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MULTIPLE STRESSOR EFFECTS OF ENVIRONMENTAL CHEMICALS IN A CHANGING CLIMATE

Sabrina Katharina Roth

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Supervisors:	Professor Alf Norkko University of Helsinki, Finland Stockholm University, Sweden
	Dr. Andreu Rico University of Valencia, Spain
Thesis advisory committee:	Professor Ulrika Candolin University of Helsinki, Finland
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Coordinating academic:	Professor Sakari Kuikka University of Helsinki, Finland
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Custos:	Professor Alf Norkko University of Helsinki, Finland Stockholm University, Sweden

Für Aurora ♥

Summary

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In the Anthropocene, dating from the beginning of significant human-induced impacts on Earth, natural ecosystems and the human population are exposed to a variety of alterations, such as those caused by anthropogenic climate change and environmental pollution. The widespread distribution of synthetic organic chemical compounds is of particular concern, as it has increased at rates that greatly exceed those of other global environmental changes. Yet, while climate change impacts are extensively studied, chemical pollution as a global change factor contributing to worldwide alterations in ecosystem functioning has received much less attention in ecosystem research so far. Importantly, despite the recognition that climate change and chemical pollution are deeply interconnected and often joint key drivers of the biodiversity crisis, the combination of their effects is rarely considered as multiple stressors in environmental research. Thus, in this thesis, I focus on the combined effects resulting from the co-occurrence of chemical pollutants and global change-induced stressors, such as extreme weather events, on ecosystem functioning across a variety of biological organizations, ranging from single species, over mixed-species communities, all the way to a human population.

As multiple stressor research focusing on climate change-chemical pollution interactions is still in its infancy, following a step-by-step approach with interdisciplinary scientific tools was imperative. Thus, at first, I commenced a literature review to identify knowledge gaps and provide recommendations for integrating climate change into multiple stressor research and chemical risk assessment at the ecosystem level (**chapter I**). Subsequent steps involved a series of laboratory experiments using heatwaves as an exemplary environmental threat of a changing climate and commonly used environmental chemicals in 2x2 full factorial experimental designs using single species and natural mixed-species communities. Specifically, in **chapter II**, I performed a controlled laboratory experiment with a single phytoplankton species for a mechanistic insight into the toxicity mode of ciprofloxacin – a common antibiotic – when simultaneously exposed to a heatwave. I progressed to a more environmentally realistic assessment in **chapter III**, using a natural mixed-species plankton community exposed to terbuthylazine – a widely-used herbicide – and a heatwave. I complete this thesis with an *in silico* modelling approach (using computer software) to explore chemical exposure pathways and their implications for human health (**chapter IV**), ultimately bridging the gap between ecological research and human health concerns.

Overall, I conclude that alterations of climate change-induced environmental conditions can have unexpected, synergistic effects on ecosystem functioning when in combination with chemical exposure. More specifically, from the scoping literature review of **chapter I**, I deduce that knowledge of the effects of combined stress through exposure to chemicals and climate change-induced environmental conditions is still scarce. Using aquatic ecosystems as a model system and heatwaves as an environmental threat of a changing climate, I showed that only ~20 % of multiple stressor studies looking at the effects of heatwaves used chemicals as a second stressor. While the combination of the two stressors

(i.e., heatwave + chemical stressor) often led to a higher sensitivity towards the chemical, mechanisms behind these observations and multitrophic species interactions were rarely explored, making it difficult to infer consistent trends. The results of **chapter II** showed that a heatwave effectively altered the mixture toxicity effect of ciprofloxacin on the Baltic Sea dinoflagellate *Apocalathium malmogiense*. While the heatwave supported growth as anticipated, the presence of ciprofloxacin at realistic exposure concentrations did not affect growth response parameters. Yet, the combination of the heatwave and ciprofloxacin resulted in a negative synergistic effect due to antibiotic-induced microbiome changes in the phycosphere, highlighting the importance of considering mechanistic interactions when studying multiple stressors, as effects may go undetected if studied in isolation only. The combination of a heatwave and chemical exposure to terbuthylazine in experiments of **chapter III** synergistically led to a complex cascade of effects throughout the food web in experiments that promoted individual species growth and altered predator-prey interactions. Such indirect effects of multiple stressor interactions can unfold through a differential sensitivity of predator and prey in a natural system and highlight the importance of performing multiple stressor experiments using multitrophic approaches under realistic conditions to comprehend their implications for ecosystem resilience. Lastly, the results of the *in silico* modelling of **chapter IV** highlight potential shifts in human exposure pathways and intake fractions of chemicals with a changing climate. Specifically, the combination of climate-induced changes in environmental conditions will significantly impact how humans are exposed to environmental chemicals, such as through drinking water, food consumption, or the indoor environment. The findings accentuate the importance of linking ecological research that explores implications for human health, for providing a more holistic perspective on the risks associated with chemical exposures under changing climate conditions.

Together, the results from **chapters I, II, III, and IV** illuminate the complex interplay between climate change-induced stressors, chemical pollutants, and ecosystem functioning. Based on the work of the various studies, I conclude this thesis by identifying critical steps for future research. Considering the increasing rate at which new chemicals are released into our environment and the pace at which climate change impacts planet Earth, this thesis shows how crucial the integration of multiple stressor effects and holistic approaches is in chemical risk assessment and management. By addressing the complexities of multiple stressor scenarios and embracing interdisciplinary collaboration – as has been shown in this thesis – future regulatory efforts can better anticipate and mitigate the adverse impacts of chemical pollutants under shifting environmental conditions inflicted by climate change.

Tiivistelmä

Tiivistelmä

Antroposeenikaudella, eli ihmiskunnan toiminnasta syntyneellä geologisella aikakaudella, ekosysteemit ja ihmiset altistuvat erilaisille stressitekijöille muun muassa ihmistoiminnasta johtuvan ilmastonmuutoksen ja ympäristön saastumisen aiheuttaminen muutosten seurauksena. Synteettisten orgaanisten kemikaalien laaja leviäminen on erityisen huolestuttavaa, sillä niiden käyttö on lisääntynyt eksponentiaalisesti ja muutos on ollut huomattavasti nopeampaa muihin globaaleihin ympäristönmuutoksiin verrattuna. Ilmastonmuutoksen vaikutuksia tutkitaan laajasti, mutta kemiallinen saastuminen globaalina muutostekijänä ja sen vaikutukset ekosysteemien toimintaan maailmanlaajuisesti ovat jääneet vähemmälle huomiolle ekosysteemitutkimuksessa. Erityisen huomionarvoista on, että tutkittua tietoa ilmastonmuutoksen ja kemiallisen saastumisen yhteisvaikutuksia on rajallisesti. Ilmastonmuutoksen ja kemiallisen saastumisen välillä tiedetään olevan merkittävä yhteys ja niiden yhteisvaikutus on usein avaintekijä useissa biologista monimuotoisuutta uhkaavissa kriiseissä. Näistä syistä johtuen tässä tutkimuksessa tarkastelen kemiallisten haitta-aineiden ja ilmastonmuutoksesta johtuvien stressitekijöiden, kuten äärimmäisten sääilmiöiden, yhteisvaikutuksia ekosysteemien toimintaan eri tasoilla, yhden- ja usean lajin kokeista ihmispopulaatio vaikutuksiin saakka.

Olemassa olevan tiedon rajallisuudesta johtuen tutkimuskysymystä ilmaston muutoksen aiheuttamien stressitekijöiden ja kemiallisen saastumisen yhteisvaikutuksista lähestyttiin vaiheittaisesti ja hyödyntäen tieteidenvälisiä menetelmiä. Väitöskirjatyöni aluksi toteutin kirjallisuuskatsauksen, jonka tavoitteena oli tunnistaa lisää tutkimusta vaativia aukkoja olemassa olevassa tutkimustiedossa, sekä ehdottaa keinoja ilmastonmuutoksen aiheuttamien stressitekijöiden sisällyttämiseksi kemikaalien haittavaikutuksia tarkastelemaan tutkimiseen ja kemikaalien riskinarviointiin ekosysteemi tasolla (**luku I**). Seuraavaksi toteutettiin sarja laboratorikokeita, joissa tutkittiin yleisesti käytössä olevien kemikaalien haittavaikutuksia yhdessä lämpöaaltoaltistuksen kanssa, jolla simuloitiin ilmastonmuutoksen todennäköisesti aiheuttamaa ympäristöuhkaa. Koeasetelmana käytettiin usean tekijän yhdistelykoetta (2x2 full factorial design) ja altistuskokeet toteutettiin sekä yhdellä lajilla että useita lajeja sisältävällä luonnollisella lajiyhteisöllä. Yksityiskohtaisemmin kuvattuna **luvussa II** toteutettiin altistuskoe kontrolloiduissa laboratorio-olosuhteissa yhdellä kasviplankton lajilla, jossa tutkittiin yleisesti käytössä olevan antibiotiinin, siprofloksasiinin, sekä samanaikaisen lämpöaaltoaltistuksen vaikutuksia. Tutkimuksen tavoitteena oli saada lisätietoa siprofloksasiinin vaikutusmekanismeista. **Luvun III** altistuskokeessa käytettiin usean kasviplanktonlajin yhdistelmää, tavoitteena mallintaa ympäristön kannalta realistisempaa altistusskenaariota. Altistuksessa käytettiin laajasti käytössä olevaa rikkakasvien torjunta-ainetta, terbutylatsiinia, sekä kuten edellä, samanaikaista lämpöaalto altistusta. Viimeisessä luvussa (**IV**) tarkastelen kemikaalien altistusreittejä ja niiden vaikutuksia ihmisten terveyteen hyödyntäen *in silico*-mallinnusta. Tuotettu tieto kuroo umpeen ekologisen tutkimustiedon ja ihmisten terveyteen liittyvien huolenaiheiden välistä kuilua.

Yleinen johtopäätökseni on, että kemikaalialtistus yhdistettynä ilmastonmuutoksen aiheuttamiin ympäristöolosuhteiden muutoksiin voi johtaa odottamattomiin, synergistisiin vaikutuksiin ekosysteemien toiminnassa. Yksityiskohtaisemmin tarkasteltuna **luvun I** laajassa kirjallisuuskatsauksessa havaitsin, että olemassa oleva tieto kemikaalialtistuksen ja ilmastonmuutoksen seurauksena muuttuneiden ympäristöolosuhteiden yhteisvaikutuksista on rajallista. Tarkastelemalla vesiekosysteemejä mallijärjestelmänä sekä lämpöaaltoja muuttuvan ilmaston ympäristöuhkana havaitsin, että monen stressitekijän tutkimuksissa, joissa toisena tekijänä oli lämpöaallot, ainoastaan ~20 % tutkimuksista toisena stressitekijänä käytettiin kemikaalialtistusta. Siitä huolimatta, että kahden stressitekijän (ts. lämpöaalto + kemikaalialtistus) yhdistelmä johti usein kohonneeseen herkkyyteen kemikaalille, vaikutusmekanismeja tai vaikutuksia eliöiden vuorovaikutussuhteisiin eri trofiatasoilla tutkittiin harvoin, jonka vuoksi yleispätevien johtopäätösten tekeminen on vaikeaa. **Luvun II** tulokset osoittivat, että lämpöaaltoaltistus vaikutti siprofloksasiinin seostoksisuuteen Itämeren panssarisiimaeliö lajilla *Apocalathium malmogiense*. Odotetusti, lämpöaaltoaltistus yksinään johti lisääntyneeseen kasvuun, ja toisaalta siprofloksasiini altistuksella yksinään ei ollut vaikutusta kasvuvasteisiin ympäristön kannalta realistisilla altistuspitoisuuksilla. Siprofloksasiinin ja lämpöaallon yhteisaltistus johti kuitenkin negatiiviseen synergistiseen vaikutukseen, joka oli seurausta antibioottialtistuksen aiheuttamasta mikrobiomin muutoksesta fykosfäärissä. Tämä havainto korostaa mekanististen vuorovaikutusten ymmärtämisen tärkeyttä useiden stressitekijöiden tutkimuksessa, sillä vaikutukset voivat jäädä havaitsematta, jos stressitekijöitä tutkitaan erikseen. **Luvun III** kokeissa lämpöaallon ja terbutylatsiini kemikaalialtistuksen yhdistelmä johti synergistisiin ja monimutkaisiin yhteisvaikutuksiin ravintoverkkokokeissa, joissa yksittäisten lajien kasvu lisääntyi ja saalis-saalistaja vuorovaikutussuhteet muuttuivat. Tällaiset epäsuorat, useiden stressitekijöiden vuorovaikutuksesta johtuvat vasteet voivat luonnon systeemeissä ilmetä saalistajien ja saaliiden erilaisen herkkyytenä. //Jotta vaikutuksia ekosysteemien sietokykyyn voidaan ymmärtää, useiden stressitekijöiden yhteisvaikutuksia on erityisen tärkeää tutkia ympäristön kannalta realistisissa altistusolosuhteissa ja käyttää useiden trofiatasojen tutkimusasetelmia. Lopuksi **luvun IV in silico**-mallinnuksen tulokset osoittavat mahdollisia muutoksia ihmisten altistusreiteissä kemikaaleille, sekä niiden saantiosuoksissa ilmaston muuttuessa. Erityisesti useat samanaikaiset ilmastonmuutoksesta johtuvat muutokset ympäristössä vaikuttavat merkittävästi siihen, miten ihmiset altistuvat ympäristön kemikaaleille, kuten juomaveden, ruuan ja sisäilman välityksellä. Tutkimuksen tulokset korostavat ekologisen tutkimuksen soveltamisen tärkeyttä ihmisten terveyteen liittyvissä kysymyksissä, jotta voidaan saavuttaa kokonaisvaltaisempi käsitys kemikaalialtistukseen liittyvistä riskeistä muuttuvissa ilmasto-olosuhteissa.

Yhteenvedon **lukuja I, II, III ja IV** tulokset havainnollistavat ilmastonmuutoksen aiheuttamien stressitekijöiden, kemikaalialtistuksen ja ekosysteemien toiminnan välisiä moniulotteisia yhteyksiä. Tutkimuksen tulosten pohjalta nostan esiin havaitsemiani kriittisiä askelia tulevia tutkimuksia varten. Ottaen huomioon ympäristöön vapautuvien uusien kemikaalien jatkuvasti kasvavan määrän sekä ilmastonmuutoksen aiheuttamien muutosten

vauhdin on ilmeistä, että useiden stressitekijöiden yhteisvaikutusten arvioiminen sekä kokonaisvaltainen lähestymistapa kemikaalien riskinarvioinnissa ja -hallinnassa ovat välttämättömiä. Kuten tässä tutkimuksessa on osoitettu, ottamalla huomioon useiden stressitekijöiden vaikutukset ja tekemällä tieteidenvälistä yhteistyötä tuotetaan tietoa, jonka avulla kemikaalien haittavaikutuksia voidaan paremmin ennakoida ja ehkäistä muuttuvissa ympäristöolosuhteissa ilmastonmuutoksen aiheuttamien paineiden alla.

Sammanfattning

Sammanfattning

Sedan början av Antropocen, då mänsklig påverkan började få betydande effekter på jorden, har naturliga ekosystem och den mänskliga befolkningen utsatts för en mängd olika förändringar. Dessa innefattar av människan orsakade klimatförändringar och miljöföroreningar. Särskilt oroande är den utbredda spridningen av syntetiska organiska kemiska föreningar, då dessa sprids med en hastighet som avsevärt överstiger andra globala miljöförändringar. Samtidigt, medan klimatförändringarnas effekter studeras noga, har effekterna som kemiska föroreningar orsakar hos ekosystems funktion globalt hittills fått mycket mindre uppmärksamhet i ekosystemforskningen. I synnerhet, trots att det är känt att klimatförändringar och kemiska föroreningar är sammankopplade och ofta gemensamma nyckelfaktorer bakom förlusten av biologisk mångfald, betraktas kombinationen av deras effekter sällan som flera stressfaktorer inom miljöforskning. Därför fokuserar jag i den här avhandlingen på hur kombinerade effekter av kemiska föroreningar och stressfaktorer orsakade av global förändring, såsom extrema väderhändelser, påverkar funktionen av ekosystem. Studierna har bedrivits på olika biologiska nivåer, från enskilda arter till samhällen med blandade arter, och slutligen till grupper av människor.

Eftersom forskning på interaktioner mellan klimatförändringar och kemiska föroreningar fortfarande är i sin linda, var det nödvändigt att tillämpa en steg-för-steg-strategi med tvärvetenskapliga verktyg. Därför började jag med en litteraturgranskning för att identifiera kunskapsluckor och ge rekommendationer för att integrera klimatförändringar i forskning om flera stressfaktorer och kemisk riskbedömning på ekosystemnivå (**kapitel I**). Efterföljande steg involverade en serie laboratorieexperiment där effekten av värmeböljor, som representation för miljöhot i ett förändrat klimat, studerades tillsammans med vanliga miljökemikalier i 2x2 fullfaktoriella experiment. Här användes både enskilda arter och naturliga artsamhällen. Specifikt, i **kapitel II**, utförde jag ett kontrollerat laboratorieexperiment med en enda växtplanktonart för att nå mekanistisk insikt i hur giftigheten hos ciprofloxacin – ett vanligt antibiotikum – tar sig i uttryck när subjektet samtidigt exponeras för en värmebölja. Jag gick därefter vidare till en mer miljömässigt realistisk bedömning i **kapitel III**, där jag studerade ett naturligt planktonsamhälle som exponerats för terbutylazin – en allmänt utbredd herbicid – under värmebölja. Jag avslutar denna avhandling med att tillämpa in silico-modellering för att utforska kemiska exponeringsvägar och deras konsekvenser för människors hälsa (**kapitel IV**), vilket kopplar dessa frågor till ekologisk forskning.

Sammantaget drar jag slutsatsen att förändringar av miljöförhållanden i kombination med kemisk exponering kan ha oväntade synergistiska effekter på ekosystem. Mer specifikt, från den omfattande litteraturgranskningen i **kapitel I**, drar jag slutsatsen att kunskapen om effekterna av kombinerad stress från kemikalieexponering och förändrade miljöförhållanden fortfarande är knapp. Genom att använda akvatiska ekosystem som modellsystem och värmeböljor som ett miljöhot i ett förändrat klimat, visade jag att endast ~20 % av studierna med flera stressfaktorer som studerat effekterna av värmeböljor använde kemikalier som en andra stressfaktor. Medan kombinationen av de två stressfaktorerna (dvs värmebölja + kemikalier) ofta ledde till en högre känslighet för kemikalien, undersöktes mekanismerna bakom dessa observationer och multitrofiska artinteraktioner sällan. Detta gjorde det svårt att urskilja konsekventa trender. Resultaten av **kapitel II** visade att en värmebölja effektivt förändrade kombinationstoxiciteten av ciprofloxacin på Östersjödinoflagellaten *Apocalathium malmogiense*. Som förväntat bidrog värmeböljan till

tillväxt, som inte påverkades av ciprofloxacin vid exponering av realistiska koncentrationer. Däremot resulterade kombinationen av värmeböljan och ciprofloxacin i en negativ synergistisk effekt på grund av antibiotika-inducerade mikrobiomförändringar i fykosfären. Dessa resultat understryker vikten av att ta hänsyn till mekanistiska interaktioner när man studerar flera stressfaktorer, eftersom effekterna kan förbli oupptäckta om dessa faktorer enbart studeras en i taget. Kombinationen av en värmebölja och kemisk exponering för terbutylazin i experiment i **kapitel III** ledde till en komplex kaskad av synergistiska effekter genom hela näringsväven i experiment som främjade individuella arters tillväxt och förändrade interaktioner mellan rovdjur och bytesdjur. Sådana indirekta effekter av interaktionen mellan multipla stressfaktorer kan utvecklas genom en skillnad i känslighet mellan rovdjur och bytesdjur i ett naturligt system. Detta belyser vikten av att utföra experiment med multipla stressfaktorer med multitrofiska artsamhällen under realistiska förhållanden för att förstå konsekvenserna av stressfaktorerna för ekosystemets motståndskraft. Slutligen belyser resultaten av modelleringen i **kapitel IV** potentiella förändringar i mänskliga exponeringsvägar och intag av kemikalier i ett förändrat klimat. I synnerhet kommer förändringar i miljöförhållanden att avsevärt påverka hur människor exponeras för kemikalier såsom genom dricksvatten, matkonsumtion eller inomhusmiljön. Resultaten accentuerar vikten av att koppla samman ekologisk forskning som utforskar konsekvenser för människors hälsa, för att ge ett mer holistiskt perspektiv på riskerna förknippade med kemisk exponering under förändrade klimatförhållanden.

Tillsammans belyser resultaten från **kapitlen I, II, III** och **IV** det komplexa samspelet mellan stressfaktorer inducerade av klimatförändring, kemiska föroreningar och ekosystemfunktioner. Utifrån arbetet i de olika studierna avslutar jag denna avhandling med att identifiera kritiska steg för framtida forskning. Med tanke på den ökande hastigheten med vilken nya kemikalier släpps ut i vår miljö och den takt med vilken klimatförändringarna påverkar planeten, visar denna avhandling hur avgörande det är att integrera effekten av multipla stressfaktorer och holistiska tillvägagångssätt i kemisk riskbedömning och hantering. Genom att ta hänsyn till komplexiteten i scenarier med flera stressfaktorer och anamma tvärvetenskapligt samarbete – som påvisats i denna avhandling – kan framtida regleringsinsatser bättre förutse och mildra de negativa effekterna av kemiska föroreningar under förändrade miljöförhållanden.

Zusammenfassung

Zusammenfassung

Im Anthropozän, das mit dem Beginn erheblicher vom Menschen verursachter Eingriffe auf die Erde beginnt, sind Ökosysteme und die menschliche Bevölkerung vielfältigen Veränderungen ausgesetzt, beispielsweise durch den menschenverursachten Klimawandel und Umweltverschmutzung. Besonders besorgniserregend ist die weite Verbreitung synthetischer organischer chemischer Verbindungen, da diese mit einer Geschwindigkeit zugenommen hat, die die anderer globaler Umweltveränderungen bei weitem übersteigt. Während die Auswirkungen des Klimawandels bereits umfassend untersucht werden, hat die chemische Verschmutzung als globaler Veränderungsfaktor, der zu weltweiten Veränderungen der Ökosystemfunktion beiträgt, in der Ökosystemforschung bisher viel weniger Aufmerksamkeit erhalten. Obwohl der Klimawandel und chemische Verschmutzung eng miteinander verbunden sind und oft gemeinsame Hauptursachen für die Biodiversitätskrise sind, wird die Kombination ihrer Auswirkungen in der Umweltforschung selten als Mehrfachstressoren betrachtet. Daher konzentriere ich mich in dieser Dissertation auf die kombinierten Auswirkungen, die sich aus dem gleichzeitigen Auftreten chemischer Schadstoffe und durch globale Veränderungen verursachter Stressfaktoren, wie z. B. extremer Wetterereignisse, auf die Ökosystemfunktion in einer Vielzahl biologischer Organisationen ergeben, die von einzelnen Arten über gemischte Gemeinschaften bis hin zu einer menschlichen Population reichen.

Da die Forschung zu mehreren Stressoren, die sich auf die Wechselwirkungen zwischen Klimawandel und chemischer Verschmutzung konzentriert, noch in den Anfängen steckt, war ein schrittweiser Ansatz mit interdisziplinären wissenschaftlichen Instrumenten unerlässlich. Daher begann ich zunächst mit einer Literaturrecherche, um Wissenslücken zu identifizieren und Empfehlungen für die Integration des Klimawandels in die Forschung zu mehreren Stressoren und die Bewertung chemischer Risiken auf Ökosystemebene zu geben (**Kapitel I**). Nachfolgende Schritte umfassten eine Reihe von Laborexperimenten, bei denen Hitzewellen als beispielhafte Umweltbedrohung eines sich ändernden Klimas und häufig verwendete Umweltchemikalien in 2x2 vollfaktoriellen Versuchsplänen mit einzelnen Arten und natürlichen Gemeinschaften gemischter Arten zum Einsatz kamen. Insbesondere habe ich in **Kapitel II** ein kontrolliertes Laborexperiment mit einer einzelnen Phytoplanktonart durchgeführt, um einen mechanistischen Einblick in die Toxizitätsart von Ciprofloxacin – einem häufigen Antibiotikum – bei gleichzeitiger Exposition gegenüber einer Hitzewelle zu erhalten. In **Kapitel III** bin ich zu einer umweltrealistischeren Bewertung übergegangen, bei der ich eine natürliche Planktongemeinschaft gemischter Arten herangezogen habe, die Terbuthylazin – einem weit verbreiteten Herbizid – und einer Hitzewelle ausgesetzt war. Ich schließe diese Arbeit mit einem *In-silico*-Modellierungsansatz ab, um chemische Expositionspfade und ihre Auswirkungen auf die menschliche Gesundheit zu untersuchen (**Kapitel IV**), und schließe letztendlich die Lücke zwischen ökologischer Forschung und Bedenken hinsichtlich der menschlichen Gesundheit.

Insgesamt komme ich zu dem Schluss, dass Veränderungen der durch den Klimawandel verursachten Umweltbedingungen in Kombination mit der Exposition gegenüber Chemikalien unerwartete, synergistische Auswirkungen auf die Ökosystemfunktion haben können. Genauer gesagt schließe ich aus der umfassenden Literaturübersicht in **Kapitel I**,

dass das Wissen über die Auswirkungen von kombiniertem Stress durch die Exposition gegenüber Chemikalien und durch den Klimawandel verursachten Umweltbedingungen immer noch lückenhaft ist. Unter Verwendung aquatischer Ökosysteme als Modellsystem und Hitzewellen als Umweltbedrohung eines sich verändernden Klimas konnte ich zeigen, dass nur etwa 20 % der Studien zu mehreren Stressoren, die sich mit den Auswirkungen von Hitzewellen befassten, Chemikalien als zweiten Stressor verwendeten. Während die Kombination der beiden Stressoren (d. h. Hitzewelle + chemischer Stressor) häufig zu einer höheren Empfindlichkeit gegenüber der Chemikalie führte, wurden die Mechanismen hinter diesen Beobachtungen und den Wechselwirkungen zwischen multitrophen Arten selten erforscht, was es schwierig machte, konsistente Trends abzuleiten. Die Ergebnisse von **Kapitel II** zeigten, dass eine Hitzewelle die gemischttoxische Wirkung von Ciprofloxacin auf den Ostseedinoflagellaten *Apocalathium malmogiense* wirksam veränderte. Während die Hitzewelle das Wachstum wie erwartet unterstützte, hatte das Vorhandensein von Ciprofloxacin in realistischen Expositionskonzentrationen keinen Einfluss auf die Parameter der Wachstumsreaktion. Die Kombination aus Hitzewelle und Ciprofloxacin führte jedoch zu einem negativen synergistischen Effekt aufgrund von Antibiotika-induzierten Mikrobiomveränderungen in der Phykosphäre, was die Bedeutung der Berücksichtigung mechanistischer Wechselwirkungen bei der Untersuchung mehrerer Stressfaktoren unterstreicht, da die Auswirkungen möglicherweise unentdeckt bleiben, wenn sie nur isoliert untersucht werden. Die Kombination aus Hitzewelle und chemischer Exposition gegenüber Terbuthylazin in den Experimenten von **Kapitel III** führte in Experimenten synergistisch zu einer komplexen Wirkungskaskade im gesamten Nahrungsnetz, die das Wachstum einzelner Arten förderte und die Interaktionen zwischen Raubtieren und Beutetieren veränderte. Solche indirekten Auswirkungen mehrerer Stressor-Interaktionen können sich durch eine unterschiedliche Empfindlichkeit von Räubern und Beutetieren in einem natürlichen System entfalten und verdeutlichen die Bedeutung der Durchführung von Experimenten mit mehreren Stressoren unter Verwendung multitrophischer Ansätze unter realistischen Bedingungen, um ihre Auswirkungen auf die Widerstandsfähigkeit des Ökosystems zu verstehen. Zuletzt verdeutlichen die Ergebnisse der *In-silico*-Modellierung von **Kapitel IV** mögliche Verschiebungen der Expositionspfade des Menschen und der Aufnahme fraktionen von Chemikalien bei einem sich ändernden Klima. Insbesondere wird die Kombination klimabedingter Veränderungen der Umweltbedingungen erhebliche Auswirkungen darauf haben, wie Menschen Umweltchemikalien ausgesetzt sind, beispielsweise durch Trinkwasser, Lebensmittelkonsum oder das Raumklima. Die Ergebnisse unterstreichen die Bedeutung der Verknüpfung ökologischer Forschung, die Auswirkungen auf die menschliche Gesundheit untersucht, um eine ganzheitlichere Perspektive auf die Risiken zu bieten, die mit der Exposition gegenüber Chemikalien unter sich ändernden Klimabedingungen verbunden sind.

Zusammengefasst beleuchten die Ergebnisse aus den **Kapiteln I, II, III und IV** das komplexe Zusammenspiel zwischen durch den Klimawandel verursachten Stressfaktoren, chemischen Schadstoffen und der Funktionsweise des Ökosystems. Basierend auf den Ergebnissen der verschiedenen Studien schließe ich diese Dissertation ab, indem ich kritische Schritte für zukünftige Forschung identifiziere. Angesichts der zunehmenden Geschwindigkeit, mit der neue Chemikalien in unsere Umwelt freigesetzt werden, und der Geschwindigkeit, mit der sich der Klimawandel auf den Planeten Erde auswirkt, zeigt diese Dissertation, wie wichtig die Integration mehrerer Stressoreffekte und ganzheitlicher Ansätze bei der Bewertung und dem Management chemischer Risiken ist. Durch die

Auseinandersetzung mit der Komplexität mehrerer Stressorszenarien und die Einbeziehung interdisziplinärer Zusammenarbeit – wie in dieser Doktorarbeit gezeigt wurde – können zukünftige Regulierungsbemühungen die negativen Auswirkungen chemischer Schadstoffe unter sich verändernden Umweltbedingungen, die durch den Klimawandel verursacht werden, besser antizipieren und abmildern.

Resumen

Resumen

En el Antropoceno, cuya era comenzó tras la evidencia de importantes impactos inducidos por el hombre en la Tierra, los ecosistemas naturales y la población humana están expuestos a una variedad de alteraciones, como las causadas por el cambio climático antropogénico y la contaminación ambiental. La distribución generalizada de compuestos químicos orgánicos sintéticos es motivo de especial preocupación, ya que ha aumentado a un ritmo que supera con creces el de otros cambios ambientales globales. Sin embargo, si bien los impactos del cambio climático se estudian ampliamente, la contaminación química como factor de cambio global que contribuye a las alteraciones mundiales en el funcionamiento de los ecosistemas ha recibido mucha menos atención hasta ahora. Es importante destacar que, a pesar del reconocimiento de que el cambio climático y la contaminación química están profundamente interconectados y, a menudo, son factores clave para la crisis de la biodiversidad, los efectos combinados de estos han sido raramente estudiados. Por tanto, en esta tesis, me centro en evaluar los efectos combinados causados por la contaminación química y los factores estresantes inducidos por el cambio global, como eventos climáticos extremos, en el funcionamiento de los ecosistemas. Esta tesis incluye la evaluación de efectos en diferentes niveles de organización biológica, que van desde especies individuales hasta comunidades, incluyendo la población humana.

Como la investigación de los factores de estrés múltiple que consideran la interacción entre el cambio climático y la contaminación química aún está en sus inicios, era importante seguir un enfoque paso a paso con herramientas científicas interdisciplinarias. Por lo tanto, al principio comencé el trabajo con una revisión de la literatura para identificar lagunas de conocimiento y brindar recomendaciones para integrar el cambio climático en la investigación de los factores de estrés múltiple y la evaluación de riesgos químicos a nivel de ecosistema (**capítulo I**). Los pasos posteriores implicaron una serie de experimentos de laboratorio que utilizaron olas de calor como una amenaza ambiental ejemplar de un clima cambiante y productos químicos de uso común en diseños experimentales factoriales completos (2x2) utilizando especies únicas y comunidades naturales. Específicamente, en el **capítulo II**, realicé un experimento de laboratorio controlado con una sola especie de fitoplancton para estudiar la acción tóxica de la ciprofloxacina (un antibiótico común) cuando se expone simultáneamente a una ola de calor. En el **capítulo III** pasé a realizar una evaluación ambientalmente más realista, utilizando una comunidad natural de plancton expuesta a terbutilazina (un herbicida ampliamente utilizado) y una ola de calor. Completo esta tesis con un enfoque de modelado *in silico* para explorar las vías de exposición a sustancias químicas y sus implicaciones para la salud humana (**capítulo IV**), y en última instancia cerrar la brecha entre la investigación ecológica y las preocupaciones sobre la salud humana.

En general, llego a la conclusión de que las alteraciones de las condiciones ambientales inducidas por el cambio climático pueden tener efectos sinérgicos inesperados en el funcionamiento de los ecosistemas cuando se combinan con la exposición química. Más específicamente, de la revisión de la literatura del **capítulo I**, deduzco que el conocimiento de los efectos del estrés combinado a través de la exposición a sustancias químicas y las condiciones ambientales inducidas por el cambio climático aún es escaso. Utilizando

ecosistemas acuáticos como sistema modelo y olas de calor como amenaza ambiental de un clima cambiante, demostré que solo ~20 % de los estudios de múltiples factores estresantes que analizaban los efectos de las olas de calor utilizaban productos químicos como segundo factor estresante. Si bien la combinación de los dos factores estresantes (es decir, ola de calor + factor estresante químico) a menudo condujo a una mayor sensibilidad hacia el químico, los mecanismos detrás de estas observaciones y las interacciones de especies multitróficas rara vez se exploraron, lo que dificulta inferir tendencias consistentes. Los resultados del **capítulo II** mostraron que una ola de calor alteró efectivamente el efecto de toxicidad de la mezcla de ciprofloxacino en el dinoflagelado *Apocalathium malmogiense* del Mar Báltico. La ola de calor apoyó el crecimiento como se esperaba, mientras que la presencia de ciprofloxacina en concentraciones realistas no afectó los parámetros de respuesta del crecimiento. Sin embargo, la combinación de la ola de calor y la ciprofloxacina resultó en un efecto sinérgico negativo debido a los cambios del microbioma inducidos por los antibióticos en la picosfera, lo que resalta la importancia de considerar diferentes factores de estrés a la vez, ya que los efectos pueden pasar desapercibidos si se estudian de forma aislada únicamente. La combinación de una ola de calor y la exposición química a la terbutilazina en los experimentos del **capítulo III** condujo sinérgicamente a una cascada de efectos en toda la cadena alimentaria, alterando las interacciones depredador-presa. Estos efectos indirectos pueden manifestarse a través de una sensibilidad diferencial entre depredadores y presas en un sistema natural y resaltan la importancia de realizar experimentos con factores de estrés múltiple utilizando enfoques multitróficos en condiciones realistas para comprender sus implicaciones sobre la resiliencia de los ecosistemas. Por último, los resultados de la modelización *in silico* del **capítulo IV** ponen de relieve posibles cambios en las vías de exposición humana y las fracciones de ingesta de sustancias químicas en un escenario de clima cambiante. Específicamente, la combinación de cambios en las condiciones ambientales inducidos por el clima afectará significativamente la forma en que los humanos están expuestos a las sustancias químicas ambientales, como a través del agua potable, el consumo de alimentos o el ambiente interior. Los hallazgos acentúan la importancia de vincular los impactos sobre procesos ecológicos y sus implicaciones para la salud humana, con el fin de proporcionar una perspectiva más holística sobre los riesgos asociados con la exposición a sustancias químicas en condiciones climáticas cambiantes.

En conjunto, los resultados de los **capítulos I, II, III y IV** destacan la compleja interacción entre los factores de estrés múltiple inducidos por el cambio climático, los contaminantes químicos, y el funcionamiento de los ecosistemas. Con base en el trabajo de los diversos estudios, concluyo esta tesis identificando pasos críticos para futuras investigaciones. Teniendo en cuenta el ritmo cada vez mayor al que se liberan nuevas sustancias químicas en nuestro medio ambiente y el ritmo al que el cambio climático afecta al planeta Tierra, esta tesis muestra cuán crucial es la integración de factores de estrés múltiple y enfoques holísticos en la evaluación y gestión de los riesgos de la contaminación. Al abordar la complejidad de los diferentes escenarios, abordando una colaboración interdisciplinaria, se garantiza que los esfuerzos regulatorios futuros puedan anticipar y mitigar mejor los impactos adversos de los contaminantes químicos bajo las condiciones ambientales cambiantes infligidas por el cambio climático.

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List of abbreviations

<i>A. malmogiense</i>	Baltic Sea dinoflagellate <i>Apocalathium malmogiense</i>
<i>Chl-a</i>	chlorophyll-a
CIP	ciprofloxacin treatment
dbRDA	distance-based Redundancy Analysis
DDT	organochlorine dichlorodiphenyltrichloroethane
e.g.	Latin: “exempli gratia” – meaning “for example”
FAO	Food and Agriculture Organization of the United Nations
HW	heatwave treatment
HW+CIP	the combined treatment of a heatwave and ciprofloxacin
i.e.	Latin: “id est” – meaning “that is to say”
<i>in silico</i>	conducted by means of computer modelling or computer simulation
IUCN	International Union for Conservation of Nature
IQR	Interquartile range
L	Liter
LMMs	linear mixed models
NH ₄ ⁺	Ammonium
NO ₃ ⁻	Nitrate
NO ₂ ⁻	Nitrogen dioxide
PAH(s)	polycyclic aromatic hydrocarbon(s)
PBDEs	polybrominated diphenylethers
PCA	Principal Component Analysis
PCB(s)	polychlorinated biphenyl(s)
PE	phycoerythrin
PFAS	per- and polyfluoroalkyl substances
REACH	the European Union regulation on the registration, evaluation, authorisation and restriction of chemicals
sp.	species (singular)
spp.	species (plural)
SSPs	Shared Socioeconomic Pathways
TER	terbuthylazine treatment
UNEP	United Nation Environment Program
USEtox® 2.0	Scientific consensus model
UV	ultraviolet
WMA	World Meteorological Association
µg	micro gram
µm	micro meter

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List of original publications

Publication I (chapter I)

Francesco Polazzo, [Sabrina K Roth](#), Markus Hermann, Annika Mangold-Döring, Andreu Rico, Anna Sobek, Paul J Van den Brink, Michelle C Jackson, 2021. Combined effects of heatwaves and micropollutants on freshwater ecosystems: Towards an integrated assessment of extreme events in multiple stressors research. *Global change biology*, 28(4), pp.1248-1267.

Publication II (chapter II)

[Sabrina K Roth](#), Catharina Uth, Iris Orizar, Andreu Rico, Per Hedberg, Alf Norkko, and Aleksandra Lewandowska. Synergistic effects of the antibiotic ciprofloxacin and a simulated heatwave on the Baltic Sea dinoflagellate *Apocalathium malmogiense*. *Manuscript submitted to Marine Pollution Bulletin*

Publication III (chapter III)

[Sabrina K Roth](#), Francesco Polazzo, Ariadna García-Astillero, Laura Cherta, Anna Sobek, Andreu Rico, 2022. Multiple stressor effects of a heatwave and a herbicide on zooplankton communities: Implications of global climate change. *Frontiers in Environmental Science* (2022):2212.

Publication IV (chapter IV)

[Sabrina K Roth](#), John D Hader, Prado Domercq, Anna Sobek, Matthew MacLeod, 2023. Scenario-based modelling of changes in chemical intake fraction in Sweden and the Baltic Sea under global change. *Science of the Total Environment* (2023):164247

Author contributions to individual publications

Publication I

Conceived and designed the study in collaboration with co-authors; performed parts of the literature review, extracted critical information from identified literature and data evaluation in collaboration with other leading co-authors; produced majority of figures; supported first author in leading the writing of the manuscript.

Publication II

Conceived and designed the study in collaboration with co-authors; designed the laboratory set-up in collaboration with co-authors; planned and coordinated the experiment; performed the experiment and the phytoplankton sample counting; processed the data; prepared all figures and tables; took the lead in writing the manuscript.

Publication III

Conceived and designed the study in collaboration with co-authors; designed the laboratory set-up in collaboration with co-authors; planned and coordinated the experiment; performed the experiment and the majority of zooplankton sample counting; processed the data; prepared all figures and tables; took the lead in writing the manuscript.

Publication IV

Structured and planned the study in collaboration with co-authors; reviewed the literature and available monitoring data; extracted critical information from identified literature; generated socio-economic pathways in collaboration with co-authors; reviewed available data and generated data set of background data to be used for the model in collaboration with second author; analysed model output in collaboration with second author; took the lead in writing the manuscript.

Other publications resulting from the doctoral work

Sabrina K Roth, Prado Domercq, Anna Sobek & Matthew MacLeod, 2022. Human exposure to chemicals in Sweden in a changing climate. Commissioned report for Naturvårdsverket (Swedish Environmental Protection Agency)

Samuel A. Welch, Taylor Lane, Alizée OS Desrousseaux, Joanke van Dijk, Annika Mangold-Döring, Rudrani Gajraj, John D Hader, Markus Hermann, Anju Ayillyath Kutteyeri Parvathi, Sophie Mentzel, Poornima Nagesh, Francesco Polazzo, Sabrina K Roth, Alistair BA Boxall, Benny Chefetz, Stefan C Dekker, Josef Eitzinger, Merete Grung, Matthew MacLeod, S Jannicke Moe, Andreu Rico, Anna Sobek, Annemarie P van Wezel, Paul van den Brink, 2022. ECORISK2050: An Innovative Training Network for predicting the effects of global change on the emission, fate, effects, and risks of chemicals in aquatic ecosystems. *Open Research Europe* (2022), 1.

1 | General introduction

1

Driven by past and current anthropogenic behaviour and actions, global change has affected the environment of Earth in a myriad of ways. The effects of global change impact nature, its ecology, ecosystem functioning (Cardinale *et al.* 2012), and, ultimately, humans (Schlesinger 2006). The epoch of the Anthropocene, dating from the beginning of significant anthropogenic impacts on Earth, is characterized, among others, by human-induced climate change, biodiversity loss, and environmental pollution (Lewis & Maslin 2015). Climate change is among the most discussed drivers of the overarching phenomena of global change (e.g., Brook *et al.* 2008; Moran & Alexander 2014) and is considered the most disturbing for natural ecosystems and the human population (Parry *et al.* 2001). Together with land/sea-use change, anthropogenic climate change is also one of the primary drivers responsible for biodiversity loss worldwide (European Commission 2020). The widespread distribution of synthetic organic chemical compounds throughout the past decades is another significant component of global change (Vitousek 1994) and has increased at rates that greatly exceed those of other global environmental changes (Bernhardt *et al.* 2017). Yet, while climate change and biodiversity are often studied together, chemical pollution as a global change factor contributing to worldwide alterations in ecosystem functioning has received much less attention in ecosystem research so far (Sigmund *et al.* 2023). Yet, in this regard, the United Nation Environment Program (UNEP) framed the most pressing societal challenges recently as: “Three interconnected planetary crises: climate change, biodiversity loss and pollution are putting global economic and social well-being at risk” (The United Nations Environment Programme 2021). An UNEP expert panel on chemical pollution has just recently been established (United Nations Environment Programme 2024). Yet, despite the recognition that climate change and chemical pollution are deeply interconnected and often joint key drivers of the biodiversity crisis, the combination of their effects is rarely considered as multiple stressors in environmental research. Thus, in this thesis, I focus specifically on exploring the multiple stressor connections between climate change, chemical pollution, and ecosystem functioning.

1.1 Chemicals in the environment

At the end of the nineteenth century, with the advance of the industrialisation came the production and spread of a variety of chemical substances used for food, cosmetics, paints, textiles, and many other consumer products. Today, both natural and manufactured synthetic chemical compounds are present in every aspect of our lives (European Environment Agency 2023). For instance, polycyclic aromatic hydrocarbons (PAHs) are released through incomplete combustion of fossil fuels or other organic material or liberated from petroleum products (e.g., petroleum spills or oil leakages) (Manahan 2022). Several industrial products, such as crude oil, coal, and roofing tar, but also consumer products, such as dyes, plastics, and pesticides, are sources of PAHs (Franck & Stadelhofer 1988). Another compound class, polychlorinated biphenyls (PCBs), is unintentionally formed by the joint combustion with chlorine and served as non-flammable and heat-resistant by-products in the colour pigment or chlorinated paraffins production (Cairns & Siegmund 1981; Hu &

Hornbuckle 2010; Takasuga *et al.* 2013). Metal industries and waste incineration are the main sources of dioxins (Dopico & Gómez 2015). Industrially produced pesticides were introduced to secure the food supply, while organic chemical compounds, like organochlorine dichlorodiphenyltrichloroethane (DDT), became popular as insecticides effective for plant pests and human parasites (Matthews 2018). Despite a ban on those legacy pollutants, such as DDT, most can still be found in the environment (e.g., Padayachee *et al.* 2023). To date, just the European Union produces more than 200 million tons of chemicals per year that are considered hazardous to the environment (Eurostat 2024), and more than 22.000 different chemicals are registered under REACH – the European Union regulation on the registration, evaluation, authorisation, and restriction of chemicals (ECHA 2023).

Importantly, chemical compounds released into the environment and exposure to even small amounts of certain chemicals can impair the health and functioning of the receiving organisms and ecosystem.

Comprehending the diverse array of chemicals present in the environment, thus, becomes an essential first step for devising effective strategies to mitigate their impact and preserve ecosystem functioning.

1.1.1 Environmental contaminants in aquatic systems

Depending on their properties, modes, and extent of use, many of these chemical substances are mobilized through air, water, or soil and accumulate in the environment or in biota. Exploring the intricate dynamics of environmental contamination reveals a profound impact, particularly on aquatic systems. Factors such as direct discharge into receiving water bodies, the solubility of chemicals in the water phase, and the interconnectedness with terrestrial systems make aquatic ecosystems highly susceptible to the effects of chemical pollutants. Yet, aquatic resources (inland water bodies such as ponds, lakes, rivers, and streams, as well as the coastal seas and oceans) are especially precious natural assets providing many ecosystem services, including their associated fisheries and food supply for humanity.

Most chemicals reach aquatic systems via direct discharges of industries, improper disposal of waste, or agricultural runoff to rivers, lakes, groundwater, or the coastal seas (United Nations 2003; Schwarzenbach *et al.* 2006). For example, 140 million tons of fertilizers and several million tons of pesticides form the pollution from agriculture runoff (Food and Agriculture Organization of the United Nations (FAO) 2006). Pesticides are a major group of toxic compounds that inflict substantial impacts on aquatic environments and are linked to human exposure, as low concentrations of pesticides in water can get magnified through the food chain (Ellgehausen *et al.* 1980; Helfrich *et al.* 2009). Biocides and resulting persistent metabolites often have toxic effects on non-targeted biota, such as

aquatic primary producers (Iwata *et al.* 1994; Solomon *et al.* 1996; Bignert *et al.* 1998; Lindström *et al.* 2002). Pharmaceuticals (including antibiotics) originating from human activities enter the aquatic environment mainly via wastewater discharges (Daughton 2016). Thereby, the increasing contamination of the environment with pharmaceuticals is not only due to their increasing consumption but also results from inefficiencies in the removal of these compounds in wastewater treatment plants (Fatta-Kassinos *et al.* 2011). Especially pharmaceuticals (Kolpin *et al.* 2002) and personal care products (Daughton & Ternes 1999) pose a risk for the aquatic environment, leading to, e.g., bacterial resistance and a multitude of (partially unknown) side effects. The extremely persistent compound group of per- and polyfluoroalkyl substances (PFAS) are formed in industrial processes and get transported to the aquatic system via, e.g., wastewater release into the environment (Spaan *et al.* 2023). PFAS are harmful for the human and environmental health and are occurring more frequently and in increasing concentrations in the environment (Brunn *et al.* 2023). Microplastics and their additives can be found nearly everywhere in the aquatic environment and on agricultural soils (Schell *et al.* 2020, 2022). Additional sources of chemical pollution in aquatic systems originate from accidental oil and gasoline spills or human-driven mobilization of heavy metals and metalloids (Schwarzenbach *et al.* 2006). Deposition of household and industrial waste (e.g., solvents, petrochemicals, intermediates) (ECETOC 1999) with subsequent runoff from landfills to the adjacent environment leads to drinking-water contamination via phthalates, PCBs, or polybrominated diphenylethers (PBDEs) (Abiriga *et al.* 2020). Further, volatile chemical compounds released via production processes or from waste combustion are transported over long ranges via the atmosphere (Lei *et al.* 2021) and contribute substantially to air pollution (Nault *et al.* 2021), ultimately entering surface waters through dry and wet deposition (Shi *et al.* 2021). Many chemicals are also subjected to numerous chemical, physical, and biological processes that further define the fate of a compound in general. Depending on a chemical compound's physiochemical properties and environmental conditions, a chemical compound can either remain as a whole ('parent' substance), meaning its structure remains unchanged, or it can be transformed into new compounds. Through environmental processes, such as precipitation, wind, ultraviolet (UV) radiation, or temperature, compounds are transported, or a compound's structure can be altered. Other compounds do not degrade at all, like heavy metals, or degrade only very slowly, e.g., persistent organic pollutants such as PCBs or DDT. Subsequently, the fate of a compound depends on its physiochemical properties, leading to a transfer between various phases or compartments, e.g., the exchange between water and air or sediment and water, adsorption to sediments or uptake in biota (Schwarzenbach *et al.* 2003). Importantly, a high proportion of synthetic chemicals, or their transformation products, are water-soluble, allowing them to be easily transported across aquatic environments and facilitating their dispersion through rivers, lakes, and the ocean. Hydrophobic contaminants released into aquatic systems adhere to particles, causing an accumulation in sediments and posing a long-term risk, as they can be released back into the water or ingested by sediment-dwelling organisms and, thus, transferred through the food web.

As a result of this multifold pollution, aquatic environments have deteriorated substantially due to the accumulation of toxic inorganic and organic pollutants that cause potentially adverse, widespread, and detrimental effects to the ecosystem and, ultimately, human health.

1.2 Multiple stressor effects

In the Anthropocene, however, ecosystems face a variety of environmental and anthropogenic stressors acting simultaneously (Simmons *et al.* 2021). The consequences of environmental changes and the occurrence of numerous pollutants in the environment can basically lead to countless possible combinations of multiple stressors for the environment and its inhabitants. Yet, traditionally, scientists have studied single stressors and their effects on single (model) organisms in isolation only. For example, if a new chemical is to be released, screening tests are mandatory for the producing company within the scope of REACH but are currently characterised by standard test conditions that are not comparable to real-world environmental conditions and the interactions with other substances in the environment or changing environmental factors (German Environment Agency 2017). Possible consequences of combined effects have largely been neglected and just gained more interest in the past decade (e.g., Côté *et al.* 2016; Birk *et al.* 2020; Orr *et al.* 2020) as the understanding increased that, in reality, most stressors do not operate in perfect synchrony (Jackson *et al.* 2021). Most often, the list of stressors acting on an ecosystem is, however, grouped by their source – so, by where the stressor comes from (see for example the International Union for Conservation of Nature (IUCN) Threats Classification Scheme; International Union for Conservation of Nature and Natural Resources 2020). While this source-based classification is effective at describing, for example, how frequently taxa are being affected by a source of stress, it provides little insight into the mechanisms and ecological scales through which these stressors act (Simmons *et al.* 2021). As an example, the two stressors ‘chemical pollution’ and ‘temperature change’ are regarded as separate under a source-based classification; yet, considering the mechanisms by which they operate, they may be grouped together, altering the metabolism and physiology of individuals, leading to more accurate estimates of the direction of their combined effects. In fact, the combination of stressors may lead to effects that are larger (synergism), smaller (antagonistic), or the same (additive) than the sum of the individual effects (Hay *et al.* 1994; Hay 1996; Folt *et al.* 1999). Further, new methods have been discussed to clarify the classification of non-additive effects in multiple stressor research, as complex synergistic or antagonistic interactions between multiple stressors present one of the largest uncertainties when predicting ecological change (Piggott *et al.* 2015; Tekin *et al.* 2020).

Chemical compounds, in particular, interact with each other and with environmental factors (e.g., a changing temperature), leading to, e.g., the break-down of a compound, the formation of a new compound, possibly to changes in toxicity, or they can modify the

interactions among various chemical compounds (Laskowski *et al.* 2010; Kazmi *et al.* 2022). Consequently, predicting the effects of combined stressors when targeting chemical pollution is extremely complex, as the number of possible combinations for stressors is nearly endless. Specifically, synergistic effects are difficult to assess as they differ from additionality assumptions. Accordingly, environmental systems can be affected in different ways when exposed to a variety of chemical pollutants and, for example, climate-change-induced environmental factors. In fact, the fate of organic substances and their risks for the environment (e.g., aquatic communities or human populations) is clearly influenced by environmental conditions, such as precipitation or temperature, meaning that a changing climate is influencing the fate of chemical pollutants in the environment. The effects of the changing climate, however, are not only limited to extreme events per se but also extend to environmental changes that, in turn, affect the fate and mobilization of chemical pollutants in the environment (de Wit *et al.* 2022). Knowledge of the environmental consequences of the combined effects of chemicals and climate change-related stressors (e.g., heatwaves) is still scarce, and more research is urgently needed to estimate environmental impacts in the future and implement mitigation actions effectively.

1.2.1 Climate change and extreme weather events

Anthropogenic behaviour, such as extensive energy use, has caused changes in the atmospheric composition, leading to climatic changes (Karl & Trenberth 2003), which, in turn, are causing significant weather changes globally, including heatwaves (Woolway *et al.* 2021), floods (Milly *et al.* 2002), or droughts (Dai 2013). Such extreme weather events have been increasing globally in the past decades and are foreseen to increase further in the future (IPCC 2021). Temperature and precipitation changes triggered by climate change are predicted to cause severe biodiversity loss by 2050, in some regions even before 2030 (Trisos *et al.* 2020). In particular, heatwaves, with their extreme temperature fluctuations, can have a more severe impact than elevated mean temperature and, thus, pose a greater risk to, e.g., change species performance and dominance (Vasseur *et al.* 2014). Understanding the impacts of climate change and its extreme weather events requires exploring interactions with other anthropogenic stressors, such as environmental chemical pollution. Currently, our knowledge of the combination of multiple stressors, particularly the effects of the combination of climate change-related environmental factors and chemical stressors, remains limited, one of the reasons being that they are separated according to common ‘source-based’ classifications of stressors. However, aquatic ecosystems are particularly prone to the risk of the combined effects of multiple stressors deriving from chemical pollution and climate change, which often co-occur in time and space.

1.2.2 Aquatic systems at the peril of multiple stressors

As a result, aquatic ecosystems are increasingly threatened by multiple human-induced stressors associated with anthropogenic changes, including warming and chemical pollution. The element water exhibits faster conduction than terrestrial media (van Rooyen

& Winterkorn 1957; Singh & Devid 2000), making the aqueous environment particularly prone to temperature fluctuations. Temperature directly controls the metabolic rate of cells and their size (Gillooly *et al.* 2001), which is particularly relevant for ectotherm animals (Brown *et al.* 2004), representing the majority of species in aquatic ecosystems. Importantly, extreme temperature fluctuations can pose a greater risk of altering species performance than elevated mean temperature (Vasseur *et al.* 2014), and heatwaves are predicted to increase in magnitude and frequency (e.g., Meehl & Tebaldi 2004). At the same time, aquatic biota are constantly exposed to water as the surrounding media, including chemical pollutants in the water phase, leading to a higher exposure in comparison to terrestrial living biota. Additionally, water is the base of life and essential for all living organisms. Modifications to the aqueous environment or the exposure within can cascade through complex food webs quickly. For instance, phytoplankton builds the base of aquatic food webs, and accumulated chemical compounds are passed on to higher trophic levels. Direct effects on phytoplankton communities, such as changes in the community composition through temperature fluctuations, may affect the surrounding environment, too, as the food supply or feeding competition may be changed. Despite being positioned at the upmost position in the food web hierarchy, humans are also influenced by changes in aquatic systems via consumption of, e.g., contaminated fish from marine (Barboza *et al.* 2020; Huang *et al.* 2020) and freshwater (Valsecchi *et al.* 2021) environments. Chemical substances can bioaccumulate in organisms and biomagnify through the food chain, achieving levels of contamination that often exceed the initial organisms uptake (Gobas *et al.* 1999; Undeman *et al.* 2022).

However, understanding multiple stressors is particularly challenging in aquatic environments when their combined effect cannot be predicted based on evidence from single-stressor studies, i.e., there are interactions that cause non-additive effects (Crain *et al.* 2008; Jackson *et al.* 2016; Tekin *et al.* 2020). A meta-analysis investigating >250 paired stressor effects in freshwater ecosystems showed that only 19 % were, in fact, additive, while 83 % comprised nonadditive interactions (i.e., positive synergistic or antagonistic) (Jackson *et al.* 2016). Thus, the sign and strength of the correlation between species sensitivities and multiple stressors must be considered when predicting their impacts on aquatic environments. The nonadditive interactions can also occur as interaction chains (one stressor increases the level of a second stressor) or interaction modifications (the impact of a stressor changes with the level of a second stressor) (Simmons *et al.* 2021). In addition, Jackson *et al.* (2016) suggest that “both stressor identity and characteristics of the ecological response (e.g., level of biological organization and organism type) are essential in predicting interactions between multiple stressors” in aquatic ecosystem research. Correspondingly, environmental stressors may affect different levels of organization within an ecological system in different ways: one group may experience extreme pressure, whilst others may not be affected at all. Biological organisations are complex concepts (Mace *et al.* 2012), and the effects of environmental disturbances can often impact an ecosystem’s structure or its function in unpredictable dimensions (Buma 2015). For example, stressors may generate impacts on the metabolism or physiology of individuals, populations, communities, and

ecosystems. At each of the different levels of biological organisation, there are different targets at which the stress can be identified, for example, at the physiological scale, the life history traits of individuals, the abundance or biomass of a population, or the diversity or structure of a community (Simmons *et al.* 2021). Disentangling the processes causing these effects, thus, requires systematic testing of different exposure orders at different levels of organization (Polazzo & Rico 2021; Polazzo *et al.* 2021), something that is currently largely lacking in order to understand the different effect mechanisms (i.e., toxicological modes of action from chemical pollutants and/or climate change-induced stress) throughout various levels of biological organization. In summary, although being particularly vulnerable to environmental stressors and extremely valuable for life on Earth (i.e., as biodiversity hotspots and the provisioning of essential ecosystem services), our knowledge of the combined effects of multiple stressors on aquatic ecosystems is still limited, with current understanding mostly restricted to a few model organisms and a small number of stressor combinations (Jackson *et al.* 2016; Orr *et al.* 2022). To identify the effects of the occurring changes in fate and re-localization of chemicals in combination with climate change-induced environmental changes, it is essential to expand from knowledge gained from exposure experiments of individuals and broaden the focus on potential target groups of various biological levels of organization from a population of individuals living together in a coalescence, multiple species living together in a community, or entire ecosystems.

Subsequently, in this thesis, I focus on the combined stress resulting from the co-occurrence of chemical pollutants and global change-induced stressors, such as extreme weather events, on different levels of biological organization, ranging from single aquatic species over to mixed-species communities, all the way to human exposure.

1.3 Knowledge gaps

An increasing number of studies describe the effects of climate change-induced environmental changes and their consequences for life on Earth (Bonan & Doney 2018; Díaz *et al.* 2019). At the same time, there is an increasing consciousness that chemicals have penetrated (almost) every biological system (Cousins *et al.* 2022), with toxicity-induced effects on its functioning. While multiple stressors research has received increasing attention in recent years following the need for a more realistic and comprehensive assessment (Orr *et al.* 2020; Simmons *et al.* 2021), knowledge of the combined effects of climate changed-induced extreme weather events and chemical pollution, as well as of the mechanisms underpinning those effects in environmentally realistic species assemblages, is still scarce. Based on the current state of research, I identified four major knowledge gaps that will be addressed in the four chapters of this thesis:

(1) Summarizing the known, identifying the unknown. In the context of global climate change, aquatic ecosystems are most sensitive to changes in temperature, precipitation, and other climate variables. At the same time, they can be impacted by various pollutants,

including chemicals from industrial processes, agriculture, and urban runoff. Climate change can influence the transport, fate, and toxicity of these chemicals in aquatic environments. Understanding the combined effects of heatwaves and chemicals is pressing because they can interact, resulting in effects larger (synergism) or smaller (antagonism) than additive effects. The combined effects also depend on the order of action, meaning that the toxicity of micropollutants may either increase for organisms previously exposed to warming or, conversely, micropollutants can reduce the heat tolerance of organisms (Hooper *et al.* 2013; Moe *et al.* 2013). Given the breadth of study designs to disentangling such responses, there is a need to critically evaluate the state of research across laboratory, semi-field, and field studies assessing the effects of heatwaves alone and in combination with micropollutants on different trophic levels and highlighting current knowledge gaps.

(2) Understanding mechanisms – focusing on multiple stressor effects on a single species. Single-species experiments are essential for gaining a comprehensive understanding of the mechanisms by which chemicals and climate change interact, as they allow isolating variables and unravel the molecular, physiological, and behavioural mechanisms underlying the effects. Phytoplankton forms the base of aquatic food webs and is specifically prone to combined stressors resulting from climate change-induced environmental changes (Winder *et al.* 2012; Anderson *et al.* 2021) and human-caused chemical pollution (Pringault *et al.* 2020). At the same time, studying toxicity effects with phytoplankton is a critical component of environmental monitoring and management, providing valuable information about the health of aquatic ecosystems and potential risks to ecosystem and human well-being. Phytoplankton respond quickly to changes in their environment, such that toxicity effects in phytoplankton can serve as an early warning system before effects occur at higher trophic levels. Yet, at present, our knowledge of the combination of multiple stressors, particularly the effects of the combination of climate change-related environmental factors and chemical stressors, remains limited for phytoplankton species but is crucial to prepare for possible future changes and efficiently improve protection measures.

(3) Increasing ecological realism – multiple stressor effects on mixed-species communities. Most research studying the effects of the combination of climate change-related environmental factors and chemical stressors is limited to experimental approaches using single-species setups. Although these studies provide valuable insights into the mode of toxicity (i.e., mechanisms), they do not account for the complexity of whole communities and the potential synergistic effects of biological interactions (Jackson *et al.* 2021; Polazzo *et al.* 2021). Studying such effects within mixed-species communities offers a more ecologically relevant and holistic perspective on the potential impacts of contaminants on aquatic ecosystems, contributing to better-informed environmental management and conservation practices.

(4) Exposure of humans to chemicals in a changing climate. Lastly, climate change — acting as a combination of multiple stressors — also has the potential to modify the

organization and functioning of environmental systems (e.g., changing trophodynamics in food webs (Polazzo & Rico 2021). This, in turn, may alter the bioaccumulation and biomagnification of persistent organic pollutants and, thereby, the risk of negative effects on the environment and humans (Noyes *et al.* 2009). In addition, a shift in human activities, such as changes in dietary patterns, may accelerate chemical exposure. To the best of my knowledge, however, a holistic approach that identifies chemical emission routes, exposure pathways, and chemical properties that may lead to increased or decreased efficiency of human exposure to chemicals in the environment under alternative pathways for global change is missing.

2 | Research aims and objectives

2

2.1 Research questions and hypotheses of this thesis

This thesis aims to understand how chemical pollutants may affect environmental systems that are simultaneously exposed to climate change considering various levels of biological organization. This thesis addresses the following main research question:

How do climate change-induced environmental conditions affect the exposure to and effects of chemicals in the environment?

More specifically, this thesis will concentrate on the following four research questions, in their order each representative for one of the four chapters included in this thesis:

- (1) How does the combined exposure to heatwaves and chemicals affect the structure and functioning of aquatic ecosystems? (chapter I)*
- (2) Does a heatwave modify the toxicity of the antibiotic ciprofloxacin on a single-species Baltic Sea dinoflagellate population? (chapter II)*
- (3) Can climate change-induced heatwaves affect the susceptibility of a freshwater mixed-species community to the herbicide terbuthylazine? (chapter III)*
- (4) How may climate change affect human exposure to chemical contaminants in the environment? (chapter IV)*

Based on these research questions, I hypothesize that:

- (1) The combined stressors of micropollutants and heatwaves generally affect freshwater ecosystems negatively, in a non-additive manner. (chapter I)*
- (2) A heatwave accentuates the effects of the chemical stressor on a single-species phytoplankton population by limiting the energy available for coping and recovering from chemical stress. (chapter II)*
- (3) A heatwave potentiates the effects of the herbicide on some species of a mixed-species freshwater community, thereby causing changes to the community structure. (chapter III)*
- (4) Climate change-induced environmental changes lead to higher human exposure to chemicals in the environment. (chapter IV)*

2.2 Objectives of this thesis

To answer the research questions mentioned above and test the resulting hypotheses, the following general objective is addressed in this thesis:

To combine interdisciplinary scientific tools required to resolve how chemical pollutants may affect environmental systems at various levels of biological organization that are simultaneously exposed to global climate change.

The general objective was further divided and specified into the following sub-objectives that are addressed in the four chapters of this thesis:

- (1) To evaluate the state of knowledge on the combined effects of heatwaves and chemicals with different toxicological modes of actions across freshwater ecosystem laboratory, semi-field, and field studies and to provide recommendations for better integration of heatwaves into multiple stressor research and chemical risk assessment. (chapter I)*
- (2) To identify the effects and disentangle the toxicological mode of action of the combination of an antibiotic and a heatwave on a single-species phytoplanktonic population using a controlled laboratory experiment. (chapter II)*
- (3) To assess the single and combined effects of a herbicide and a heatwave on the structure of a mixed-species freshwater plankton community using microcosm facilities. (chapter III)*
- (4) To identify chemical emission routes, exposure pathways, and chemical properties that may lead to increased or decreased efficiency of human exposure to chemicals in the environment under alternative pathways for global change using in silico modelling tools. (chapter IV)*

3 | Approach and methods

3

3.1 General approach and thesis outline

This thesis includes four chapters presented as a “summary of results” that, along with a general introduction and a general conclusion, provide a holistic view of multiple stressor effects of environmental chemicals in a changing climate. As multiple stressor research focusing on the combined effects of climate change-induced environmental change and chemical pollution is still in its infancy, following a step-by-step approach with interdisciplinary scientific tools was imperative to resolving how chemical pollutants may affect environmental systems at various levels of biological organization that are simultaneously exposed to global climate change (Fig. 1). Thus, at first, I present a literature review to identify knowledge gaps and provide recommendations for integrating climate change into multiple stressor research and chemical risk assessment (**chapter I**). Subsequent steps involve controlled laboratory experiments with a single phytoplankton species (**chapter II**), progressing to a more realistic assessment using a mixed-species freshwater community (**chapter III**), and concluding with *in silico* modelling tools to explore chemical exposure pathways and their implications for human health (**chapter IV**), ultimately bridging the gap between ecological research and human health concerns. In this emerging research field, with limited ability to cover all possible outcomes of combined stressors, systematically progressing from controlled laboratory studies to broader ecological models and human exposure assessments enhances our ability to make informed decisions for the protection of ecosystems and human populations in the face of changing environmental conditions.

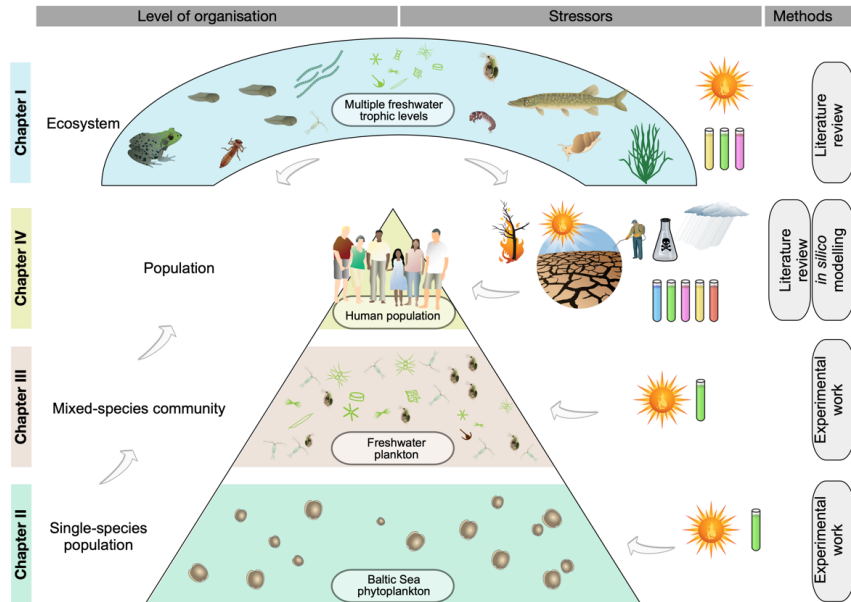


Figure 1. Schematic overview of projects included in this thesis. The literature review of **chapter I** focuses on the ecosystem level (blue). Experimental work of **chapter II** targets a mechanistic understanding at the single-species population level (turquoise), whereas **chapter III** aims at resolving species interactions using a natural mixed-species freshwater plankton community (brown). *In silico* modelling work of **chapter IV** concentrates on the human population level (yellow). The filling of the pyramid indicates the target organisms to which the stressors were applied within each level of organisation: multiple freshwater trophic levels (ecosystem level), freshwater plankton (mixed-species community), Baltic Sea phytoplankton (single-species), and a human population (population level). The stressors indicated with symbols show the stressors applied in each chapter: various chemicals + heatwave (**chapter I**), one chemical + heatwave (**chapter II + III**), and a variety of chemicals, heat stress, droughts, forest fires, heavy precipitation (**chapter IV**). Methods applied in the thesis are indicated by the grey ellipses: literature review (**chapter I + IV**), experimental work (**chapter II + III**), and *in silico* modelling (**chapter IV**).

3.2 Methods

Answering the main research question of this thesis required a step-by-step approach with different interdisciplinary scientific tools for each of these steps (**chapters I-IV**). As the methods vary considerably across the four chapters, they are presented below for each chapter individually. The description of the methods has been kept to a minimum in the following, as all details are provided in the attached papers (**chapter I-IV**).

3.2.1 Methods of chapter I: Multiple stressor effects of heatwaves and micropollutants on aquatic ecosystems – a synthesis

A scoping review was performed to identify the impacts of heatwaves alone and in combination with micropollutants on different trophic levels within freshwater ecosystems (Fig. 2). In contrast to a systematic review attempting to collate empirical evidence from a small number of studies relating to a focused research question, the scoping review sought to map a large and diverse body of existing literature, identify the key concepts, sources of evidence, and research gaps (Munn *et al.* 2018).

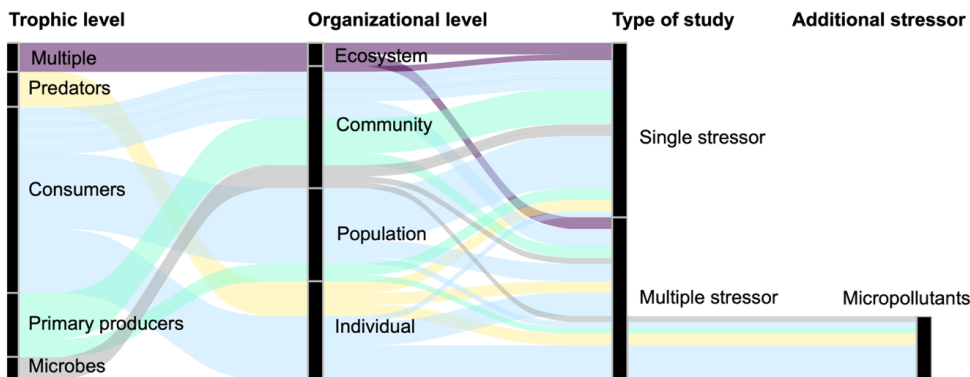


Figure 2. Alluvial plot showing the studies available for the scoping review of chapter I. From left to right: presented are the studies available at the time of the review focusing on heatwaves, the trophic levels evaluated, the level of biological organization, and whether the experiment was conducted as a single stressor (heatwave only) or a multiple stressor (heatwaves in combination with other stressors) experiment, and, lastly, how many of the multiple stressor studies included micropollutants as additional stressor. The blocks represent clusters of nodes, and bands between the blocks represent changes in the composition of these clusters. The bandwidth is proportional to the number of studies evaluated (total number of studies included = 61). The band colour continues from the first node (i.e., trophic level) throughout all others. Figure adapted from Polazzo et al. (2021).

Micropollutants that were expected to have the largest direct impact on the various trophic levels under investigation included: antibiotics for bacteria, herbicides for primary producers (phytoplankton, cyanobacteria, and macrophytes), insecticides for arthropods (micro- and macro-crustacea, and insects), and all of the above for vertebrate predators. Based on the existing literature, we predicted single and combined effects of heatwaves and micropollutants on the different trophic levels and identified key topics for future research.

3.2.2 Methods of chapter II: Combined effects of a heatwave and the antibiotic ciprofloxacin on a single-species phytoplankton population

This experiment was performed at Tvärminne Zoological Station of Helsinki University, Finland. The Baltic Sea dinoflagellate *Apocalathium malmogiense* from the Tvärminne Zoological Station’s algae culture collection (as part of the FINMARI Culture Collection/Syke Marine Research Laboratory) was used as study organism to test for the individual and combined effects of an artificial heatwave and the pharmaceutical antibiotic ciprofloxacin. *A. malmogiense* was incubated in F/2 medium (Guillard & Ryther 1962; Guillard 1975) in the research station’s climate chambers according to the following four treatments: 1) control (no treatment), 2) heatwave exposure, 3) ciprofloxacin exposure, and 4) the exposure to the combination of a heatwave and ciprofloxacin, with six replicates each, respectively. The control water temperature was set at 4°C, resembling the natural spring conditions for *A. malmogiense* in the Gulf of Finland, while the treatments that included the heatwave were set at 9°C for 7 consecutive days, following the World Meteorological Association (WMA) definition of a heatwave. Culture flasks of treatments that included

ciprofloxacin exposure were spiked with a ciprofloxacin stock solution to a final concentration of 0.1 µg/L, resembling a realistic but conservative concentration expected in Baltic waters. Incubations ran for 14 days, with sampling for dependent response variables on days 1, 4, 10, and 14 (Fig. 3).

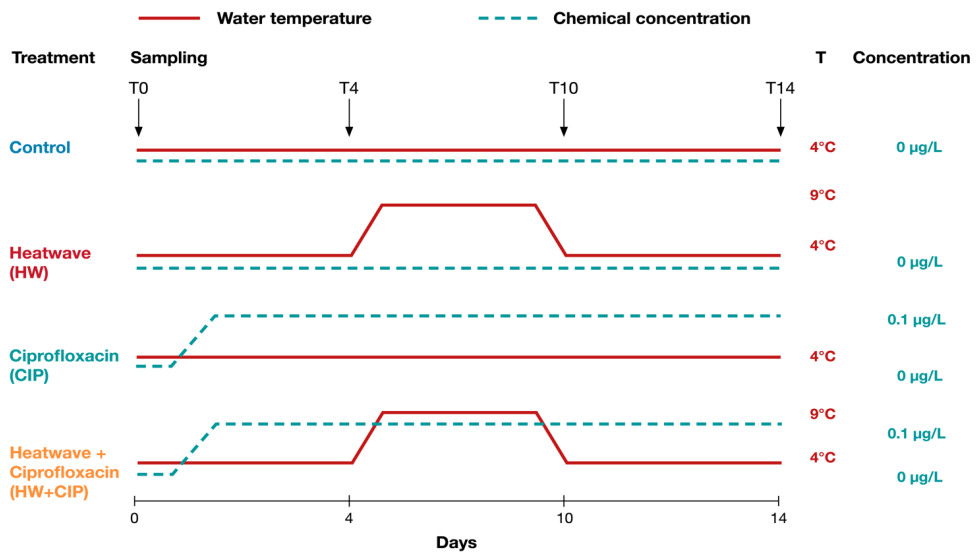


Figure 3. Experimental design for the multiple stressor study of chapter II. *Apocalathium malmogiense* cell cultures were incubated for 14 days and exposed to the following four treatments: control, heatwave (HW), ciprofloxacin (CIP), and the combined heatwave and ciprofloxacin (HW+CIP) treatment. Each treatment comprised six replicates, totalling 24 incubation chambers. The heatwave was induced in the HW and HW+CIP treatment on day 4 and terminated at the end of day 10. During this period, the water temperature in the HW and HW+CIP treatment was increased by 5°C, from 4°C to 9°C. The exposure to the chemical compound ciprofloxacin was initiated in the CIP and HW+CIP treatment on day 1. The CIP and HW+CIP cultures were spiked with ciprofloxacin to reach a final concentration of 0.1 µg/L.

Measured response variables included: chlorophyll-a fluorescence as proxy for growth, microscopic cell counting and cell size measurements, as well as samples for dissolved inorganic nutrients (i.e., nitrate, nitrite, ammonium, and phosphate). Based on these measurements, calculated response variables included: nutrient uptake rates and specific growth rates. The dependent response variables were explored using linear mixed models (LMMs) with the factor ‘treatment’ (i.e., control, heatwave, ciprofloxacin, and the combined heatwave and ciprofloxacin) as fixed factor and the factor ‘day’ (day 1 – 14) using a repeated-measures structure.

3.2.3 Methods of chapter III: Combined effects of a heatwave and the herbicide terbuthylazine on a mixed-species zooplankton community

This experiment was performed at IMDEA Water Institute, Science and Technology Campus of the University of Alcalá, Madrid, Spain. A natural mixed-species freshwater plankton community from an artificial lagoon at the IMDEA Water Institute was used as study system to test for the individual and combined effects of an artificial heatwave and the herbicide terbuthylazine. After collection, the plankton community was allowed to

acclimatise to the environmental conditions in the microcosms for two weeks prior to the start of the experiment. The mixed-species plankton community was then incubated in cylindrical glass microcosms in unpolluted water from the lagoon according to the following four treatments: 1) control (no treatment), 2) heatwave exposure, 3) terbuthylazine exposure, and 4) the exposure to the combination of a heatwave and terbuthylazine, with four replicates each, respectively. The control water temperature was set at 22°C during the experiment, which resembled the water temperature from the origin water body at the time of the experiments. The treatments that included the heatwave were set at around 27°C for 7 consecutive days, following the WMA definition of a heatwave. Microcosms of treatments that included terbuthylazine exposure were spiked with a terbuthylazine stock solution to a final concentration of 15 µg/L, resembling a realistic but conservative concentration estimated for freshwater ecosystems of Southern European agricultural areas. Incubations ran for 21 days, with sampling for dependent response variables at days 0, 7, 14, and 21, relative to the start of the experiment (Fig. 4).

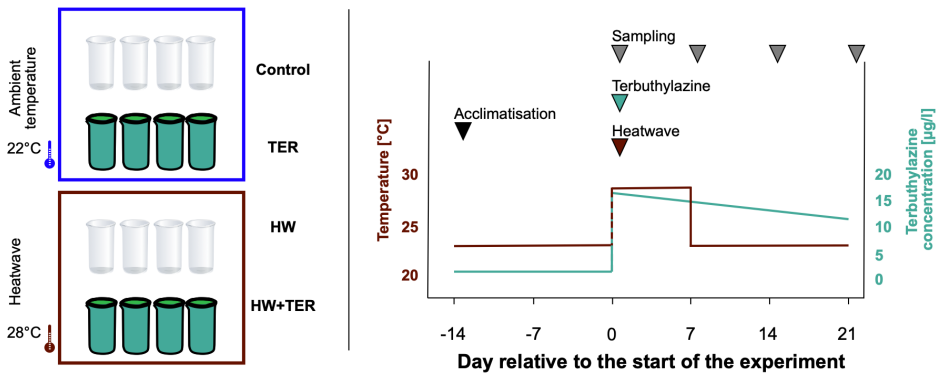


Figure 4. Experimental design for the multiple stressor study of chapter III. Left panel: overview of the four treatments; right panel: schematic timeline of the experiment. After an acclimatisation period of 14 days at 22°C, the experiment was started

with the simultaneous application of the heatwave and the herbicide terbuthylazine on day 0. The coloured lines represent the temperature (red) and the terbuthylazine (green) dynamics in the stressed microcosms. The grey arrows mark the sampling days (i.e., day 0, 7, 14, and 21, relative to the start of the experiment). Figure adapted from Roth et al. (2022).

Measured variables included: zooplankton samples (mesh size: 55 µm), phycoerythrin (PE), chlorophyll-a, dissolved oxygen, electrical conductivity, salinity, pH, ammonium, nitrate, phosphate. The chlorophyll-a concentration was used as an indicator of the total phytoplankton biomass, while the pigment PE was used as an indicator for the biomass of cyanobacteria. We calculated zooplankton total abundance, taxa richness, and taxa diversity and used two-factorial ANOVAs followed by post-hoc tests to identify differences between the treatments and days. Principal Component Analysis (PCA) was used to visualise differences in community structure between the controls and the treatments, while differences in multivariate community composition between the treatments were assessed by means of distance-based Redundancy Analysis (dbRDA).

3.2.4 Methods of chapter IV: Climate change-related chemical exposure on a human population level

We explored chemical emission routes, exposure pathways, and chemical properties that may lead to increased or decreased exposure of humans to chemicals in the environment under alternative pathways for global change using *in silico* modelling tools. At first, a literature review was performed to synthesize information on future human exposure to chemicals occurring in the environment and how these exposures may be influenced by climate change. The outcomes of the literature review of potential drivers of change in chemical exposure related to climate change were used to identify the most relevant exposure pathways for the Swedish population, i.e., exposure through indoor air, drinking water, and food and consumption. For each of these exposure pathways, three possible scenarios of human exposure to chemicals in Sweden have been developed. The scenarios are based on the Shared Socioeconomic Pathways (SSPs), a scenario framework used for global change research (O'Neill *et al.* 2014). The SSPs were defined as reference pathways to depict plausible alternative situations in the development of ecosystems and society over the period of a century (O'Neill *et al.* 2014). The first scenario (SSP1), *Taking the Green Road*, simulates a global community that has developed into a sustainable society with sustainable and equal use of common resources. SSP1 comprises low challenges to mitigation and adaptation due to climate change. The second scenario (SSP2) describes medium challenges to mitigation and adaptation and relays to *Taking the Middle of the Road*, the third scenario (SSP5) compares to *Taking the Highway* in a fossil-fuel development with high challenges to mitigation and high challenges to adaptation (Riahi *et al.* 2017). The scenarios are dependent upon and defined by the measures that the world takes to reduce greenhouse gas emissions. Further, the identified scenarios served as a basis for conducting an exposure model with spatial and temporal resolution using the far-field- and indoor exposure model USETox® 2.0 (Fantke *et al.* 2018) to identify the future human exposure to chemical emissions in Sweden in times of the changing climate (Fig. 5).

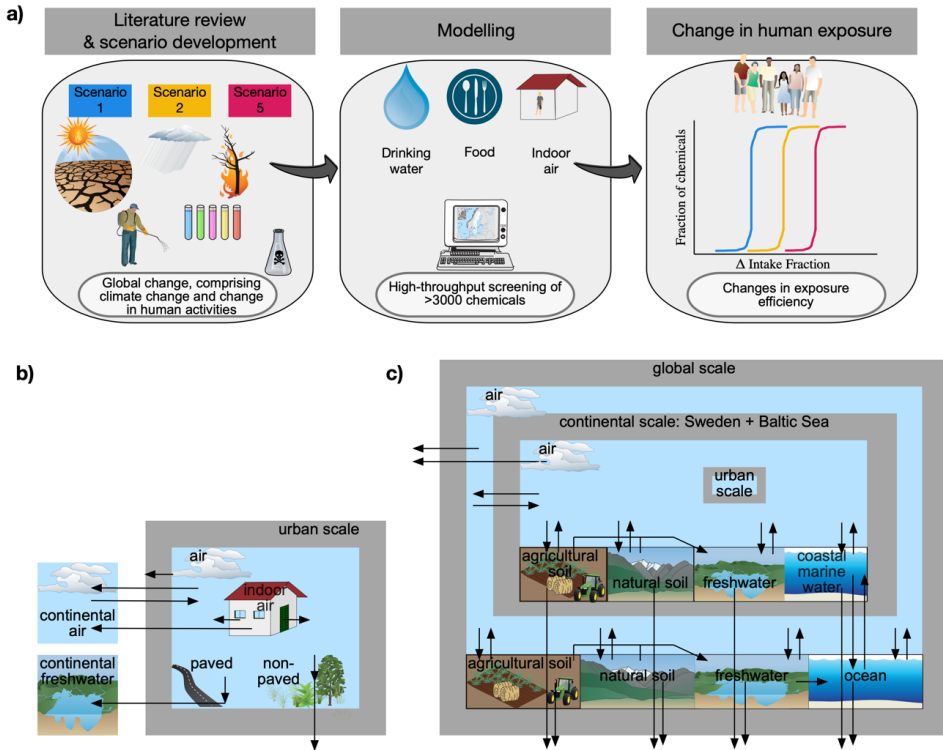


Figure 5. Research procedure for chapter IV and the USEtox® 2.0 box compartments. a) Overview of the research procedure of **chapter IV**, b) overview of USEtox® 2.0 modelling framework showing the urban scale environment, and c) continental and global scale environments, with the urban scale embedded in the continental scale. Adapted and modified from Roth et al. (2023), graphics b) and c) adapted and modified from the original of Fantke et al. (2018).

4 | Summary of results

4

4.1 Multiple stressor effects of heatwaves and micropollutants on aquatic ecosystems – a synthesis

Multiple stressor research focusing on the combined effects of climate change-induced environmental change and chemical pollution is still in its infancy, rendering a step-by-step approach with interdisciplinary scientific tools imperative to resolve how chemical pollutants may affect environmental systems at various levels of biological organization that are simultaneously exposed to global climate change (Fig. 1). As such, in this thesis, my colleagues and I first commenced a literature review to avoid redundancy, build on existing knowledge, identify critical knowledge gaps, and, finally, provide recommendations for a better integration of climate change-induced environmental change into multiple stressor research and chemical risk assessment (**chapter I**). Freshwater aquatic ecosystems were selected as study systems to identify the effects of multiple stressors. Heatwaves served as an exemplary extreme weather event caused by climate change, and its effects in combination with chemical pollutants as case to investigate multiple stressor effects at the ecosystem level. The literature search resulted in 61 studies fitting the definition of a heatwave following the WMA (i.e., “five or more consecutive days of prolonged heat in which the daily maximum temperature is higher than the average maximum temperature by 5 °C or more”). Other studies were conceived as conventional temperature raise studies and not included. Of the 61 studies, only 13 assessed the combined effects of heatwaves and chemicals, with only 5 articles looking at the combination of both stressors using a multitrophic approach (Fig. 6). Overall and across all trophic levels (i.e., microbes, primary producers, primary consumers, vertebrate predators), heatwaves (or heatwave treatments) were generally found to increase the metabolic activity of organisms, often causing changes in the community structure due to different thermal sensitivities. Chemical stressors mainly had negative physiological effects. The combination (i.e., heatwave + chemical stressor) often led to a higher sensitivity towards the chemical stressors. Importantly, however, the mechanisms behind these multiple stressor observations remain speculative in all studies and were never explored in detail. Thus, it becomes apparent that the scarcity of available studies does not allow to draw general conclusions on how heatwave-driven changes may affect the sensitivity towards different chemical classes – let alone how different responses by single species of mixed-species assemblages can cascade through the food web. The lack of testing in complex species assemblages appears particularly problematic since ecological realism, and more community/ecosystem-oriented studies have recently been recognized as essential for the advancement of multiple stressors research (Orr et al. 2020). Overall, based on the (limited) available literature, we come to the following four main conclusions/recommendations: (1) At the species level, multiple stressor effects need to be mechanistically resolved to better understand the mode of action of chemicals in combination with climate-related changes, which have often shown to be non-additive, and, thus, non-predictable; (2) We further suggest that the assessment of the effects of extreme events combined with other stressors should involve investigations that go beyond one trophic level, as species-specific responses can result in shifts in the community structure, alter predator-prey interactions and, ultimately, cascade through the food web; (3)

Experimental designs also need to consider biologically typical nonlinear responses through, for example, gradient designs. Gradient testing would consent to different realistic disturbance intensities (i.e., multiple temperature regimes or chemical concentrations); (4) Lastly, the effects of multiple stressors (and their interactions) are temporal-scale dependent (Garnier et al. 2017). That is, interactions between stressors can appear right after disturbance's application, as well as in the recovery phase.

Therefore, studying how the order, duration, or reoccurrence of stressors affect the studied system may help to solve current managing conflicts, protect biodiversity in the long-term, and guarantee the delivery of ecosystem functions and services.

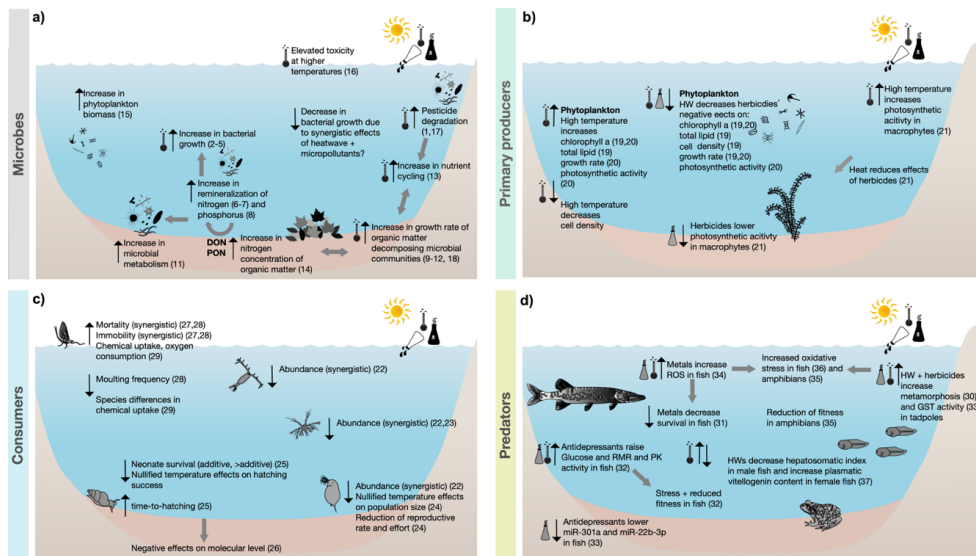


Figure 6. Conceptual overview of the potential combined effects of heatwaves and chemicals on a) microbial communities, b) primary producers, c) consumers, and d) predators. Black upward or downward arrows indicate an increase or decrease in the respective processes. Grey arrows indicate direct and indirect effects on other processes. The thermometer symbol indicates an effect related to temperature only, whereas the symbols of the thermometer and of the chemical together indicate a combined effect of both. (1) (Bighiu & Goedkoop 2021), (2) (Zeng et al. 2014), (3) (Zamarreño et al. 2009), (4) (Höfle 1979), (5) (White et al. 1991), (6) (Berthelot et al. 2019), (7) (Hayes et al. 2019), (8) (Klausmeier et al. 2004), (9) (Duarte et al. 2013), (10) (Donnelly et al. 1990), (11) (Stelzer et al. 2003), (12) (Fernandes et al. 2012), (13) (Phillips et al. 2017), (14) (Kaushik & Hynes 1971), (15) (Pomeroy & Wiebe 1988), (16) (Delnat et al. 2021), (17) (Wickham et al. 2020), (18) (Arias Font et al. 2021), (19) (DeLorenzo et al. 2013), (20) (Tasmin et al. 2014), and (21) (Wilkinson et al. 2017), (22) (Arenas-Sánchez et al. 2019), (23) (Van de Perre et al. 2018), (24) (Miracle et al. 2011), (25) (Kimberly & Salice 2013), (26) (Baag et al. 2021), (27) (Macaulay et al. 2021), (28) (Macaulay et al. 2020), and (29) (Camp & Buchwalter 2016), (30) (Freitas et al. 2016), (31) (Park et al. 2020), (32) (Mehdi et al. 2019), (33) (Freitas et al. 2017), (34) (Prophete et al. 2006), (35) (Gripp et al. 2017), (36) (Birmie-Gauvin et al. 2017), and (37) (Hani et al. 2019). The figure was adapted and modified from (Polazzo et al. 2021).

4.2 Combined effects of a heatwave and a chemical stressor on a single-species population – mechanistic insights into toxicity modes

At the species level, multiple stressor effects need to be mechanistically resolved to better understand the mode of action of chemicals in combination with climate-related changes,

which have often shown to be non-additive and, thus, very difficult to predict (**chapter I**). As a response and second step of this thesis, I used a single phytoplankton species and controlled laboratory incubations to test the combined effects of a climate change-induced heatwave and a chemical stressor (**chapter II**). By focusing on a single species with a 2x2 full factorial experimental design, this experimental approach allowed to investigate the individual and interactive effects of a heatwave and chemical exposure on the response variables, helping to establish cause-and-effect relationships identified as a major knowledge gap in **chapter I**. Specifically, in **chapter II**, we examined the effects of a heatwave and one of the most commonly used antibiotics in the Nordic countries – ciprofloxacin – and the combination of both stressors (heatwave + ciprofloxacin) on the Baltic Sea spring bloom dinoflagellate *Apocalathium malmogiense*. Cell counts, cell size, growth rates, chlorophyll-a (Chl-a) contents, and nutrient uptake rates served as study parameters to identify the effects of a more realistic stressor scenario where both the heatwave and the antibiotic pollution are present. The results showed that, as expected by thermal response curves (Hinners *et al.* 2017), a simulated heatwave (+5 °C above control for 7 consecutive days) increased the growth and Chl-a content of *A. malmogiense* (Fig. 7). Within the temperature range of normal activity, metabolic rates increase with temperature according to the Boltzman–Arrhenius function (Brown *et al.* 2004), and, consequently, coin temperature as a key variable that controls biological activity (Perkins *et al.* 2010). Our results also showed a decrease in cell size with increasing temperature (i.e., the heatwave treatment compared to the control), which is typical for protists (Atkinson *et al.* 2003). These findings align with experimental tests using *A. malmogiense* (Hinners *et al.* 2017) and in-situ observations (Moore & Folt 1993; Zohary *et al.* 2021), where temperature increases led to smaller cell sizes of phytoplankton species. In contrast, the presence of ciprofloxacin at an environmentally realistic concentration of 0.1 µg/L did not affect the dinoflagellate growth, aligning with many studies that did not find changes in phytoplankton growth under ciprofloxacin exposure (e.g., Johansson *et al.*, 2014). The assumption based on common additivity models would be that the individually non-active pollutant ciprofloxacin would not contribute to the stressor mixture effect (Rodea-Palomares *et al.* 2015), and that the combination of both factors (heatwave + ciprofloxacin) would, therefore, resemble the heatwave treatment in its response. Yet, when both factors were applied together in the multiple stressor treatment (heatwave + ciprofloxacin), significantly lower cell counts (-17 %) and Chl-a content (-34 %) at the end of the experiment and a by >20 % reduced specific growth rate compared to the heatwave treatment were observed. However, despite showing no effect on growth response parameters, ciprofloxacin in isolation reduced *A. malmogiense* nitrogen uptake rates (NH₄⁺, NO₃⁻ and NO₂⁻) by >40 % compared to the heatwave and control treatment. An antibiotic-induced microbiome change within the phycosphere (i.e., the region immediately surrounding individual phytoplankton cells, Seymour *et al.* 2017) with a resulting breakdown of the nutrient exchange relationship of *A. malmogiense* with associated bacteria may have led to this observation (Tungaraza *et al.*, 2003). As a consequence, reduced nitrogen uptake rates induced by ciprofloxacin with increased growth stimulated by the heatwave may have led to an interruption of a balanced growth response in the combined ‘heatwave + ciprofloxacin’ treatment. Indeed, Marañón *et*

al. (2018) showed that the nutrient availability controls the temperature dependence of phytoplankton metabolism, such that the direct effect of increasing temperature on growth is absent under more nutrient-limited conditions.

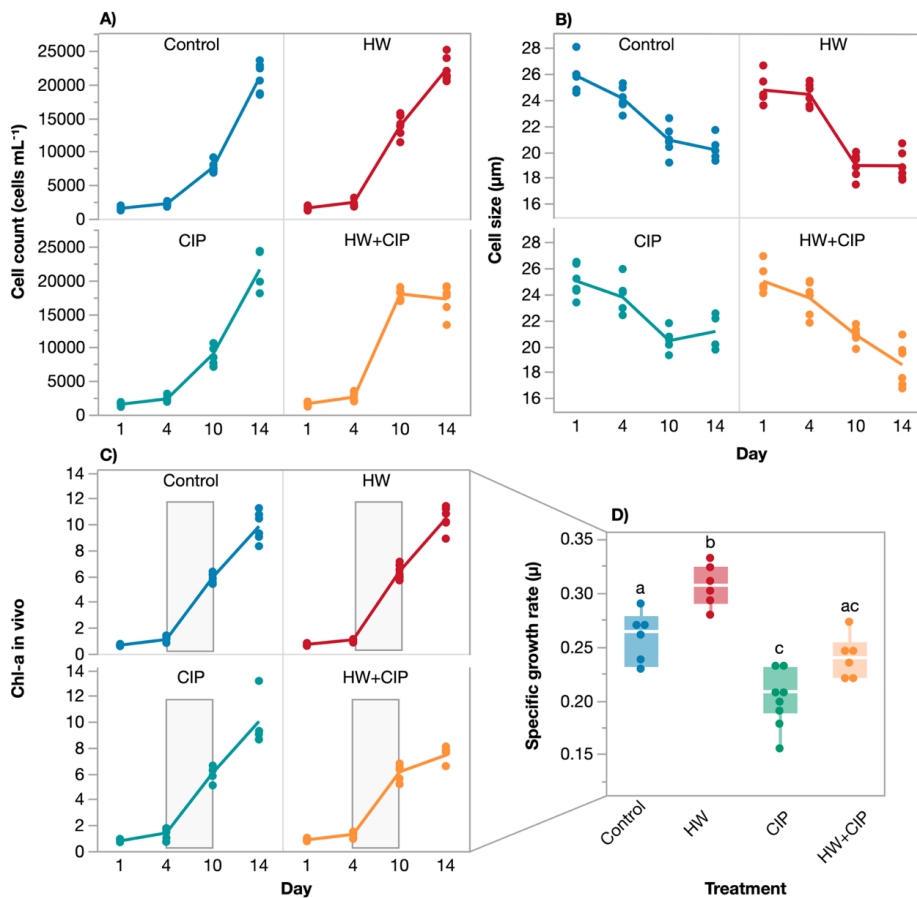


Figure 7. Temporal evolution of *Apocalathium malmogiense* growth response parameters. Development of cell counts (A), cell size (B), and Chl-a in-vivo content (C) during experimental incubations, and the specific growth rate (D) between day 4 and 10 in the control, heatwave (HW), ciprofloxacin (CIP) and the combined heatwave and ciprofloxacin (HW+CIP) treatment. Grey boxes in (C) represent the exponential phase between day 4 and 10 during which the specific growth rates (in D) were calculated. Boxplots in (D) display the median (horizontal white line), first (Q1) and third quartiles (Q3; lower and upper end of box, respectively) and the minimum and maximum data value (lower and upper whisker, respectively) and its variability in comparison to the interquartile range ($Q1 - 1.5 \cdot IQR$; and $Q3 + 1.5 \cdot IQR$). Individual data points are shown by dots. In (D): treatments that have different letters are significantly different, whereas treatments that have the same letter are not significantly different. Summary of the results from the ANOVA tests for the specific growth rate (μ) are presented in the supplementary material of chapter II.

Overall, the results point towards a negative synergistic (inhibition) effect of ciprofloxacin when in combination with heat stress, highlight that individually (seemingly) non-active chemicals, such as ciprofloxacin, can substantially modify mixture toxicity, i.e., when applied in combination with another stressor, i.e., a heatwave. These findings show that the antibiotic ciprofloxacin can affect the growth of *A. malmogiense* if applied together

with a second stressor, highlighting the importance of multiple stressor application in research, as effects may otherwise go undetected if only studied in isolation. As the Baltic Sea is heavily impacted by legacy chemical pollutants and the transport of new pharmaceutical residuals at present day, as well as an increasing frequency of marine heatwaves, our study demonstrates that the risk assessments of chemical compounds on aquatic life should consider environmental change as a co-existing factor (**chapter I**), and provides some first mechanistic insights for predicting multiple stressor effects in aquatic ecosystems using the antibiotic ciprofloxacin and a simulated heatwave on the dinoflagellate *A. malmogiense*.

4.3 Combined effects of a heatwave and a chemical stressor on a mixed-species community – seeking a higher grade of environmental realism

A key finding of **chapter I** was that the assessment of the effects of extreme events combined with other stressors should involve investigations that go beyond one trophic level, as species-specific responses can result in shifts in the community structure, alter predator-prey interactions and, ultimately, cascade through the food web. Accordingly, in **chapter II**, we found that a single species phytoplankton population can be negatively influenced by the combined stressor effects of a chemical and a heatwave; yet, from this experiment, it remains unclear how the effects would change under natural conditions within a natural mixed-species community. As such, in the next step of this thesis, I sought a higher grade of environmental realism, performing a 2x2 full factorial experiment to investigate the individual and interactive effects of a heatwave and chemical exposure on a natural mixed-species planktonic community in a microcosm facility (**chapter III**). Working with a natural mixed-species community provides a more realistic representation of the ecosystem, where organisms coexist and interact with each other and their environment. This experimental approach also shows how stressors can cause resilient species to outcompete sensitive ones, with effects for the community structure. Specifically, in **chapter III**, we evaluated the effects of a heatwave and the herbicide terbuthylazine and the combination of both stressors (heatwave + terbuthylazine) on a freshwater mixed-species plankton community using indoor microcosms. Unlike the results of **chapter II**, we did not observe an increase in total Chl-a concentration in the heatwave treatment, which would have pointed towards an increase in the metabolic activity with temperature (Brown *et al.* 2004) and subsequent growth of phytoplankton (Chl-a as proxy for growth of the whole phytoplankton community). Yet, the heatwave induced a 3-fold higher total abundance of zooplankton as compared to the control treatment. This observation was likely related to a reorganisation of the phytoplankton community structure with warming (Henson *et al.* 2021) with an increase of more palatable phytoplankton for the zooplankton rather than an overall increase in the total abundance of phytoplankton. Also, smaller zooplankton individuals, which usually have shorter reproductive cycles, may have responded faster to the metabolic increase caused by warming (Gillooly *et al.* 2001; Brown *et al.* 2004; Dam 2013), explaining the observed general increase in smaller zooplankton taxa in the heatwave treatment. Interestingly, the increase in the abundance of zooplankton may have caused the

observed decline in Chl-a after the heatwave was terminated due to the amplified grazing pressure (O'Connor *et al.* 2009). Terbutylazine, a photosynthesis inhibiting herbicide, can have lethal effects on phytoplankton communities. Thus, in the terbutylazine treatment, we expected to see a decrease in phytoplankton abundance (Chl-a as proxy), which would limit the food availability for the zooplankton community and, consequently, indirectly cause a decline in zooplankton abundance (Pereira *et al.* 2017). Yet, although we did not detect a decline in Chl-a in the terbutylazine treatment, a decline in zooplankton abundance and significant shift in the zooplanktonic community composition was observed. While we did not analyse the community structure of the phytoplankton, a previous study described a phytoplankton community composition shift from dominant (i.e., *Chloromonas angustissima*, *Navicula lanceolata*, and *Gomphonema parvulum*) towards less-sensitive (i.e., *Chroococcus turgidus*, *Peridinium willei*, and *Trachelomonas* spp.) species when exposed to terbutylazine (Wijewardene *et al.* 2021). Therefore, it seems likely that edible phytoplankton taxa (e.g., *Scenedesmus* spp. or *Cyclotella* sp.) were replaced by more grazing-resistant phytoplankton (e.g., *Anabaena* or *Leptolyngbya* spp.) in our set-up, concluding that both the decline in zooplankton abundance and the shift in community composition were likely driven indirectly by the direct effect of terbutylazine on phytoplankton community structure. Hence, the combined application of both stressors (heatwave and terbutylazine) was expected to cause additive or synergistic effects on the zooplankton community, owing to the cumulative energy demand caused by both stressors (Dinh *et al.* 2016). To our surprise, the combination of both stressors caused a significant increase in Chl-a concentration, which could be classified as a positive synergistic effect, since neither the heatwave nor terbutylazine influenced the Chl-a levels when applied as single stressors (Piggott *et al.* 2015). This positive synergistic effect may, again, have been caused by a shift in the phytoplankton community composition towards terbutylazine resistant species that simultaneously were stimulated in their growth by the heatwave application. Interestingly, despite the increase in Chl-a, no significant effect on the zooplankton community was observed, contrasting the expectations of a higher food availability. However, we also observed an increase in the pigment PE (indicative of cyanobacteria growth (Sidler 1994)) in the combined heatwave + terbutylazine treatment. Consequently, the simultaneous increase in PE and Chl-a may have been a result of cyanobacteria growth, as high temperatures also decrease their sensitivity to herbicide toxicity (Bérard *et al.* 1999). Hence, we assume that cyanobacteria benefited from the combined heatwave + terbutylazine treatment, leading to a rapid growth in the lack of strong competition with other algae taxa (Carey *et al.* 2012; Paerl & Otten 2013). An increase in cyanobacteria may, however, lead to a spread of toxic bioactive compounds that can prevent zooplankton grazing on them (Lyu *et al.* 2016). The heatwave and terbutylazine treatment could also have led to a reduction of primary productivity in green algae and to a lower relative abundance of palatable phytoplankton, as observed in comparable studies (Allen *et al.* 2021; Rumschlag *et al.* 2021), together causing food limitation for zooplankton, and, consequently, a decrease in zooplankton abundance (Rumschlag *et al.* 2021) (Fig. 8). While it seems that the planktonic community used in this experiment was resilient to (or even positively influenced by) the combined heatwave and

terbuthylazine application when considering growth only, our results point towards varying responses of individual species (both phytoplankton and zooplankton) within the natural mixed-species community. The consequential shifts in the community structure resulted in a complicated cascade of propagating shifts along the food web structure (from phytoplankton to zooplankton). Further, spatiotemporal variation in the stress events can yield asynchronous exposure scenarios, whose impacts are even more challenging to predict (**chapter I**). Future research should thus be dedicated to assessing multiple stressor effects applied in different sequences and intensities, as well as to providing mechanistic understanding and models to quantify their propagation across different levels of biological organization (**see chapter IV**).

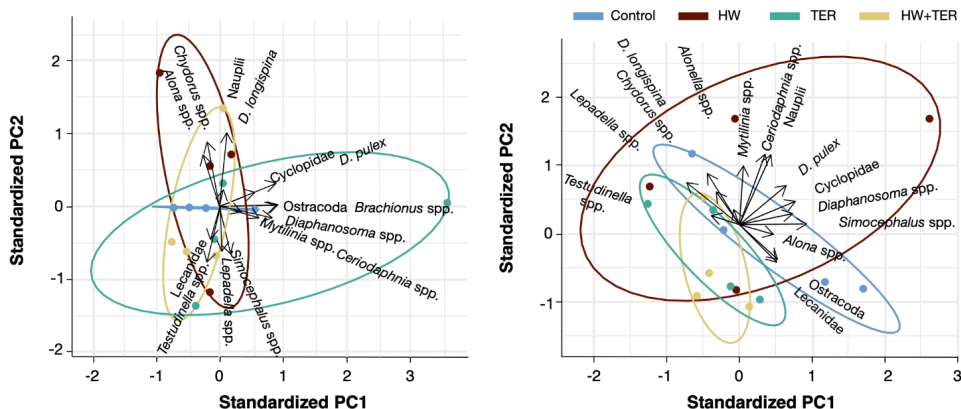


Figure 8. Principal Component Analysis (PCA) indicating the relationship between the treatments (control, heatwave (HW), terbuthylazine (TER), and the combination (HW+TER)) and the individual taxa of the mixed-species zooplankton community. The length and direction of the arrows indicate the affinity of each stressor for each taxon, respectively. (left panel) PCA of day 7, (right panel) PCA of day 14. Adapted from (Roth *et al.* 2022).

4.4 Climate change-related chemical exposure on a human population level

Building upon the insights gained from the preceding **chapters (I, II, and III)**, **chapter IV** serves as a natural follow-up by extending the focus from aquatic ecosystems to the exposure of a human population. Having looked at the individual and interactive effects of climate change-induced stressors and chemical pollution on aquatic ecosystems, **chapter IV** extends these findings by examining how human exposure is intricately linked to the transfer of chemicals through aquatic environments, for example, via pathways such as the food chain. This step-by-step approach from a single aquatic species assessment to human exposure modelling was used to bridge the gap between ecological research and human health implications. It provides a more holistic perspective on the potential risks associated with chemical exposures under changing climate conditions, facilitating informed decision-making and actions to safeguard both ecosystems and human populations. Specifically, we explored chemical emission routes, exposure pathways, and chemical properties that may

lead to increased or decreased efficiency of human exposure to chemicals in the environment under alternative pathways for global change using *in silico* modelling tools (**chapter IV**). We assessed how climate change-related chemical exposure may change in the future, with a focus on drivers of change in exposure of the Swedish population to chemicals in the indoor and outdoor environment. First, we formulated three alternative exposure scenarios that are inspired by three of the SSPs (O'Neill *et al.* 2014). We then conducted scenario-based exposure modelling of the >3000 organic chemicals in the USEtox® 2.0 chemical library, and further selected three chemicals (terbutylazine, benzo[a]pyrene, PCB-155) from the USEtox library that are archetypical pollutants of drinking water and food as illustrative examples. The different exposure pathways assessed were via the ingestion of 1) drinking water, 2) food, and 3) the inhalation of indoor air. Overall, our results demonstrate that changes in intake fractions of chemicals are possible by up to twofold increases or decreases under different development scenarios (Fig. 9).

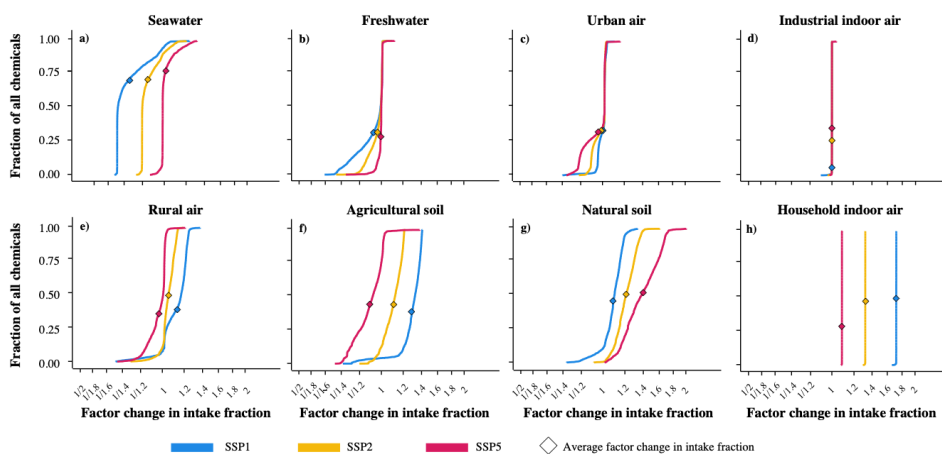


Figure 9. Full variant cumulative distributions for all 3077 chemicals of the ratio of the total intake fraction for each SSP relative to the baseline for chemical emissions. a) seawater, b) freshwater, c) urban air, d) industrial indoor air, e) rural air, f) agricultural soil, g) natural soil, and h) household indoor air. The individual panels are arranged in approximate order of increasing ratio of the total intake fraction. Adapted from (Roth *et al.* 2023).

Changes in intake fraction in the most optimistic SSP1 scenario are mostly attributable to a shift by the population towards a more plant-based diet, while changes in the pessimistic SSP5 scenario are driven by environmental changes such as rainfall and runoff rates. More specifically: (1) Drinking water. With a changing climate, Sweden is likely to suffer from more floods and droughts in the future. Floods may transport chemical contaminants into groundwater and surface water. More frequently occurring droughts will increase the mobilization of heavy metals and leakage of chemicals into groundwater. The extent of a changing climate will, thus, largely determine the extent of changes in human exposure to chemicals through drinking water. (2) Food consumption. Pesticide usage is forecasted to increase due to climate change, leading to higher human exposure to pesticide residues

through the consumption of contaminated crops. Chemical uptake via food can be influenced by pesticide treatment of crops and is modelled to increase with climate change. More widespread adoption of indoor agriculture for high-value specialty crops could result in new pathways for human exposure to pesticides. And, in a scenario where application of wastewater and/or sewage sludge to agricultural fields becomes more extensive in Sweden and Europe, new pathways for chemical exposure through agricultural will be opened. (3) Indoor environment. With a warming climate, increasing demand for energy-efficient buildings is expected. The use of air-conditioning in private households is likely to increase, which will translate into lower ventilation rates in buildings in the future. With poorer ventilation, concentrations of chemicals in indoor air are likely to increase. Additionally, extreme weather events and wildfires will drive the population (particularly vulnerable subgroups such as the elderly and children) to spend more time indoors, which will increase their exposure to contaminants indoors. Results suggest that the human exposure to environmental chemicals through intake fraction in Sweden will change due to climate change and are mainly driven by changes in the human diet and in the mobilization of chemicals through changing environmental conditions such as increasing precipitation, soil erosion, and runoff.

5 | Conclusion

5

5.1 Conclusion

Climate change and chemical pollution are planetary-scale emergencies that are deeply interlinked and need to be faced in an integrative manner. In this thesis, using a step-by-step approach with interdisciplinary scientific tools, I provide evidence that the multifaceted effects of chemicals in the environment are posing a growing threat to ecosystem functioning at various levels of biological organization when simultaneously exposed to climate-induced changes. Taking into account the main research question of this thesis, I can conclude that:

Alterations of climate change-induced environmental conditions can have non-predictable, synergistic effects on ecosystem structure and functioning when in combination with chemical exposure.

More specifically, I can draw the following conclusions that answer the four specific research questions of this thesis:

(1) Knowledge on the effects of the combined stress through the exposure to chemicals and climate change-induced environmental conditions is still scarce.

Using aquatic ecosystems as model system and heatwaves as an environmental threat of a changing climate, I showed in **chapter I** that only ~20 % of multiple stressor studies looking at the effects of heatwaves used chemicals as a second stressor. Only five of these articles used a multitrophic approach, highlighting that disentangling pollution impacts in a comprehensive manner remains challenging, especially in realistic, complex ecosystem settings. While the combination of the two stressors (i.e., heatwave + chemical stressor) often led to a higher sensitivity towards the chemical stressors, mechanisms behind these multiple stressor observations were rarely explored, making it difficult to deduce consistent trends. In light of an increasing frequency and magnitude of heatwaves under climate change and the simultaneous growing exposure to a multitude of chemicals in the environment, it is alarming that minimal attention to chemical pollution as a global change factor has been given in chemical risk assessments so far.

(2) A heatwave can synergistically affect the toxicity and mode of action of an environmentally relevant concentration of a chemical on a single species population.

As deduced from **chapter I**, the mechanisms behind multiple stressor observations, using climate-induced environmental change and chemical pollution as factors, are rarely assessed in detail. In **chapter II**, I, thus, showed that a heatwave effectively altered the mixture toxicity effect of ciprofloxacin – a common antibiotic – on the Baltic Sea dinoflagellate *A. malmogiense*. While the heatwave supported growth as anticipated, the presence of ciprofloxacin at realistic exposure concentrations did not affect growth response parameters. Yet, the combination of the heatwave and ciprofloxacin resulted in a negative synergistic effect due to

antibiotic-induced microbiome changes in the phycosphere. The results underscore the importance of considering mechanistic interactions when studying multiple stressors, as effects may go undetected if studied in isolation only. The study also highlights the non-linearity of responses below the thresholds of observable monotonic responses, such as that seemingly non-active chemicals can induce changes in stressor mixtures.

- (3) **Climate change-induced heatwaves can alter the susceptibility to the herbicide terbuthylazine of individual species within a mixed-species community, leading to unpredictable shifts in the community structure.** In **chapter III**, I highlighted the complexities arising from multiple-species studies, contrasting the single-species cause-and-effect focus of **chapter II**. Specifically, the combination of a heatwave and chemical exposure to terbuthylazine – a widely-used herbicide – synergistically led to a complex cascade of effects throughout the food web by promoting the growth of individual species and altering predator-prey interactions. Such indirect effects of multiple stressor interactions can unfold through a differential sensitivity of predator and prey in a natural system or through reactions of environmental microbiomes, as has been shown in **chapter II**. The results highlight the importance of performing multiple stressor experiments using multi-trophic approaches under realistic conditions for comprehending their implications for ecosystem resilience.
- (4) **The combination of climate-induced changes in environmental conditions will significantly impact human exposure to environmental chemicals, emphasizing the importance of considering climate change in chemical risk assessment and mitigation strategies.** Building upon the insights gained from the preceding **chapters (I, II, and III)**, **chapter IV** served as a natural follow-up by extending the focus from aquatic ecosystems to the exposure of a human population. By assessing various exposure scenarios, such as drinking water, food consumption, and the indoor environment, the results of *in silico* modelling highlight potential shifts in exposure pathways and intake fractions of chemicals with a changing climate. The findings underscore the intricate linkages between ecological research and human health implications, providing a more holistic perspective on the risks associated with chemical exposures under changing climate conditions.

The results from **chapters I, II, III, and IV** illuminate the complex interplay between climate change-induced stressors, chemical pollutants, and ecosystem functioning (Fig. 10).

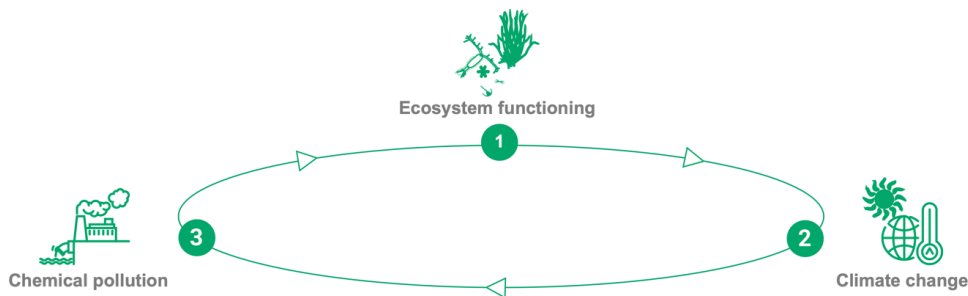


Figure 10. Schematic overview of the interplay between ecosystem functioning (1), climate change-induced stressors (2), and chemical pollution (3) as assessed in this thesis. The graphic is inspired by and adapted from The United Nations Environment Programme (2021).

Thereby, the statement by the UNEP asserting that "Three interconnected planetary crises: climate change, biodiversity loss, and pollution are putting global economic and social well-being at risk" resonates deeply with the findings outlined in this thesis (The United Nations Environment Programme 2021).

In essence, the findings here underscore the critical need for holistic approaches in chemical risk assessment and management. Specifically, through experimental cause-and-effect investigations at different levels of biological organization, the thesis showcases how climate-induced changes in environmental conditions can alter the susceptibility of organisms to chemical contaminants and impact human exposure pathways in unpredictable ways. Thus, by understanding the mechanisms underlying the responses to multiple stressors, future risk assessment strategies can be refined to better anticipate and mitigate the impacts of climate change on chemical exposures. Current regulatory frameworks rely heavily on single-species, single-stressor studies, while multiple-stressor approaches remain inadequately addressed. Transparency in the selection and evaluation of key studies used for regulatory purposes is, thus, essential to foster credibility and user-friendliness in decision-making processes. By addressing the complexities of multiple stressor scenarios and embracing interdisciplinary collaboration, future regulatory efforts can better anticipate and mitigate the adverse impacts of chemical pollutants under changing environmental conditions.

5.2 Future directions

Chemical pollution can have significant and far-reaching effects on ecosystem functioning (Schwarzenbach *et al.* 2006; Bernhardt *et al.* 2017; Groh *et al.* 2022), but disentangling pollution effects under multiple stressor impacts considering climate-induced environmental changes remains an even greater challenge, especially in complex and realistic ecosystem scenarios. Based on the work of this thesis, I identified some critical considerations for future research:

- (1) **Consider chemical concentrations at or near toxicological thresholds:** It is crucial to acknowledge that even low doses of chemicals can result in synergistic interactions when combined with other stressors, such as climate-induced changes. Future research should thus focus on understanding the thresholds of observable effects and how they are influenced by varying chemical concentrations. This aligns with the conclusion drawn in **chapters II and III** that alterations of climate change-induced environmental conditions can lead to synergistic effects on ecosystem functioning when combined with low-dose chemical exposure.
- (2) **Explore sub-organismal level responses and indirect effects:** Traditional toxicity testing often focuses on apical endpoints (e.g., mortality, growth inhibition) but future research should also explore sub-organismal responses, like gene expression, protein synthesis, or energy allocation – in which lag effects may only be detectable at much later stage or in multigenerational studies. Additionally, understanding indirect effects through changes in predator-prey or other competitive interactions is crucial, as they can have cascading effects through the food web. This aligns with findings of **chapters II and III**, accenting the importance of considering mechanistic interactions when studying multiple stressors.
- (3) **Assess effects on multiple levels of biological organization:** Assessing effects on multiple levels of biological organization is crucial for understanding the complexity of interactions among stressors (**chapters I and III**). Investigations at the community and ecosystem levels can reveal the combined impacts of extreme events and other stressors on functional processes and species interactions. This approach, underscored by the importance of considering species performances within communities, allows for a more nuanced understanding of how stressors cascade through food webs and affect species assemblages.
- (4) **Maintain consistency in experimental design:** From single-organism testing to multi-species approaches, using the same organisms and environmental/chemical factors ensures consistency and comparability across studies. This consistency facilitates the integration of findings and allows for a more comprehensive understanding of the effects of multiple stressors (unlike **chapters II and III**).
- (5) **Address the evolving landscape of chemical pollution:** With an increasing number of potentially harmful chemicals in the environment and countless combinations in which they can interact with environmental changes, future research must adapt to this evolving landscape. This included developing scenarios for the use and emission rates of chemical substances to forecast changes in bulk intake of chemicals under global change (**chapter IV**).
- (6) **Refinement of modelling frameworks:** Future research should consider employing alternative modelling frameworks beyond USEtox (**chapter IV**), which may provide

more comprehensive insights into chemical fate, transport, and exposure mechanisms, especially in response to global change factors. Spatially, temporally, and mechanistically resolved modelling can help to better understand changes in exposure efficiency, particularly concerning changes in runoff and diet. This could involve integrating more sophisticated models that capture finer-scale environmental dynamics and environmental or human behaviours.

- (7) **Enhance risk assessment and management procedures, improve decision support for policy and mitigation measures:** Despite their complexity, risk assessment and management procedures need to be efficient and flexible enough to handle different types of chemicals, environmental changes, and data availability. Further guidance for decision-making and policy development should be provided to mitigate global change impacts and implement effective chemical exposure protection measures. This includes informing policymakers and stakeholders about potential risks and strategies for managing chemical exposures in changing environments.

By addressing these future directions, research can advance our understanding of the complex interactions between climate change-induced stressors, chemical pollutants, and ecosystem functioning, ultimately informing more effective strategies for environmental management and conservation.

6 | References

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References

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