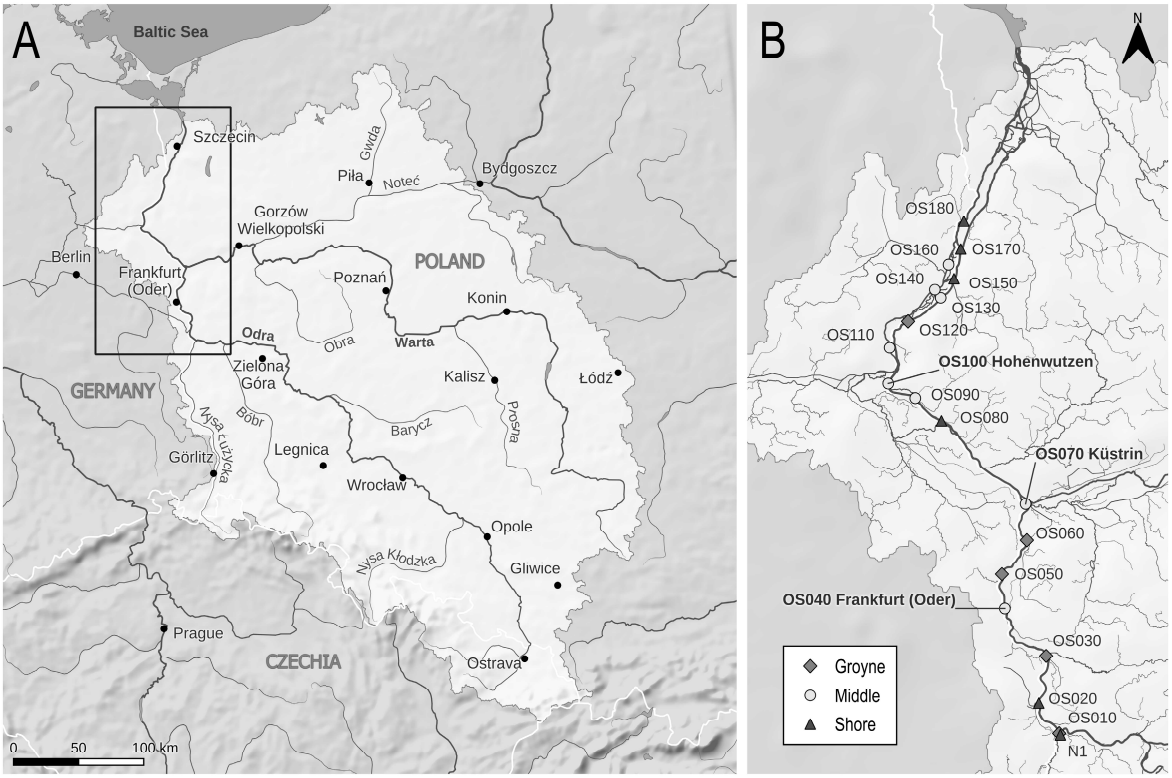


Figure 1. Sampling sites in the Oder River catchment (used with permission from Cell Press, Kuhl et al. 2024 [12]). (**A**). Overview map; (**B**). sampling sites along the main stem, the West Oder River branch, and the Hohensaaten-Friedrichsthaler-Wasserstra ße (HoFriWa).

Additional water samples were taken during the bloom in 2024. We assessed a vertical profile using water samples taken at different depths (surface, middle of the water column (0.75 m), bottom (1.5 m)). These samples were obtained using a motorboat. The samples were taken using a diaphragm pump (Typ: VETUS Water pressure pump 12 V—13 L/min, WP1213B) via a 3 m long hose (Typ: EHEIM Hose, green, Ø 12/16 mm) attached to a 2.5 m long pole with corresponding depth markings. A round plate with a diameter of 10 cm and a thickness of 0.5 cm was attached to the lower end of the pole to prevent it from sinking into the sediment. The suction opening of the hose was located 5 cm above the end of the pole, which prevented sediment from being sucked in. The first 500 mL of each sample was discarded to prevent water from higher water layers from contaminating the sample. Since no major difference was observed between the respective depths, all samples were subsequently taken near the surface. More frequent event sampling was carried out at three sampling sites (Frankfurt (Oder) OS040, Küstrin OS070, and Hohenwutzen OS100) between the 6th of May and the 21st of July.

2.2. DNA Extraction

Water samples were rigorously mixed for 2 min; two replicates of 10 mL were transferred into Falcon vials and centrifuged for 1 h 45 min at 4600× *g* and 4 °C. After the complete removal of the supernatant, the pellet was extracted with the QIAmp mini kit (Qiagen, Hilden, Germany). In brief, the pellet was resuspended in 400 µL (360 µL ATL + 40 µL proteinase K) prewarmed (56 °C) lysis buffer (Qiagen) and pulse-vortexed, followed by shaking at 56 °C for 10 min. The liquid was then transferred to 2 mL vials, pulse-vortexed, and incubated in a thermomixer at 56 °C for 2 h until the sample was completely lysed. After addition of200 µL prewarmed (56 °C) AL buffer and vortexing for 15 s, 200 µL Ethanol (EtOH 99%p.a.) was added, followed by vortexing for 15 s and incubation for 5 min at room temperature (RT). The lysate was transferred to the QIAamp MinElute column and centrifuged at 6000× *g* for 1 min, followed by washing with 500 µL AW1 and centrifugation at 6000× *g* for 1 min. A second washing step was carried out with 500 µL AW2 and centrifugation at 6000× *g* for 1 min. After centrifugation at 20,000× *g* for 3 min, 50 µL of nuclease-free PCR water was added. The column was incubated at 70 °C for 2 min, and the eluted DNA was collected by centrifugation at 20,000× *g* for 3 min. Elution was repeated with the eluate without incubation at 70 °C. Inhibitors were removed with the OneStep PCR inhibitor removal kit (Zymo Research, Irvine, CA, USA). In brief, for column preparation, 600 µL Prep solution was added, centrifuged at 8000× *g*, and discharged. Then, 50 µL DNA solution was added to the column and centrifuged at 14,000× *g* for 3 min. Finally, 4 µL of the filtered DNA solution was used for QPCR analysis.

2.3. QPCR Quantification

Each DNA replicate (Section 2.2) was assessed in duplicate QPCR measurements. Species-specific primers (PrymF2239 (5′-CACATCCGATCGTGTCTGC-3′) and PrymR2384 (5′-GGCACAACGACTTGGTAGG-3′) for the Internal Transcribed Spacer 2 (ITS2) were designed using the NCBI sequence information of all strains available. The specificity of the assays was confirmed by direct sequencing (SeqLab, Göttingen, Germany) of the amplicon (142 bp) and monitored by melting curve analysis. Real-time PCR was performed with a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). For the QPCR reaction, 4 µL of DNA solution (diluted if >200 ng/µL) was used as template in 25 µL PCR mix (1× PCR buffer, 1*SYBR-Green I [Invitrogen, Carlsbad, CA, USA], 1.5 mmol/L MgCl2, 200 µmol/L of each dNTPs [Qiagen dNTP mix], BSA 9.6 pg/µL, 400 nM of each primer and 1 U Invitrogen Platinum Taq polymerase). PCR was carried out with an initial denaturation at 96 °C for 3 min, followed by 40 cycles of denaturation at 96 °C for 30 s, primer annealing at 67 °C for 30 s, and elongation at 72 °C for 30 s. The ITS2 copy number was calculated from a PCR standard dilution (1:10) series using a PCR standard diluted in AE buffer (Qiagen) that was quantified with a QuantiFluor dsDNA System (Promega, Madison, WI, USA) and a DeNovix DS-11 spectrometer (Promega). The dilution series was freshly prepared for each run. The cell equivalents were calculated from the ITS2 copy number using a correlation factor (6.0956) obtained from 60 environmental samples from the Oder in summer 2022 and counted microscopically. As a control, 5% of all samples were counted microscopically in parallel.

2.4. Total Nitrogen and Phosphorus Analyses

Total nitrogen was determined in original water samples by chemiluminescence after catalytic oxidation according to DIN EN 1484 (Shimadzu TOC-L, Shimadzu, Berlin, Germany). Total phosphorus was determined in original water samples by phosphomolybdenum blue spectral photometry after wet chemical digestion according to EN ISO 6878 (modified oxidation reagent with hydrogen peroxide and sulfuric acid). Limit of quan-

tification was 0.1 mg L^{-1} for TN and 3 µg L^{-1} for TP. Analytical precision was <3%. For calculation purposes, values below LOQ were substituted with a value of 0.5*LOQ.

2.5. B-Type Prymnesin Toxin Assessment

Analysis of B-type prymnesins, the toxins of the Oder strain of *P. parvum* [10], was carried out via LC-QTOF-MS (Agilent 6550 series, Agilent, Waldbronn, Germany), similarly to in Varga et al. [14]. Samples were extracted according to Svenssen et al. [21] and Medić et al. [22], with modifications to speed up the process.

In brief, water samples of a recorded volume were filtered onto 25 mm glass fibre filters (Whatman GF/F). The filters were transferred to a 15 mL Falcon tube, and 12 mL CL-grade methanol was added. The tubes were well vortexed and transferred into an ultrasonic bath for 30 min. Afterwards, they were centrifuged for 5 min at 10 °C and 4000× *g*. The supernatant was transferred to a G12 HPLC glass vial (CS-Chromatographie, Langerwehe, Germany) to evaporate the solvent at 30° C on a nitrogen evaporator (Barkey vapotherm basis mobil II, Barkey, Griesheim, Germany) to an approximate volume of 1.5 mL. For LC-QTOF-MS analysis, a 100 µL sample was transferred to an HPLC-vial with a microinsert. After HPLC gradient separation (Kinetex 2.6 µm F5 100 Å, LC Column 100 × 2.1 mm size, Eluent A: LCMS H20 + 0.1% formic acid + 1 mM ammonium formate, Eluent B: acetonitrile–water 90:10 *v*/*v* + 0.1% formic acid + 1 mM ammonium formate, gradient 20% to 67% B in 15 mins) in positive ionization, full-scan mode was used and samples were analysed for target m/z 918.4365, 920.4117, 999.4629, and 1001.4382.

3. Results

3.1. Monthly Monitoring of Prymnesium

From March 2023 to February 2024, following the same protocols, no mass proliferation of the algae was observed [12]. During May and June 2023, *Prymnesium* increased from low abundances to 90–160 CE/mL and 310–910 CE/mL, respectively, but returned to low abundances thereafter.

In March 2024 (Figure 2), *Prymnesium* abundances varied between 0 (confluence of the Neiße River) and 10 CE/mL, slightly increasing to a maximum of 18 CE/mL in April. On the 6th of May, abundances had drastically increased to 7000–10,000 CE/mL during a first bloom of *Prymnesium* in the main stem, described below (Section 3.2). Following the bloom (Figure 3), abundances in the main stem decreased to 850–4250 CE/mL in July to 5–500 CE/mL in August.

3.2. Prymnesium Bloom in May/June 2024

During the bloom in the main stem in 2024 (Figure 3), abundances rapidly increased from 7000 to 10,000 CE/mL on 6th May to over 240,000 CE/mL (7th June in Küstrin). This bloom collapsed within a week to concentrations of 50,000–100,000 CE/mL and returned to relatively low abundances of 1800 CE/mL in Frankfurt, 3400 CE/mL in Küstrin, and 1400 CE/mL in Hohenwutzen on the 4th July.

During the bloom (Figure 4), a transect of the river was studied from the inner to the outer bank with five sampling points, revealing a homogenous distribution of algal densities between sites (Figure 4). Also, a vertical profile did not reveal significant differences (Tukey's test) between samples taken from near the bottom (approximately 1.5 m), the middle water column (0.75 m), and the surface.

Following the bloom (Figure 3), abundances in the different main-stem locations sampled decreased to 850–4250 CE/mL in July and further to 5–500 CE/mL in August.

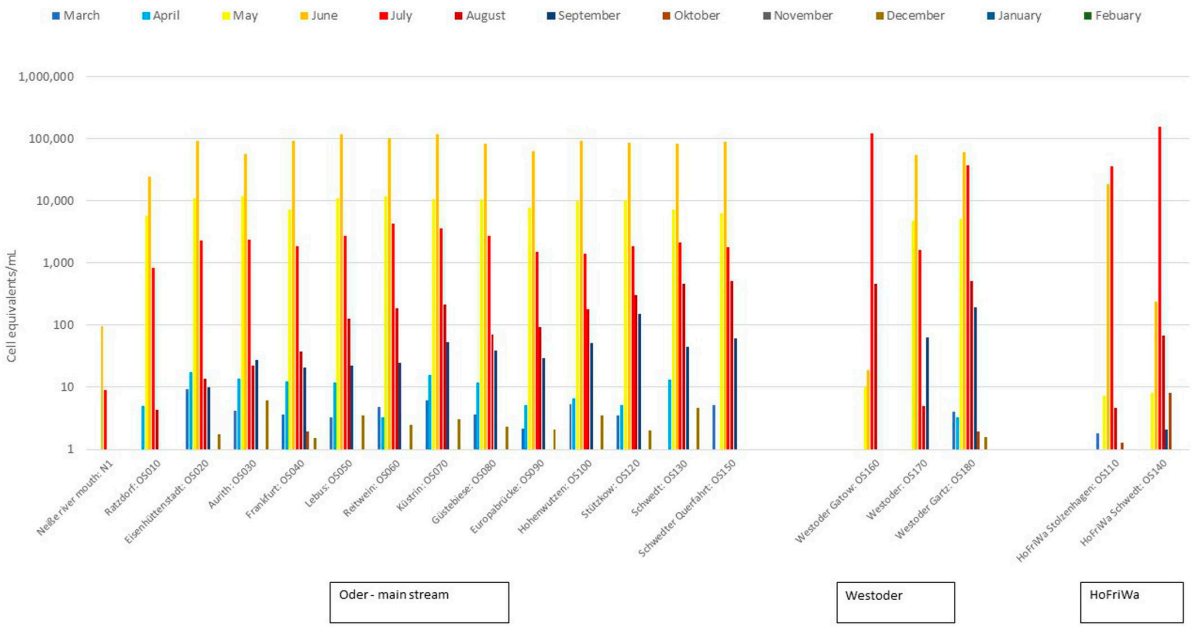


Figure 2. Monthly monitoring of the abundances of *Prymnesium parvum* in the Oder catchment from March 2024 until February 2025 using QPCR quantification. Cell equivalents were determined as ITS2 copies/mL in the Neisse, the Oder Main Stream, the West Oder River branch, and the Hohensaaten-Friedrichsthaler-Wasserstraße (HoFriWa).

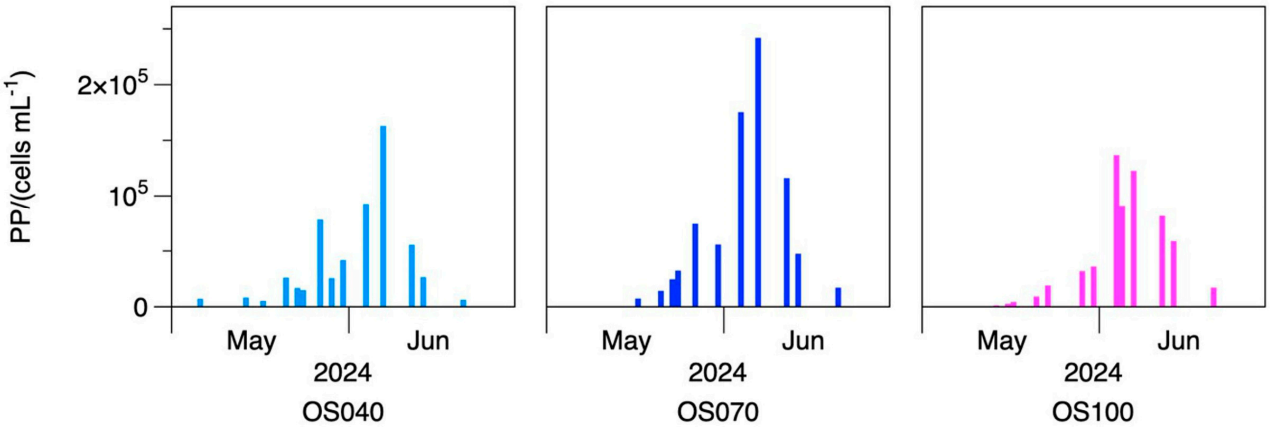


Figure 3. Abundances of *Prymnesium parvum* in the extra samples taken during the blooming event in May/June 2024 at three selected sites (Frankfurt, OS040, Küstrin, OS070, Hohenwutzen, OS100) using QPCR quantification. Cell equivalents were determined as ITS2 copies per mL.

3.3. Prymnesium Bloom in July/August 2024

In contrast to the findings in the main-stem river in July, high abundances (37,000–150,000 CE/mL) were observed in the West Oder and the Hohensaaten-Friedrichsthaler Wasserstrasse (HoFriWa), which decreased to 500 CE/mL in August. In September, again, low abundances around 50 CE/mL were recorded. Between October 2024 and February 2025, *Prymnesium* abundances fluctuated at or below the detection limit (1 CE/mL) and 3 CE/mL. At the maximum densities, local mortalities of single species (pike, Esox lucius) were reported in small numbers, which might have been due to the low O2 concentration in the early-morning hours during the intensive blooms.

3.4. Dynamics of Nutrients Nitrogen and Phosphorus During 2024–2025

The total nitrogen in all four river sections, namely the main-stem Oder, Neisse, and West Oder, and the Hohensaaten-Friedrichsthaler Wasserstrasse (HoFriWa) decreased

considerably during spring 2024 (Mar–May) and remained at low concentrations during the vegetation period until September (Figure 5). During autumn and winter, until February 2025, concentrations steadily increased, revealing a substantial increase in the main-stem Oder, Neisse, and Western Oder, and a less pronounced one in the HoFriWa. The average TN concentrations were typically lowest in the HoFriWa, higher in the Neisse and Western Oder, and highest in the Main Oder (Figure 5). The total phosphorus shows a somewhat contrasting pattern, with an increase in TP from March towards the summer months, and a decrease towards winter (Figure 5, middle). The four river sections are not clearly differentiated in average TP levels, with the highest concentrations being identified for the HoFriWa and West Oder in July, and the lowest for the Neisse in November (Figure 5, middle). The molar ratio of TN over TP as an indicator of ecological stoichiometry seemed largely defined by the nitrogen dynamics, and was highest (up to >200) in Mar 2024, strongly decreased to low levels during the entire vegetation period until Sep 2024, and increased again, but to lower levels, in winter 2024–2025 (Figure 5, bottom).

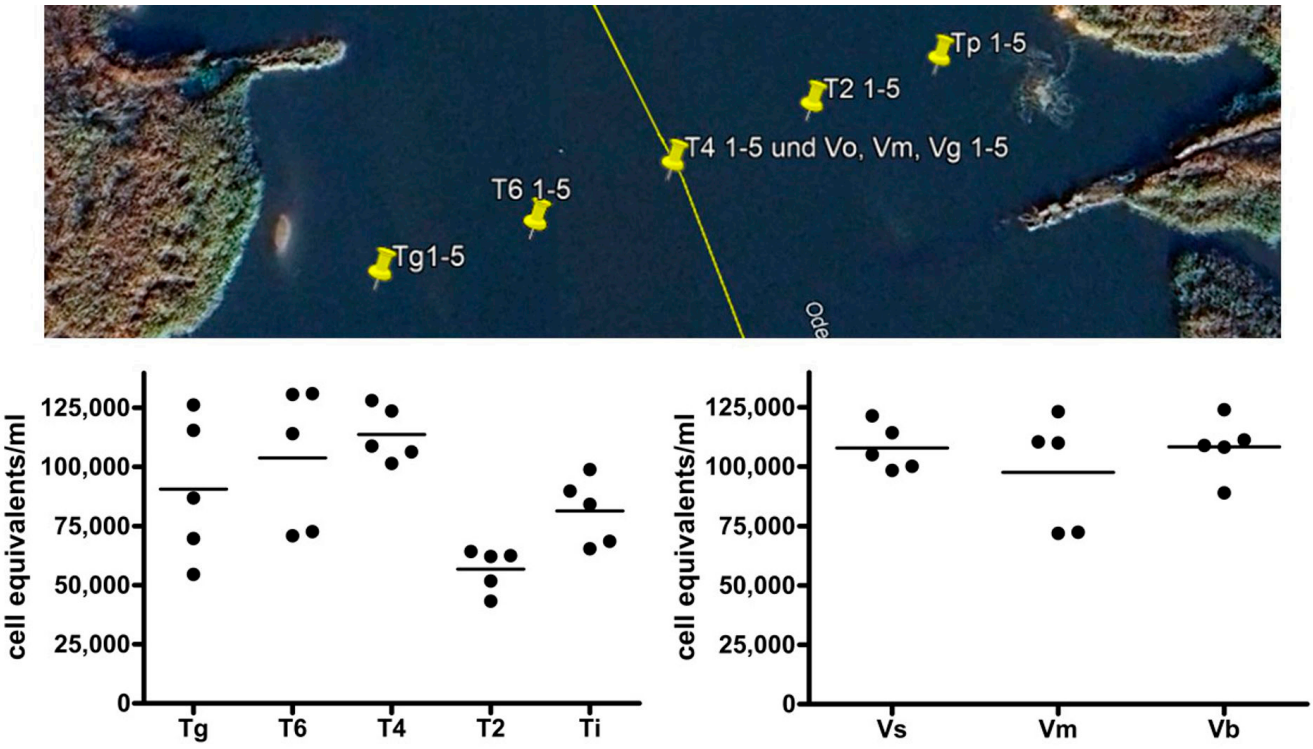


Figure 4. Transect from the gliding slope (Tg) to the impact slope (Ti) and vertical profile of samples taken from the surface (Vs), middle (Vm) water column (0.5 m), and near the bottom (1 m). Samples were taken near Reitwein, km 604.

3.5. Assessment of B-Type Prymnesins

In all extracted samples taken from the Frankfurt Oder during the bloom in the main stem in May/June 2024, no B-type prymnesins were identified with the protocol and method applied.

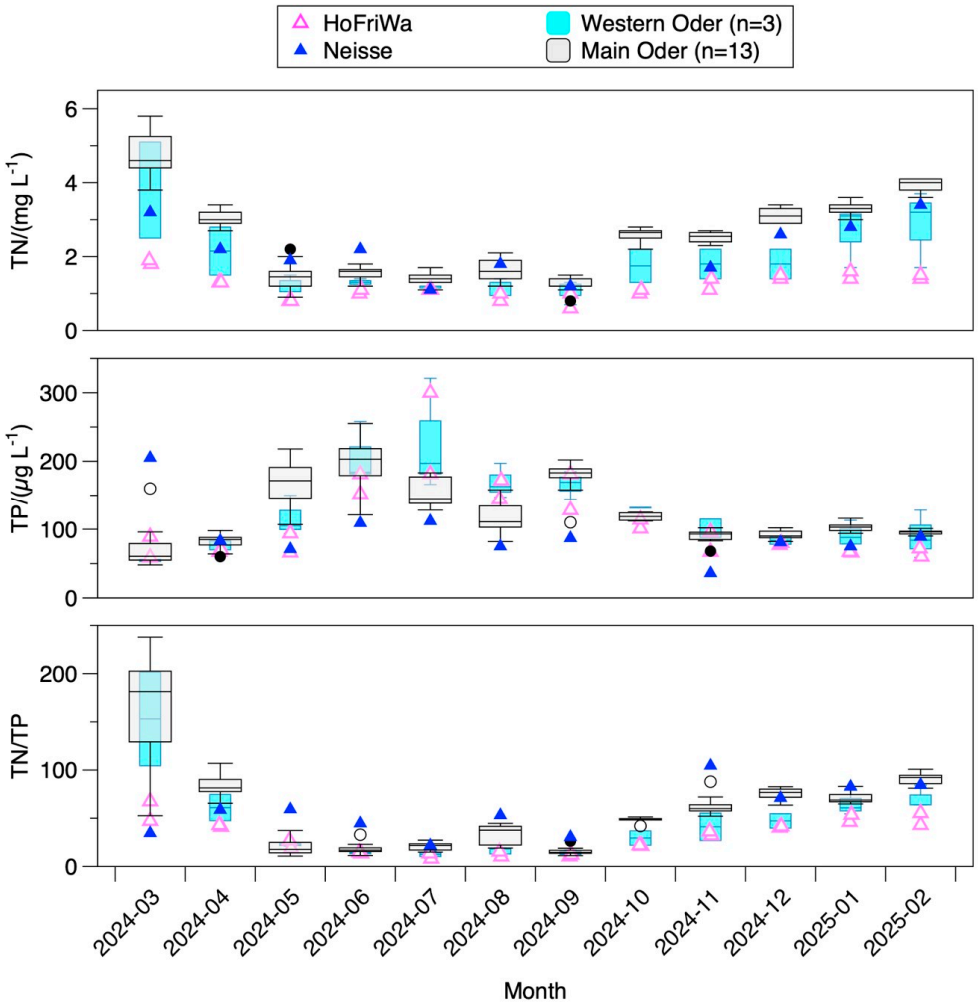


Figure 5. Nutrient dynamics in the Oder River in 2024–2025. Top to bottom: Total nitrogen (TN), total phosphorus (TP), and the TN:TP ratio for the sampling sites in the Neisse (blue filled triangles), HoFriWa (pink open triangles), Western (cyan narrow bars), and Main Oder (grey wide bars). Note that the number of sampling sites differs in each river branch.

4. Discussion

P. parvum has been reported to cause devastating mortalities of gill-breathing organisms (fish, mussels, snails) in both coastal and inland waters in the past [23]. The Oder was first affected by such a mass fish death in 2022 [10,12], and fears persist that such a catastrophe could repeat itself. Since the Oder is a well-known recreational area, embedded in the German National Park Lower Oder Valley and a landscape park on Polish territory (Dolina Dolnej Odry), extensive economic and ecological impacts are to be expected.

Here, for the first time, we report a massive bloom of *P. parvum* in spring 2024, which locally exceeded the abundances recorded during the catastrophe in 2022 substantially. Despite high abundances no substantial fish death was observed, either in spring or summer 2024.

Monthly monitoring of *Prymnesium* in 2024 revealed pronounced temporal variability with two major bloom events occurring in May/June in the main-stem Oder and July/August in the West Oder (Figure 2). The early spring observations (March–April) indicated only minor population increases, typical of low-temperature and low-light conditions constraining algal growth. The first bloom in May/June was the most intense, reaching concentrations over 240,000 CE/mL in Küstrin by early June (Figure 3). The rapid upsurge from 10–18 CE/mL in April to 7000–10,000 CE/mL in early May suggests a steep

transition toward favourable conditions, likely influenced by rising temperature, enhanced light intensity, and increasing TP but decreasing TN in the water (Figure 5). The uniform distribution of *Prymnesium* across river transects and water depths implies homogeneous environmental conditions and strong mixing of the water column, preventing stratification effects (Figure 4).

The abrupt collapse of this bloom within a week, with cell densities declining to <50,000 CE/mL, aligns with the typical boom-and-bust dynamics of *Prymnesium* species [16,24]. Factors likely contributing to this collapse include nutrient depletion, self-shading, or toxin-mediated auto-inhibition, as well as potential grazer activity or viral infection [19,25,26].

Following this collapse, *Prymnesium* abundances remained comparatively low throughout July in the main stem but increased again in the West Oder and HoFriWa regions, forming a second, spatially distinct bloom. This secondary bloom (37,000–150,000 CE/mL) is an indicator of the limited hydrological connectivity and nutrient transport between river sections. The main Oder discharge is directed through the East Oder branch, while the West Oder discharge is controlled by the Widuchowa Weir, resulting in average flow velocities of 0.8 m/s vs. 0.2 m/s, respectively, which increases retention time in the West Oder markedly. In contrast, the HoFriWa only receives water from the Oder Havel Canal and the dewatering ditches connected to it, rendering the HoFriWa almost stagnant. As a result of its low slope and limited discharge, northerly winds tend to slow the West Oder and push water into the HoFriWa, which can increase the water level by up to 50 cm. This hydrological isolation creates local conditions that permitted *Prymnesium* proliferation even after main stream densities had declined.

The subsequent decrease to low concentrations ($\leq$500 CE/mL) in August and near detection limits during autumn and winter supports the strong seasonal control on *Prymnesium* abundance, with environmental conditions (temperature, light, nutrient ratios) becoming unfavourable for bloom persistence in the Oder.

Considering the bloom dynamics in May in the main stem and in August in the West Oder, taking approximately 14 d to go from basic levels to peak levels, a higher sampling frequency (every 7–10 d) is recommended to avoid missing rapid-bloom dynamics in future monitoring activities. Although the main stem revealed quite uniform abundances of *Prymnesium*, monitoring needs to include sampling sites from the West Oder, as indicated by the local bloom in August. During blooming events (at abundances > 10,000 CE/mL), sampling frequency should at least consider sampling every 2 days to describe the bloom dynamics.

The nutrient data from 2024 provide additional context. The total nitrogen (TN) showed a consistent seasonal decline during spring and summer, reflecting uptake by primary producers, including *Prymnesium*, during the vegetation period. Nitrogen replenishment in autumn and winter likely stems from reduced biological demand and increased runoff or internal recycling. Conversely, the total phosphorus (TP) concentrations increased from spring to summer, before decreasing toward winter. This divergence in TN and TP trends produced a marked shift in nitrogen-to-phosphorus (TN:TP) ratios—from high (>200) in early spring to much lower values in summer. These lower ratios suggest relative nitrogen limitation, a condition that may favour mixotrophic species like *Prymnesium*, which can exploit alternative nutrient sources through phagotrophy or organic uptake when inorganic nitrogen becomes scarce. Recent work on protist responses to light and nutrient availability suggests that nutrient limitation is a major environmental driver for mixotrophic feeding behaviour (e.g., increased bacterivory or phagotrophy) when inorganic nutrients are low [27].

Spatially, the HoFriWa and West Oder systems, with low discharge originating from intensive farming terrain and massively impacted by slowing effects of northerly winds due to their very low slopes (<0.1 ppt), recorded higher TP levels during summer, coinciding with intense *Prymnesium* activity, implying that local phosphorus enrichment and slower water exchange promoted favourable conditions for bloom development. Alternatively, lower TN:TP ratios and weaker gradients in these systems may have limited competitive phytoplankton and provided a niche for *Prymnesium* dominance.

Prymnesium blooms and their toxicity are controlled by a complex interaction between chemical stressors (micropollutants), resource availability (biomass and organic matter), and environmental drivers such as light and temperature, arising from both natural variability and anthropogenic pressure. Hartmann et al. [28] and Escher et al. [29] emphasize that micropollutants (e.g., pesticides, pharmaceuticals, industrial chemicals) can act as sub-lethal stressors in aquatic systems. While not always directly toxic to Prymnesium, these compounds can selectively suppress competitors (such as zooplankton or other phytoplankton), indirectly favouring *Prymnesium* dominance. Micropollutants also accumulate in biomass and organic matter, altering microbial activity and nutrient recycling. This can shift trophic interactions and promote conditions under which Prymnesium increases toxin production, particularly when chemical stress coincides with nutrient imbalance (e.g., nitrogen or phosphorus limitation).

Sobieraj et al. [30] suggest that light and temperature are key modulators of both bloom formation and toxicity. Elevated temperatures generally accelerate *Prymnesium* growth rates and can intensify toxin production, particularly under fluctuating or stressful conditions. Light availability affects photosynthetic efficiency and metabolic balance; low or variable light can promote mixotrophic behaviour, increasing reliance on prey capture and potentially enhancing toxin release. These physical drivers often interact with anthropogenic influences such as climate warming, altered hydrology, and nutrient enrichment, increasing bloom frequency and severity.

Overall, the 2024 *Prymnesium* dynamics illustrate complex interactions between hydrology, nutrient stoichiometry, seasonal cycles, and internal impacts. The progression from low spring abundances to intense early-summer blooms, followed by spatially constrained late-summer occurrences, points to both environmental and possibly anthropogenic drivers. The strong correspondence with nutrient availability and TN:TP ratio underscores the potential for nutrient management and flow regulation to mitigate future *Prymnesium* bloom events in the Oder system.

Nevertheless, no mass fish deaths were registered despite the increased attention from the general public and official authorities. In a previous report the hypothesis was raised that fish primarily die due to suffocation as a consequence of severely damaged fish gills [31]. In particular, these authors correlated interlamellar hyperplasia as a consequence of prymnesin exposure with the species-specific susceptibility, which accounted for the largest number of casualties. It is important to know that actual gill damage is reversible within days to weeks [31], and may therefore restore oxygen uptake after the blooming event. Interestingly, during the 2024 blooming event, oxygen levels remained high, ranging between 8 and 12 mg/L during the day and 7 and 9 mg/L at night. Similar values were observed during the blooming event of 2022. Here, when the fish death was noticed on the 9th August 2022, elevated oxygen was measured, ranging between 7 and over 12 mg/L during the day. Therefore, the elevated oxygen concentration would have compensated for suffocation to a certain extent. As a consequence, damage to the gill epithelium has to be regarded as the main reason for the fish death observed in 2022. In addition to the absence of fish mortality, we could not detect any B-type Prymnesins—the toxins of the Oder strain of Prymnesium parvum—in the samples that were taken during 2024, supporting

our conclusion that the 2024 bloom was indeed a non-toxic bloom. Prymnesins are a group of highly toxic polyethers and possess an amphipathic character (both hydrophilic sugar/amide moieties and a hydrophobic polyether backbone), which enables membrane interactions [32]. They have been reported to cause lysis of cells, particularly the gill and blood cells of gill-breathing aquatic organisms, acting as ionophores and increasing the permeability [32]. Therefore, ion leakage and loss of membrane integrity leading to increased energy expenditure are considered the main mode of action of these toxins.

The ITS2 region resides within nuclear ribosomal DNA repeats (rDNA). In eukaryotes, rDNA operons (18S–ITS1–5.8S–ITS2–28S) occur in tandem arrays with many copies per genome, so multiple ITS2 copies exist per cell as part of those repeats. However, in *Prymnesium parvum* the number of ITS2 copies per genome/cell is relatively low compared to many other eukaryotes, where rDNA repeats can sometimes number in the hundreds–thousands per cell. Studies that use qPCR/ddPCR targeting the ITS2 region estimate that approximately ~6–15 ITS2 copies per *Prymnesium parvum* cell exist [12,18,32–34]. Variation may be a result of strain-to-strain variability due to genome plasticity or evolutionary gain/loss of rDNA repeats, cell-to-cell variation due to ploidy variation, cell cycle stage, or unequal segregation of rDNA repeats. In addition, a haplo-diplontic life cycle has been discussed in *Prymnesium* that may alter the ITS2 copy number/cell further. All this may contribute to limitations in the estimation of cell numbers from ITS2 copy numbers quantified by qPCR/ddPCR. Therefore, conventional counting should be included in monitoring studies at 10% of the samples.

5. Conclusions

Prymnesium parvum has repeatedly caused severe fish deaths in both coastal and inland waters, but the 2024 Oder bloom shows that very high cell densities do not inevitably lead to similar ecological disasters to the one in 2022. Taken together, the results highlight that bloom toxicity and the resulting impacts depend not only on *Prymnesium* abundance but also on environmental context and exposure conditions, ultimately leading to the expression of toxin-producing genes. Still, the induction of toxin synthesis remains mostly unknown. In the future, as soon as a quantification standard is available, monitoring of toxins needs to included in future studies.

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