

A Standardised Multi-Temperature Toxicity Framework for Freshwater Primary Producers: Assessing Climate-Driven Modulation of Chemical Toxicity

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Abstract

Freshwater ecosystems provide essential ecological and socio-economic benefits but are increasingly subjected to the combined pressures of climate change and chemical pollution. Although multiple stressor interactions are recognised as a major challenge in ecotoxicology, the extent to which climate-related warming modifies contaminant toxicity in freshwater primary producers remains poorly resolved. This thesis investigates how moderate increases in temperature influence the toxicity of established and emerging contaminants to freshwater primary producers and whether responses differ among taxonomic groups. The study represents a feasibility assessment for studying multi-stressor bioassays using a repeatable, standardised method.

Representative species of green algae (*Pseudokirchneriella subcapitata*), cyanobacteria (*Anabaena flos-aquae*), and diatoms (*Nitzschia palea*) were exposed to toxicologically relevant concentrations of five contaminants, including the metals copper and zinc, the pharmaceuticals erythromycin and sulfamethoxazole, and the pesticides metazachlor. Experiments were conducted under three temperature regimes (15, 20, and 25 °C) using a standardised 96-hour growth inhibition assay in accordance with OECD Test Guideline 201. Concentration–response relationships were modelled using four-parameter log-logistic functions, from which EC_{50} values were derived to quantify changes in sensitivity across temperatures. Targeted chemical analysis was performed alongside biological testing to confirm exposure concentrations and assess contaminant stability across temperature treatments.

Across taxa and contaminants, moderate warming did not result in increased toxicity. Instead, EC_{50} values frequently increased with temperature, indicating a modest reduction in sensitivity at higher temperatures. These patterns suggest that organisms were operating closer to their thermal optima within the tested range, consistent with the inverted V-shaped temperature–toxicity relationships described in the ecotoxicological literature. While clear differences in toxicity were observed among contaminant classes, chemical identity remained the primary driver of toxic potency, with temperature exerting a comparatively minor and context-dependent influence.

Overall, this study demonstrates that moderate warming within ecologically realistic limits does not necessarily amplify chemical toxicity in freshwater primary producers. By applying a controlled and standardised experimental framework, the research demonstrates that temperature effects on toxicity are non-linear and species-specific rather than uniformly synergistic. These findings have important implications for ecological risk assessment, biomonitoring, and regulatory frameworks seeking to incorporate climate-sensitive toxicity data in a warming freshwater environment.

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Author's declaration

I acknowledge that I received acceptable proofreading and editing assistance from a family member and from third party software, in accordance with the University's PGR Programmes Policy. All research ideas, discussion points, data collection, and data interpretation are entirely my own work.

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for a degree or other qualification at this University or elsewhere. All sources are acknowledged as references.

Signed:



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Chapter 1. Introduction

1.1 Importance of the freshwater ecosystem

Freshwater is a fundamental, finite resource for the human population and the global economy (Ingrao et al., 2023). Ecologically, freshwater in the form of rivers, lakes, and wetlands makes up ecosystems covering less than 1% of the Earth's surface area (Myers et al., 2000), yet supports over 10% of all known species. Freshwater ecosystems provide a range of fundamental ecosystem services (ES) (Hanna et al., 2018a; Kaval, 2019) which connect human well-being and natural systems to ecological and economic development, providing a link between nature and society (Xue et al., 2019). Freshwater provides a variety of provisioning and regulating ESs, both directly and indirectly. These include contributions to food supply, water supply, water quality regulation, habitat provision, erosion control, and climate regulation (Albert et al., 2019; Hanna et al., 2018b; Reynaud and Lanzanova, 2017; Tomscha et al., 2017).

The periphyton is a key component of freshwater ecosystems. It is a keystone assemblage of lower trophic-level species that maintain freshwater ecosystems and the subsequent ES that benefit human well-being (Lamprecht et al., 2022). Periphyton is composed of primary producers that form the foundation of many food webs, stabilise substrates, and provide habitat for numerous organisms (Los Huertos, 2020). It comprises of a complex community of algae, bacteria, fungi, and a variety of invertebrates (Hendrey, 2001). The phototrophic component of the periphyton, comprising eukaryotic algae and cyanobacteria, acts as the main primary producers in marine and freshwater ecosystems (Helliwell et al., 2016; Mazard et al., 2016). Phytoplankton also provide a global service by mitigating climate change through the photosynthetic fixation of approximately 5.0×10^{10} tons annually (Marañón, 2009). These organisms, which form the foundation of the freshwater ecosystems, experience natural pressures such as predation, competition, and disease, alongside increasing stressors from pollution arising from both natural and anthropogenic sources.

1.2 Major sources and classes of pollution in freshwater ecosystems

Freshwater ecosystems are increasingly exposed to a wide range of pollutants derived from both point sources (e.g., industrial effluents, municipal wastewater treatment plants) and non-point sources (e.g., agricultural runoff, urban stormwater, atmospheric deposition). Pollutants vary greatly in terms of chemical composition, environmental persistence, and ecological toxicity. They frequently co-occur in aquatic systems, where they interact to produce cumulative impacts on ecosystem structure and function (Jackson et al., 2016; Ormerod et al., 2010). The main classes of pollutants affecting freshwater ecosystems are discussed below. The major sources and pathways through which pollutants enter freshwater ecosystems are summarised in Figure 1. This conceptual diagram illustrates point and non-point source inputs from agricultural, urban, industrial, and atmospheric origins, highlighting their convergence into rivers and lakes.

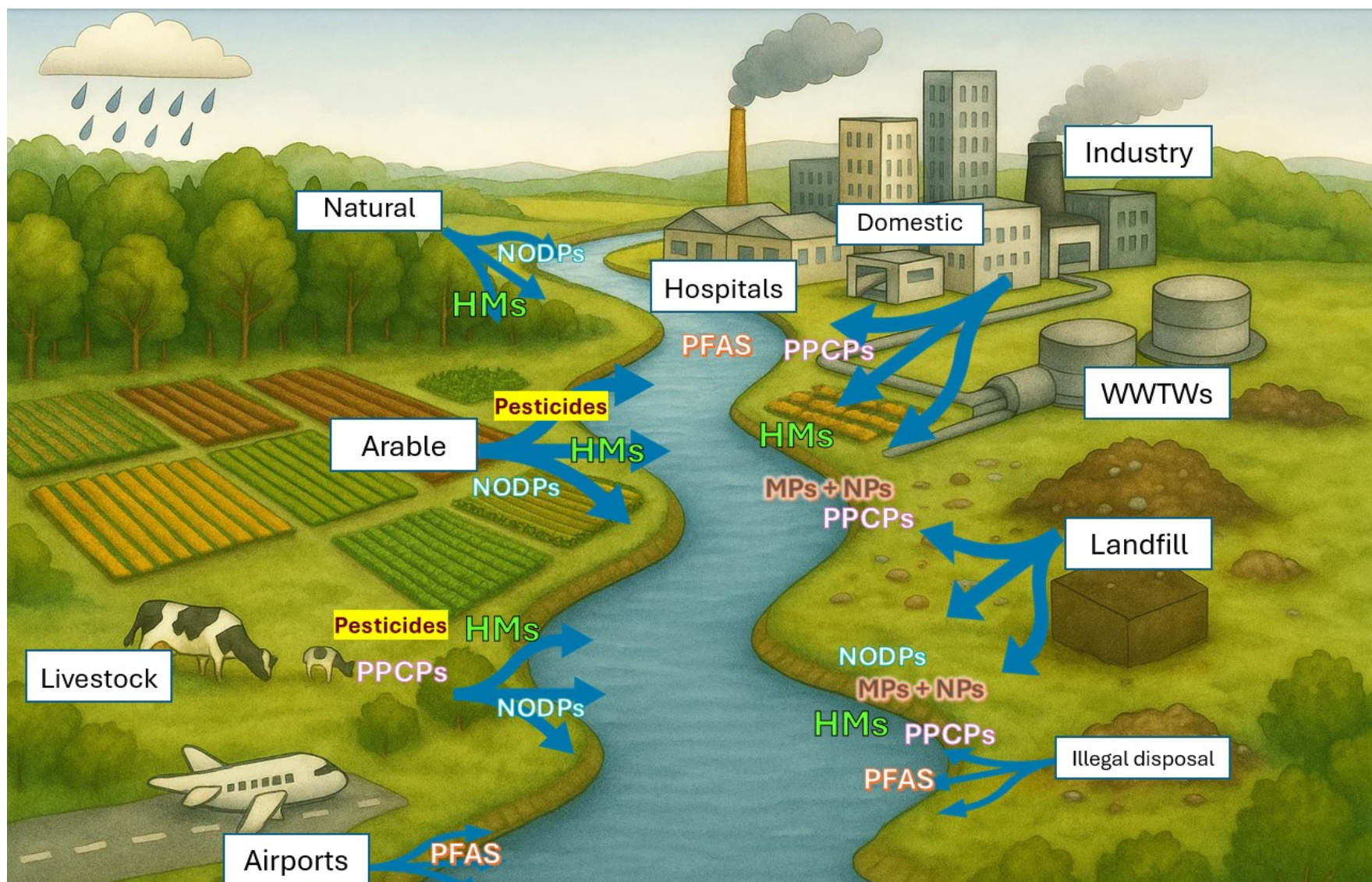


Figure 1. Point and diffuse sources of emerging contaminants entering freshwater ecosystems.

1.2.1 Nutrient and oxygen-demanding pollutants

Nitrogen (N) and phosphorus (P) are essential macronutrients that regulate primary production in freshwater ecosystems. Anthropogenic inputs, particularly from agriculture (e.g., fertilisers, livestock manure) and municipal wastewater discharges, frequently exceed ecological thresholds, resulting in nutrient over-enrichment or eutrophication (Elser, Marzolf and Goldman, 1990; Schindler, 1971). Among these inputs, ammonia represents a particularly important source of nitrogen, commonly entering aquatic environments via untreated or partially treated effluents. Once present, ammonia undergoes nitrification, a microbially mediated process in which autotrophic nitrifying bacteria sequentially oxidise ammonia to nitrite and then nitrate. This transformation consumes significant amounts of dissolved oxygen (DO), contributing to hypoxic or anoxic conditions (Ruiz, Jeison and Chamy, 2003).

Excessive nutrient enrichment (increases in N and P) promotes the rapid proliferation of algae and cyanobacteria, disrupting the natural balance between primary producers and consumers. This shift results in large reductions in DO concentrations, driven by both elevated algal respiration and the microbial decomposition of organic matter following algal senescence. Such processes generate pronounced diurnal oscillations in oxygen availability, substantial fluctuations in phytoplankton biomass, and shifts in species composition toward dominance by bloom-forming taxa (Cottingham, Rusak and Leavitt, 2000). Over time, these physicochemical alterations induce hypoxic or anoxic conditions, rendering habitats unsuitable for many native aquatic species and triggering ecological succession toward degraded, low-diversity ecosystems (Baxa et al., 2021).

The consequences of eutrophication extend beyond ecological impairment to include significant socioeconomic costs. For example, hypoxia-induced fish kills can dramatically reduce fishery yields (Potužák, Hůda and Pechar, 2007), while excessive algal growth can obstruct irrigation systems, clog drinking water infrastructure, and impair hydropower generation by fouling turbines (Akinnowo, 2023). Even in the absence of visible algal blooms, chronic nutrient enrichment can alter elemental stoichiometry (e.g., C:N:P ratios) within primary producers, degrading food quality for grazers and causing cascading effects through higher trophic levels (Schulhof et al., 2019).

Overall, nutrient and oxygen-demanding pollutants drive a continuum of ecological disturbances. These range from community-level changes in species composition to ecosystem-scale functional impairments, while simultaneously imposing considerable economic burdens on agriculture, fisheries, and water resource management sectors.

1.2.2 Pesticides and herbicides

Pesticides represent a major class of synthetic organic pollutants introduced into freshwater ecosystems primarily through agricultural runoff, spray drift, and improper disposal practices. Their extensive application in modern agriculture and domestic pest control has intensified over recent decades in response to escalating food production demands (Tudi et al., 2021). Although specifically designed to control target pests, including insects (insecticides), weeds (herbicides), and fungi (fungicides), pesticides often exert toxic effects on a wide range of non-target aquatic organisms, including invertebrates, fish, and primary producers, even at environmentally realistic concentrations (Aktar, Sengupta and Chowdhury, 2009; Oerke and Dehne, 2004).

Field-based investigations consistently demonstrate strong relationships between pesticide exposure (expressed either as measured concentrations or as toxicity weighted indices) and observed biological impairment across diverse freshwater ecosystems (Liess and Ohe, 2005; Schäfer et al., 2007; Singh et al., 2023).

Pesticides affect not only community composition through lethal toxicity but also ecosystem function through sublethal impacts. These include behavioural modifications, reproductive impairment, developmental delays, and physiological stress, all of which are frequently reported in aquatic biota (Elskus, 2012; Villarroel et al., 2003).

Although such effects rarely precipitate immediate population collapses, their cumulative and chronic manifestation can lead to long-term biodiversity loss and reduced ecosystem resilience (Holden, 1972).

At the continental scale, Malaj et al. (2014) analysed monitoring data from more than 4,000 European sites and demonstrated that pesticides accounted for 81%, 87%, and 96% of acute risk threshold exceedances for fish, invertebrates, and algae, respectively.

Among the pesticides studied, diuron, atrazine, and isoproterenol were most frequently detected above both chronic and acute risk thresholds (Malaj et al., 2014), with many compounds exhibiting photosystem II (PSII) inhibition in primary producers, thereby directly suppressing photosynthesis and primary productivity (Oropesa, Floro and Palma, 2017).

Furthermore, the environmental persistence of many pesticides is exacerbated by their hydrophobicity and strong affinity for sediment adsorption, allowing residues to persist in aquatic environments long after initial application events (Bonmatin et al., 2015; Pathak et al., 2022).

Particularly concerning is the detection of banned pesticides in contemporary freshwater systems. Several compounds prohibited under EU Regulation (EC) No 1107/2009 continue to appear in environmental monitoring data, with one recent study reporting that 38% of detected residues originated from pesticides no longer approved for use within the European Union (Navarro et al., 2024).

This highlights the regulatory challenges posed by legacy contamination and the long-term ecological risks associated with the environmental persistence of synthetic pesticides.

1.2.3 Pharmaceuticals and personal care products (PPCPs)

Pharmaceuticals and personal care products (PPCPs) constitute a broad and chemically diverse group of biologically active contaminants. These contaminants originate from multiple anthropogenic sources, including hospital effluents, municipal wastewater discharges, veterinary treatments, and livestock production facilities (Lin et al., 2010). Based on their functional applications, PPCPs can be broadly divided into three main categories: (1) prescription and over-the-counter pharmaceuticals; (2) personal care products such as surfactants, artificial sweeteners, preservatives, and antimicrobial agents including triclosan; and (3) insect repellents such as N,N-diethyl-meta-toluamide (DEET) (Chen and Akhtar, 2022). Conventional wastewater treatment plants (WWTPs) are not specifically designed to remove these compounds, resulting in incomplete

degradation and continuous release into receiving freshwater environments (Pal et al., 2014).

The ecological risks associated with PPCPs are primarily linked to their pharmacological potency at low concentrations and resistance to environmental degradation (Wang et al., 2021). The combination of these characteristics leads to chronic exposure of aquatic organisms across multiple trophic levels (Ebele, Abou-Elwafa Abdallah and Harrad, 2017; Osuoha, Anyanwu and Ejileugha, 2023). Empirical monitoring studies across Europe have consistently detected PPCPs such as propranolol, sulfamethoxazole, carbamazepine, indomethacin, and diclofenac in both surface waters and wastewater effluents (Thomas and Hilton, 2004; Zhou et al., 2009).

A particularly serious concern is the role of antibiotic pollution in the emergence and dissemination of antimicrobial resistance (AMR). Freshwater systems receiving high antibiotic loads combined with dense microbial populations provide ideal conditions for the selection, maintenance, and horizontal transfer of antibiotic resistance genes (ARGs), turning these ecosystems into environmental reservoirs of AMR pathogens (Larsson and Flach, 2022; Samreen et al., 2021). Given that recreational activities such as swimming and bathing facilitate direct human contact with contaminated waters, this represents a significant public health threat (Leonard et al., 2022).

Another dimension of concern arises from the chemical mode of action (MoA) of many PPCPs. These compounds are specifically designed to interact with conserved biochemical pathways in humans, many of which are also present in non-target aquatic organisms. Fluoxetine provides an example of this non-target effect. Fluoxetine disrupts serotonin (5-HT) signalling pathways in aquatic invertebrates, leading to altered reproduction, impaired metabolic regulation, and reduced locomotor activity at concentrations approaching or even below those measured in the natural environment (Ford and Fong, 2015; Franzellitti et al., 2013).

Furthermore, several PPCPs function as endocrine-disrupting chemicals (EDCs). This constitutes a broad range of compounds including natural oestrogens, synthetic hormones such as diethylstilbestrol (DES), and industrial compounds like bisphenol A

and phthalates. These substances interfere with hormone-regulated developmental and reproductive processes in aquatic organisms, raising concerns about population-level impacts on biodiversity (Marlatt et al., 2022).

Finally, increasing evidence shows that primary producers at the base of freshwater food webs are also vulnerable and currently exposed to effective concentrations of PPCPs. For instance, carbamazepine has been shown to bioaccumulate in freshwater algae such as *Pseudokirchneriella subcapitata* (Hindak 1990), while sulfamethoxazole, nicotine, and clarithromycin inhibit algal growth and photosynthetic efficiency, thereby reducing ecosystem productivity and altering energy flow across trophic levels (Shan et al., 2021; Wilkinson et al., 2022; Yamashita et al., 2006).

1.2.4 Heavy metals

Heavy metals (HMs) represent a broad class of pollutants encompassing naturally occurring metallic elements characterised by high atomic weight and densities significantly greater than that of water (Tchounwou et al., 2012). Elements such as copper, iron, zinc, manganese, and cobalt function as essential micronutrients at trace concentrations, supporting enzymatic activity, electron transport, and other critical metabolic pathways in aquatic organisms. Low levels of certain metals have been shown to promote the growth of submerged macrophytes, highlighting their ecological significance under natural background conditions (Wei et al., 2021).

HMs enter freshwater ecosystems through both natural processes, including soil erosion, volcanic activity, and biogenic cycling (Zaynab et al., 2022), and anthropogenic activities such as mining, metal smelting, industrial effluents, intensive agriculture (via fertilisers, pesticides, and herbicides), and urban runoff (Sharma et al., 2024).

Anthropogenic inputs often elevate concentrations far above natural background levels, particularly in areas with rapid industrialisation and inadequate wastewater treatment infrastructure.

The principal environmental concern stems from the fact that, unlike many organic contaminants, heavy metals are non-biodegradable. Once released, they persist in

aquatic systems, where they undergo bioaccumulation in tissues of aquatic organisms and biomagnification through food webs, thereby amplifying ecological and human health risks (Shou et al., 2022). Metals such as arsenic, cadmium, lead, and mercury are particularly hazardous because they exhibit toxic effects even at low concentrations (Tchounwou et al., 2012). Numerous studies have documented significant tissue accumulation in freshwater fish, providing clear evidence of both ecological toxicity and implications for human populations consuming contaminated aquatic food resources (Ray and Vashishth, 2024).

Toxicological research highlights a range of sublethal and lethal effects. For instance, arsenic (As) exposure has been associated with vascular collapse in fish gills, leading to severe hypoxic stress and mortality (Ahmed et al., 2013). Mercury contamination induces profound structural, physiological, and biochemical alterations in the central nervous system, while Lead exposure has been linked to behavioural abnormalities, reduced fertility, impaired growth, and developmental retardation in fish populations (Jamil Emon et al., 2023). These cases represent only a fraction of the reported toxicological endpoints, as numerous other metals and exposure pathways have been extensively documented in the literature.

In a global context, Kumar et al. (2023) conducted the first meta-analysis of toxic metal contamination in continental surface waters. Their findings revealed that concentrations of chromium (Cr), arsenic (As), and mercury (Hg) exceeded World Health Organization (WHO) drinking water thresholds across all continents. Importantly, statistical analyses demonstrated strong correlations implicating both anthropogenic activities and natural geochemical processes as significant contributors to the observed contamination patterns (Kumar et al., 2023).

1.2.5 Plastics and microplastics

Plastic particles, including microplastics (less than 5 mm) and nanoplastics (less than 1 μm), have recently emerged as contaminants of growing concern within freshwater environments (Cole et al., 2015). Their light weight, durability, versatility, and low production cost have driven widespread use across modern society, encompassing applications in clothing, personal care products, packaging, and infrastructure (Andrady and Neal, 2009). These same properties, combined with inadequate waste management practices, have led to increasing contamination of freshwater systems. Plastics enter aquatic environments either as primary microplastics, manufactured at small sizes for use in products such as cosmetics, or as secondary microplastics generated through the degradation and fragmentation of larger plastic debris via physical, chemical, and photochemical processes (Bexleitova et al., 2024).

A comprehensive review on the occurrence, ecological implications, and research needs associated with microplastics in freshwater systems reported widespread contamination across multiple continents (Jolaosho et al., 2025). Microplastic abundances were recorded at levels ranging from 293 ± 83 to 19,860 items/ m^3 in Chinese rivers (Yan et al., 2019), 0.28 ± 0.18 to 1265 items/ m^3 in European freshwater systems (Uurasjärvi et al., 2020), and comparably high levels across North and South America (Lenaker et al., 2019), demonstrating the global scale of this emerging pollutant (Sarijan et al., 2021).

The chemical composition of microplastics plays a critical role in determining their ecotoxicological risks. During manufacturing, plastics are formulated with numerous additives such as flame retardants (e.g., polybrominated diphenyl ethers), plasticisers, and stabilisers. Upon environmental degradation, these additives can leach into freshwater systems, contributing to a complex chemical mixture of known toxic contaminants (Campanale et al., 2020; Gore, Khraisheh and Kandasubramanian, 2018; Rani et al., 2015). Even when chemically inert polymers dominate, the sheer abundance of particles poses bioaccumulation and physical hazard risks comparable to those of persistent inorganic contaminants such as heavy metals.

Experimental studies illustrate significant biological impacts. For instance, laboratory exposures have shown that polystyrene microplastics impair reproduction and feeding in freshwater mussels and oysters by disrupting normal energy intake and assimilation (Sussarellu et al., 2016). Similarly, zooplankton exposed to microplastic particles experienced a 40% reduction in carbon biomass, accompanied by substantial declines in energy availability and growth rates (Cole et al., 2015).

A further emerging concern is the role of microplastics as vectors for microbial colonisation and the dissemination of antimicrobial resistance (AMR). Biofilms forming on microplastic surfaces near wastewater treatment plants frequently harbour antimicrobial resistance genes (ARGs). Through horizontal gene transfer mechanisms such as conjugation, ARGs can propagate among environmental and pathogenic bacteria, thereby increasing the public health risks associated with AMR dissemination (Ochman, Lawrence and Groisman, 2000; Yuan et al., 2022; Zarei-Baygi and Smith, 2021).

Finally, the progressive fragmentation of microplastics into nanoplastics represents an additional research frontier. Nanoplastics possess greater surface area-to-volume ratios, enhanced chemical reactivity, and potentially greater bioavailability than larger particles. However, empirical data on their environmental behaviour, ecological toxicity, and interactions with other stressors remain scarce, prompting calls for urgent research into nanoplastic-specific risks to freshwater organisms (Trevisan et al., 2022).

1.2.6 Per- and polyfluoroalkyl substances (PFAS) and their degradation products

The Environment Agency defines poly- and perfluoroalkyl substances (PFAS) as a large and chemically diverse group of synthetic organofluorine compounds that have been widely manufactured and used since the 1940s (Dewapriya et al., 2023). They are unified by the presence of at least one fully fluorinated methyl ($-\text{CF}_3$) or methylene ($-\text{CF}_2-$) functional group (Pemberton, 2021). These anthropogenic chemicals rapidly gained prominence because they introduced properties that were previously unattainable in industrial materials. Their exceptional thermal stability, hydrophobicity, and lipophobicity endowed PFAS-containing products with heat-resistant, waterproof, and

non-stick characteristics (McKeen, 2013). Consequently, PFAS became integral to numerous industrial, commercial, and domestic applications ranging from surface coatings and textiles to food packaging and firefighting foams. The Organisation for Economic Co-operation and Development (OECD) has identified and manually classified 4,730 distinct PFAS-related CAS numbers, reflecting the chemical diversity within this group (OECD, 2018). Their widespread and long-term use has created multiple pathways for PFAS to enter freshwater environments. These include conventional pollution routes such as landfill leachates, wastewater treatment plant effluents, industrial discharges, and agricultural runoff where PFAS-containing pesticides are applied. However, PFAS contamination is also linked to unique, point-source incidents. Studies have repeatedly documented localised but severe contamination arising from uncontrolled releases of aqueous film-forming foams (AFFFs) used in firefighting. Training facilities at airports and military bases, in particular, have been identified as persistent contamination hotspots (Drenning et al., 2023; Liu et al., 2023).

A comprehensive review commissioned by the Drinking Water Inspectorate reported that PFOS, PFOA, and PFPeS frequently exceeded concentrations of $\geq 0.1 \mu\text{g L}^{-1}$ in protected raw water sources, including both surface waters and groundwater (Jarvis et al., 2025). Greater concentrations of PFAS have identified 'Hotspots' from spatial freshwater sampling analyses. For example, screening data from 17 UK airports submitted to the Environment Agency reported total PFAS concentrations up to $12,845 \text{ ng L}^{-1}$, with PFOA alone reaching 441 ng L^{-1} in surface water samples taken from trade effluents (Neill, 2025).

A recurring theme of PFAS is their extreme environmental persistence, primarily attributed to the strong carbon-fluorine bonds that resist chemical, thermal, and biological degradation. This stability promotes bioaccumulation in freshwater organisms, raising concerns about long-term ecological and human health risks. Furthermore, certain PFAS compounds, notably PFOA, exhibit endocrine-disrupting properties (White et al., 2011).

The World Health Organization defines an endocrine disruptor as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently

causes adverse health effects in an intact organism, or its progeny, or (sub)populations” (Damstra et al., 2002, p.1). This classification highlights the dual environmental and toxicological significance of PFAS contamination in freshwater ecosystems.

Ecotoxicological research on PFAS remains limited, particularly in freshwater ecosystems. Soltanighias et al. (2024) investigated the combined effects of perfluoroalkyl substances and microplastics on *Daphnia magna* (Straus, 1820). Their study showed that environmentally relevant exposure to PET, PFOS, and PFOA reduced size at maturity and fecundity, increased the interval between broods, and increased mortality (Soltanighias et al., 2024). Conversely, Razak et al., (2025) performed a risk assessment using integrated predictive and empirical toxicity models and concluded that PFAS posed a negligible risk in freshwater ecosystems. However, it is noted that sampling was not performed temporally and cannot be taken as a homogeneous conclusion. A summary of the major classes of emerging contaminants discussed above is provided in Table 1.

Table 1. Summary of major pollutant classes in freshwater ecosystems: sources, effects, persistence, and challenges

Pollutant Class	Defining Characteristics	Representative Sources	Persistence	Key Ecological Effects	References
Nutrient and Oxygen-Demanding Pollutants	Macronutrients (N, P); oxygen-demanding organic matter stimulating eutrophication processes	Agricultural runoff (fertilisers, manure), municipal wastewater, untreated effluents	Moderate; excess loading sustains enrichment despite natural cycling	Eutrophication, algal blooms, hypoxia/anoxia, shifts in species composition, loss of biodiversity, altered food-web stoichiometry	Elser et al., 1990. Schindler, 1971. Ruiz et al., 2003. Cottingham et al., 2000. Baxa et al., 2021. Potužák et al., 2007. Akinnawo, 2023. Schulhof et al., 2019
Pesticides and Herbicides	Synthetic organic biocides designed to control pests (insects, weeds, fungi) but toxic to non-target species	Agricultural runoff, spray drift, improper disposal, legacy contamination	Often persistent; hydrophobicity leads to sediment adsorption and long-term exposure	Acute/chronic toxicity to fish, invertebrates, algae; sublethal behavioural, reproductive, and developmental impacts; PSII inhibition reducing primary productivity	Tudi et al., 2021. Aktar et al., 2009. Oerke and Dehne, 2004. Liess and Ohe, 2005. Schäfer et al., 2007. S. Singh et al., 2023. Elskus, 2012. Villarroel et al., 2003. Holden, 1972. Malaj et al., 2014. Oropesa et al., 2017. Bonmatin et al., 2015. Pathak et al., 2022. Navarro et al., 2024
Pharmaceuticals and Personal Care Products (PPCPs)	Biologically active compounds designed to interact with specific molecular targets; include pharmaceuticals, antimicrobials, surfactants, personal care additives	Hospital wastewater, domestic sewage, livestock production, WWTP effluents	Moderate–high; continuous inputs maintain chronic exposure	Endocrine disruption, altered reproduction/metabolism, behavioural impacts, antimicrobial resistance gene selection, toxicity to algae and primary producers	Lin et al., 2010. Chen and Akhtar, 2022. Pal et al., 2014. Ebele et al., 2017. Osuoha et al., 2023. Thomas and Hilton, 2004. Zhou et al., 2009. Larsson and Flach, 2022. Samreen et al., 2021. Leonard et al., 2022. Ford Fong, 2015. Franzellitti et al., 2013. Marlatt et al., 2022. Shan et al., 2021. Wilkinson et al., 2022. Yamashita et al., 2006

Heavy Metals (HMs)	Metallic elements with high atomic weight and density; essential at trace levels but toxic above thresholds	Mining, smelting, industrial discharges, urban runoff, agricultural inputs, natural weathering	Very high; nonbiodegradable, bioaccumulation and biomagnification in food webs	Gill damage, neurotoxicity, reproductive/developmental effects, biodiversity loss, trophic transfer risks	Tchounwou et al., 2012. Wei et al., 2021. Zaynab et al., 2022. Sharma et al., 2024. Shou et al., 2022. Ray and Vashishth, 2024. Ahmed et al., 2013. Jamil Emon et al., 2023. Kumar et al., 2023
Plastics, Microplastics and Nanoplastics	Synthetic polymer particles; microplastics <5 mm, nanoplastics <1 µm; additives leach over time	Personal care products (primary MPs), fragmentation of larger plastics (secondary MPs), wastewater, landfill leachate, stormwater	High; particles persistent, NPs highly mobile	Ingestion impacts: reduced feeding, energy budgets, growth; reproduction impairment; vectors for biofilms and antimicrobial resistance genes	Cole et al., 2015. Andrady and Neal, 2009. Bexeitova et al., 2024. Yan et al., 2019. Uurasjärvi et al., 2020. Lenaker et al., 2019. Sarijan et al., 2021. Campanale et al., 2020. Gore et al., 2018. Rani et al., 2015. Sussarellu et al., 2016. Ochman et al., 2000. Q. Yuan et al., 2022. Zarei-Baygi Smith, 2021. Trevisan et al., 2022
Per- and Polyfluoroalkyl Substances (PFAS)	Synthetic organofluorine chemicals with strong C–F bonds giving extreme stability and mobility	Firefighting foams (AFFFs), industrial effluents, wastewater, landfill leachates, agricultural runoff	Extremely high; resistant to chemical, thermal, biological degradation	Bioaccumulation, endocrine disruption, long-term contamination of ecosystems and drinking-water sources	Poly- and perfluoroalkyl substances (PFAS): sources, pathways and environmental data. McKeen, 2013. Summary Report on the New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs), 2018. Drenning et al., 2023. Liu et al., 2023. Jarvis et al., 2025. Neill, 2025. White et al., 2011. Damstra et al., 2002. Soltanighias et al., 2024. Razak et al., 2025

1.3 Climate change and temperature effects on freshwater systems

The United Nations Framework Convention on Climate Change (UNFCCC) defines climate change as “a change in climate attributed directly or indirectly to human activity that alters the composition of the global atmosphere, in addition to natural variability observed over comparable time periods” (UNFCCC, 1992, Article.1, p.3). The Intergovernmental Panel on Climate Change (IPCC) Sixth Assessment Report (AR6) concluded, with high confidence, that climate change, land-use change, and chemical pollution are key and interacting drivers of freshwater ecosystem degradation (IPCC, 2023).

Anthropogenic climate change is largely driven by the combustion of fossil fuels used for energy production (Höök et al., 2013). This process releases substantial quantities of carbon dioxide (CO₂) and other greenhouse gases into the atmosphere, which accumulate and intensify the greenhouse effect by absorbing and re-emitting infrared radiation, thereby trapping thermal energy and contributing to rising global surface temperatures (Chanda et al., 2020).

Climate change stressors rarely act in isolation; instead, they interact with existing environmental pressures to produce complex, often non-linear effects on freshwater quality, biodiversity, and ecosystem function (Whitehead et al., 2009; Noyes et al., 2009). Among these stressors, temperature is a dominant driver influencing both environmental processes and biological responses.

1.3.1 Physical and hydrological changes

Climate change alters hydrological regimes at both regional and global scales (Nimma et al., 2025). It has been estimated that seasonal flow regimes will change by more than 10% across approximately 90% of the global land area (excluding Greenland and Antarctica) (Döll et al., 2010). These changes manifest as shifts in runoff patterns, with

increases or decreases depending on geographic location and season (Alcamo, Flörke and Märker, 2007).

Such hydrological alterations influence river discharge, sediment transport, and channel morphology, thereby affecting substrate stability and habitat structure within freshwater ecosystems (Bunn and Arthington, 2002). In addition, changes in precipitation patterns, including more frequent extreme rainfall events and prolonged droughts, further disrupt freshwater systems by altering dilution capacity and contaminant transport pathways (Tabari, 2020).

1.3.2 Physicochemical responses to warming

Rising temperatures represent one of the most significant climate-driven stressors in freshwater systems. Increased thermal energy enhances stratification in lakes and reservoirs, reducing vertical mixing and limiting the transport of oxygen and nutrients. This often leads to hypoxic or anoxic conditions in deeper waters (Jane et al., 2021).

Temperature also influences key physicochemical properties of freshwater systems. Elevated temperatures reduce dissolved oxygen concentrations, alter pH under certain conditions, and increase the mobilisation of dissolved organic carbon (DOC) (Sawicka et al., 2016; Singh et al., 2021). Increased DOC inputs, often linked to climate-intensified precipitation events, contribute to reduced water clarity, commonly referred to as freshwater “browning”, which limits light penetration and can reduce primary productivity.

Furthermore, temperature-dependent changes in chemical processes influence pollutant behaviour. For example, reduced summer flows may decrease dilution potential, increasing contaminant concentrations, while elevated temperatures may enhance degradation rates of certain chemicals, resulting in complex and sometimes opposing effects on contaminant dynamics (Bloomfield et al., 2006).

1.3.3 Ecological responses and species-specific effects

Temperature plays a fundamental role in regulating biological processes, and climate-driven warming can shift species beyond their thermal optima. This can lead to changes in community composition, often favouring thermally tolerant or generalist taxa at the expense of more specialised species (Nelson et al., 2017).

The influence of temperature on biological performance is well established and is typically characterised by a unimodal thermal performance curve (TPC) (Huey and Stevenson, 1979). This framework defines an optimum temperature (T_o), at which performance is maximised, and an operative thermal range bounded by a critical minimum (CT_{min}) and critical maximum (CT_{max}). Thermal performance curves are widely used to assess the sensitivity of ectothermic organisms to climate warming, providing a mechanistic basis for understanding how deviations from thermal optima influence physiological performance, growth, and survival (Amarasekare and Savage, 2012).

Such shifts can alter species interactions, including competition and predation, and may facilitate the establishment of invasive species (Rahel and Olden, 2008). Temperature increases have also been associated with changes in organismal physiology, including reduced body size in some invertebrates and increased growth rates, reflecting broader metabolic responses to warming.

Importantly, these responses are highly species-specific, with different taxa exhibiting varying sensitivities to temperature changes. This highlights the importance of considering multiple species when assessing ecological responses to climate change, as single-species approaches may fail to capture the variability observed across trophic levels and taxonomic groups.

Additionally, warming can exacerbate existing environmental pressures such as eutrophication. Elevated temperatures promote more frequent and intense algal blooms, compounding the ecological and water quality challenges associated with nutrient enrichment (Nazari Sharabian, Ahmad and Karakouzian, 2018).

1.3.4 Temperature effects on pollutant behaviour and toxicity

Temperature is a key factor influencing the fate, behaviour, and toxicity of contaminants in freshwater systems. A growing body of research demonstrates that climate change not only alters contaminant transport but also affects their chemical speciation, bioavailability, and biological effects.

Noyes et al. (2009a), provided one of the first comprehensive reviews on how climate change-induced temperature increases influence contaminant toxicology, highlighting that warming can increase contaminant bioavailability and uptake rates in aquatic organisms. This is largely driven by temperature-dependent increases in metabolic rates, where higher temperatures lead to increased resource uptake and, consequently, greater contaminant exposure. At the same time, elevated temperatures may reduce physiological tolerance, thereby amplifying toxic effects (Noyes et al. 2009a).

However, temperature–toxicity relationships are not uniform. A further review by Jackson et al. (2016), demonstrated that interactions between warming and additional stressors can be synergistic, additive, or antagonistic depending on the system and stressor combination. In some cases, moderate warming may reduce toxicity by enhancing metabolic or detoxification processes, while in others it may exacerbate toxic effects.

Temperature-driven changes in environmental conditions also influence contaminant behaviour. For example, pH shifts can modify the speciation and toxicity of metals such as mercury and lead, while thermal stratification can promote anoxic conditions that enhance the release of phosphorus and trace metals from sediments (Xiao et al., 2024).

Empirical studies have demonstrated that higher temperatures can exacerbate the toxic effects of pesticides and other contaminants in aquatic organisms, although the magnitude and direction of these effects vary across species and chemical classes (Jacquin et al., 2019; Lacy et al., 2023; Lacy and Rahman, 2022; Laetz et al., 2014).

1.3.5 Socio-economic consequences of climate change on pollution dynamics

Climate change is projected to exert profound socio-economic pressures on freshwater systems by altering both water availability and agricultural practices. Prolonged drought periods are expected to increase irrigation demands within the agricultural sector (Ingrao et al., 2023). As water abstraction rates from rivers intensify to meet irrigation needs, overall river discharge volumes decline, leading to higher concentrations of existing pollutants and thereby exacerbating their known toxic effects on aquatic ecosystems (Nilsson and Renöfält, 2008).

Conversely, the increased frequency and intensity of extreme rainfall events under climate change scenarios will accelerate nutrient and contaminant runoff from agricultural landscapes into freshwater systems (Bieroza et al., 2024). This process can also create a feedback mechanism. Nutrient leaching following heavy rainfall often necessitates additional applications of nitrogen and phosphorus fertilisers to maintain crop productivity, further elevating the risk of eutrophication in receiving waters (Han et al., 2023).

Moreover, global food insecurity driven by climate change is expected to increase pressure for agricultural intensification, involving higher usage of pesticides, fertilisers, and water resources to maximise yields from increasingly stressed croplands (Yang et al., 2024). Such intensification will likely amplify pollutant loads entering freshwater ecosystems, compounding the combined effects of climate change, chemical pollution, and land-use change on water quality and ecosystem health.

1.4 Multi-stressor and chemical mixture interactions

Freshwater ecosystems are increasingly exposed to multiple, co-occurring stressors, including climate-driven changes (e.g., warming and altered hydrology), chemical mixtures (e.g., pesticides, PPCPs, heavy metals, PFAS), and habitat modifications such as eutrophication and sedimentation. Unlike single-stressor experiments, natural environments expose organisms to dynamic combinations of stressors that may interact additively, synergistically, or antagonistically. Understanding these interactions is crucial because they frequently generate non-linear and unpredictable biological responses (Morris et al., 2022), thereby challenging conventional risk-assessment frameworks that rely heavily on single-chemical toxicity thresholds (Lima, Sayanda and Wrona, 2023).

Synergistic effects occur when the combined impact of stressors exceeds the expected additive response, resulting in amplified toxicity. In contrast, antagonistic effects arise when one stressor reduces or counteracts the effect of another. For example, certain metals may precipitate under specific pH conditions, reducing their bioavailability. These conceptual relationships are illustrated in Figure 2.

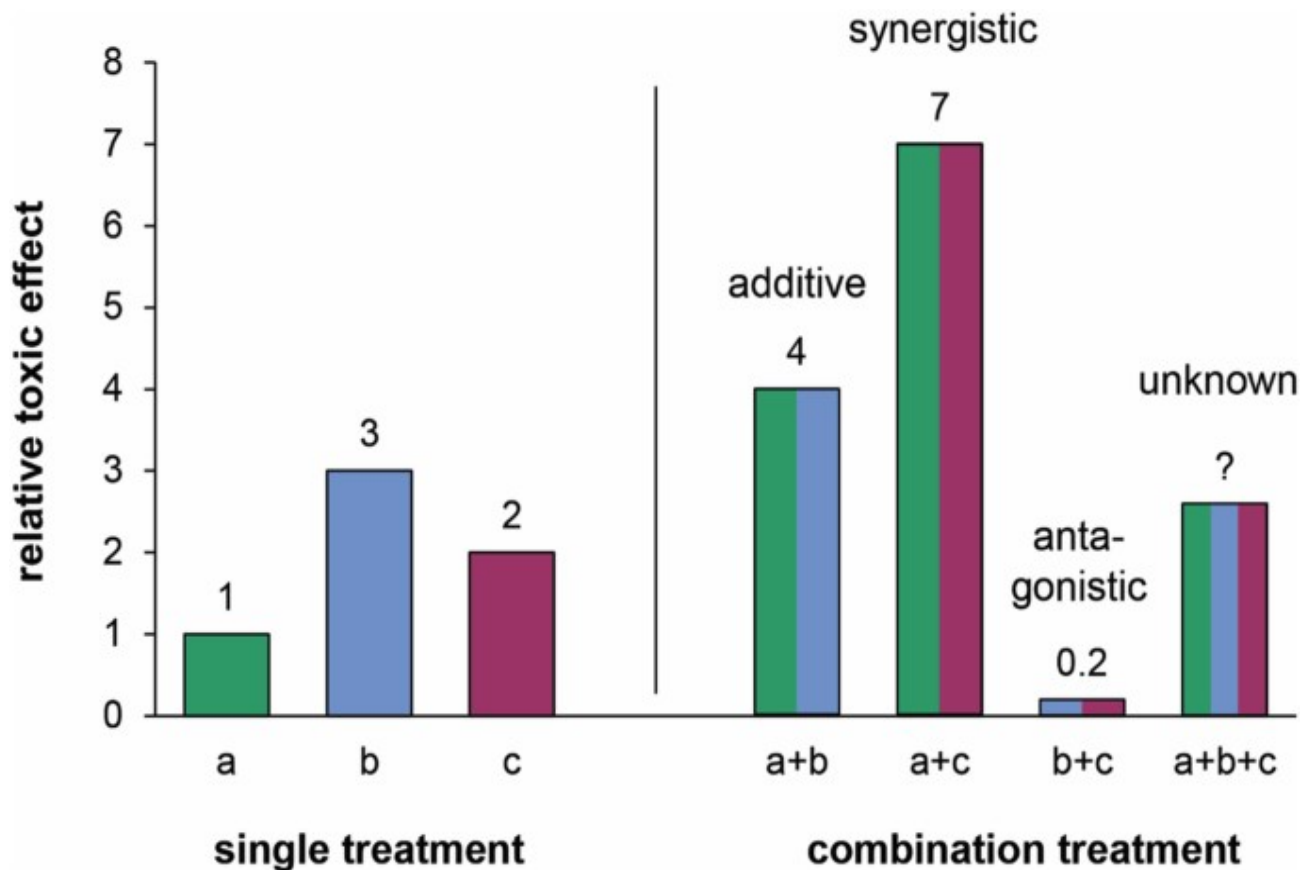


Figure 2. Conceptual representation of additive, synergistic, and antagonistic interactions in multi-stressor studies, illustrating their effects on relative toxicity. Adapted from Büch et al. (2021).

Jackson et al. (2016) conducted a meta-analysis examining the net effects of multiple stressors in freshwater ecosystems. Their findings indicated that the mean interaction effects across stressor pairs and response metrics were predominantly antagonistic or additive, contrasting with marine systems, where synergistic interactions are more commonly reported (Jackson et al., 2016). The authors suggested that the smaller spatial scale of many freshwater ecosystems may allow greater acclimatisation and co-adaptation to co-occurring stressors. Notably, warming was the stressor most frequently associated with reversal interactions (19.05% of warming interactions), likely due to its stimulatory physiological effects (Jackson et al., 2016). However, the study highlighted limitations linked to comparing results across a heterogeneous collection of studies with minimal methodological standardisation.

Despite ongoing research efforts, identifying and predicting multi-stressor interactions remains challenging. The complexity of community-level responses, variation in ecological endpoints, and difficulty in reproducing realistic environmental conditions hinder accurate modelling and verification. Bao et al. (2024) performed a quantitative analysis on freshwater macroinvertebrates and found that additive interactions were the most common outcome of multiple stressor exposures. Similarly, Orr et al. (2024) evaluated the current landscape of multi-stressor research in freshwater systems and noted that, although experimental diversity enhances ecological insight, it can also lead to fragmented research efforts. This fragmentation makes it difficult to synthesise findings across studies or integrate them into predictive models or meta-analyses (Orr et al., 2024).

1.4.1 Critically reviewing the regulatory system

The diverse range of pollutants originating from both point and non-point sources (Figure 1) interact with each other and with environmental stressors, resulting in complex degradation pathways and non-linear effects, including synergistic and antagonistic interactions (Whitehead et al., 2009; Noyes et al., 2009). These interactions present significant challenges for accurately quantifying toxicity within predictive models used in environmental risk assessment. Furthermore, each freshwater catchment possesses a unique combination of geological, hydrological, and socio-economic characteristics, complicating the development of generalisable, large-scale risk models (McDonnell et al., 2007).

The protection of human health and the environment from chemical risks is achieved through regulatory frameworks and risk assessment approaches, including the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH; European Commission, 2006), the Water Framework Directive (WFD; European Commission, 2000), and conceptual frameworks such as the Driver–Pressure–State–Impact–Response (DPSIR) model (European Environment Agency, 1999), alongside biomonitoring tools such as the Trophic Diatom Index (TDI) (Whitton and Kelly, 1995). While water quality legislation has evolved over recent decades towards more

biologically based assessment approaches, it has been argued that current frameworks still lack the holistic integration required to fully account for multiple interacting stressors (Fischer, Pomati and Eggen, 2013).

Ecotoxicology plays a fundamental role in informing these regulatory processes. Under REACH, ecotoxicological data derived from standardised laboratory studies are used to establish safe concentration thresholds for chemicals (European Commission, 2006). These studies typically follow OECD Test Guidelines, which prioritise standard test organisms such as fish, *Daphnia*, and algae, and rely on endpoints such as EC_{50} and LC_{50} values (European Chemicals Agency, 2023). However, while lower trophic level organisms are often considered more sensitive indicators of toxicity, the prevailing reliance on single-species tests, particularly algal assays, risks oversimplifying responses across diverse primary producer communities.

In addition, current OECD Test Guidelines do not incorporate standardised approaches for assessing multi-stressor interactions, such as those involving temperature and chemical exposure. This lack of methodological standardisation presents a significant limitation for ecotoxicological research and contributes to inconsistencies across studies, making it difficult to compare results or integrate findings into regulatory frameworks.

From an applied perspective, climate change has the potential to further challenge existing assessment frameworks. Many freshwater monitoring approaches, including those underpinning current regulatory systems, are primarily designed to assess responses to organic pollution and often do not explicitly account for climate-driven variables such as temperature. Kazmi et al. (2022) highlight that temperature variation can influence not only chemical toxicity in aquatic organisms but also the derivation of Water Quality Guidelines (WQGs), potentially introducing bias into risk assessments intended to protect freshwater ecosystems.

1.5 Importance of primary producers and current ecotoxicology knowledge

Historically, ecotoxicological research has focused predominantly on higher trophic level organisms, particularly fish, due to their ecological and regulatory relevance. However, increasing evidence suggests that these organisms may not always represent the most sensitive indicators of chemical exposure, owing to their comparatively higher tolerance and position within the food web (Thoré et al., 2021). In contrast, organisms at lower trophic levels, including primary producers and invertebrates, often exhibit greater sensitivity to chemical stressors, making them more suitable for detecting early ecological impacts (Tokatli, 2022). This distinction is particularly important in the context of multi-stressor environments. For example, de Souza et al. (2023) conducted a meta-analysis of 60 studies examining the combined effects of warming and pesticide exposure across freshwater taxa. Their findings demonstrated that microalgae and invertebrates were generally more sensitive than vertebrates, with combined stressor effects often producing greater impacts than when stressors were assessed independently (de Souza et al., 2023). These findings reinforce the importance of lower trophic levels in assessing climate–pollutant interactions.

Primary producers, including diatoms (Bacillariophyceae), green algae, and cyanobacteria, are key components of freshwater ecosystems. As phytoplankton, they play a fundamental role in the global carbon cycle, contributing significantly to carbon fixation (Bonny and Jones, 2007), while also supporting ecosystem services such as nutrient cycling, habitat provision, and energy transfer through aquatic food webs (B-Béres et al., 2023). As the base of the food web, primary producers form the entry point for energy transfer and provide a pathway through which environmental stressors can propagate to higher trophic levels.

Recent mesocosm studies further demonstrate that primary producers play a central role in mediating ecosystem-level responses to multiple stressors. For instance, Cheng et al. (2026) shows that changes in phytoplankton diversity and resource use efficiency

under multi-stressor conditions can influence consumer dynamics and overall food web stability, highlighting the potential for cascading effects across trophic levels.

Crucially, primary producers are directly exposed to contaminants in the water column, providing a clear and immediate link between environmental concentrations and biological response. In contrast, higher trophic level organisms are influenced by multiple exposure pathways, including both direct uptake from the water and indirect exposure through dietary intake and trophic transfer (Sefali et al. 2026). While these processes can lead to bioaccumulation and biomagnification, they also introduce additional complexity when interpreting toxic effects. As a result, primary producers, particularly microalgae, are widely used as model organisms in ecotoxicological studies due to their relative biological simplicity and suitability for controlled experimental conditions (Chen et al. 2026). Their use enables more controlled investigation of contaminant toxicity, particularly in multi-stressor contexts where isolating individual effects is critical.

Temperature further modulates the responses of primary producers. Certain diatom species, including eurythermal taxa, may become dominant under long-term climate change scenarios (Tatters et al., 2013). Elevated temperatures have been shown to enhance metabolic processes and growth rates up to species-specific optima (Montagnes and Franklin, 2001; Sheehan et al., 2020; Taucher et al., 2015). However, these responses are highly context-dependent, and temperature effects are unlikely to act independently of other environmental stressors (De Nicola, 1996; Schindler, 1971; Woodward et al., 2010).

Climate change is also expected to alter the biodiversity and structure of primary producer communities. For example, changes in water retention and stratification may favour smaller-sized phytoplankton species, leading to shifts in community composition and potential reductions in overall biomass (Abonyi et al., 2018; Bopp et al., 2005). Such morphological and compositional changes may ultimately influence ecosystem functioning and the provision of ecosystem services.

Importantly, primary producers already play a central role in biomonitoring frameworks. Benthic diatoms, in particular, are widely used as bioindicators in freshwater assessment. The Trophic Diatom Index (TDI) forms the basis of classification tools such as DARLEQ under the Water Framework Directive (WFD), using diatom communities as proxies for broader ecological status (Taylor et al., 2023). Species such as *Nitzschia palea* are widely distributed and commonly used in ecotoxicological studies, with documented tolerance to both metals and pesticides (Bagmet et al., 2020; Kim Tiam et al., 2018; Arini et al., 2012; Morin et al., 2008; Larras et al., 2012; Moisset et al., 2015). These organisms are used globally in biomonitoring programmes, including in Russia (Barinova et al., 2023), Canada (Lavoie et al., 2014), the USA (Potapova and Charles, 2007), Africa (Mangadze, Dalu and William Froneman, 2019), and India (Alakananda et al., 2011), highlighting their widespread applicability and ecological relevance.

Given their sensitivity, ecological importance, and regulatory relevance, primary producers represent a critical group for assessing the impacts of climate change and chemical pollution. Importantly, changes in their sensitivity under warming conditions may influence the reliability of existing biomonitoring frameworks. Therefore, focusing on primary producers provides a proactive and ecologically relevant approach to environmental risk assessment, complementing assessments based on higher trophic level organisms.

1.6 Knowledge gaps

Despite the widespread use of freshwater primary producers in biomonitoring programmes and ecological risk assessments worldwide, several critical knowledge gaps remain unresolved. First, there is currently no standardised ecotoxicological testing framework specifically designed to assess temperature–contaminant interactions in a controlled and repeatable manner. Although primary producers are routinely tested under OECD guidelines, these protocols are largely limited to single-stressor exposures conducted at fixed temperatures. As a result, outcomes from temperature–toxicity studies are often not directly comparable across experiments, limiting their utility for synthesis, meta-analysis, and regulatory application.

Second, existing multi-stressor studies examining the combined effects of temperature and emerging contaminants frequently suffer from experimental design limitations. Many investigations focus on a single species or incorporate multiple concurrent stressors (e.g., nutrients, competition, or predation) alongside temperature and chemical exposure. While ecologically relevant, such complexity often obscures the isolated role of temperature as a modifier of chemical toxicity, making it difficult to disentangle causal mechanisms or identify consistent patterns across studies.

Finally, substantial gaps persist in the toxicological knowledge base for emerging contaminants, particularly pharmaceuticals and pharmaceutically active compounds. Compared with pesticides and metals, these substances remain underrepresented in ecotoxicological studies involving freshwater primary producers, despite their continuous environmental release and biological activity at low concentrations. Consequently, there is limited understanding of how warming may alter pharmaceutical toxicity at the base of freshwater food webs.

Addressing these gaps requires controlled, standardised experimental approaches that isolate temperature as a key modifying factor while maintaining ecological relevance. By applying a repeatable multi-temperature toxicity framework across multiple primary producer taxa and contaminant classes, this study aims to improve comparability with existing datasets and contribute novel evidence on the temperature-dependent toxicity of emerging contaminants, particularly pharmaceuticals, within freshwater ecosystems.

1.7 Overall aim, hypotheses, and objectives of the thesis

The overarching aim of this thesis is to determine how climate change–induced warming influences the toxicity of emerging contaminants to freshwater primary producers, using three taxonomic groups: diatoms, green algae, and cyanobacteria. Primary producers form the foundation of aquatic food webs, and their sensitivity to combined thermal and contaminant stress has significant implications for ecosystem functioning, water quality, and ecological resilience under future climate scenarios. This study aims to assess the effects of temperature across multiple contaminant categories and evaluate the

feasibility of a repeatable multi-stressor methodology to address the gap in a standardised ecotoxicology test method.

1.7.1 Hypotheses

This thesis tests the following hypotheses:

H₁: Rising temperatures alter the toxicity of emerging contaminants to freshwater primary producers.

H₂: Sensitivity to combined thermal and contaminant stress varies among taxonomic groups of primary producers, with differential responses expected between diatoms, green algae, and cyanobacteria.

1.7.2 Specific objectives

To address these hypotheses, the thesis pursues four specific objectives:

Literature Synthesis: Critically review multi-stressor ecotoxicology studies using climate-driven warming and emerging contaminants to assess current understanding of effects and evaluate the methodologies employed.

Experimental Assessment: Conduct controlled laboratory experiments exposing representative taxa of diatoms, green algae, and cyanobacteria to selected emerging contaminants under simulated warming conditions in a standardised, repeatable experiment setup.

Quantitative Toxicity Responses: Assess primary producer responses (growth rate) by modelling dose–response curves to each contaminant.

Determine whether the toxicity of different contaminants is influenced by warming and varies among primary producer species.

Potential Ecological Implications: Evaluate the potential consequences of these interactions for ecosystem functioning, water quality, and environmental risk assessment under future climate change scenarios.

Chapter 2. Identifying knowledge gaps in temperature–multi-stressor freshwater ecotoxicology

2.1 Introduction

The interactive effects of climate change and chemical pollution on freshwater ecosystems have become a key focus in ecotoxicology, with recent studies increasingly recognising the multi-stressor nature of environmental change (Noyes et al., 2009). Multiple stressors, including climate-induced warming, altered hydrology, and chemical mixtures of pesticides, pharmaceuticals, and other emerging contaminants, frequently co-occur, producing ecological effects that are non-linear and often unpredictable.

While several meta-analyses and review studies have attempted to synthesise evidence on multi-stressor interactions, progress in modelling and predicting outcomes has been hindered by substantial methodological variability across studies (e.g., differences in experimental designs, exposure regimes, species selection, and response metrics). This lack of standardisation complicates efforts to compare findings, identify clear patterns, and establish repeatable approaches for assessing interactive effects (discussed in 1.6).

Moreover, there remains a critical academic call for more controlled, repeatable experiments investigating the isolated effects of climate drivers, such as warming

and its effects on contaminant toxicity, before scaling up to multi-stressor scenarios (see Section 1.6). Such studies are essential for disentangling the mechanistic pathways through which climate change influences contaminant fate, bioavailability, and ecological impact. This review aims to evaluate how temperature influences the toxicity of emerging contaminants and to assess the methodological approaches used in existing studies.

2.1.1 Aim of the chapter

This chapter aims to:

- Assess how temperature influences the toxicity of chemical stressors in freshwater systems, and whether these responses are pollutant-specific or generalisable across chemical classes.
- Critically evaluate experimental designs used in multi-stressor studies, with a focus on how methodological choices influence observed temperature–contaminant interactions.
- Identify key methodological limitations and gaps within the current literature, and propose recommendations to improve the standardisation and comparability of multi-stressor ecotoxicological studies.

2.2 Methods

The systematic review was conducted in accordance with the principles outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Page et al., 2021). Literature searches were conducted on 1 October 2024. Relevant literature was identified from two major scientific literature databases, the Web of Science (search field = topic) and Scopus (search field = article title, abstract, keywords), using the following Boolean search operators: ("climate warming*" OR "temperature increase*" OR warming*) AND (multiple* OR combined*) AND (stressor* OR pollutant*) AND (freshwater* OR river* OR lake*) where * denotes a wildcard, which is used to represent any group of characters.

Temperature-only studies were excluded as they do not provide evidence on how warming influences toxicological endpoints in the presence of chemical contaminants. The focus of this thesis is explicitly on climate-relevant warming as a modifier of contaminant toxicity, rather than temperature as an isolated stressor.

2.3 Selection of studies

A total of 605 journal articles were identified in the initial database search (Web of Science: 479, Scopus: 126). Duplicate articles ($n = 103$) were subsequently removed. All screening was conducted using Excel, with the following binary primary inclusion criteria being applied to the titles and abstracts of the remaining articles ($n = 502$):

- studied freshwater organism(s)
- studied the freshwater organism(s) to one or more pollutant(s) (not including only biotic stressors, e.g., parasitism, algal blooms, invasion) with temperature increases, in a field or controlled environment (where controlled environments were mesocosms or similar);
- presented empirical data (i.e. not solely from a model);
- not a review paper

To maximise coverage, papers with any ambiguities based on the primary screening criteria were included for full text screening for clarification. Following the primary screening, 185 journal articles were selected for full text screening. The following secondary screening criteria were applied before subsequent data extraction:

- data available for species studied, pollutant type, exposure methods and subsequent concentrations, growth conditions, analytical methodology.
- numerical data for exposure concentrations of pollutants presented in a graph, table, or stated in the text.

- was an ecotoxicological study (no paleolimnological studies or models)
- invasive species were not included
- sufficient detail to fully interpret data.

Following secondary screening, the experimental methodologies, and resultant data from 96 journal articles were systematically extracted into a Excel spreadsheet. The exclusion reasons and frequency count for the studies rejected during the secondary screening stage (n = 89) are given in the Supporting information, figure S1. Any discrepancies or uncertainties encountered during the article selection process were resolved through discussion with a second reviewer.

2.4 Data extraction

The data extracted from each article are summarised in Table 2. A quantitative meta-analysis was not conducted due to the heterogeneity in endpoints, exposure durations, and temperature regimes across studies, as well as the limited timescale available for this study. However, the review provided valuable insights into the current state of research in this area to address the aims of this chapter. The effect of temperature on pollutant toxicity was determined by assessing whether interactions were synergistic, additive, antagonistic or mixed effect was observed on the biomarker/s studied. Biomarkers were categorised into level of observation from genes up to community level. The determination of whether study outcomes was synergistic, antagonistic or additive was based on Büch et al. (2021) model for defining ecological synergies and other interaction between multiple stressors (see figure 2). From the extracted data, a key focus of this study was to assess whether community-level relationships influence the reported experimental outcomes. Attention was given to identifying whether specific aspects of experimental design contributed to positive or negative outcomes, and how these factors influenced the interpretation of results across studies.

Table 2: Stored data of the articles that passed the primary and secondary screening criteria

Data extracted for all articles	
Article identification details (title, authors, publication year,journal, DOI)	
Type(s) of organism studied	
Pollutant(s) Studied	
Type(s) of biomarker studied	
Types of effect Temperature had on the study (synergistic, addititve, antagonisitc)	

2.5 Results

The review looked at 96 journals published between 2006 and 2024 and includes studies that looked at a range of trophic levels and variety of biomarker scales from genes to community assemblage shifts. Almost half (48%) of the studies observing the combined stressor interaction of warming and pollutants looked at pesticides (insecticide 21%, herbicide 17%, fungicide 10%). Nutrient enrichment through increase Nitrogen and phosphorus was also significantly studied (26%), often used for simulation of eutrophication. Studies looked at species across trophic levels with 23% of studies studying fish species (n = 22). Phytoplankton and zooplankton were however both frequently studied (n = 20) often together (n = 9). The frequency of pollutant types and types of organism studied are displayed in figure 3.

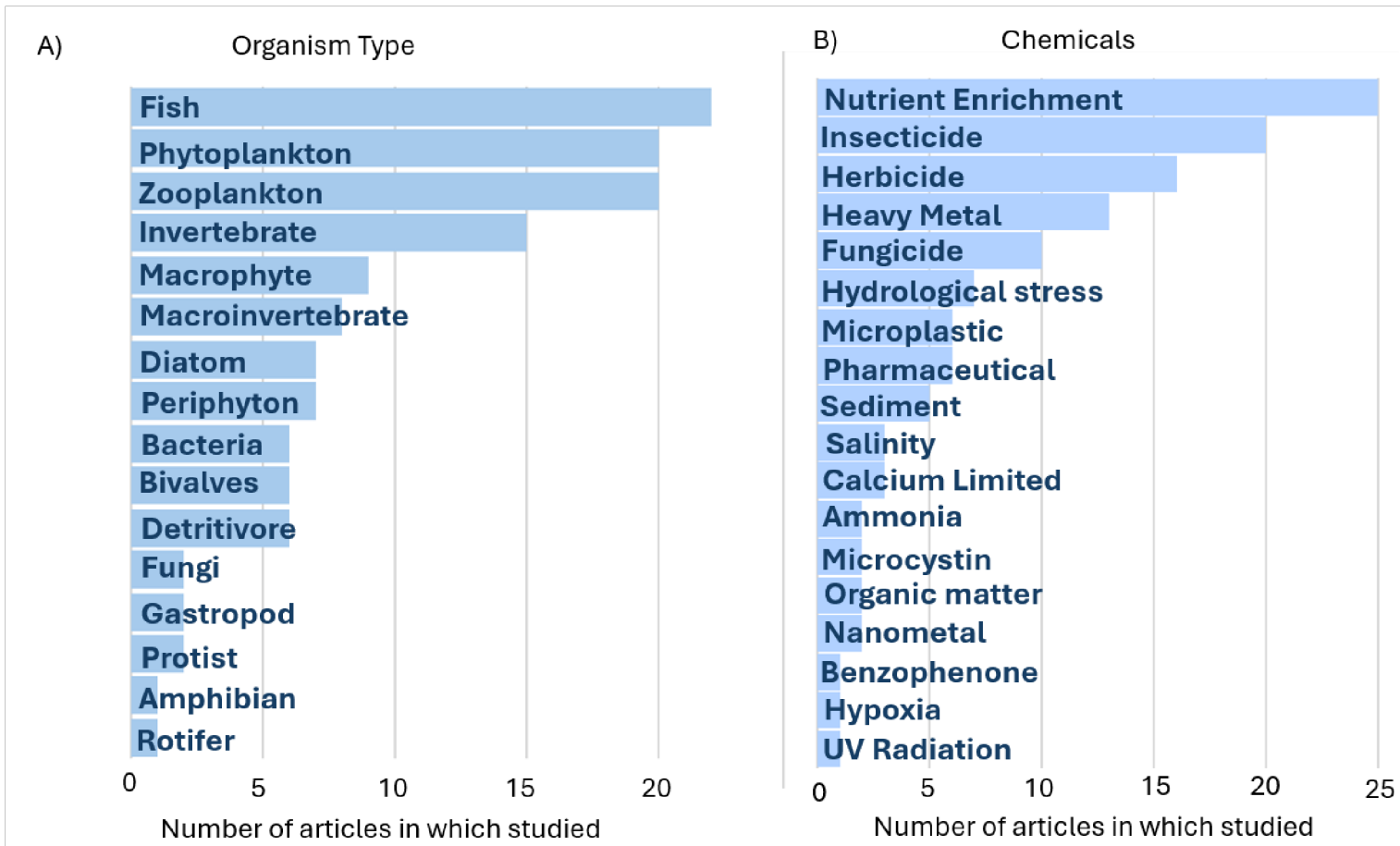


Figure 3. Frequency of (A) organism groups and (B) additional stressors investigated in studies assessing interactions between temperature and co-occurring stressors.

2.6 Trends between experiment design and results observed

The experimental design of studies was compared with the conclusions and observations reported in each study. Overall, 56% of study experimental designs included two or more organism types ($n = 53$). When comparing results between single-species and multi-species studies, single-species studies were significantly more likely to report synergistic interactions between temperature and chemical toxicity (i.e., combined stressors produced a greater negative effect than expected based on individual effects) ($F = 7.771$, $p = 0.006$). Studies looking at multiple organisms found 4.1 times more mixed interactions ($n = 22$) and antagonistic interactions ($n = 11$). A summary of these trends are displayed in Figure 4.

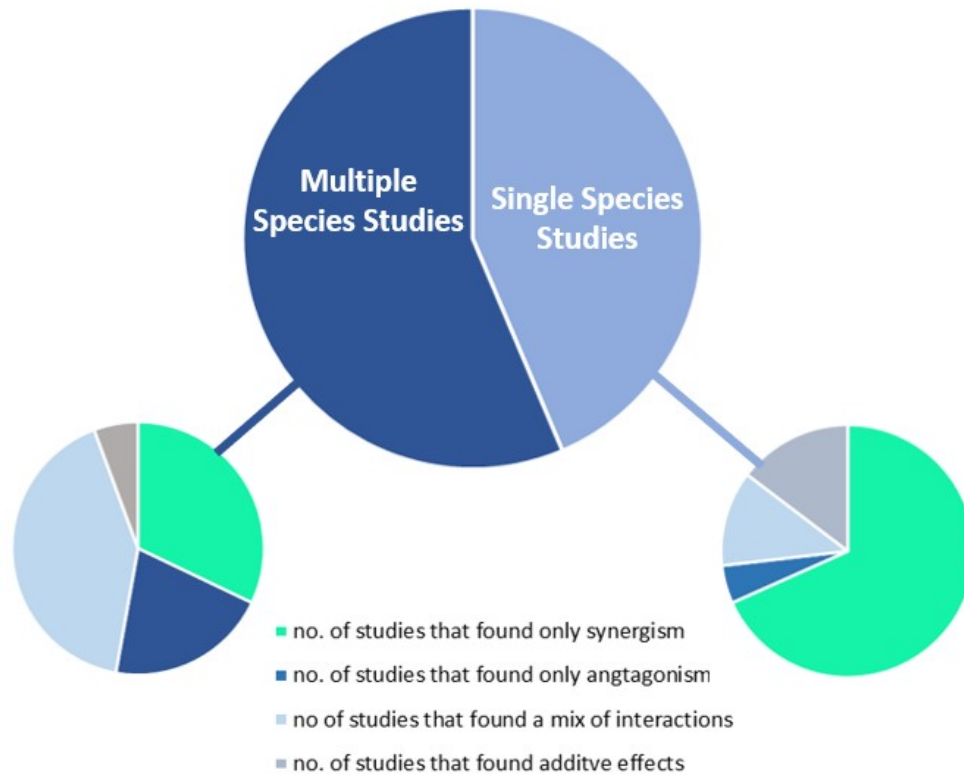


Figure 4. Distribution of interaction outcomes in studies comparing single-species and multi-species experimental designs.

Multiple organism studies reporting antagonistic interactions considered shifts in community composition to more tolerant species acted as a ‘buffer’ in the results to the stressors (Blake and Duffy, 2010; Lambert et al., 2016; Morin et al., 2017). The high number of multiple species studies that found mixed effects of temperature on stressors interactions was often trophic level dependent, with biotic factors such as “interspecific competition” and “community assemblage” responses having the ability to mask true effects of chemical toxicity.

2.7 Influence of nutrient enrichment on stressor interactions

Although nutrient enrichment can and has been used to simulate the stressor of eutrophication, nutrient enrichment was the most frequent stressor type to be coupled with temperature (Figure 4). Nutrient supply (N+P) affected the way stressors interacted on the studies examining both factors together ($n = 15$). Nutrient enrichment showed no consistent trends, with some studies reporting buffering effects due to increased

biomass and diversity, while others reported no effect or even synergistic responses (López Moreira Mazacotte et al., 2023; Piggott, Townsend and Matthaei, 2015; Schulhof et al., 2019; Yuan et al., 2024) however, others discuss there was no clear effect or even synergistic effect of the presence of increased concentrations of N +P. Xie et al. (2024) observed lower biomass of macrophytes from nutrient loading treatments but overall concluded antagonisms are more common than synergies among multiple stressors (Xie et al., 2024). This highlights a key challenge with attempting to simplify multi-stressor community species studies.

Stressors often interact with each other as discussed in these papers which in turn complicates the isolation of individual stressor effects making final interpretation difficult particularly when modelling freshwater ecotoxicology (Oginah et al., 2023). It is also important to note that this review found nutrient enrichment to be the most studied with warming, however this is expected due to the environmental concern of eutrophication being long standing compared to the other emerging contaminants.

2.7.1 Pesticide interaction with warming temperatures

The review identified pesticides and warming temperatures to be one of the most significant areas of multi-stressor studies (n = 32). Herbicides (n = 16) were the most studied category of pesticide with 60% of the studies concluding temperature-herbicide effect to be synergistic in nature. Vijayaraj et al. (2022) used a mesocosm experiment to study multiple-stressor exposure of aquatic food webs, they discussed the synergistic relationship being linked to the community interactions through warming temperatures promoting increased bacteria growth causing competition for resources, providing an additional stress in addition to the toxicity of the pesticide contributing to the observed synergistic response (Vijayaraj et al., 2022).

Fiori and Pistocchi, (2014) discussed the synergistic link of the two stressors could be due to lower temperatures producing low division rates, combined with the high nutrient uptake rates at (15°C), allowing the phytoplankton to gradually adapt its photosynthetic apparatus to the PS II inhibition caused by the herbicide exposure (Fiori and Pistocchi,

2014). A recent mesocosm study that attempted to disentangle the combined effects of agricultural pesticide runoff and climate warming illustrates several methodological constraints inherent to this approach. The authors were unable to confirm one of their central hypotheses, that warming would promote phytoplankton dominance, because the system did not produce a sufficiently clear ecological signal (J.Allen et al. 2021). This ambiguity highlights a broader limitation of mesocosm experiments. This is because interacting stressors often generate mixed or variable responses across trophic levels, making it difficult to attribute observed patterns to specific drivers with confidence.

The methodological challenges are not limited to interpreting community responses. The review found mesocosms may also restrict the ability to capture temporal dynamics, particularly the sequence and timing of stressor introduction, which are known to influence ecological trajectories. When stressors such as pesticides and warming occur simultaneously in nature but must be operationalised in controlled experimental phases, important temporal interactions may be lost or altered.

Moreover, logistical constraints related to sampling and laboratory processing can introduce additional sources of uncertainty. For example, in a recent study examining trophic interactions under pesticide exposure, zoobenthos collected for stable isotope analysis were held alive in laboratory conditions for up to 12 hours prior to processing (Xie et al., 2024b). Such delays may influence physiological status or isotopic signatures, thereby confounding the interpretation of trophic relationships. Collectively, these issues demonstrate that while mesocosms offer valuable ecological realism, their methodological limitations can significantly affect the strength and reliability of inferences in multi-stressor ecotoxicology.

2.7.2 Pharmaceuticals interaction with warming temperatures

Across the six studies that investigated the combined effects of warming and pharmaceutical or pharmaceutically active compounds ($n = 6$), approximately half reported clear synergistic interactions, where warming amplified the toxicological effects of the chemical stressor. Both mesocosm experiments and controlled OECD assays demonstrated that elevated temperatures intensified pharmaceutical toxicity.

For example, Barbosa et al. (2017) showed that exposure to fluoxetine under variable temperature regimes resulted in a 37% reduction in reproductive success and a 17.9% decline in population growth rate, alongside significant changes in physiological biomarkers such as total lipid content and lipid profile variation in the aquatic insect *Micropterna nycteribiid* (Barbosa et al., 2017).

Studies that did not detect synergistic responses often attributed this to taxon-specific sensitivities or the inherent complexity of ecological responses such as predator–prey interactions, which can obscure simple stressor relationships (Ward et al., 2020). In these cases, the absence of synergy does not necessarily indicate a lack of interaction but may instead reflect compensatory biotic processes or species-specific thermal tolerances. Conversely, studies reporting synergistic outcomes frequently interpreted these effects as the result of temperature-induced increases in organismal growth, development rates, and metabolic maintenance costs, which heighten susceptibility to the direct toxic effects of pharmaceutical mixtures (Kokotović et al., 2024). Collectively, these findings support the difficulty in establishing generalisable conclusions across taxa and chemical classes, highlighting the need for more standardised and mechanistically informed experimental designs.

2.7.3 Critical review of experimental designs and protocols

The full-text screening highlighted substantial variability in the experimental methodologies employed in multi-stressor ecotoxicology studies. A key challenge identified across the literature is the trade-off between environmental realism and experimental control. While mesocosm studies aim to replicate natural conditions, this often comes at the cost of increased variability and reduced interpretability of results. Several studies demonstrated that temperature regimes used to simulate climate change are not always ecologically realistic or consistently justified. For example, one mesocosm study applied a rapid heatwave scenario ($+0.18\text{ }^{\circ}\text{C h}^{-1}$ from 18 to 26 °C), with justification based partly on site-specific observations rather than standardised climate projections (Hermann, Peeters and Van den Brink, 2023). Such approaches may confound the distinction between acute thermal stress (e.g. heatwaves) and gradual

climate warming, making it difficult to attribute observed synergistic effects specifically to long-term climate change processes.

Similarly, laboratory-based studies often introduce temperature changes during organism transfer between conditions (e.g. $\pm 1\text{ }^{\circ}\text{C h}^{-1}$), which may induce short-term thermal stress responses rather than stable acclimatisation to new temperature regimes (Kratina et al. 2019). Although final exposure temperatures may align with projected climate scenarios, the transition phase itself may influence experimental outcomes.

Another limitation identified is the issue of replication and system variability in mesocosm experiments. For example, factorial mesocosm designs with limited replication may be affected by “aquarium individuality”, where initially identical systems diverge due to stochastic processes, reducing statistical power and confidence in observed treatment effects (Sanderson 2002; Kokotović et al. 2024). This variability complicates the detection of consistent stressor interactions and may contribute to the high frequency of mixed or context-dependent results reported in the literature.

Methodological constraints related to organism handling and sample processing were also identified as potential sources of bias. For instance, studies involving field collection and laboratory transport of organisms (e.g. chironomid larvae) introduce additional stressors, such as handling and environmental change, which may influence behavioural or physiological responses prior to experimental exposure (Lencioni, Di Nica and Villa, 2021). While such approaches improve ecological relevance, they may reduce experimental control and introduce confounding effects.

Finally, studies incorporating community-level responses often reported buffering effects due to increased biodiversity, where tolerant species compensate for sensitive taxa. While this reflects ecological realism, it can mask the direct effects of individual stressors, making it difficult to isolate temperature–contaminant interactions (Xie et al. 2024). Additionally, variations in sampling methods, such as periphyton collection and homogenisation, further reduce methodological consistency across studies.

Overall, these findings demonstrate that methodological variability remains a major limitation in multi-stressor ecotoxicology. Inconsistent temperature regimes, differences in acclimatisation protocols, limited replication, and variability in sampling approaches all contribute to reduced comparability across studies. This highlights the need for more standardised experimental frameworks to improve the reliability and interpretability of findings in this field.

A further limitation within current ecotoxicological frameworks is the absence of standardised test guidelines for assessing multi-stressor interactions involving climate variables. While the Organisation for Economic Co-operation and Development (OECD) provides well-established protocols for single-stressor toxicity testing (e.g. OECD Test Guidelines 201, 202, and 203) (OECD, 2004, 2006; 2019), these standardised methods are typically conducted under fixed environmental conditions and do not explicitly incorporate temperature variation or combined stressor scenarios. As a result, studies investigating temperature–contaminant interactions often rely on bespoke experimental designs, contributing to the methodological variability identified throughout this review.

Consequently, the lack of standardisation limits the comparability and reproducibility of findings and presents a barrier to integrating multi-stressor effects into regulatory risk assessment frameworks. Therefore, there is a clear need for the development of standardised experimental approaches that incorporate climate-relevant variables. The experimental design adopted in this thesis contributes to addressing this gap by evaluating the feasibility of controlled, repeatable methods for assessing temperature–contaminant interactions in freshwater primary producers.

2.8 Summary and recommendations

The findings of this review demonstrate that experimental outcomes are highly context-dependent and influenced by methodological design. The effects of combined stressors were not homogeneous across communities; instead, outcomes were strongly dependent on the specific biomarkers or ecological endpoints measured. Studies that

exposed multiple species within the same treatment frequently reported mixed or ambiguous interactions, often attributing this to community-level processes, such as compensatory dynamics, interspecific competition, or shifts in species composition that can mask or dilute the true effects of individual stressors.

A recurring challenge across the literature is the lack of clear, generalisable conclusions. Many studies employ heterogeneous terminology and report context-dependent outcomes, which complicates synthesis and reduces the utility of these findings for broader ecological modelling or management applications. Although laboratory-based single-species experiments benefit from high levels of control, they typically test only one pollutant type and therefore yield pollutant-specific responses that are difficult to compare across chemical classes. Conversely, mesocosm experiments offer greater ecological realism but often suffer from limited statistical power, restricted replication, and insufficient environmental gradients, reducing confidence in the magnitude and direction of observed effects (Chapman, 2002).

Furthermore, studies attempting to mirror natural conditions face additional methodological uncertainties arising from sample handling, including transportation delays, storage conditions, and laboratory preparation, all of which can inadvertently alter physiological responses or biochemical markers. Collectively, these issues highlight a pressing need for standardised, replicable experimental frameworks that incorporate multiple contaminant categories and clearly defined temperature treatments. Such an approach would improve comparability across studies and help determine whether multi-stressor responses reflect general ecological principles or remain tightly linked to the toxicological properties of individual pollutants. These findings informed the methodological design of this study.

Based on these findings, several key recommendations can be made for improving experimental design in multi-stressor ecotoxicology. First, studies should aim to minimise confounding variables by using controlled experimental systems that isolate the effects of temperature and chemical exposure, rather than relying solely on

community-level responses where interactions may obscure underlying mechanisms. Second, temperature treatments should be applied in a consistent and ecologically relevant manner, with appropriate acclimatisation periods to avoid introducing unintended thermal shock effects. Third, where possible, experiments should prioritise real-time or minimally invasive measurement techniques to reduce artefacts associated with organism handling and sample preparation.

Furthermore, experimental designs should aim to improve comparability across studies by adopting standardised protocols, including consistent exposure regimes, clearly defined temperature gradients, and comparable endpoints. The use of factorial designs across multiple contaminant classes may also enhance the generalisability of findings and improve statistical robustness. These findings informed the methodological design of the experimental work presented in this thesis.

Chapter 3. Materials and methods

3.1 Introduction

This chapter presents a controlled laboratory experiment designed to isolate the effects of moderate warming on contaminant toxicity. The systematic review highlighted key limitations in existing multi-stressor studies, including inconsistent temperature regimes, variability in experimental designs, and a lack of standardised methodologies for assessing temperature–contaminant interactions.

To address these limitations, the experimental design adopted in this study incorporates several methodological improvements. First, temperature treatments were applied in a controlled and standardised manner, with defined exposure conditions and acclimatisation periods to minimise thermal shock effects identified in previous studies. Second, a single-species exposure approach was used to reduce confounding effects associated with community-level interactions, enabling clearer interpretation of toxicological responses. Third, a factorial design was implemented across multiple contaminant classes, allowing for direct comparison of responses under consistent

experimental conditions. Collectively, these approaches aim to improve the reproducibility and comparability of multi-stressor ecotoxicological experiments.

A key consideration in the experimental design was the trade-off between experimental breadth and replication. Chapter 2 identified that many existing studies focused on single contaminant classes or individual test species, limiting the ability to compare responses across different chemical classes and reducing the generalisability of findings. In contrast, relatively few studies have applied a consistent framework across multiple contaminant types within the same study.

To address this gap, the present study prioritised the inclusion of multiple contaminant classes (pharmaceuticals, pesticides, and metals) and representative primary producer taxa within a unified experimental design. This approach enables direct comparison of temperature–contaminant interactions across different modes of action, which would not be possible if each contaminant class were investigated in isolation. Furthermore, the inclusion of well-characterised metal contaminants provided a reference baseline against which responses to emerging contaminants could be interpreted.

However, this broader experimental scope necessarily constrained the level of replication achievable within the available time and resources of this project. As such, this study is positioned as an exploratory and methodological investigation, aimed at evaluating the feasibility and comparability of a standardised multi-contaminant framework rather than providing highly replicated toxicity estimates for individual compounds.

Primary producer species were selected as model organisms due to their ecological relevance and established use in ecotoxicological testing. Periphyton communities are widely recognised as robust indicators of environmental state and are frequently used in freshwater assessment and management (De Nicola and Kelly, 2014; La Hée and Gaiser, 2012; McCormick and Stevenson, 1998). In addition, their short generation times enable observable responses within practical experimental timeframes.

The study focused on three representative freshwater primary producers: the green alga *Pseudokirchneriella subcapitata* (Korshikov) Nygaard, Komárek, Kristiansen and Skulberg 1987, the diatom *Nitzschia palea* (Kützinger) Smith 1856, and the cyanobacterium *Anabaena flos-aquae* Brébisson ex Bornet and Flahault 1886 (Figure 5). These taxa represent key functional groups within freshwater ecosystems and are commonly used in ecotoxicological research.

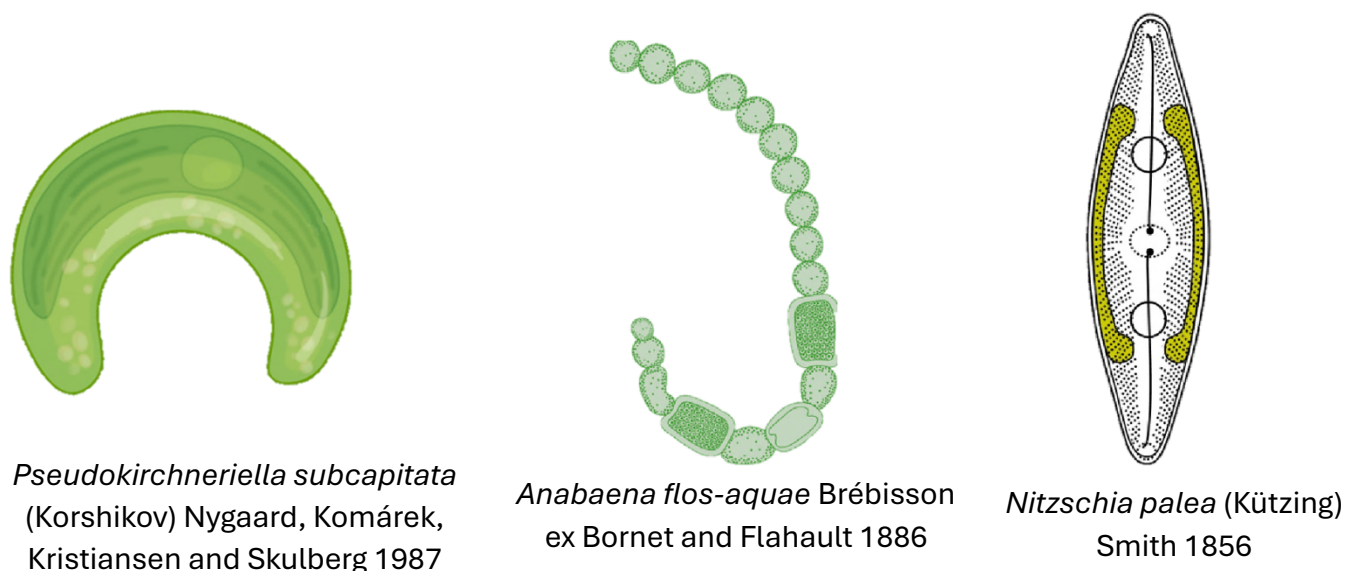
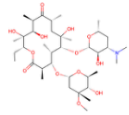
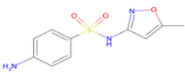
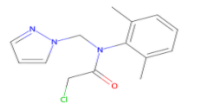
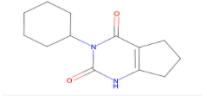


Figure 5. Freshwater primary producer species used in this study.

Two chemicals were selected from each of three contaminant categories: pharmaceuticals, pesticides, and heavy metals. Categories were selected for the study due to their known persistence in freshwater and chemical toxicity established from our systematic literature review and the academic literature (Kumar et al., 2023; Malaj et al., 2014; Wilkinson et al., 2022). These were copper chloride, zinc sulphate, erythromycin, sulfamethoxazole, metazachlor and lenacil (Table 3)

Table 3, Physical characteristics from ¹PubChem (Kim et al., 2025) and effective concentrations of selected test contaminants obtained from the ²EPA’s ECOTOX database (Environmental Protection Agency, 2025), alongside corresponding environmental concentrations extracted from the ³Waterbase dataset (European Environment Agency, 2025).

	Compound	CAS-No.	Structure ¹	Molecular Weight (g/mol) ¹	Log Kow ¹	PKa ¹	Solubility in water (mg/L ⁻¹) ¹	Median EC ₅₀ values for primary producers (m/ L ⁻¹) ²			Concentration in Europe Surface water bodies (mg/L ⁻¹) ³	
								Green Algae	Diatom	Cyanobacteria	Range	Median
Metal	copper chloride	1344-67-8		134.5	N/A	N/A	7.06 × 10 ⁵	0.605	0.615	No Data	0 – 2.77 × 10 ⁻¹	2.67 × 10 ⁻³
	zinc sulphate	7733-02-0		161.4	N/A	N/A	5.45 × 10 ⁵	0.221	0.710	0.0101	0 – 1.55 × 10 ⁻¹	1.19 × 10 ⁻²
Pharmaceutical	erythromycin	7704-67-8		733.9	3.06	7.0	2.00 × 10 ³	0.140	No Data	0.160	1.00 × 10 ⁻⁵ – 1.15 × 10 ⁻⁴	1.00 × 10 ⁻⁵
	sulfamethoxazole	723-46-6		253.3	0.89	1.6	>3.80 × 10 ¹	1.54	No Data	0.550	6.00 × 10 ⁻⁶ – 4.66 × 10 ⁻⁴	2.49 × 10 ⁻⁵
Pesticide	metazachlor	67129-08-2		277.8	0.30	1.5	4.30 × 10 ²	0.044	No Data	No Data	3.00 × 10 ⁻⁷ – 2.21 × 10 ⁻³	1.87 × 10 ⁻⁵
	lenacil	2164-081		234.3	2.31 (pKow)	10.3	6.00	0.012	No Data	No Data	5.00 × 10 ⁻⁶ – 1.00 × 10 ⁻⁴	5.00 × 10 ⁻⁶

3.2 Objectives and aims of the study

Aim

To investigate how temperature influences the toxicity of selected emerging contaminants on freshwater primary producers, with a focus on comparing responses across contaminant classes and taxonomic groups.

Objectives

- To determine the effect of temperature on the toxicity of selected contaminants, including emerging contaminants (pharmaceuticals and pesticides) and reference contaminants (heavy metals), to freshwater primary producers.
- To compare the responses of different primary producer taxa (green algae, diatoms, and cyanobacteria) to combined temperature and contaminant exposure.
- To isolate species-specific toxicological responses by conducting single-species exposures under controlled conditions.
- To generate empirical data to inform ecotoxicological risk assessment frameworks under climate warming scenarios.

3.3 Methods

Three freshwater primary producer species (see Section 3.1), representing each organism group, were used in accordance with the recommended test species outlined in OECD Test Guideline 201 (OECD, 2006). Stock cultures of the primary producers were obtained from the Culture Collection of Algae and Protozoa (CCAP) who had previously collected the species from Cassop Pond, Tynn and Wear (Culture Collection of Algae and Protozoa, 2021). Once obtained, cultures were maintained on a freshwater growth medium based on Beakes et al., (1988) medium and blue green algae were maintained on freshwater growth medium described by Allen and Stanier, (1968). The medium was produced from 1 mL stocks (see supplementary materials figure 3 for stocks) that were mixed and diluted with one litre of sterilised ultrapure water (Milli-Q) and subsequently autoclaved. The pH of the medium was adjusted to 6.9 and monitored using a pH meter

(Accumet AB150). These primary cultures were cultured in tissue culture flasks (Greiner 50 mL T-25 flask, canted neck) in a controlled environment at 20 °C control room with the light intensity 5,320 lux.

3.4 Emerging contaminant selection

This study examined the influence of temperature on contaminant toxicity using six representative compounds spanning three major classes of persistent freshwater pollutants: pesticides (metazachlor and lenacil), pharmaceuticals (sulfamethoxazole and erythromycin), and heavy metals (copper chloride and zinc sulphate). These contaminant groups were selected because they are routinely detected in environmental monitoring programmes (European Environment Agency, 2025) and have been identified as priority or high-risk substances in freshwater environmental risk assessments (Kumar et al., 2023; Malaj et al., 2014; Wilkinson et al., 2022). The literature review further highlighted that pesticides, pharmaceuticals, and metals are among the most prevalent and persistent contaminant types in aquatic ecosystems, with documented effects on primary producers. PFAS and microplastics were not included due to additional analytical and validation constraints.

Testing Concentrations of these were selected from the Environmental Protection Agency's ECOTOX Knowledgebase with the stated species included (Environmental Protection Agency, 2025). Searches on the Knowledgebase included the chemical name, genera of the three primary producer testing species. The test condition (freshwater) was always included in the search. Data from the endpoints (ID50, IC50, EL50, EL50EC50, LD50, LC50, LL50) were extracted from the knowledge base and the average values were calculated for each compound for each species (references can be found in supplementary materials table). Where sufficient studies were available to be analysed for the compound, final testing concentrations for each species was logarithmically selected around the EC₅₀ (centralised in the range) to help produce an accurate dose response curve. Where insufficient studies were available, concentrations were selected with a standardised range covering a range from $\mu\text{g L}^{-1}$ up to mg L^{-1} while considering the solubility limits of each compound. Testing concentrations were in accordance with the concentration requirements outlined in OECD Test Guideline 201

Freshwater Alga and Cyanobacteria, Growth Inhibition Test (OECD, 2006). See table 4 for final concentrations.

Although six contaminants were originally selected for experimental exposure, analytical and resource constraints during the experimental period meant that Lenacil could not be included in the final toxicity analysis. All subsequent results and dose–response calculations therefore reflect five chemicals: copper, zinc, erythromycin, sulfamethoxazole, and metazachlor. This decision was made prior to data analysis and was unrelated to observed biological responses.

Table 4. Final testing concentrations for each chemical studied

Chemical	Control	1	2	3	4	5	6	7	Units	Source
metazachlor	0	0.200	0.342	0.585	1.000	1.710	2.924	5.000	mg L ⁻¹	Standardised range
erythromycin	0	0.010	0.022	0.046	0.100	0.215	0.464	1.000	mg L ⁻¹	Standardised range
sulfamethoxazole	0	0.200	0.330	0.543	0.894	1.474	2.428	4.000	mg L ⁻¹	Standardised range
zinc sulphate	0	0.023	0.069	0.116	0.231	0.463	0.694	1.157	mg L ⁻¹	EPA ECOTOX knowledgebase
copper chloride	0	0.043	0.128	0.213	0.427	0.853	1.280	2.133	mg L ⁻¹	EPA ECOTOX knowledgebase

3.5 Test sample preparation

Subcultures of each species were transferred into each temperature condition with fresh medium at a 1:3 dilution. After 72 hours, samples were taken of each culture and the chlorophyll NA absorbance measured using a fluorometer (Turner, Trilogy). The cultures were then diluted with diatom media to give a cell count of 10,000 cells/ml with dilutions calculated (OECD, 2006). This step was included to minimise variability in initial biomass across treatments, a limitation identified in previous studies.

Cell cultures were adjusted to the same RFU value across each temperature culture (+/- 10 RFU). 0.5 mL of culture was pipetted into eight glass test tubes (3ml), to meet the target concentrations of the testing contaminants, stock concentrations were diluted with growth medium and added proportionately to achieve testing concentrations using equation 2 ensuring that the added stock solution comprised less than 0.05% of the total volume.

Equation 2.0

$$\text{Testing concentrations} = \left(\frac{T_{1(\text{top dose})}}{S_{(\text{conc})}} \right) \times N_{(\text{conc})}$$

Where;

$S_{(\text{conc})}$ = Stock concentration of stressor

$T_{1(\text{top dose})}$ = Total volume of experiment (including media and stressor)

$N_{(\text{conc})}$ = Nominal Concentrations selected for testing

This was repeated three times for each testing species across the 8 concentrations. Test tubes were sealed with perforated lids to permit gas exchange while preventing microbial contamination; the perforations were small enough to act as a barrier to airborne microorganisms. All transfers were performed under aseptic conditions under laminar flow hoods, this included autoclaving media, test tubes and pipette tips in order to minimise contamination of the samples. To ensure the absence of contamination and

interspecific competition, samples of the test tubes were taken and inspected under microscope to ensure no contamination from other algal species or bacteria.

3.6 Method procedure

The ecotoxicity tests were performed at three temperatures (15, 20, and 25 °C) with 5-degree increments selected due to the 4.34 degree rise predicted by 2100 for annual daily maxima in the Northern Hemisphere (Wanders et al., 2019). Samples were exposed to a 16:8 h light:dark cycle. Light intensity was maintained within the OECD 201 guidelines of 4440 – 8880 lux. After the test tubes had been prepared (see 3.4), a 300 µL sample from each test tube was vortexed (Mini Vortex Mixer 100) and transferred into a 96 well plate (Greiner 96 F-Bottom) and analysed for optical density (OD) (CLARIOstar Plus) every 24 hours up to 96 hours across all temperature treatments. The well plate reading protocol consisted of measuring absorbance at three optic settings (682,720,750) in triplicate then identifying an average with the well plates receiving a double orbital shaking for 30 seconds before plate reading. Blanks consisting of media were first run before taking readings for correction.

3.7 Chemical analysis

Quantitative analysis of metazachlor, erythromycin, and sulfamethoxazole was performed using liquid chromatography coupled with mass spectrometry (LC-MS) following an internally validated university method (Ieuan et al., 2024). Chromatographic separation was achieved using an Agilent InfinityLab LC/MSD system equipped with a reverse-phase C18 analytical column (Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm).

Compounds were detected in positive electrospray ionization (ESI) mode under selected ion monitoring (SIM) conditions. The monitored m/z values were 278.1 for metazachlor, 734.4 for erythromycin, and 254.1 for sulfamethoxazole. The fragmentor voltage was 110 V, and the detector gain was 1.0. The injection volume for all samples was 10 µL.

The mobile phase consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1 % formic acid, operated under a gradient elution from 10 % B to 90 % B over 15 minutes, providing retention times of approximately 7.6 min (sulfamethoxazole), 10.6 min (erythromycin), and 11.8 min (metazachlor).

Each analyte was quantified using external calibration curves prepared at seven concentration levels. Curve models were linear for erythromycin and sulfamethoxazole and quadratic for metazachlor, with forced-origin fitting. Calibration ranges and regression models are listed in Table 5 below.

Table 5 Calibration ranges and curve models for organic target analytes

<i>Compound</i>	<i>Curve model</i>	<i>Calibration levels (mg L⁻¹)</i>	<i>Range (mg L⁻¹)</i>
sulfamethoxazole	Linear (forced origin)	0.0004, 0.004, 0.04, 0.2, 0.4, 2, 4	0.0004 – 4
erythromycin	Linear (forced origin)	0.0001, 0.001, 0.01, 0.05, 0.1, 0.5, 1	0.0001 – 1
metazachlor	Quadratic (forced origin)	0.0004, 0.004, 0.04, 0.2, 0.4, 2, 4	0.0004 – 4

Chromatographic peak integration employed Agilent ChemStation with the standard tangent-skim mode and automatic baseline correction. No chromatogram or spectral smoothing was applied. Blank subtraction was not used, and analyte identity was confirmed based on retention time ($\pm 4\%$) and peak shape. The method used asymmetric mass windows (0.3 mDa low, 0.7 mDa high).

Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to quantify copper (as copper chloride) and zinc (as zinc sulphate) concentrations. Analyses were carried out on a Thermo Fisher Scientific ICP-OES instrument. Calibration standards ranged from 0.0001 $\mu\text{g L}^{-1}$ to 1.0 mg L^{-1} , and calibration curves showed excellent linearity with R^2 values ≥ 0.9999 . Procedural blanks, drift checks, and wash solutions were run alongside samples to ensure data quality and instrument stability throughout the analysis. Degradation of each chemical throughout the experiment was monitored and compared across temperatures to assess temperature-dependent variation and is presented in Figure 8. The key methodological improvements implemented in this study, based on the limitations identified in Chapter 2, are summarised in Table 6

Table 6. Methodological improvements implemented in this study based on limitations identified in Chapter 2.

Limitation identified in literature	Common issue in previous studies	Approach adopted in this study	Improvement achieved
Lack of comparability across chemical classes	Studies often focus on single pollutants or separate experiments	Three contaminant classes (metals, pesticides, pharmaceuticals) tested under a unified experimental framework	Enables direct comparison of responses across chemical classes and modes of action
Limited use of reference compounds	Emerging contaminants often studied without baseline comparison	Inclusion of well-characterised metals (copper chloride, zinc sulphate) alongside emerging contaminants	Provides a benchmark for interpreting responses to less-studied compounds
Lack of standardised experimental conditions	Variability in light, media, and exposure protocols	Adoption of OECD TG 201-aligned conditions (e.g. media composition, light regime)	Improves reproducibility and facilitates comparison with regulatory studies
Inconsistent experimental design	Experimental design limited comparison	Fully factorial design across species, temperature, and contaminant treatments	Enables systematic comparison of temperature–contaminant interactions
Uncertainty in exposure concentrations	Lack of analytical confirmation of contaminant levels	Use of ICP-OES and LC-MS to verify exposure concentrations	Increases confidence in exposure accuracy and experimental reliability
Thermal shock effects	Rapid temperature shifts without acclimatisation	Species acclimated at experimental temperatures for 72 hours prior to exposure	Reduces confounding stress responses and improves ecological relevance
Inconsistent starting biomass	Variable initial cell densities across treatments	Standardised cell counts across species prior to exposure	Improves comparability of growth and toxicity responses
Pseudo replication	Sequential or pooled experimental setups	Independent replication of each species–contaminant combination	Improves statistical robustness and reduces experimental bias
Unrealistic temperature regimes	Temperature changes not aligned with climate projections	Temperature treatments selected based on climate warming scenarios (e.g. Wanders et al., 2019)	Enhances ecological relevance of temperature treatments
Lack of culture validation	Limited assessment of organism health prior to testing	Pre-experimental culture checks to confirm healthy, exponential growth phase	Ensures reliability of biological responses
Inconsistent modelling approaches	Variable or unclear dose–response modelling	Use of four-parameter log-logistic (LL.4) models across all datasets	Provides consistent and comparable toxicity metrics
Limited integration with regulatory metrics	Difficulty comparing results with existing frameworks	Extraction of EC ₅₀ values as standard toxicity endpoints	Enables comparison with regulatory thresholds and meta-analyses

3.8 Data Analysis

Data analysis was conducted in accordance with OECD Test Guideline 201 (OECD, 2006). Optical density readings from the 96-well plate reader were exported to Microsoft Excel in plate format across the three temperatures and 96 hours. Each plate reader dataset was compiled into a master spreadsheet containing measurements recorded at 24-hour intervals over 96 hours across three replicates for each species and temperature treatment. For the three replicates, mean values were calculated and used to generate growth curves of each temperature across nominal concentration. This was to assess whether exponential growth was achieved and identify any anomalies for exclusion during initial data processing.

Optical density (OD) measurements were converted to cell counts for each species using calibration equations derived from preliminary growth studies. These calibration curves were established by a preliminary experiment, culturing each species over a one-week period, during which absorbance at 600 nm was recorded at 24-hour intervals.

At each time point, well-mixed (vortexed) samples were collected and analysed microscopically (Olympus BX51 fluorescence microscope) using a haemocytometer to determine the corresponding cell count. The resulting linear relationships between absorbance and cell count (defined by regression slope and intercept values) were subsequently used to convert optical density readings in experimental samples to estimated cell counts. Preliminary calibration results for each species are shown in Figure 6.

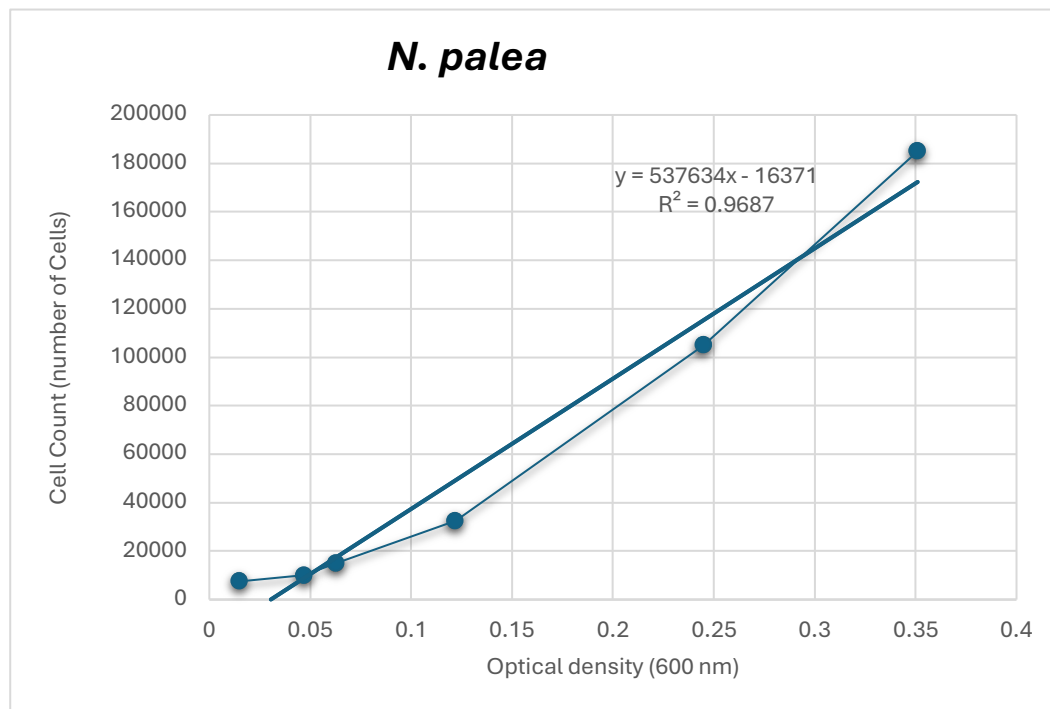
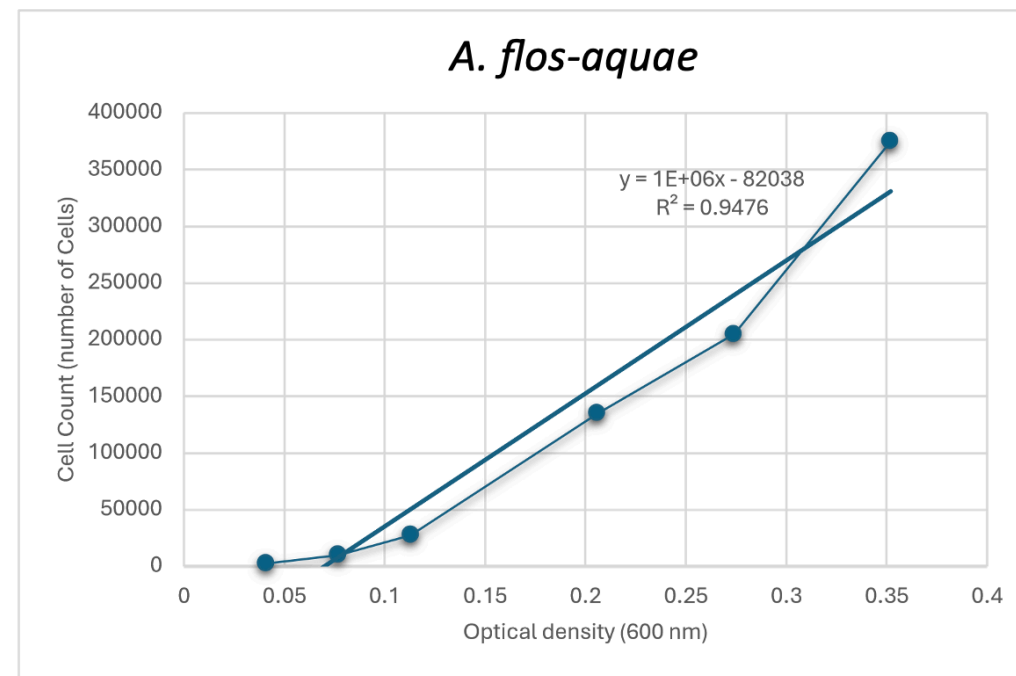
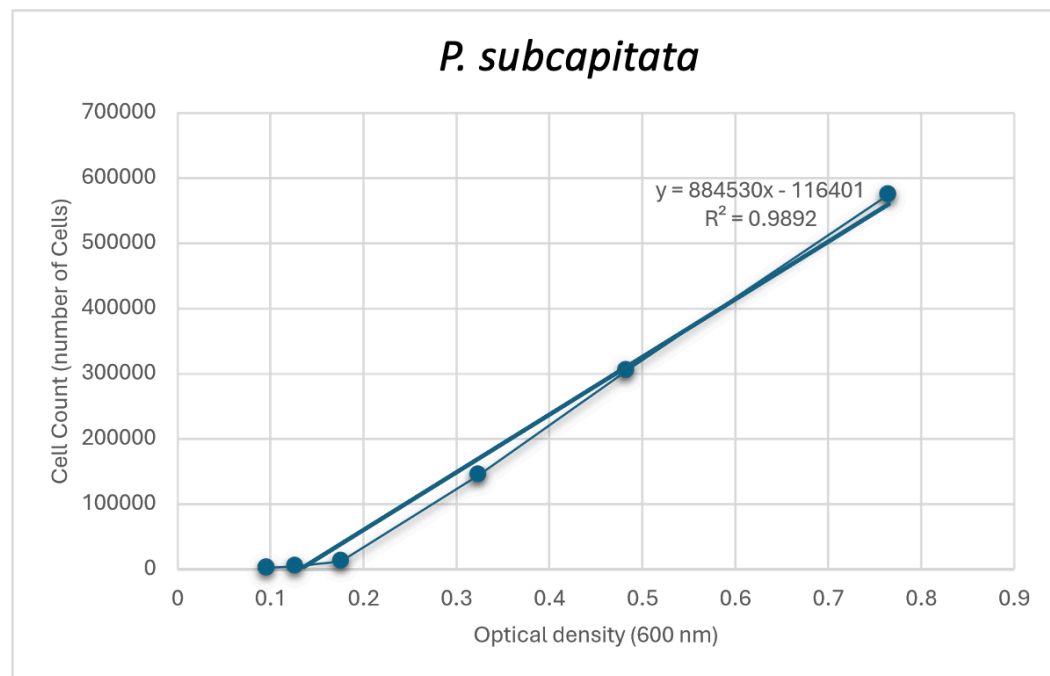


Figure 6. Linear calibration graphs of the preliminary growth experiments to convert optical density to cell count across the three selected species for testing.



Growth rates were calculated for the intervals 0–24, 24–48, 48–72, and 72–96 hours to capture exponential growth across the test period. The mean growth rate across these intervals was taken and used in Equation 3 to produce percentage inhibition.

Equation 3

$$\%I_r = \frac{\mu_C - \mu_T}{\mu_C} \times 100$$

where percent inhibition in average specific growth rate;

μ_C mean value for average specific growth rate (μ) in the control group;

μ_T average specific growth rate for the treatment replicate.

Once percentage inhibition (%I_r) had been calculated from mean values of triplicate replicates for the 6 chemicals across the three species and three temperatures, concentration–response relationships were modelled in accordance with OECD Test Guideline 201 using nonlinear regression. Percent growth inhibition was modelled as a function of measured exposure concentration using four-parameter log-logistic models (LL.4) implemented in the drc package. Prior to model fitting, inhibition values were constrained to the biologically meaningful range of 0– 100%. Control treatments (zero concentration) were excluded from model fitting but retained for graphical presentation.

To ensure comparability across temperature treatments, the same model structure was fitted independently for each temperature. In all models, the lower and upper asymptotes were fixed at 0% and 100% inhibition, respectively, while the slope and median effect concentration (EC₅₀) parameters were estimated from the data. Model fitting was conducted using the drm() function from the drc package in R. Model adequacy was assessed using visual inspection of fitted curves and Akaike’s Information Criterion (AIC).

Effect concentrations (EC_{50}) were derived by inverse prediction from the fitted concentration–response models. Standard errors and confidence intervals were estimated using the delta method. EC_{50} values were reported only where the corresponding effect level was observed within the experimental concentration range. Where inhibition did not reach the target effect level, EC_{50} values were reported as not reached and excluded from inferential statistical analyses but retained for descriptive interpretation. EC_{50} was selected as the primary comparative endpoint due to more robust model convergence across temperature treatments.

Nominal exposure concentrations were replaced with analytically verified concentrations obtained from chemical analysis (Section 3.6), ensuring that effect estimates reflected actual exposure conditions. All statistical analyses and visualisations were conducted in R (Posit team, 2025).

3.9 Statistical analysis for temperatures effects

Temperature effects on specific growth rate (calculated in accordance with OECD Test Guideline 201) were analysed separately for each species using a one-way Analysis of Variance (ANOVA), with temperature (15, 20, 25 °C) as a fixed factor. Prior to analysis, the assumptions of ANOVA were assessed using the Shapiro–Wilk test for normality and Levene’s test for homogeneity of variances.

Growth rate data met assumptions for *A. flos-aquae* and *P. subcapitata*. For *Nitzschia palea*, residuals deviated from normality; however, given the small temperature effects and equal variance structure, ANOVA results were considered robust for interpretation.

When the overall ANOVA indicated differences among temperatures, Tukey’s Honest Significant Difference (HSD) test was used for pairwise comparisons between 15–20 °C, 20–25 °C, and 15–25 °C.

3.9.1 Chemical-specific statistical analysis of EC₅₀ values

Chemical-specific differences in toxicity were evaluated using EC₅₀ values derived from concentration–response modelling. Only EC₅₀ estimates with finite numerical values were included in inferential statistical analyses. EC₅₀ values reported as not reached at the highest tested concentration were excluded from statistical testing and retained for descriptive purposes only, as they do not represent quantified effect concentrations.

To stabilize variance and improve normality, EC₅₀ values were natural log-transformed prior to analysis. A linear model was then fitted with log-transformed EC₅₀ as the response variable. Chemical identity was included as a fixed effect to test for overall differences in toxicity among chemicals. Species identity was included as an additional fixed effect to account for interspecific variation in baseline sensitivity, and a species × chemical interaction term was included to evaluate whether chemical-specific toxicity patterns differed among taxa. Experimental temperature was included as a continuous covariate to control for temperature-related variation in EC₅₀ values but was not interpreted as a primary factor in this analysis.

The model was specified as:

$$\ln(\text{EC}_{50}) = \beta_0 + \beta_1(\text{Temperature}) + \beta_2(\text{Species}) + \beta_3(\text{Chemical}) + \beta_4(\text{Species} \times \text{Chemical}) + \varepsilon$$

Statistical significance of chemical effects was assessed using analysis of variance on the fitted linear model. A significant main effect of chemical identity was interpreted as evidence of systematic differences in toxicity among chemicals across species, while a significant species × chemical interaction indicated taxon-specific sensitivity to individual chemicals. Model residuals were inspected to verify assumptions of homoscedasticity and approximate normality. All analyses were conducted in R (version 4.5.1).

3.9.2 Statistical analysis of temperature-dependent shifts in species sensitivity

Temperature-dependent changes in species sensitivity were assessed using estimated median effect concentrations (EC_{50}) derived from dose–response modelling. Analyses were conducted separately for each species–chemical combination to avoid confounding effects among taxa or compounds.

For each species–chemical combination, EC_{50} values were estimated at three experimental temperatures (15, 20 and 25 °C). Inferential analyses were restricted to EC_{50} estimates with finite numerical values, consistent with OECD guidance that EC_x estimates should not be treated as independent replicates in factorial analyses unless they are derived from replicated concentration–response experiments (OECD, 2006). EC_{50} values reported as “not reached” at the highest tested concentration were retained for descriptive reporting but were not included in hypothesis tests because they represent censored (unquantified) effect concentrations. This decision was stated explicitly to avoid misinterpretation of censored values in statistical inference. To stabilise variance and meet model assumptions, EC_{50} values were natural-log transformed prior to analysis.

Given the experimental design, which yielded a single EC_{50} estimate per temperature for each species–chemical combination, formal factorial analysis of variance (ANOVA) was not appropriate due to the absence of within temperature replication and residual degrees of freedom. Instead, temperature effects were assessed using linear regression treating temperature as a continuous predictor, thereby testing for monotonic trends in EC_{50} across the temperature gradient.

For each species–chemical combination, a linear model of the form:

$$\log(EC_{50}) = \beta_0 + \beta_1 \times \text{Temperature} + \varepsilon$$

was fitted, where β_1 represents the direction and magnitude of the temperature dependent change in sensitivity. The statistical significance of temperature effects was evaluated using F-tests on the regression slope (β_1). This approach allowed detection of directional changes in sensitivity while minimising over-parameterisation of the model.

Because each temperature level was represented by a single EC_{50} estimate, these analyses were considered exploratory rather than confirmatory. Resulting p-values were interpreted as indicative of temperature-associated trends rather than definitive evidence of differences among discrete temperature treatments. Particular caution was applied where model fits were near-perfect due to limited degrees of freedom, as reflected by diagnostic warnings during model evaluation. Despite these limitations, the regression-based approach provides a statistically defensible framework for assessing temperature-dependent shifts in species sensitivity and is consistent with approaches commonly applied in ecotoxicological studies where replication at the EC_{50} level is constrained.

All statistical analyses were performed in R (version 4.5.1). Data manipulation and modelling were conducted using the packages dplyr, tidyr, and stats, with regression outputs inspected using base R diagnostics.

4. Results

4.1. Chemical analysis

Observed concentrations from chemical analysis of samples used in the exposure studies followed the nominal concentration pattern for all five tested chemicals (shown in Figure 7). Copper chloride and sulfamethoxazole displayed particularly close agreement between nominal and actual concentrations across all treatment levels, indicating close agreement between nominal and measured concentrations during preparation.

For zinc sulphate, erythromycin, and metazachlor, actual concentrations were slightly lower than the nominal targets, especially at the higher exposure levels. Despite these deviations, all three compounds still demonstrated a consistent increase in measured concentration with increasing nominal dose. This monotonic relationship confirms that the exposure gradient was effectively maintained across treatments.

The observed patterns indicate that the experimental system achieved a well-structured concentration range suitable for subsequent inhibition modelling and analysis. Even in cases where absolute values deviated from targets, the proportional increase ensures that concentration–response analyses in the study remain robust for interpretation.

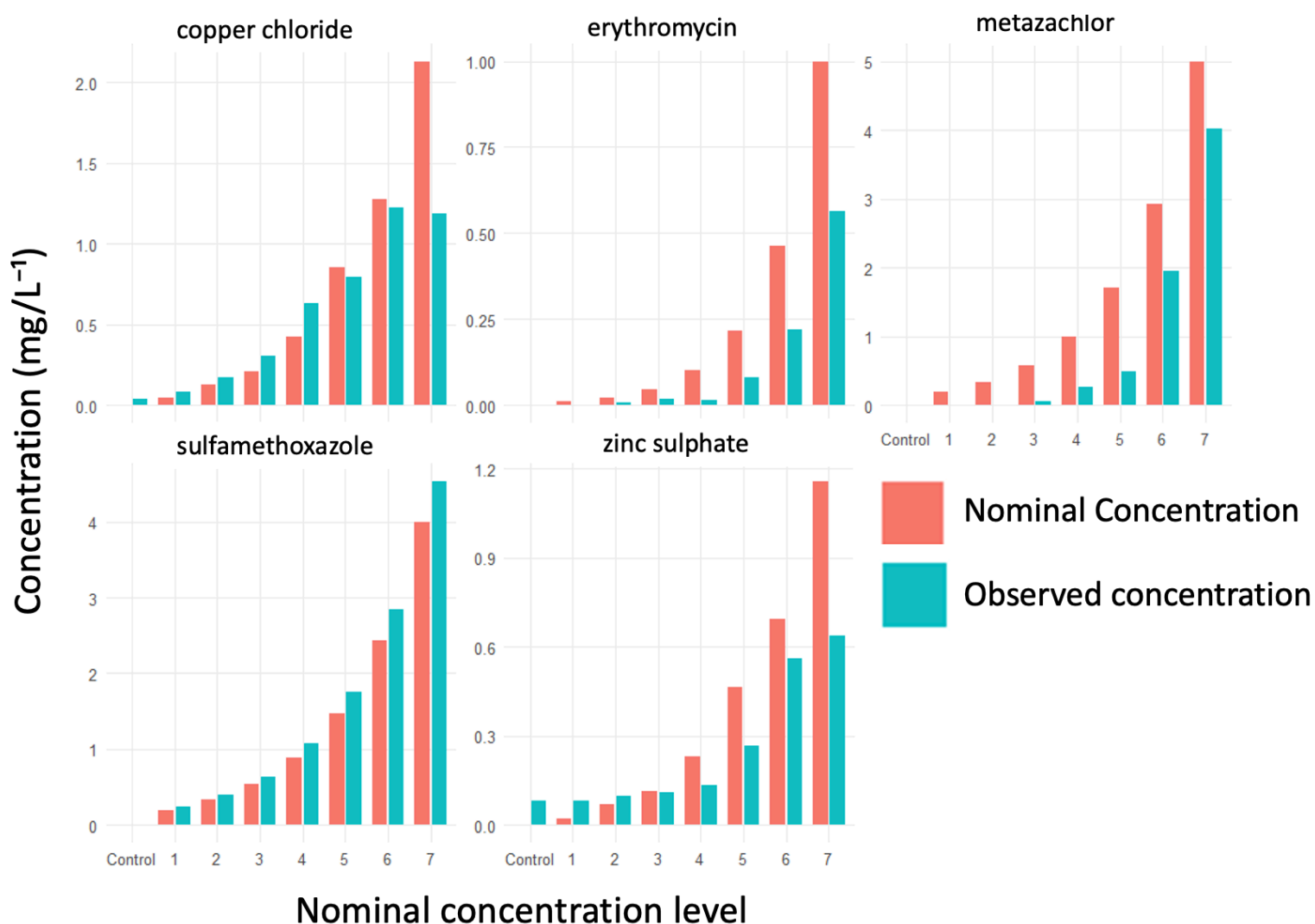


Figure 7. Comparison of nominal and measured concentrations for five contaminants following chemical analysis of experimental samples. Error bars are not shown due to the absence of analytical replication.

4.1.1 Temperature effect on chemical degradation

Measured concentrations of the five contaminants; sulfamethoxazole (SMZ), metazachlor (MTZ), erythromycin (ERY), zinc (as ZnSO₄), and copper (as CuCl) were evaluated at 15, 20, and 25 °C over a 96-h exposure at two dose tiers (lowest and highest nominal concentrations; Figure 8). Across chemicals and temperatures, concentrations were stable and showed no consistent change attributable to temperature-driven degradation.

At the lowest concentration (Figure 8a), SMZ and MTZ means were closely aligned across all temperatures. MTZ displayed greater dispersion (wider standard deviation bars), but central tendencies remained comparable among temperatures. ERY remained near the analytical lower range, while Zn and Cu showed only minor between-temperature differences. For all compounds, inter-temperature variation overlapped with the standard deviation error bars.

At the highest concentration (Figure 8b), no systematic temperature-dependent trends were observed across all compounds. Any minor variations between temperatures were small and within, or close to, analytical uncertainty and were therefore not interpreted as evidence of thermal degradation.

Overall, the results indicate no clear temperature-dependent degradation for any compound within 15–25 °C over 96 h. Measured concentrations remained comparable across temperatures at both dose tiers, confirming a stable and reproducible exposure regime suitable for dose–response analyses (e.g., inhibition modelling). Apparent minor differences are considered to reflect routine experimental variability rather than true temperature effects. Samples for zinc sulphate at 15 °C and erythromycin at the highest concentration at 20 °C were damaged during storage and could not be recovered for analysis; these missing data are indicated in Figure 8.

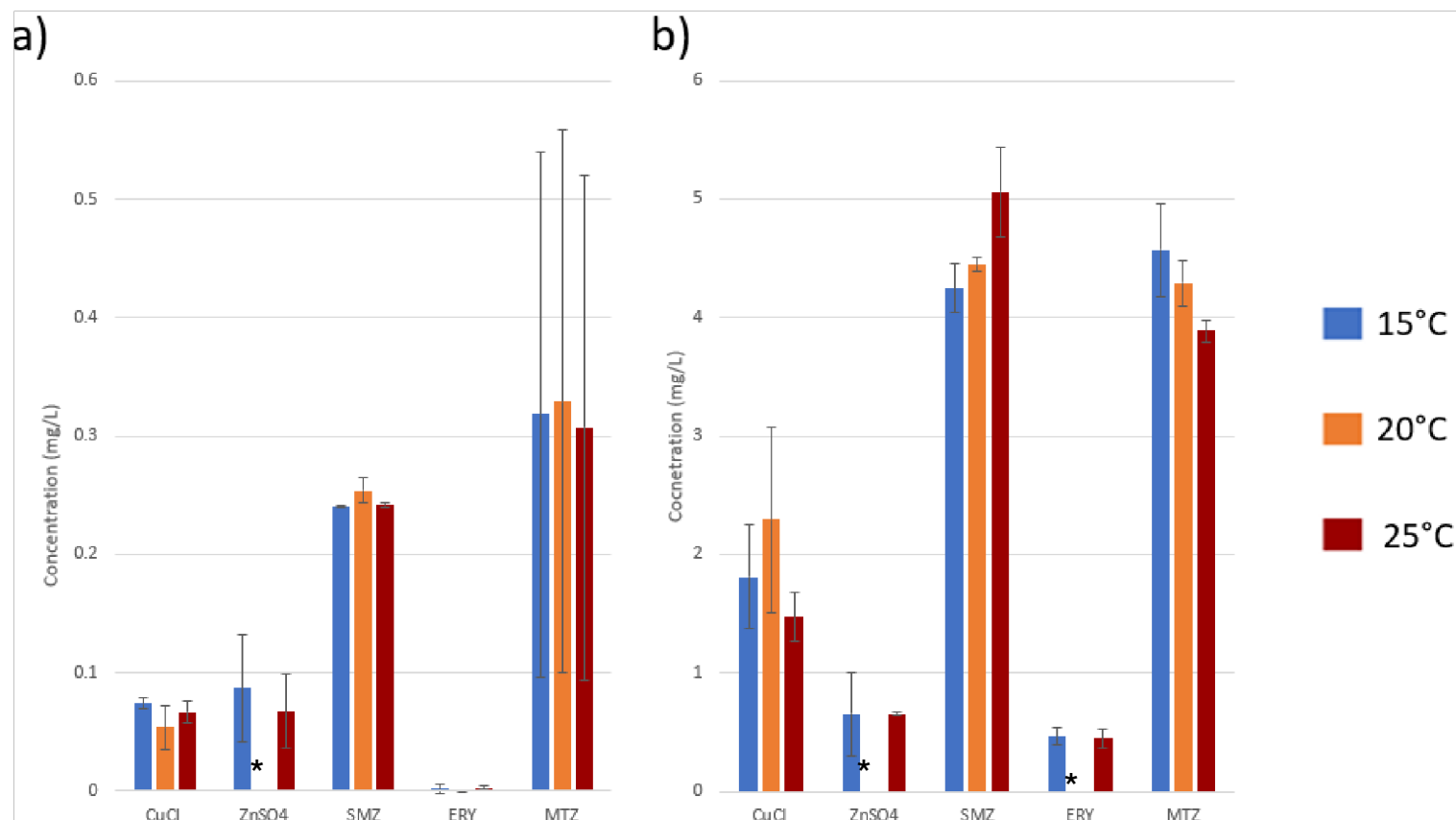


Figure 8. Measured concentrations (mean $\pm$ SD) of five contaminants; sulfamethoxazole (SMZ), metazachlor (MTZ), erythromycin (ERY), zinc (ZnSO₄), and copper (CuCl) at 15, 20, and 25 °C after 96 h exposure. a) Lowest nominal concentration; (b) highest nominal concentration. Error bars represent standard deviation from triplicate measurements at each temperature and concentration. Concentrations were quantified using ICP-OES for metals (Cu, Zn) and LC-MS for organic compounds (SMZ, MTZ, ERY). * indicates absence of data.

4.2 Temperature influence on growth

All three primary producers (*A. flos-aquae*, *P. subcapitata*, and *Nitzschia palea*) showed overall increases in cell counts over the 96-hour experimental period shown in Figure 9(a). Growth consistently appeared temperature-dependent, with the lowest cell counts observed at 15 °C, and the highest at 25 °C across all taxa.

For *A. flos-aquae*, mean cell counts increased from approximately $13,681 \pm 11,711$ (SD) at 0 h to $142,932 \pm 11,574$ at 96 h under 25 °C, compared with $46,408 \pm 19,035$ at 15 °C. Similarly, *P. subcapitata* showed substantial growth differences across the different temperature treatments, rising to over 240,000 cells at 25 °C by 96 h, while *N. palea*

exhibited the lowest temperature response. In all species, the steepest increases occurred between 48–96 hours, particularly at 20 °C and 25 °C, whereas growth was minimal or plateaued at 15 °C.

In contrast to cell counts, growth rate trends in Figure 9(b) revealed more complex temporal patterns that differed among species. For *A. flos-aquae*, growth rates were highest during the 0–24 h interval at all temperatures (average $\approx 0.51\text{--}1.12 \ln(\text{cells}) \text{ day}^{-1}$), followed by a decline in later intervals (48–96 h). Growth rates converged across temperatures after 72 h, indicating early exponential growth followed by a slower phase.

P. subcapitata exhibited the highest average growth rates overall, particularly at 25 °C during the 0–24 h phase ($\sim 1.0\text{--}1.3 \ln(\text{cells}) \text{ day}^{-1}$), but experienced a mid-experiment dip (24–72 h) before recovering toward the end. In contrast, *N. palea* displayed more variable responses with its growth rate at 15 °C peaked later (24–48 h), suggesting delayed acclimatisation to low temperature, while at higher temperatures (20–25 °C) it showed sustained or fluctuating high growth rates across all intervals.

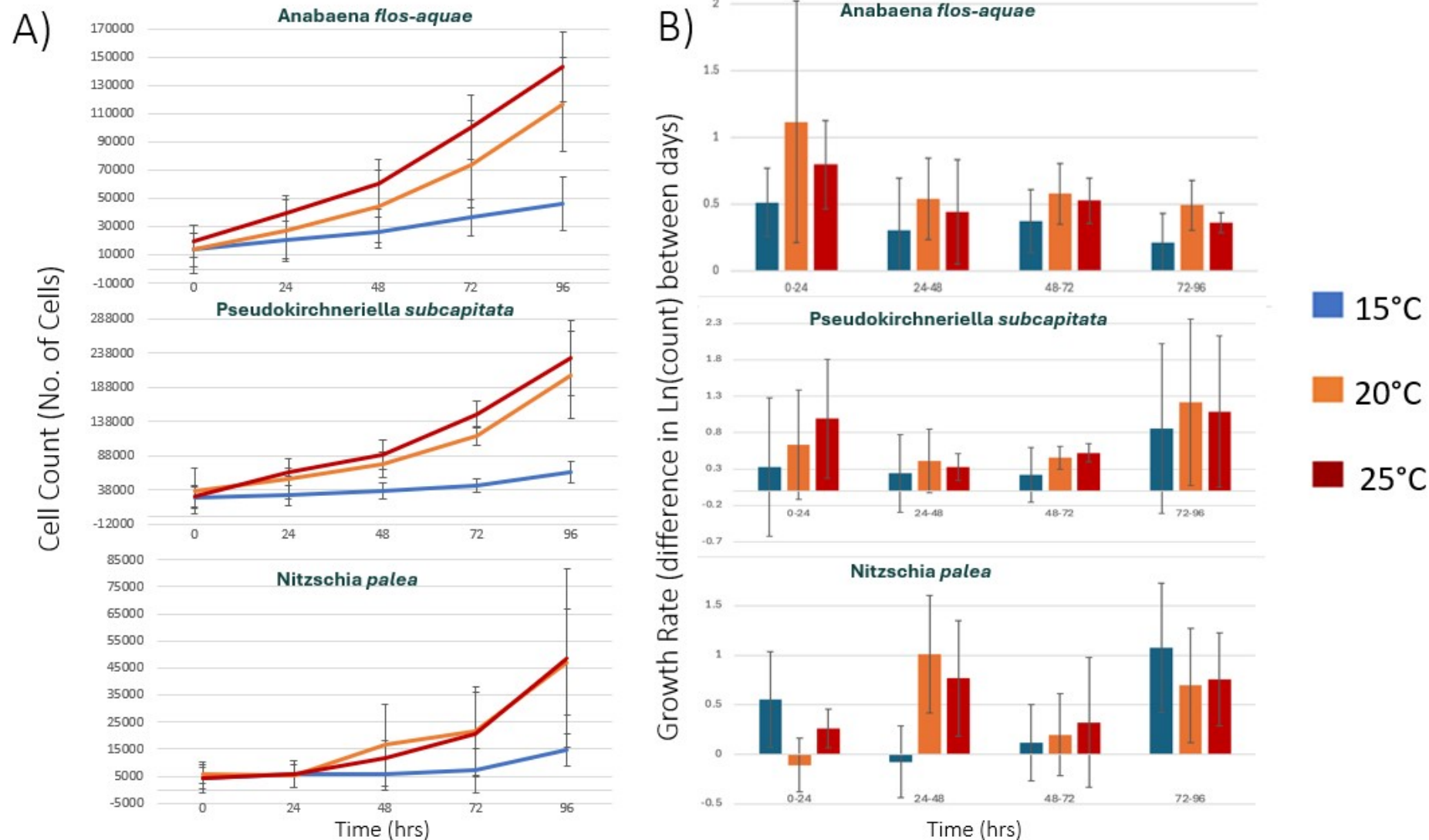


Figure 9. Effects of temperature on growth dynamics of the three test species. (a) Mean growth trajectories during the 96-h exposure at 15, 20 and 25°C (n = 5 replicates per treatment; error bars denote $\pm$ SD). (b) Temperature-specific growth rates derived from 24-h time intervals across the exposure period. Error bars denote $\pm$ SD

4.3.1 Chemical-specific patterns

Clear chemical-specific patterns in species sensitivity were observed across all taxa, as reflected by both the fitted dose–response curves (Figure 10) and the derived EC_{50} values (Table 7). For each chemical, species differed consistently in their position along the concentration–response gradient, indicating intrinsic differences in sensitivity that were largely conserved across temperatures.

Copper exhibited relatively similar toxicity across the three taxa, although subtle differences were evident. Algae generally showed the lowest EC_{50} values, indicating the highest sensitivity, followed by cyanobacteria, while diatoms tended to be less sensitive where EC_{50} values could be quantified. These patterns are apparent in the steeper dose–response curves observed for algae in Figure 10 and are supported by consistently lower EC_{50} estimates in Table 7.

For zinc, species sensitivity was more variable. Algae again tended to display lower EC_{50} values than diatoms and cyanobacteria, although overlap in EC_{50} ranges was observed among taxa. In some cases, dose–response curves did not reach 50% inhibition within the tested concentration range for certain species, indicating comparatively lower sensitivity to zinc. This incomplete response is reflected in shallow or truncated curves (Figure 10) and in EC_{50} values reported as not reached (Table 7).

Erythromycin showed pronounced interspecific differences in sensitivity. Cyanobacteria were consistently the most sensitive taxon, with EC_{50} values substantially lower than those observed for algae and diatoms. This heightened sensitivity is evident from the leftward shift of cyanobacterial dose–response curves relative to other species (Figure 10). In contrast, diatoms frequently failed to reach 50% inhibition at the highest tested concentrations, indicating comparatively low sensitivity to this compound.

A similar pattern was observed for sulfamethoxazole, where cyanobacteria again exhibited the greatest sensitivity, followed by algae, while diatoms were the least sensitive. Several diatom dose–response curves did not achieve 50% inhibition,

consistent with EC_{50} values reported as not reached in Table 7. These results highlight a strong taxon-specific response to this antibiotic.

For metazachlor, algae and cyanobacteria generally displayed measurable EC_{50} values across temperatures, whereas diatoms frequently showed no detectable EC_{50} within the tested concentration range. The corresponding dose–response curves for diatoms remained relatively flat across concentrations (Figure 10), indicating limited inhibition. Cyanobacteria exhibited particularly low EC_{50} values, suggesting high sensitivity to this herbicide.

Overall, these results demonstrate that chemical identity strongly influenced species sensitivity patterns, with consistent rankings among taxa observable across multiple compounds. These chemical-specific sensitivity differences are clearly captured by both the shape and position of the dose–response curves (Figure 10) and the magnitude of EC_{50} values reported in Table 7, providing clear evidence for intrinsic, taxon-dependent responses to individual chemicals.

Across all taxa, EC_{50} values differed significantly among chemicals ($F_{4,20} = 53.2$, $p < 0.001$), indicating marked differences in toxicity. Species identity also had a strong effect on EC_{50} values ($F_{2,20} = 109.1$, $p < 0.001$), with diatoms generally exhibiting higher EC_{50} values (lower sensitivity) than algae.

Importantly, a significant Species $\times$ Chemical interaction was detected ($F_{7,20} = 23.7$, $p < 0.001$), demonstrating that chemical toxicity varied strongly among taxa. In particular, cyanobacteria displayed substantially lower EC_{50} values for erythromycin, metazachlor, and sulfamethoxazole relative to algae, consistent with the steep dose–response curves observed in Figure 10 and the EC_{50} estimates reported in Table 7. Diatom responses differed from both algae and cyanobacteria, with selective sensitivity to sulfamethoxazole and zinc.

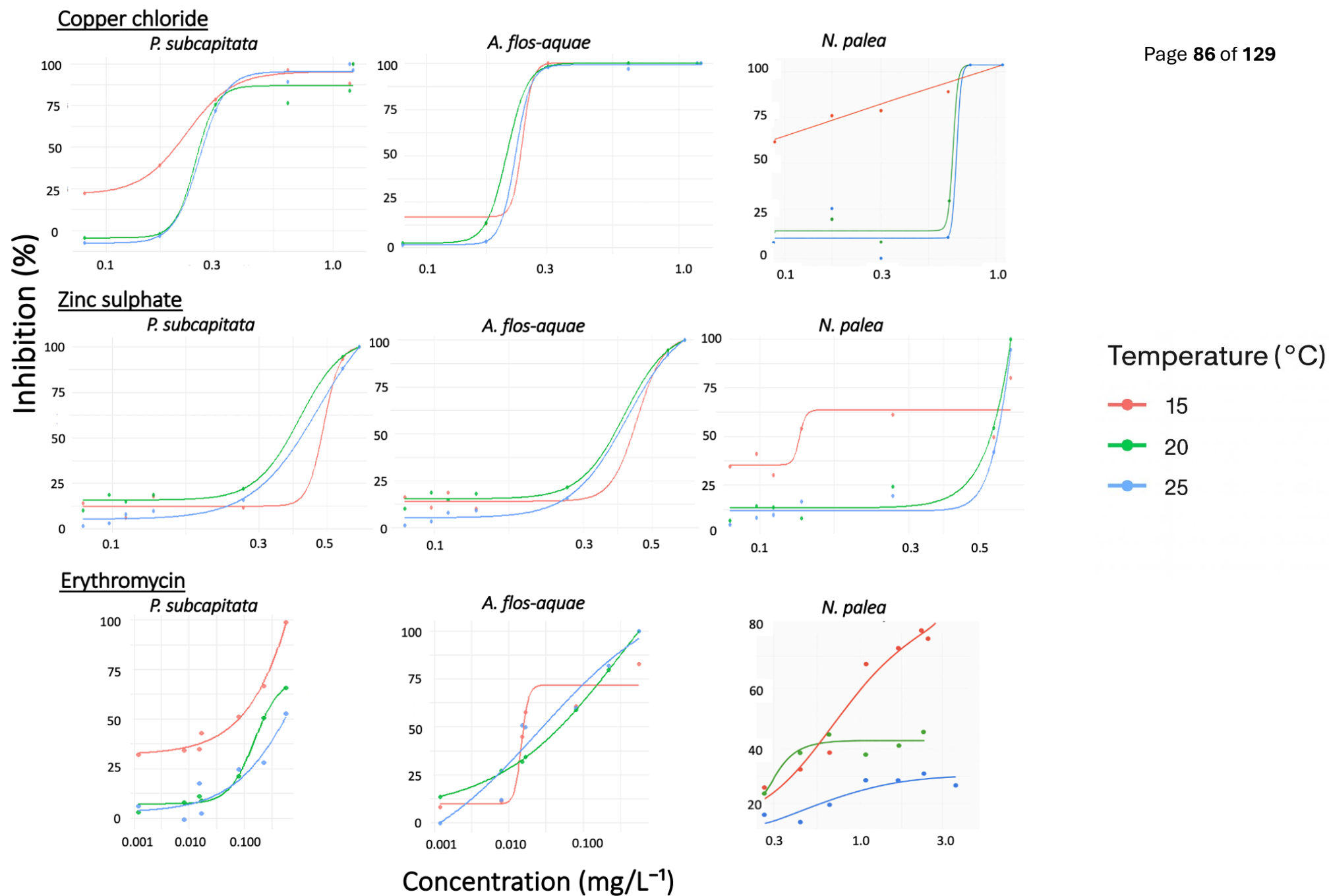
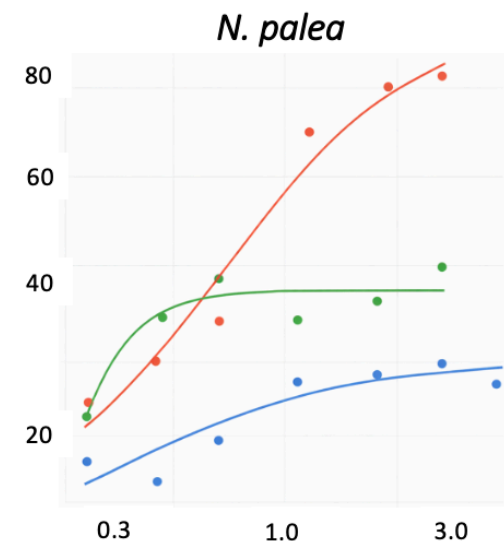
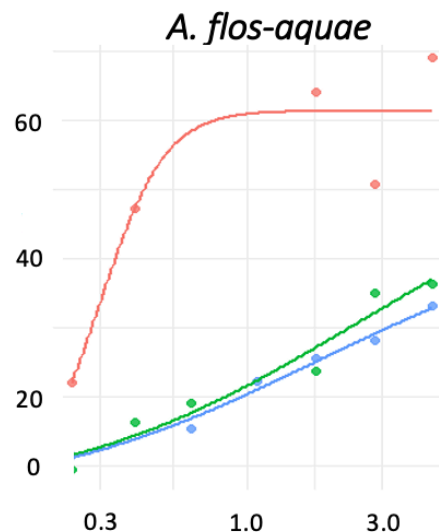
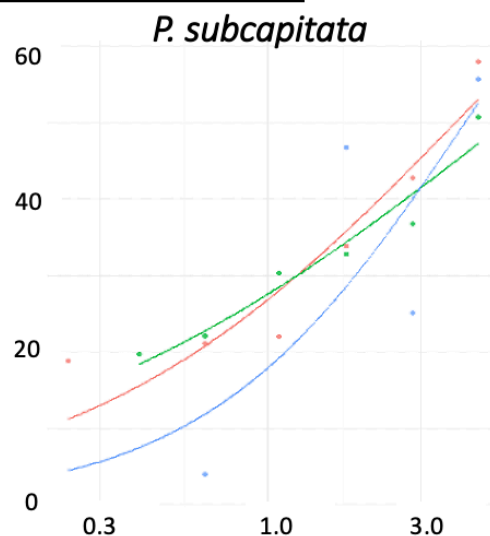
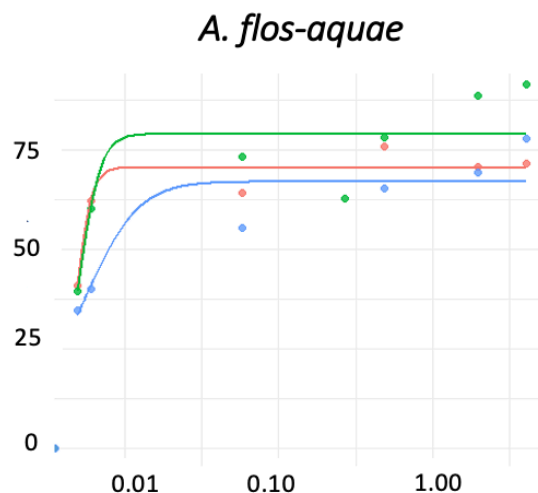
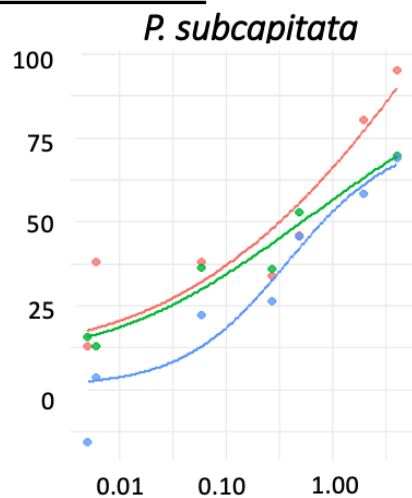


Figure 10. Concentration–response (dose–response) curves for three freshwater primary producers exposed to five contaminants across three temperature treatments (15, 20, and 25 °C). Panels show modelled growth inhibition as a function of measured concentration using four-parameter log-logistic models (LL.4). Error bars represent standard deviation from triplicate measurements.

Sulfamethoxazole



Metazachlor



N. palea

NO DATA

Figure 10 (continued). Concentration- response curves for remaining species-contaminant combinations under the same experimental conditions.

4.4 Temperature-chemical interaction

Across all taxa and chemicals, EC_{50} values showed variation across the temperature range, with both the direction and magnitude of responses differing among taxonomic groups (Figure 11; Table 7). For several taxon–chemical combinations, EC_{50} values increased with increasing temperature, indicating reduced sensitivity at higher temperatures, whereas in other cases EC_{50} values remained relatively stable across the tested range (15–25 °C). These temperature-related patterns were evident in the concentration–response curves, which showed shifts in curve position rather than changes in overall shape (Figure 10).

Instances where EC_{50} values were not reached at the highest tested concentration occurred more frequently at higher temperatures for certain taxa, particularly for cyanobacteria and diatoms exposed to sulfamethoxazole and metazachlor (Table 7). These cases suggest a reduced apparent toxicity under warmer conditions, although they could not be incorporated into inferential statistical analyses due to censoring.

When evaluated across the full dataset using a linear model controlling for taxonomic and chemical effects, temperature alone explained a relatively small proportion of the overall variability in log-transformed EC_{50} values. The effect of temperature was marginal and did not reach conventional levels of statistical significance (ANOVA, $F_{1,20} = 4.10$, $p = 0.056$), indicating that temperature did not act as a consistent, unidirectional driver of toxicity across taxa.

Overall, these results demonstrate that temperature did not produce a consistent shift in chemical toxicity across the experimental range; instead, variation in EC_{50} values was primarily driven by species identity and chemical type.

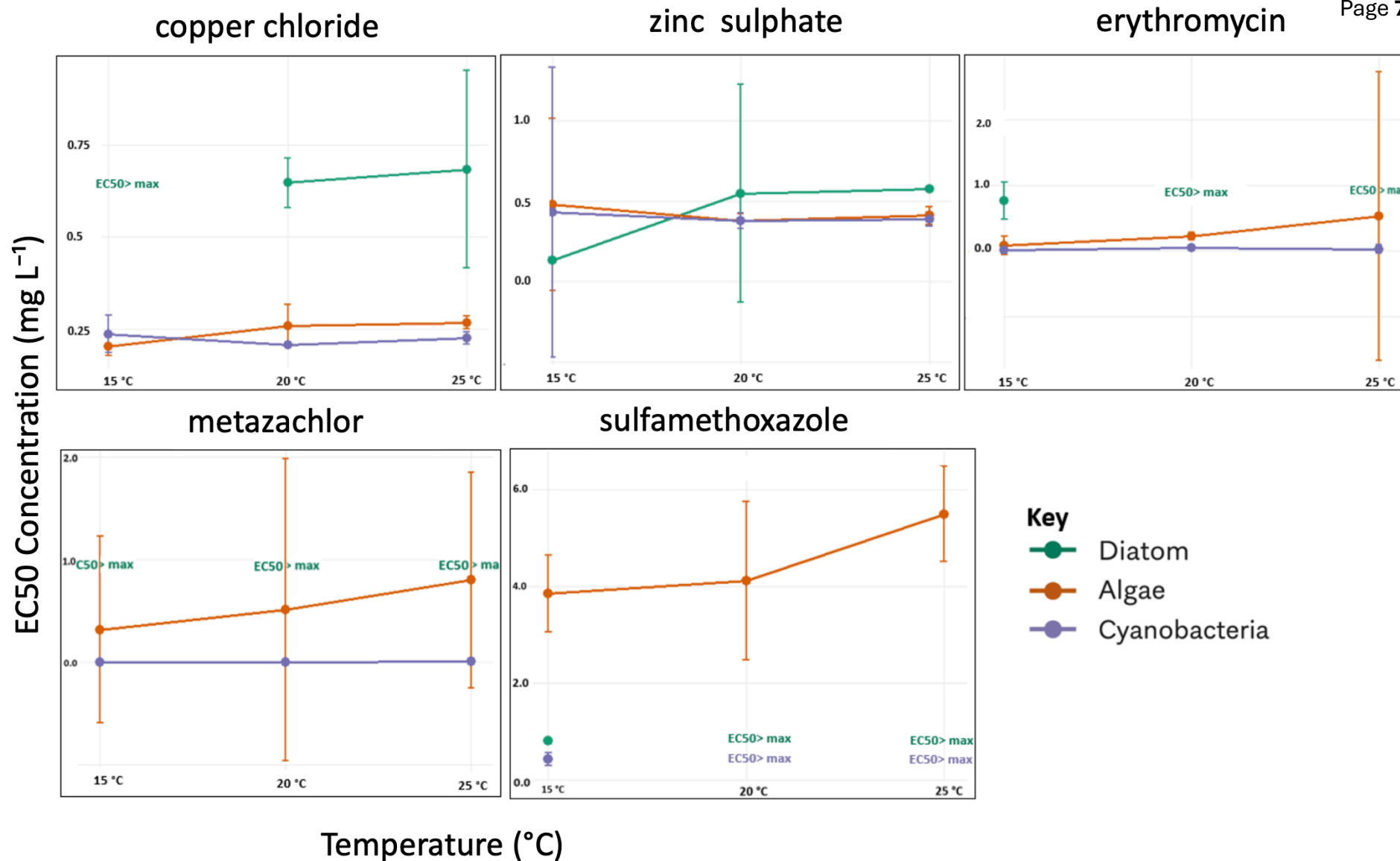


Figure 11. Temperature dependence of median effect concentrations (EC_{50}) for three aquatic taxa (diatom, green alga, and cyanobacterium) exposed to 5 different contaminants. Points represent mean EC_{50} estimates at each test temperature (15, 20, and 25 °C), with vertical error bars indicating $\pm$ standard error where available. Solid lines connect EC_{50} values across temperatures within each taxon to illustrate temperature-related trends and do not imply interpolation beyond the tested range. Text annotations (“ $EC_{50} > \text{max}$ ”) indicate cases where 50 % inhibition was not reached at the highest tested concentration, and EC_{50} values therefore exceed the experimental concentration range. EC_{50} values were derived from concentration–response modelling following OECD Test Guideline 201 principles.

Table 7 - EC_{50} values are reported as mean $\pm$ standard error (SE). “<” indicates that 50 % inhibition was not achieved at the highest test concentration. All concentrations are expressed in $mg L^{-1}$. Values of 0.000 indicate that variability was below the rounding precision and should not be interpreted as true zero variance.

Table 7.1 *Pseudokirchneriella subcapitata* EC_{50} values

Chemical	EC_{50} (15 °C, $mg L^{-1}$)	EC_{50} (20 °C, $mg L^{-1}$)	EC_{50} (25 °C, $mg L^{-1}$)
copper chloride	0.203 ± 0.025	0.258 ± 0.058	0.267 ± 0.0176
zinc sulphate	0.478 ± 0.535	0.375 ± 0.047	0.409 ± 0.0574
erythromycin	0.078 ± 0.142	0.220 ± 0.058	0.533 ± 2.20
sulfamethoxazole	3.853 ± 0.792	4.120 ± 1.645	5.507 ± 0.989
metazachlor	0.318 ± 0.908	0.516 ± 1.477	0.803 ± 1.052

Table 7.2 *Anabaena flos-aquae* EC_{50} values

Chemical	EC_{50} (15 °C, $mg L^{-1}$)	EC_{50} (20 °C, $mg L^{-1}$)	EC_{50} (25 °C, $mg L^{-1}$)
copper chloride	0.236 ± 0.051	0.207 ± 0.000	0.225 ± 0.017
zinc sulphate	0.432 ± 0.900	0.377 ± 0.046	0.388 ± 0.044
erythromycin	0.016 ± 0.001	0.049 ± 0.015	0.029 ± 0.059
sulfamethoxazole	0.430 ± 0.126	<	<
metazachlor	0.005 ± 0.000	0.005 ± 0.000	0.008 ± 0.003

Table 7.3 *Nitzschia palea* EC_{50} values

Chemical	EC_{50} (15 °C, $mg L^{-1}$)	EC_{50} (20 °C, $mg L^{-1}$)	EC_{50} (25 °C, $mg L^{-1}$)
copper chloride	<	0.647 ± 0.068	0.684 ± 0.269
zinc sulphate	0.134 ± 0.016	0.548 ± 0.677	0.576 ± 0.009
erythromycin	0.764 ± 0.286	<	<
sulfamethoxazole	0.805 ± 0.054	<	<
metazachlor	<	<	<

5. Discussion

5.1 Overview

This study investigated how climate warming influences the toxicity of contaminants to three freshwater primary producers: *Pseudokirchneriella subcapitata*, *Nitzschia palea*, and *Anabaena flos-aquae*. These taxa were exposed to representative contaminants from three chemical classes (metals, pharmaceuticals, and pesticides) across a temperature gradient (15–25 °C) to assess whether warming alters toxicity and to identify interspecific differences in sensitivity. Overall, the results revealed that temperature did not significantly alter contaminant toxicity overall, although slight increases in tolerance were observed in some cases. Toxicity was driven primarily by chemical identity and species-specific traits, with temperature exerting a secondary, modulatory effect.

5.2 Effect of temperature on controls

Growth rates of the unexposed control cultures varied significantly across temperature treatments, reflecting the physiological temperature dependence of photosynthetic organisms. Optimal growth for *P. subcapitata* and *N. palea* occurred at 20–25 °C, consistent with previous reports for temperate freshwater taxa. A thermal response analysis of 424 strains of microalgae, cyanobacteria, and diatoms reported mean optimal temperatures of 28.8 ± 4.3 °C for cyanobacteria, 23.1 ± 6 °C for diatoms, and 26.1 ± 6 °C for green algae (Rossi, Carecci and Ficara, 2023).

Growth at 15 °C was reduced, likely due to reduced enzymatic activity and lower membrane fluidity, which slow nutrient assimilation and photosynthetic efficiency (Los and Murata, 2004). Of the three taxa, the cyanobacteria *A. flos-aquae* exhibited comparatively reduced variability in growth across all three temperatures, consistent with its cyanobacterial physiology that enables adaptation to wide thermal ranges and efficient use of light under variable conditions due to the species capacity for metabolite induction (Touceda et al., 2024). This may be associated with the production of stress-related metabolites that support physiological resilience

Overall, a mild temperature-dependent increase in growth was observed across all taxa can be attributed to a climate change warming scenario run in this experiment (demonstrated in figure 11). Increased temperatures can cause changes in plankton structure (size distribution) and function (productivity and turnover) that are essential to support food web functioning, maturation and stability (Rasconi et al., 2015). However, when taking these results into the freshwater environment and hence re-introducing species interaction, the increase in temperature resulting from climate change may favour surface-floating algae, giving a competitive advantage over benthic algae (such as the diatom *N. palea*), leading to increased potential for eutrophication, primary producer homogenisation and therefore deterioration in water quality and reduced ecosystem resilience (Mei et al., 2022).

This established baseline temperature–growth relationship is important for interpreting toxicity outcomes, as temperature-driven changes in metabolic rate will directly influence the nutrient uptake and, consequently, contaminant uptake. This has been referred to as important toxicokinetic parameters in the literature (Raths et al. 2023).

5.3 Chemical toxicity

Across the contaminants tested, erythromycin exhibited high toxicity, reflected by consistently low EC_{50} values across taxa, especially for cyanobacteria and green algae (Table 7). This was followed by the herbicide metazachlor, then sulfamethoxazole, while the heavy metals copper and zinc generally exhibited higher EC_{50} values, indicating lower apparent toxicity under the experimental conditions. These patterns are broadly consistent with previous laboratory studies. For example, Machado and Soares, (2019) demonstrated that erythromycin, even at low $\mu\text{g L}^{-1}$ concentrations, significantly reduced growth rates and disrupted cellular physiology in *P. subcapitata*. The strong sensitivity observed here supports the interpretation that pharmaceuticals designed to target prokaryotic biochemical pathways can exert pronounced, unintended toxic effects on non-target primary producers, particularly cyanobacteria, which retain prokaryotic cellular machinery.

Sulfamethoxazole demonstrated consistently high EC_{50} values with 50% inhibition not achieved at 4 mg L^{-1} for *N. palea* and *A. flos-aquae*. This resilience has been observed in the literature with Zhang et al., (2023) where they observed both green algae (*Pseudokirchneriella subcapitata*) and the cyanobacteria (*Synechococcus sp.*), green algae was able to alleviate SMX toxicity by degrading it to low eco-toxic transformation products and the cyanobacteria was able to increase the activity of glutathione-S-transferase (GST) and glutathione peroxidase to resist oxidative stress (Zhang et al., 2023). However, interpretation of sulfamethoxazole toxicity in the present study warrants caution. In several cases, 50% growth inhibition was not achieved within the tested concentration range, resulting in censored EC_{50} estimates and increased uncertainty in concentration–response modelling.

Differences in toxicity among chemical classes likely reflect intrinsic physicochemical properties and modes of action, as outlined in Section 1.2. For heavy metals such as copper and zinc, toxicity is strongly mediated by bioavailability rather than total dissolved concentration. Their inorganic nature enables precipitation, adsorption to organic matter, or complexation with ligands in solution, thereby reducing the proportion of free, biologically available metal ions (Adams et al., 2020). This highlights the importance of integrating water chemistry into ecological risk modelling and supports the relevance of mechanistic tools such as the Biotic Ligand Model (BLM) when interpreting metal toxicity. Although no decline in total dissolved metal concentrations was detected during the experiment (ICP-OES results; Figure 7), the analytical methods employed were not able to quantify changes in bioavailable metal fractions. This methodological limitation should be considered when interpreting the comparatively lower toxicity of metals observed in this study.

Metazachlor displayed substantial toxicity towards both cyanobacteria and green algae, with low EC_{50} values observed across temperatures (Table 7). These results align with (Mohr et al., 2007) who reported EC_{50} values below $5\text{ }\mu\text{g L}^{-1}$ for similar freshwater phototrophic taxa. While Mohr et al. (2007) employed biomass-based endpoints and the present study quantified growth inhibition, the convergence of sensitivity estimates across different biological endpoints supports the reliability of metazachlor toxicity

assessments. The pronounced sensitivity of cyanobacteria in particular may reflect the herbicide's interference with photosystem II and downstream energy metabolism, pathways that are highly conserved among photosynthetic microorganisms.

Overall, the chemical-specific toxicity patterns observed in this study highlight the importance of mechanistic understanding when comparing contaminant classes. Compounds with targeted biochemical modes of action such as pharmaceuticals and herbicides, tend to elicit pronounced effects at lower concentrations, particularly in taxa sharing conserved molecular pathways. In contrast, the toxicity of inorganic contaminants such as metals is strongly conditioned by environmental chemistry and bioavailability rather than intrinsic potency alone. These findings emphasise the need to integrate chemical mode of action, physicochemical behaviour, and organismal physiology into ecological risk assessments, especially when extrapolating laboratory-derived toxicity thresholds to natural freshwater systems.

5.4 Thermal modulation and mechanistic interpretation

Overall, EC_{50} values more often increased or remained unchanged with warming, rather than decreasing. This suggests that moderate temperature elevation did not consistently exacerbate chemical stress. This observation is compatible with established physiological theory for ectothermic organisms, in which metabolic performance and stress tolerance are strongly temperature dependent (Huey and Kingsolver, 1989). Within an organism's optimal thermal window, modest warming can coincide with improved physiological functioning, rather than intensifying their effects. In the context of this study, this would be small due to non-significant shifts in EC_{50} values.

This interpretation aligns with the broader literature on temperature-dependent chemical toxicity (TDCT) (shown in figure 12), which increasingly recognises non-linear responses rather than simple linear increases in toxicity with warming. Reviews and meta-analyses consistently report inverted V-shaped relationships (often termed “Model II”), in which tolerance is maximised near the thermal optimum and declines at temperatures above or below this range (Kazmi et al., 2022b). The absence of a strong negative temperature

effect in the present study is therefore consistent with the tested temperatures likely falling within, or close to, the physiological optima of the studied taxa. Similar non-linear TDCT patterns have been documented in both freshwater and marine systems under ecologically realistic warming scenarios (Zhou et al., 2014; Y. Y. L. Wang et al., 2023).

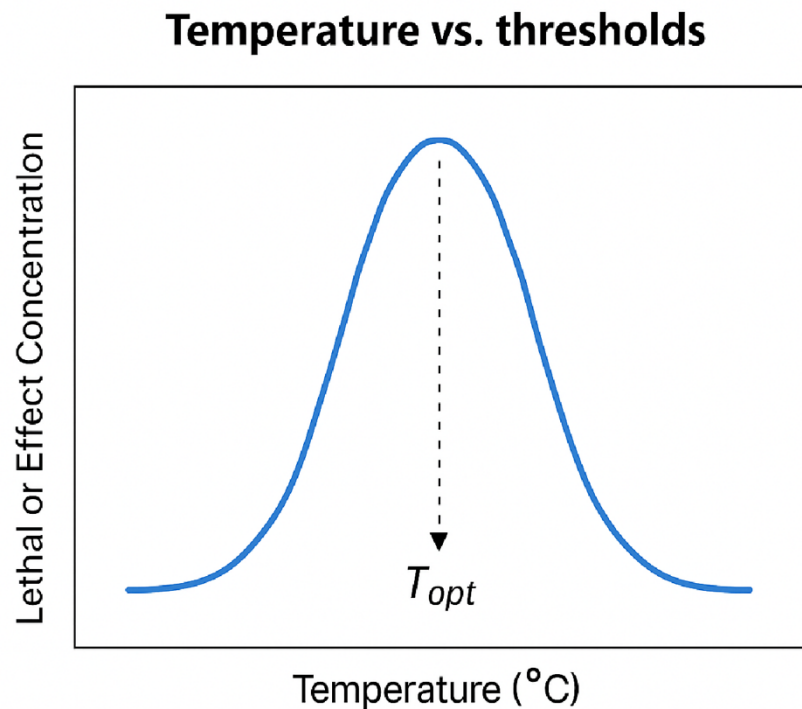


Figure 12. Conceptual inverted V-shaped temperature-dependent chemical toxicity model (Model II), adapted from Wang et al. (2023), illustrating reduced tolerance outside the thermal optimum.

Importantly, this study did not directly quantify metabolic rate, enzyme activity, or cellular repair processes, and therefore mechanistic explanations for the observed tendency towards reduced sensitivity remain inferential. However, temperature is known to influence acclimatisation capacity (Burleson and Silva, 2011), resource acquisition efficiency (Van Der Have, 2002) and overall organismal performance, all of which can indirectly shape sensitivity to chemical stress. The results here are consistent with these frameworks but should not be interpreted as direct evidence of enhanced detoxification or repair mechanisms.

Instances where EC_{50} values were not reached at the highest tested concentration occurred more frequently at higher temperatures for certain taxon–chemical

combinations, particularly for cyanobacteria and diatoms exposed to sulfamethoxazole and metazachlor (Table 7). These censored responses are suggestive of reduced apparent toxicity under warmer conditions and were therefore excluded from inferential analyses. Their occurrence nonetheless supports the conclusion that warming did not systematically increase sensitivity within the tested range.

Chemical analyses confirmed that contaminant concentrations remained stable throughout the 96-h assays under all temperature treatments, indicating that the observed patterns were not driven by chemical degradation. However, changes in chemical bioavailability were not assessed. This represents an important limitation, as temperature can influence metal speciation and solubility, as well as the ionisation or partitioning behaviour of organic contaminants (Khan et al., 2025). Such temperature-mediated shifts in bioavailability could contribute to apparent changes in toxicity without altering total measured concentrations and may partially explain the inability to achieve 50% inhibition in some treatments.

Sub-lethal physiological acclimatisation may also play a role in shaping short-term responses to combined thermal and chemical stress. For example, induction of heat shock proteins and other stress-response pathways has been documented in algae and cyanobacteria exposed to elevated temperatures and chemical stressors (Sathasivam and Ki, 2019). While such mechanisms were not directly measured here, their potential involvement provides a plausible explanatory context for the modest buffering effects observed.

Overall, the findings support a context-dependent view of thermal modulation of chemical toxicity. Within the moderate temperature range examined, warming did not intensify toxicity and may, in some cases, coincide with reduced sensitivity, consistent with the central region of an inverted V-shaped TDCT model. However, this outcome should not be generalised beyond the tested temperature range, particularly as only the ascending limb of the model was tested. Many freshwater primary producers already experience temperatures near their upper thermal limits, and even modest additional warming could shift them onto the declining limb of the TDCT curve, resulting in heightened sensitivity. Consequently, while the present study provides mechanistic

insight into short-term responses under moderate warming, extrapolation to natural systems requires careful consideration of species-specific thermal physiology, exposure duration, and the cumulative effects of multiple environmental stressors.

5.5 Species-specific differences

Marked interspecific differences in sensitivity were observed among the three primary producer taxa (Tables 7.1–7.3). However, these differences were strongly chemical-specific, rather than reflecting a single taxon that was consistently most or least sensitive across all contaminants.

P. subcapitata generally exhibited intermediate sensitivity, with EC_{50} values falling between those of the cyanobacterium *A. flos-aquae* and the diatom *N. palea* for most chemicals. For example, EC_{50} values for copper and zinc ranged from 0.20–0.27 mg L⁻¹ and 0.38–0.48 mg L⁻¹ across temperatures, respectively (Table 7.1). In contrast, substantially higher EC_{50} values were recorded for sulfamethoxazole (3.85–5.50 mg L⁻¹), indicating lower sensitivity to this compound relative to the other taxa. These patterns are consistent with the relatively permeable cell membranes and high surface-area-to-volume ratio of green algae, which can facilitate chemical uptake but do not confer strong protection against contaminants with diverse modes of action (Abdelfattah et al., 2023). *P. subcapitata* already experiences substantial toxicological stress at all three temperatures tested. Consequently, moderate warming may not confer additional metabolic or detoxification advantages for this species (Ma, 2005).

A. flos-aquae displayed high sensitivity to certain organic contaminants, particularly erythromycin and metazachlor, for which EC_{50} values were an order of magnitude lower than those observed for the other taxa (Table 7.2). Metazachlor EC_{50} values for cyanobacteria remained below 0.01 mg L⁻¹ at all tested temperatures, indicating pronounced sensitivity. This high sensitivity has been observed in the literature with Wijewardene et al. (2021) finding even traces of metazachlor 0.5, 5 and 50 µg L⁻¹ led to declines in cyanobacterial populations. In contrast, for sulfamethoxazole, EC_{50} values were not reached at 20 and 25 °C, suggesting reduced toxicity or limited bioavailability

under warmer conditions. This tolerance has been observed in the literature with Dias et al. (2015) concluding cyanobacteria might be naturally non-susceptible to these compounds, or that they might become non-susceptible due to antibiotic contamination pressure, or to the transfer of genes from resistant bacteria present in the environment (Dias et al., 2015). This compound-specific response highlights that cyanobacterial sensitivity cannot be generalised across contaminant classes, particularly for pharmaceuticals with different molecular targets.

The diatom *N. palea* generally exhibited the highest apparent tolerance, especially for organic contaminants. EC_{50} values for erythromycin, sulfamethoxazole, and metazachlor were not reached at higher temperatures, while higher EC_{50} values were observed for copper and zinc compared with the other taxa (Table 7.3). This reduced sensitivity may partly reflect the presence of a silicified frustule, which can act as a physical and chemical barrier to contaminant uptake, particularly for metals and hydrophobic organic compounds (Pančić et al., 2019). However, the protective capacity of the frustule is species-specific and may vary with pore structure and silica deposition and therefore should be interpreted cautiously. Additionally, the frustule (siliceous cell wall) of the oval morphotype is particularly efficient at binding and retaining metal ions on its surface (Gélabert et al., 2007). This may contribute to the comparatively lower sensitivity (higher EC_{50} values) observed for metals. The absence of diatom results for metazachlor due to time constraints, which limits full comparison across all chemicals and should be acknowledged as a minor methodological limitation.

Overall, these findings demonstrate that species-specific sensitivity is governed by an interaction between organismal traits and chemical mode of action, rather than by taxonomic identity alone. This reinforces the need to consider multiple taxa and contaminant classes when deriving ecotoxicological thresholds, particularly when extrapolating laboratory results to natural freshwater communities.

5.6 Ecological and regulatory implications

The observed cross-species tolerance shifts under moderate warming may have implications for ecological monitoring and regulatory decision-making. Current

regulatory frameworks, most notably Environmental Quality Standards (EQS) component of the Water Framework Directive (European Commission, 2022), and REACH (European Commission, 2006), are largely built upon toxicity data generated under standardised laboratory conditions, typically at 20 °C. If organismal sensitivity systematically changes with warming, as suggested by the moderate tolerance increase observed in this study, then risk thresholds derived from single temperature assays may either under- or overestimate toxicity under future climatic conditions. This raises concerns regarding the long-term robustness of regulatory limits that do not explicitly incorporate climate-adjusted toxicity responses.

5.6.1 Implications for biomonitoring and ecological status assessment

Many WFD biological indices rely on taxa with well-characterised pollution sensitivity, including diatom-based metrics such as the Trophic Diatom Index (TDI). Temperature-induced shifts in baseline sensitivity, especially for *N. palea*, could distort ecological status classifications by obscuring early warning signals of contamination. For example, if moderate warming enhances diatom tolerance, pollutant impacts may be underestimated, reducing the sensitivity of biomonitoring programmes and potentially delaying remediation actions. Conversely, in systems where warming pushes species toward the declining limb of the inverted V-shaped toxicity curve, sensitivity could increase, falsely signalling deterioration unrelated to pollutant pressure.

These findings highlight the need for temperature-contextualised interpretation of biological indices and potentially the development of climate-adjusted scoring thresholds within diatom and phytoplankton assessment tools.

5.6.2 Implications for environmental quality standards and risk thresholds

The EC₁₀ values obtained in this study, when compared with measured environmental concentrations from European surface waters displayed in Table 3 (EEA, 2025), indicate that contaminants such as copper, zinc, and metazachlor frequently occur at levels

capable of eliciting chronic biological effects. Under warming scenarios, increased metabolic demand and climate-induced shifts in nutrient cycles may amplify ecological vulnerability even where direct chemical–temperature synergism is limited.

Current EQSs typically assume fixed toxicity values. However, if climatic warming modulates species sensitivities, EQSs may require species-specific adjustment factors (particularly for sensitive taxa like *P. subcapitata*). Climate safety factors may be required to address uncertainty in TDCT responses, and risk characterisation may benefit from incorporating temperature-explicit approaches. These suggested modifications would align risk assessment practice with emerging scientific evidence that chemical toxicity is environmentally contingent.

5.6.3 Implications for chemical regulation and mixture toxicity frameworks

The study also highlights the need for improved integration of temperature interactions into mixture toxicity models, particularly for contaminants such as pharmaceuticals and pesticides, which exhibited strong baseline toxicity but weak thermal modulation. Existing models (e.g., concentration addition, independent action) assume temperature-invariant toxicity, yet our findings and literature synthesis indicate that thermal optima and species traits significantly influence toxicity outcomes.

To address this thermal modulation, regulators may need to consider: incorporating thermal performance curves into mixture toxicity modelling, updating technical guidance under REACH (European Commission, 2006) to include TDCT considerations and encourage climate-relevant test designs (e.g., multi-temperature assays) in standard protocols such as OECD 201, 202, and 203 (OECD, 2004, 2006, 2019).

5.7 Experimental realism and limitations

This study provides a reproducible framework for assessing temperature-induced multi-stressor interactions within a controlled ecotoxicological setting, adapting established OECD test guidelines (OECD 201, 202, 203) to incorporate thermal treatments. Although

each chemical–species combination was tested once, the experimental design ensured high comparability across taxa by standardising culture media, light intensity, aeration, inoculum density, and acclimatisation procedures. This methodological consistency strengthens confidence in the relative patterns observed among species and contaminants. Repetition of each assay would undoubtedly increase statistical power and narrow uncertainty in dose–response modelling. However, alignment with validated laboratory protocols and consistency with trends reported in the literature help mitigate concerns associated with limited replication.

A key limitation of this study is the prioritisation of experimental breadth over replication. While each species–contaminant combination was tested under standardised conditions, the design did not include extensive replication at the level of independent experimental runs, which limits the statistical power to detect subtle differences in temperature-dependent toxicity. Each treatment was conducted in triplicate, providing within-experiment replication and improving the reliability of measured responses. However, this does not fully substitute for replication across independent experimental setups.

This trade-off was made deliberately to address a gap identified in Chapter 2, where most studies focus on single contaminants or narrow taxonomic groups, limiting cross-study comparability. By incorporating multiple contaminant classes and representative taxa within a unified experimental framework, this study enables direct comparison of temperature–contaminant interactions across different modes of action. However, this broader experimental scope necessarily reduced the number of replicates achievable within the available experimental constraints.

As a result, the findings are sufficient to identify indicative cross-species and cross-contaminant patterns, although limited replication reduces sensitivity to resolve smaller or non-linear temperature effects with high statistical confidence. While the consistency of responses across species and chemicals supports the reliability of the observed trends, caution is required when interpreting the magnitude of temperature effects. Future studies should build upon this framework by increasing replication within

individual species–contaminant combinations to strengthen statistical robustness while maintaining cross-contaminant comparability.

Despite these strengths, several limitations must be acknowledged regarding ecological realism. Laboratory tests unavoidably simplify natural complexity by excluding key biotic interactions, such as interspecific competition, grazing pressure, allelopathy, and microbial community dynamics, that strongly modulate contaminant sensitivity in real freshwater ecosystems. Similarly, environmental factors such as diel temperature cycling, nutrient fluctuations, light variability, and natural organic matter are absent in standardised assays but can significantly influence toxicity outcomes and bioavailability.

Furthermore, the study employed short-term (96 h) exposures, restricting inference to acute and early sublethal effects. Chronic responses, acclimatisation trajectories, evolutionary adaptation, and community-level restructuring remain beyond the scope of this design. While mesocosm and outdoor experiments provide greater ecological realism, our literature review highlighted that such systems frequently produce ambiguous or inconsistent outcomes due to limited replication, environmental heterogeneity, and difficulty standardising conditions. In contrast, few existing studies have successfully examined multiple species and multiple classes of emerging contaminants under a single, standardised methodological framework (see Chapter 2), underscoring the value of the controlled, cross-taxa comparison achieved here.

Chemical analysis confirmed stability of contaminant concentrations across treatments, however, this study did not quantify potential changes in chemical speciation or bioavailability under different temperatures. Such shifts, particularly for metals and ionisable pharmaceuticals, could influence organism responses independently of total concentration, and this represents an important avenue for future refinement of the experimental design. A limitation of this study is the incomplete analytical profiling of Lenacil due to time constraints. While biological response data were generated, the absence of complete chemical confirmation restricts the depth of interpretation for this compound.

Despite these constraints, the results provide a useful and internally consistent quantitative baseline for incorporating temperature effects into ecological risk modelling, although limited replication restricts the strength of statistical inference. These findings should therefore be interpreted as indicative rather than definitive. Nonetheless, they support the growing recognition that regulated toxicity tests would benefit from temperature-explicit or multi-stressor OECD-style guidelines, ensuring that chemical risk assessments remain fit for purpose under future climate warming scenarios.

5.8 Future research directions

This study provided a reproducible and comparable framework for examining how moderate climatic warming influences the toxicity of multiple emerging contaminants. By standardising conditions and removing community-level interactions, it generated empirical data that help clarify species-specific thermal–toxicological responses. However, several important avenues for future work remain.

First, replication of the experimental methodology will allow for further statistically robust interpretation. Additionally, longer-term experiments are required to identify sublethal, adaptive, or cumulative effects that cannot be captured within the 96-hour design used here. Such studies would help determine whether the short-term tolerance patterns observed persist, strengthen, or reverse under chronic exposure.

Second, although isolating single species allowed clearer interpretation of mechanistic responses, real freshwater ecosystems involve complex trophic interactions that may amplify, buffer, or entirely reshape thermal–chemical effects. Incorporating multispecies or simplified trophic assemblages will therefore be essential for understanding whether the patterns identified here scale to higher ecological levels.

Third, future research should investigate the intracellular fate and bioaccumulation behaviour of contaminants across temperatures. While this study confirmed external concentration stability, it did not quantify changes in chemical speciation or internal dose, both of which are likely to influence toxicity under warming.

Finally, integrating experimentally derived toxicity thresholds with measured environmental concentrations, particularly for contaminants such as copper, zinc, and metazachlor, would allow a clearer assessment of their real-world relevance under climate change. Developing models that combine thermal physiology with contaminant behaviour may help bridge laboratory findings and regulatory needs.

Together, these steps would extend the present study into a more comprehensive framework capable of informing ecological risk assessment and supporting temperature-explicit approaches in future ecotoxicology testing.

6. Conclusion

This thesis examined how climate-change-associated temperature increases influence the toxicity of emerging contaminants to freshwater primary producers, and whether green algae, diatoms, and cyanobacteria exhibit distinct thermal-toxicological responses. By integrating a systematic literature review with a OECD-aligned, standardised experimental framework, the study addressed key methodological limitations in multi-stressor ecotoxicology and generated controlled empirical data that isolated temperature effects from community-level interactions.

Across five contaminants representing metals, pharmaceuticals, and pesticides, toxicity patterns were primarily associated with chemical identity rather than temperature alone. Pharmaceuticals and pesticides generally exhibited greater apparent potency than metals, while copper and zinc displayed comparatively lower toxicity, consistent with reduced bioavailability and complexation in freshwater media. Contrary to the first hypothesis, moderate warming across the tested range (15, 20, and 25 °C) did not significantly alter toxicity. Instead, EC_{50} values often increased slightly with temperature or remained stable, indicating no uniform temperature-driven intensification of toxic effects. Statistical analysis confirmed that temperature explained only a small

proportion of the overall variability in toxicity and did not act as a consistent, unidirectional driver across taxa and chemicals.

These findings align with inverted V-shaped temperature–toxicity models described in the literature, in which organisms exhibit maximal tolerance near their thermal optimum. Within the moderate thermal window tested, the observed patterns suggest partial physiological compensation rather than synergistic stress. Consequently, the hypothesis that rising temperatures would alter the toxic effects of emerging contaminants on freshwater primary producers is not supported under the experimental conditions employed in this study.

In contrast, the second hypothesis (predicting interspecific differences in sensitivity) was supported. However, sensitivity patterns were chemical-specific rather than taxonomically uniform. *P. subcapitata* generally exhibited intermediate sensitivity across contaminants, with relatively high tolerance to sulfamethoxazole but lower EC_{50} values for some metals and pharmaceuticals. *A. flos-aquae* showed pronounced sensitivity to erythromycin and metazachlor, but reduced sensitivity to sulfamethoxazole at higher temperatures, where EC_{50} values were not reached. *N. palea* displayed the highest apparent tolerance for several organic contaminants, particularly at elevated temperatures, while remaining sensitive to metals. These differences are consistent with species-specific traits such as cell wall structure, physiological plasticity, and interactions between chemical mode of action and cellular architecture, rather than a simple ranking of inherent tolerance. The observed interspecific variability in sensitivity challenges regulatory approaches that rely on a limited number of representative primary producer species as indicators of overall contaminant impact. This is further supported by the variation in EC_{50} values observed among species, highlighting that reliance on a single representative primary producer may not adequately capture the range of sensitivities present within freshwater ecosystems.

By isolating species responses under rigorously standardised conditions, this study reduced confounding community-level effects that often obscure mechanistic interpretation in mesocosm or field studies. The resulting EC_{50} estimates provide a useful

temperature-explicit basis for toxicity assessment which is directly relevant to ecological risk assessment and regulatory contexts, particularly as frameworks such as the Water Framework Directive and REACH, increasingly recognise the need to account for climate-sensitive toxicity.

Overall, the findings indicate that moderate warming did not produce a consistent shift in toxicity, with variation more strongly associated with species identity and chemical type. Importantly, this should not be interpreted as reduced ecological risk. Instead, the results suggest a narrow and temperature-specific window of tolerance, beyond which further warming or extreme thermal events may shift organisms onto the declining limb of the inverted V-shaped response curve, leading to rapid increases in toxicity. As climate variability intensifies, incorporating temperature-explicit toxicity data into risk assessment frameworks will be essential for accurately predicting future impacts on freshwater primary producers.

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