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| **Part A – Applicant** |

1. **Main applicant**

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| Name (titles, initials, first name, last name): | Chong Zhang |
| Affiliation (university/institute + department): | Wageningen University & Research  Aquatic Ecology and Water Quality Management Group |

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| **Part B – Scientific proposal** |

1. **Basic details**
2. **Title**

Assessing multiple stressors on freshwater ecosystems:

Combining mechanistic models of different levels of biological organization

1. **Abstract**

Among the 17 sustainable development goals (SDGs), the SDG 6 “clean water and sanitation” and SDG 13 “climate action” call for a protection of freshwater ecosystem, the major source of drinking water and the habitat of over 140,000 species. The dissatisfying ecological status of European surface water is mainly due to the widespread presence of stressors associated with anthropogenic activities. Moreover, current environmental assessment on stressors have not yet included the combined effects of multiple stressors on aquatic ecosystems. The difficulty in the risk assessment of multiple stressors is caused by the intertwined and spatiotemporal-dependent interactions between and the dependency on levels of biological organizations. To deal with the complexity of stressor interaction effects on freshwater ecosystems, modelling is preferred because of the limited capacity of the experimental evaluation of the numerous combinations of various stressors, especially for higher levels of biological organizations, e.g., ecosystem level. Additionally, the widely used null models function poorly at community and ecosystem level given that the underlying mechanisms of stressor interactions were neglected by it. Therefore, mechanistic ecological models might provide a better assessment of the interactive effects of multiple stressors than simply assuming the prevalence of interaction type of stressors. In the proposed research, I will investigate the multiple stressor effects on the freshwater ecosystem in two typical Dutch freshwater landscapes (catchment in agriculture areas and ditches receiving effluents from sewage treatment plants). Firstly, the important stressor types, physicochemical variables and ecological characteristics will be identified for the two scenarios. Secondly, the combined effects of stressors on representative species will be tested in the laboratory or collected from literature. Thirdly, TKTD (toxicokinetic-toxicodynamic) models describing the chemical effects on the individual level and the interaction with the non-chemical stressor will be parameterized using the laboratorial results. Subsequently, the results from TKTD models will be extrapolated to the population level using IBMs (individual-based models) and then to the ecosystem level using simple food web models. The results will be validated (partly) by mesocosm experiments in which the interactive effects of the stressors will be studies at the ecosystem level. Fourthly, the models will be applied for other scenarios with different stressors to develop a meta-model for future use. This research will provide a further understanding of multiple stressor interaction effects on freshwater ecosystems through the connection of mechanistic models of different levels of biological organization, supported with experimental results. Besides, it will provide a potential tool for multiple stressors assessment for better ecological and chemical status of freshwater ecosystems as required by the EU Water Framework Directive.

1. **Summary**

One of the key aims of the EU Water Framework Directive is to achieve “good status” of all water body. However, the widespread stressors associated with anthropogenic activities lead to the dissatisfying ecological status in the Europe surface water. For instance, pesticides and drought may affect freshwater in agricultural areas, and pharmaceuticals and heat waves may affect ditches connected to sewage treatment plants. Additionally, the risk assessment for water protection is scattered for various stressors rather than assembled for the combined effects of multiple stressors. The difficulty in the risk assessment of multiple stressors is caused by 1) the interaction between stressors can result in a stronger, weaker, or equal combined effect of respective stressors (synergism, antagonism, and additivity); 2) the stressor interactions are spatiotemporal-dependent; 3) the prevalent interactions between stressors may vary between different levels of biological organizations. To understand the stressor interaction effects on ecosystems, experimental evaluation can provide limited information because of the numerous combinations of various stressors. Null models are widely used for stressors interactions research. Null models are mathematic models that test the deviation of joint effect from the additivity assumption with the absence of the interaction. Nonetheless, the null models neglect the mechanisms underlying the stressor effects and provide various results when different classification framework and types of models were used, especially for the complex ecosystem levels. Instead, mechanistic models might be more appropriate for unravelling the stressors interactions for ecosystem level as they start from the mechanisms behind the assumed prevalence of stressor interaction types. In the proposed research, I will investigate the multiple stressor effects on the freshwater ecosystem through experiments and combining mechanistic models for different levels of biological organizations. This research will burrow into mechanisms of the multiple stressor interaction effects thereby providing further understanding and more reliable prediction models, and a potential tool for multiple stressors assessment.

1. **Keywords**

environmental risk assessment, multiple stressors, freshwater ecosystem, mesocosm experiment, TKTD model, individual

based model, food web model

1. **Scientific proposal**
2. **Research topic**

**Introduction**

To achieve “good status” (environmental and chemical status) of all water bodies before 2027 is one of the major goals of the WFD (Water Framework Directive), the central Directive of sustainable water usage and freshwater ecosystem protection in the EU [1]. In the recent assessment report in 2018 of the European Environment Agency, it is stated that around 60% of the surface water in Europe did not reach the aforementioned goal [2]. The main reason for the insufficient status is the presence of stressors associated with anthropogenic activities [3, 4]. A stressor is a variable in the environment that exceeds its range of normal variation and may adversely affect individual taxa, community composition, or ecosystem function. It can be an environmental stressor like drought and heatwave, or a chemical stressor like pesticides and pharmaceuticals that leach to freshwater systems, or a biological stressor like invasion species [5]. Multiple stressors are widely present in the freshwater ecosystems, for example, 97% of the monitoring sites of rivers in Germany are exposed to more than one stressor [6]. In another research, among 9330 sampling sites of lotic ecosystems in 14 European countries, 47% were impacted by more than one of the investigated stressors (water quality, hydrology, morphology, and connectivity) and around 90% of lowland rivers were impacted by a combination of all four pressure groups [7].

Interactive stressors would not only influence properties and magnitudes of each other, but also pose influence on the impacts caused by other stressors on ecosystems [8]. How multiple stressors can affect an ecosystem is illustrated in Figure 1. For example, water scarcity can influence the exposure pattern and degradation rate of fungicides in agricultural streams, and the presence of fungicides can exaggerate the effects of water scarcity on the ecosystem by altering the fungal community on leaves thereby decreasing the food consumption of detritivores [9, 10]. When exposed to more than one stressor, the biological responses are not always linear [11]. The combined stressor effects can be equal to (additivity), larger than (synergism), or smaller than (antagonism) the sum of their individual effects [12]. The prevalence and magnitude of stressor interactions are dependent on the level of biological organisation, trophic levels of the affected species [13], temporal differences (e.g. duration and frequency of stressors occurrence), and spatial differences (e.g. application locations of the pesticides) [14, 15]. Stressors like pesticides can pose influence on individuals by affecting their survival and mobility, or by affecting their life-history traits (e.g. reproduction, growth) [16]. The recovery of the populations from exposure depends on the reproduction rate and spatial avoidance of chemicals by the individuals through immigration [17]. At the community and ecosystem level, the difference in species sensitivity leads to different species responses, thereby leading not only to direct effects on populations of various species but also indirect effects through food web interactions and intra- and interspecies competition. Consequently, the ecological functioning and structure of the ecosystem are altered. [18, 19].

*Figure 1. Illustration of multiple stressors effects on the freshwater ecosystem. The stressors are in yellow, the yellow arrow is the impacts of stressors on the magnitudes of each other, e.g., increasing temperature magnifies droughts and the chemical concentration in the environment. The example food web is in green, the green arrow stands for energy flow in different trophic levels, and the grey arrow stands for the excretion and corpse of the grazers and predators that become the detritus. The impacts from the stressors on the ecosystem are in orange.*

Several projects have been conducted to study the effects of multiple stressors on aquatic ecosystems, for example, the MARS (Managing Aquatic ecosystems and water Resources under multiple Stress) project aims to provide information on the most relevant stressors for water resources in Europe [20], and the ECORISK2050 project aims to address chemical risk under different global change scenarios to deal with changes and interactions between chemicals and climate [21]. However, current regulations have not yet successfully addressed the multiple stressors issue. From the retrospective risk assessment perspective, monitoring data are gathered to assess the general ecological and chemical status of the water body [22], with highlighting heterogeneity issue [23]. From the prospective risk assessment perspective, the chemical regulations like the pesticides environmental risk assessment (ERA) and REACH for general chemicals do not included other stressors [4]. Furthermore, the need for ecological realism in the ERA of chemicals has been highlighted, which would be solved partly by the development of refined scenarios for ERA [24]. Hence, the regulations of multiple stressors need to be performed using different scenarios representative for various combination of key stressors [8, 25].

**Knowledge gap and objectives**

Multiple stressors widely occur in freshwater ecosystems and might have unpredictable effects. The experiments of multiple stressors, especially for higher levels of biological organizations, are rare because of the difficulty in manipulation of more than one stressor and replication needed to study multiple stressors in semi-field and field experiments [26]. Despite their difficulty, experiments, especially field experiments, are needed to provide insight into the mechanisms behind the stressor interaction, and to provide ecosystem-level data for parameterization and validation of ecological models. The usefulness of classifying stressor interactions into the three types (additivity, synergism, and antagonism) to predict their impacts on ecosystems has been questioned as these interaction types may depend on e.g. temporal heterogeneity of stressor levels, spatial heterogeneity of stressor levels and ecosystem structure and functioning [27]. The widely used null models for multiple stressors are mainly based on the above classification, which evaluate the deviation of joint effect from the additivity. Moreover, the validation of null models at community and ecosystem levels are questioned because of the uncertain prevalence of stressor interaction types. Under capricious environment, the same mechanism can lead to different deviations and the same deviation can be caused by different mechanisms [28]. For example, interactive stressors A and B have synergistic effects on preys and antagonistic effects on predators, since the population of predators also dependent on the food source (preys), the stressor interaction on predators not only rely of the impact on the grazers and predators individually, but also rely on the predation intensity. Thereby, once we include the preys into consideration, the interaction effects on predators could be any kind, regardless of the prevalent type for each species. As an alternative, mechanistic ecotoxicological and ecological models for different levels of biological organizations have been proposed to describe and predict the interactive effects of multiple stressor [25]. Even though previous research has connected the models of the individual level to the population level [29], the various mechanistic ecotoxicological and ecological models available for the individual, population, and ecosystem level are not uniform and linked in a systematic way. To sum up, the experimental and modelling exploration of the mechanisms of multiple stressor interactions for specific scenarios is in urgent need and would provide potential solutions for integrating multiple stressors in the regulation of chemicals and water quality.

The objective of the research is to investigate the effects of multiple stressors and their interactions on Dutch freshwater ecosystems by developing an integrated mechanistic model that connect models of different levels of biological organization.

This will be achieved by the following sub-objectives:

(1) To identify significant stressor types, physicochemical variables, and ecological characteristics of agricultural catchments and ditches receiving effluents from sewage treatment plants in the Netherlands.

(2) To investigate the mechanism behind the combined effects of stressors on different key species and ecosystems of the chosen scenarios by laboratory and semi-field experiments.

(3) To develop an integrated mechanistic model to investigate the combined effects of stressors on chosen scenarios by combining mechanistic models at the individual, population, and ecosystem level parameterized and validated with the experimental results.

(4) To develop a simple meta-model for non-experts by applying the integrated mechanistic models for different scenarios with different stressors combination

1. **Approach**

*Figure 2. Summary of the approaches taken in the proposed research. Objective 1, 2, and 3 is about investigating the multiple stressor effects on chosen freshwater scenarios. Objective 4 is a meta-model with the results of applying the developed models on other scenarios 4.*

The approaches of the proposed research go along with the objective mentioned above and are shown in Figure. 2. To start with, for the chosen scenarios, the stressor types, the magnitude of the stressors, the temporal and spatial characteristics of the stressors, and the ecological characteristics of the scenario will be gained from literature review or previous data from colleges, if the data is not available, it will be measured in the field. The range of variables measured in the scenarios will be used for variables setting in the experiments and models, and the representative species chosen for each scenario will be used for building food web structure when applying models for the chosen scenarios. The combined effects and individual effects of stressors will be investigated for key species in the laboratory and mesocosm experiments or gained from previous research or databases. As for modelling, the data from the laboratory experiments will be used as input for the TKTD models (the GUTS (General Unified Threshold model of Survival) models for mortality and the DEBtox (Dynamic Energy Based) models for sublethal endpoints). The TKTD model output will be used as input for Individual Based Models (IBMs) to assess the effects of multiple stressors on populations of each key species. Then IMBs output of the key species will be used as input of the food web model to evaluate the multiple stressor effects on the ecosystem. The results will be partly validated by the mesocosm experiments. The connected model framework will then be applied to the chosen scenarios to test the combined stressor effects. Since the mesocosm sites will probably have different food web structure than the chosen scenarios, the integrated model validated by the mesocosm experiments will have different food web structure setups when applying the models to the chosen scenarios, based on their own physicochemical and ecological characteristics. Lastly, the developed models will be used for other scenarios with different stressors, summarize in a meta-model.

**I Scenario identification**

The ditches in the Netherlands are not only for water drainage, but they also play an important role in biodiversity maintenance [30-32]. In this research, we choose two freshwater scenarios representing a common agricultural catchment and a ditch receiving effluent from a sewage treatment plant representing the worst case in the Netherlands. The chosen area for the agricultural catchment scenario is a typical agricultural landscape located in the north-east Klazinaveen-Zwartemeer region. The area is around 10 km2 and the major crop in the area is potato [33, 34]. The chosen area of the ditch receiving effluents from sewage treatment plants scenario is an open ditch connected to the urban wastewater treatment plant (UWWTP) in the village Bennekom in the Netherlands. In the UWWTP, although nitrogen and phosphorus are being removed, not specific removal actions have been taken for pharmaceuticals and personal and home care products.

The scenario identification will be majored based on the guideline from research of Perujo et al. (2021) [8], mainly done by a literature review and field measurements. The representative stressor types will be identified through a literature review for the scenarios. We speculate that pesticides and drought will be two major stressors of the agricultural catchment scenario, pharmaceuticals and heatwaves will be the two major stressors of the sewage treatment plant. The water volumes of both scenarios are comparably small and experience comparably large temperature and chemical concentration fluctuations. The above speculation will be confirmed by the literature review. If other significant stressors be identified, we will start with the two most important stressors and include other stressors after the models are validated.

The magnitude and its spatiotemporal differences of the stressors, and physicochemical variables will be obtained through monitoring data available in the literature and databases like Water Quality ICM (<https://www.eea.europa.eu/data-and-maps/data/waterbase-water-quality-icm>). Data that we cannot find in the public database, e.g., the chemical compounds in ditches and the recent temperature and flow velocity values, will be requested from the regional water authority of the Netherlands (<https://www.waterschappen.nl>) or be monitored by ourselves.

The ecological characteristics of the scenarios will be based on a field study and data already available in previous research. The specific protection goals of different landscapes decide the selection of biological indicators [35]. After collecting the samples from the field, the species in the field and the populations will be identified and enumerated. The representative focal taxa within each trophic level in the food webs will be selected on basis of their abundance, sensitivity to stressors (direct and indirect), ecological interactions and functions. The chosen taxa will be used for experiments and food web built in models.

**II Experiments**

Some experiments have done for pesticides and temperature effects on some species in the Dutch streams by colleges and in previous studies. Most of the toxicity results of chemical are available in the GUTS framework and Add-my-Pet collection as mentioned below. Besides, the early stage researcher 7 in the ECORISK2050 program have tested the combined effect of pesticides and rising temperature at the same mesocosm research site of the propose research (<https://ecorisk2050.eu/markus-hermann-esr-7-wageningen-university-and-research/>). Therefore, the laboratory experiments will be conducted for certain species at individual level when the pesticides or the pharmaceuticals toxicity effects and combination of temperature rising, or droughts have not been investigated. The mesocosm experiments will be conducted for certain stressors and combinations when the stressors have not been tested by colleges at the same research site.

For the laboratory experiments, the effects of the single and combined stressors will be evaluated for the identified representative species of the chosen scenarios, if the similar experiments have not been conducted by previous research. For the agricultural catchment scenario, the effect of decreasing water availability will be evaluated with the corresponding increase in intraspecies competition combined with pesticide exposure. For the ditches of the sewage treatment plants scenario, the effects of pharmaceuticals and heatwaves (rising temperature) will be evaluated. The test species will be collected from the field. The measured endpoints will include survival, growth, feeding behaviour, reproduction and maturity. The decision of measured endpoints of each specie also depends on the feasibility of the experiments in the laboratory, e.g., the maturity of species with comparably long-life cycle might not be able to be measured. The toxicity results of chemicals under different non-chemical variables from the tests will be used for parametrization of the TKTD models.

The mesocosm experiment(s) will be conducted at the outdoor research site ‘De Sinderhoeve’ located in Renkum, the Netherlands ([www.sinderhoeve.org](http://www.sinderhoeve.org)). Most of the mesocosm systems present at this research site mimic shallow ditches. It will not be feasible to mimic the ecological structure of the chosen scenarios in the mesocosm experiments exactly because of the different abiotic environment and available species pools. We, therefore, will not change the original taxa in the mesocosm site, but test the chosen combined stressor effects on the local community which structure (planktonic, macrophyte dominated, etc.) will be similar to the chosen scenarios. The temperature can be manipulated with the temperature-altering equipment developed within the ECORISK2050 project ([www.ecorisk2050.eu](http://www.ecorisk2050.eu)). The droughts can be mimicked by reducing the water level in the ditches. Chemicals can be introduced into the ditches, and the spatial differences of chemical concentration can be mimicked by differentiating the proportion of the ditch that is sprayed with the chemicals [36]. In this research, we will study the individual and combined effects of pesticides and drought, and pharmaceuticals and heatwaves on the ditch communities for 3 months. The invertebrate, microbial and primary producer abundance, diversity, and recovery will be measured, when possible, also use DNA metabarcoding [37], together with the dynamics of the stressors and physicochemical variables like dissolved oxygen and pH. The changes of different species populations in the ecosystem under different stressors can imply how the single and multiple stressors change the structure and function. The results of the mesocosm experiments will be used for validation of the models.

**III Eco(toxico)logical modelling**

GUTS is a framework that unifies (almost) all toxicokinetic-toxicodynamic (TKTD) models for survival [38]. DEBtox models are the TKTD models based on dynamic energy budget (DEB) theory and can be used for sublethal endpoints [39]. GUTS and DEBtox models have been proven to function well for toxicant effects at the individual level of organisms [40, 41]. However, the actual toxicity of toxicants may also dependent on abiotic variables like temperature and food availability [40]. For both the GUTS and the DEBtox models, the parameter values for different species and their dependency on other stressors can be estimated from the results of the laboratory experiments and available databases (Add-my-Pet collection: <https://www.bio.vu.nl/thb/deb/deblab/add_my_pet/>). At the individual level modelling, the toxicity of chemicals under different temperature and population density (corresponding to the water column difference caused by droughts) will be parameterized with the data from laboratory experiments and database.

Individual-based models (IBMs), or agent-based models, are models that simulate populations or meta-populations as being composed of discrete individual organisms [42]. Each individual within the population has a set of state variables or attributes and behaviors, includes spatial location, physiological traits and behavioral traits which vary among the individuals and time [43]. To assess the effects at the population level, the IBMs will be connected to a GUTS and/or DEBtox model. The GUTS model will be used for calculating the survival probability of each individual in a set sequence in daily time steps, as shown in Dohmen et al. (2016) [29]. In this research, we will use the GUTS-IBM and DEB-IBM that follows the overview, design and details (ODD) protocol for describing IBMs for population level modelling [44-46]. The DEBtox models which are parameterized for various temperatures, species, and intraspecific competition levels will be used for providing individual-level effects as input for IBMs. At population level, the fluctuation of populations of each species under the combined and individual stressors will be simulated with the IBMs integrated with a GUTS or DEBtox model.

The food web structure will be based on the ecological structure of the chosen scenario, a simple example is shown in Figure 1. The predation and competition effects will be determined by the growth rate, intake rate and mortal rate of grazers and predators [47]. The producers and the detritivores in the system will be described with a set of ordinary differential equations as shown in previous study [48]. The example of how the linked TKTD-IBM-food web model is applied for grazers and predators is demonstrated in Figure 3. The food availability of autotrophs and detritivore will not be considered as an influencing factor. The food web model will be computed at a daily execution, with input of initial population density, the rate of growth, reproduction, intake and mortality from the IBMs modelling. The change in water quantity caused by stressors like drought will lead to different initial population density. The predation strength is dependent on the population of predators, and the population of preys is thereby affected by the predation strength, the resulting food availability (preys) will be returned to the food consumption of predators [49]. The intraspecies and interspecies competition depend on the population of a certain trophic level. The competition strength will alter the food availability of species thereby alter the biotic variables [49-51].

*Figure 3. Summary the modelling process for a grazer and a predator in the linked TKTD-IBM-food web model. The green blocks are the abiotic variables, the yellow blocks are the endpoints of organisms, the orange blocks are the influence factors from the other species.*

The spatial landscape of the ditches receiving effluents from sewage treatment plants scenario will be adapted from the available MASTEP IBM model (<http://www.mastep.wur.nl>) to describe the invertebrate populations affected by periodic exposure to the point pollution of the effluents [52]. The spatial landscape of the agricultural catchment will be adapted from the Cascade model to describe the invertebrate populations affected by the non-point pollution of pesticides in a changing surface water hydrology [33, 34]. Previous research have already linked the spatiotemporally exposure concentration to IBM [33], or include spatiotemporal difference in IBM for landscape scale management [53, 54]. Each simulated landscape will contain equal size patches with individual abiotic parameters thereby the stressors effects differ at different patches. The movement of various species in the spatial landscape will be determined by species-specific movement capability. The relatively abundance of each species will be calculated by dividing the specific stressors scenarios to the control scenarios where no stressors were introduced. The proportion of abundance of each species among the ecosystem will be calculated to imply the effects on ecosystem structure and function. The recovery times can be estimated by the relatively abundance returned to 90% of control scenarios after introduced the stressors. Thereby, the temporal and spatial change of ecosystem under multiple stressors effects models will be built up.

The results of historical and the planned mesocosm experiments will be used to validate the models. The validated models will be used for the two chosen scenarios to evaluate the multiple stressor effects in their respective environments with the field measured parameters e.g., chemical concentration, representative species.

**IV Use of the models in water management**

As mentioned before, even though the need for a more ecological realistic approach has been recognized in WFD and the scientific communities [8, 21, 24, 25, 55-57], the assessment of multiple stressors have not been included in the ERA framework [58]. To provide a practical tool for interactive stressor evaluation, I will apply the models for several scenarios identified in the Netherlands. For the pesticides, I will select typical pesticides exposure scenarios of surface water under various environmental characteristics developed in FOCUS Surface Water Scenarios combined with non-chemical stressors [59, 60]. For the pharmaceuticals, since the ditch we simulated is receiving effluent from UWWTP and represent the worst case, the safety of the chosen ditch implies the safety of other ditches. Therefore, we will combine other non-chemical stressors with pharmaceuticals for the chosen case for application of the models. Then the results of modelling the stressor interaction effects on different scenarios will be summarized in a meta-model for easily use by other users like regulators. If time is available, it is possible to provide a graphical depiction integrating the meta-modal data to Bayesian network [61].

**V timetable of the project**

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| year | | 1 | | 2 | | 3 | | 4 | |
| semester | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| objective 1: scenario identification | |  |  |  |  |  |  |  |  |
|  | 1.1 literature review and data collection to identify stressor type and physicochemical variables |  |  |  |  |  |  |  |  |
|  | 1.2 field study of ecological characteristics |  |  |  |  |  |  |  |  |
| objective 2: experiments | |  |  |  |  |  |  |  |  |
|  | 2.1 laboratory experiments |  |  |  |  |  |  |  |  |
|  | 2.2. mesocosm experiments |  |  |  |  |  |  |  |  |
|  | 2.3 preparation of scientific publication of the experiments |  |  |  |  |  |  |  |  |
| objective 3: modelling | |  |  |  |  |  |  |  |  |
|  | 3.1 model building and validation |  |  |  |  |  |  |  |  |
|  | 3.2 preparation of scientific publication of modelling |  |  |  |  |  |  |  |  |
| objective 4: regulation protocol | |  |  |  |  |  |  |  |  |
|  | 4.1 model application for other scenarios |  |  |  |  |  |  |  |  |
|  | 4.2 meta-model built up |  |  |  |  |  |  |  |  |
| PhD thesis writing | |  |  |  |  |  |  |  |  |

1. **Justification**

This proposed research is for a fulltime PhD student (the applicant). The funding from the WIMEK program will cover the salary of a PhD student. The budget from the Aquatic Ecology and Water Quality Management (AEW) chair group contribute to experimental and research cost. No further investment for equipment is needed. My Master’s thesis focuses on chronic toxicity of pesticides to freshwater invertebrates, measuring endpoints including survival, mobility, growth, and food consumption. To gain more experience in the realistic ecological risks of chemicals, the expansion to combining them with other stressors effects on higher levels of biological organizations is needed. The topic is well-suited for the profession of Prof. Paul van den Brink who is involved in several international projects of interactive stressor effect on ecosystems and studying the risks of chemicals and other stressors in aquatic ecosystems through experiments and modelling.

1. **Embedding**

The major research activities will be conducted in the AEW chair group in Wageningen University & Research. The AEW group is a multidisciplinary group that focus on the aquatic ecosystem function, surface water quality and the relation between ecosystem and substances with the methods range from laboratory to field study, experiments to modelling. The proposed research is well fitted within the major issues addressed by the AEW chair group. To develop the potential application of our research for future scenarios under global change, we will collaborate with the ECORISK2050 project (<https://ecorisk2050.eu>). The ECORISK2050 project study the effect of global change on the emission, fate, effects, and risks of chemicals in aquatic ecosystems of agricultural and urban scenarios up to 2050. It includes various stakeholders to deliver interdisciplinary research on chemical impacts, potential adaptive strategies, and tools for industrial and regulations. The proposed research objectives can provide more information and tools to study how non-chemical stressors caused by the global change influence the chemical risks. I will join the conferences hosted by the project and might conduct an internship.

1. **Risk assessment**

For chosen scenarios, if it is not allowed to collect samples from the chosen scenarios, we will choose another similar sampling site for the scenarios. For the experiment and modelling, the parameterization of the mechanistic models is highly dependent on previous data. If no data is available for the identified stressors, more laboratory experiments will be needed for parameterization. Consequently, to deal with the increasing workload in the experiment, only one scenario would be tested, and the integrated model will not be applied to other scenarios for the meta-model.

1. **Scientific and/or societal impact**

**Innovative aspect**

The proposed research attempts to unravel the mechanistic background of the effects of multiple stressors without using the conventional simplified classification of stressor interactions. Although a guideline has been developed for choosing null models under different assumptions [62], the mechanistic inference could link interaction at different levels of biological organizations directly, thereby predict the stressors interaction at ecosystems more accurately. For the methods, the proposed research is the first to connect TKTD models, IBMs, and food web models that link the multiple stressor effects from the individual level to ecosystem level. The combined model is a potential new tool for multiple stressors regulations to meet the environmental sustainability goals.

**Impact**

Under the Anthropocene, several serious anthropogenic environmental issues arise. The proposed research can provide more comprehensive insights and tools for stressors, contribute to better chemical and ecological status of freshwater, thereby provide cleaner waters (SDG 6) under the climate change (SDG 13). The speculated non-chemical stressors (droughts and heatwaves) are highly correlated with climate change. With the increasing chemical use, the risks of pesticides and pharmaceuticals might also increase in freshwater ecosystems. Echoed by the ECORISK2050 project, this research provides predictive tools for ERA of chemicals under global change for future scenarios and identified two freshwater scenarios in the Netherlands that provide references for ecological realism regulations. Especially with the deteriorating water quality and decreasing biodiversity in freshwater ecosystems, the proposed research contributes to a more realistic impact evaluation and guidance for regulatory bodies and water managers [2, 63].

1. **Literature/references (in this proposal)**

1. Communities, C.o.t.E., *Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy*. 2000: Office for Official Publications of the European Communities.

2. European Environment Agency, E., *European waters—Assessment of status and pressures*, E.E.A. (EEA), Editor. 2018: EEA Report No 7/2018.

3. IPBES, *Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.*, in *IPBES secretariat*, J.S. E. S. Brondizio, S. Díaz, H. T. Ngo., Editor. 2019: Bonn, Germany.

4. Posthuma, L., et al., *Mixtures of chemicals are important drivers of impacts on ecological status in European surface waters.* Environmental Sciences Europe, 2019. **31**(1): p. 1-7.

5. Townsend, C.R., S.S. Uhlmann, and C.D. Matthaei, *Individual and combined responses of stream ecosystems to multiple stressors.* Journal of Applied Ecology, 2008. **45**(6): p. 1810-1819.

6. Schäfer, R.B., et al., *Contribution of organic toxicants to multiple stress in river ecosystems.* Freshwater Biology, 2016. **61**(12): p. 2116-2128.

7. Schinegger, R., et al., *Multiple human pressures and their spatial patterns in e uropean running waters.* Water and Environment Journal, 2012. **26**(2): p. 261-273.

8. Perujo, N., et al., *A guideline to frame stressor effects in freshwater ecosystems.* Science of The Total Environment, 2021: p. 146112.

9. Arenas-Sánchez, A., A. Rico, and M. Vighi, *Effects of water scarcity and chemical pollution in aquatic ecosystems: State of the art.* Science of the Total Environment, 2016. **572**: p. 390-403.

10. Pesce, S., et al., *Combined effects of drought and the fungicide tebuconazole on aquatic leaf litter decomposition.* Aquatic Toxicology, 2016. **173**: p. 120-131.

11. Bundschuh, M., et al., *Multiple Stressors in Aquatic Ecosystems: Sublethal Effects of Temperature, Dissolved Organic Matter, Light and a Neonicotinoid Insecticide on Gammarids.* Bulletin of Environmental Contamination and Toxicology, 2020. **105**(3): p. 345-350.

12. Jackson, M.C., et al., *Net effects of multiple stressors in freshwater ecosystems: a meta‐analysis.* Global change biology, 2016. **22**(1): p. 180-189.

13. Crain, C.M., K. Kroeker, and B.S. Halpern, *Interactive and cumulative effects of multiple human stressors in marine systems.* Ecology letters, 2008. **11**(12): p. 1304-1315.

14. Jackson, M.C., S. Pawar, and G. Woodward, *The Temporal Dynamics of Multiple Stressor Effects: From Individuals to Ecosystems.* Trends in Ecology & Evolution, 2021.

15. Sabater, S., A. Elosegi, and R. Ludwig, *Defining multiple stressor implications*, in *Multiple Stressors in River Ecosystems*. 2019, Elsevier. p. 1-22.

16. Ashauer, R., et al., *Toxicokinetic‐toxicodynamic modeling of quantal and graded sublethal endpoints: A brief discussion of concepts.* Environmental Toxicology and Chemistry, 2011. **30**(11): p. 2519-2524.

17. Araújo, C.V., M. Moreira-Santos, and R. Ribeiro, *Active and passive spatial avoidance by aquatic organisms from environmental stressors: a complementary perspective and a critical review.* Environment international, 2016. **92**: p. 405-415.

18. Sánchez-Bayo, F., K. Goka, and D. Hayasaka, *Contamination of the aquatic environment with neonicotinoids and its implication for ecosystems.* Frontiers in Environmental Science, 2016. **4**: p. 71.

19. Zhao, Q., F. De Laender, and P.J. Van den Brink, *Community composition modifies direct and indirect effects of pesticides in freshwater food webs.* Science of the Total Environment, 2020. **739**: p. 139531.

20. Hering, D., et al., *Managing aquatic ecosystems and water resources under multiple stress—An introduction to the MARS project.* Science of the total environment, 2015. **503**: p. 10-21.

21. *ECORISK2050*. [cited 2021 March]; Available from: <https://ecorisk2050.eu>.

22. Directive, W.F., *Common implementation strategy for the water framework directive (2000/60/EC).* Guidance document, 2003. **7**.

23. Wolfram, J., et al., *Water quality and ecological risks in European surface waters–Monitoring improves while water quality decreases.* Environment International, 2021. **152**: p. 106479.

24. Franco, A., et al., *Toward refined environmental scenarios for ecological risk assessment of down‐the‐drain chemicals in freshwater environments.* Integrated environmental assessment and management, 2017. **13**(2): p. 233-248.

25. Van den Brink, P.J., et al., *Towards a general framework for the assessment of interactive effects of multiple stressors on aquatic ecosystems: Results from the Making Aquatic Ecosystems Great Again (MAEGA) workshop.* Science of the total environment, 2019. **684**: p. 722-726.

26. Galic, N., et al., *When things don't add up: quantifying impacts of multiple stressors from individual metabolism to ecosystem processing.* Ecology Letters, 2018. **21**(4): p. 568-577.

27. Côté, I.M., E.S. Darling, and C.J. Brown, *Interactions among ecosystem stressors and their importance in conservation.* Proceedings of the Royal Society B: Biological Sciences, 2016. **283**(1824): p. 20152592.

28. De Laender, F., *Community‐and ecosystem‐level effects of multiple environmental change drivers: Beyond null model testing.* Global change biology, 2018. **24**(11): p. 5021-5030.

29. Dohmen, G.P., et al., *Population-level effects and recovery of aquatic invertebrates after multiple applications of an insecticide.* Integrated Environmental Assessment and Management, 2016. **12**(1): p. 67-81.

30. Verdonschot, R.C., H.E. Keizer‐vlek, and P.F. Verdonschot, *Biodiversity value of agricultural drainage ditches: a comparative analysis of the aquatic invertebrate fauna of ditches and small lakes.* Aquatic Conservation: Marine and Freshwater Ecosystems, 2011. **21**(7): p. 715-727.

31. Melman, D.C. and A.J. Van Strien, *Ditch banks as a conservation focus in intensively exploited peat farmland*, in *Landscape ecology of a stressed environment*. 1993, Springer. p. 122-141.

32. Blomqvist, M., et al., *Declining plant species richness of grassland ditch banks—a problem of colonisation or extinction?* Biological conservation, 2003. **109**(3): p. 391-406.

33. Focks, A., et al., *Integrating chemical fate and population-level effect models for pesticides at landscape scale: New options for risk assessment.* Ecological modelling, 2014. **280**: p. 102-116.

34. Kruijne, R., et al., *Surface water hydrology for the Cascade model: study area" Drentsche Veenkoloniën"*. 2008, Alterra.

35. Rico, A., et al., *Developing ecological scenarios for the prospective aquatic risk assessment of pesticides.* Integrated environmental assessment and management, 2016. **12**(3): p. 510-521.

36. Brock, T.C., et al., *Impact of a benzoyl urea insecticide on aquatic macroinvertebrates in ditch mesocosms with and without non‐sprayed sections.* Environmental Toxicology and Chemistry: An International Journal, 2009. **28**(10): p. 2191-2205.

37. Elbrecht, V., et al., *Assessing strengths and weaknesses of DNA metabarcoding‐based macroinvertebrate identification for routine stream monitoring.* Methods in Ecology and Evolution, 2017. **8**(10): p. 1265-1275.

38. Jager, T., et al., *General unified threshold model of survival-a toxicokinetic-toxicodynamic framework for ecotoxicology.* Environmental science & technology, 2011. **45**(7): p. 2529-2540.

39. Jager, T., *Revisiting simplified DEBtox models for analysing ecotoxicity data.* Ecological Modelling, 2020. **416**: p. 108904.

40. Accolla, C., et al., *DEB-tox and Data Gaps: Consequences for individual-level outputs.* Ecological Modelling, 2020. **431**: p. 109107.

41. Brock, T., et al., *Application of General Unified Threshold Models of Survival Models for Regulatory Aquatic Pesticide Risk Assessment Illustrated with An Example for the Insecticide Chlorpyrifos.* Integrated Environmental Assessment and Management, 2021. **17**(1): p. 243-258.

42. Railsback, S.F. and V. Grimm, *Agent-based and individual-based modeling: a practical introduction*. 2019: Princeton university press.

43. DeAngelis, D.L. and V. Grimm, *Individual-based models in ecology after four decades.* F1000prime reports, 2014. **6**.

44. Martin, B.T., et al., *Dynamic Energy Budget theory meets individual‐based modelling: a generic and accessible implementation.* Methods in Ecology and Evolution, 2012. **3**(2): p. 445-449.

45. Diepens, N.J., et al., *Dynamics and recovery of a sediment-exposed Chironomus riparius population: A modelling approach.* Environmental Pollution, 2016. **213**: p. 741-750.

46. Grimm, V., *The ODD protocol: An update with guidance to support wider and more consistent use.* Ecological Modelling, 2020. **428**: p. 109105.

47. Shoemaker, L.G., et al., *Quantifying the relative importance of competition, predation, and environmental variation for species coexistence.* BiorXiv, 2019: p. 797704.

48. De Laender, F., et al., *Theoretically exploring direct and indirect chemical effects across ecological and exposure scenarios using mechanistic fate and effects modelling.* Environment international, 2015. **74**: p. 181-190.

49. Van den Brink, P.J., S.L. Klein, and A. Rico, *Interaction between stress induced by competition, predation, and an insecticide on the response of aquatic invertebrates.* Environmental toxicology and chemistry, 2017. **36**(9): p. 2485-2492.

50. Del Arco, A.I., et al., *Effects of intra-and interspecific competition on the sensitivity of aquatic macroinvertebrates to carbendazim.* Ecotoxicology and environmental safety, 2015. **120**: p. 27-34.

51. Viaene, K.P., et al., *Species interactions and chemical stress: combined effects of intraspecific and interspecific interactions and pyrene on Daphnia magna population dynamics.* Environmental toxicology and chemistry, 2015. **34**(8): p. 1751-1759.

52. Van den Brink, P.J. and J.H. Baveco, *MASTEP—An individual based model to predict recovery of aquatic invertebrates following pesticide stress.* Ecological models in support of regulatory risk assessments of pesticides: developing a strategy for the future. Pensacola (FL): SETAC, 2008.

53. Topping, C.J., et al., *Towards a landscape scale management of pesticides: ERA using changes in modelled occupancy and abundance to assess long-term population impacts of pesticides.* Science of the Total Environment, 2015. **537**: p. 159-169.

54. Topping, C.J., et al., *ALMaSS, an agent-based model for animals in temperate European landscapes.* Ecological Modelling, 2003. **167**(1-2): p. 65-82.

55. Directive, W.F., *Water Framework Directive.* Journal reference OJL, 2000. **327**: p. 1-73.

56. Van den Brink, P.J., et al., *New approaches to the ecological risk assessment of multiple stressors.* Marine and Freshwater Research, 2016. **67**(4): p. 429-439.

57. Navarro-Ortega, A., et al., *Managing the effects of multiple stressors on aquatic ecosystems under water scarcity. The GLOBAQUA project.* Science of the Total Environment, 2015. **503**: p. 3-9.

58. Dafforn, K., et al., *Big data opportunities and challenges for assessing multiple stressors across scales in aquatic ecosystems.* Marine and Freshwater Research, 2016. **67**(4): p. 393-413.

59. FOCUS, E., *FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC.* Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001–rev, 2001. **2**.

60. Authority, E.F.S., et al., *Scientific report of EFSA on the ‘repair action’of the FOCUS surface water scenarios.* EFSA Journal, 2020. **18**(6): p. e06119.

61. Mitchell, C.J., et al., *Integrating metapopulation dynamics into a Bayesian network relative risk model: Assessing risk of pesticides to Chinook salmon (Oncorhynchus tshawytscha) in an ecological context.* Integrated Environmental Assessment and Management, 2021. **17**(1): p. 95-109.

62. Schäfer, R.B. and J.J. Piggott, *Advancing understanding and prediction in multiple stressor research through a mechanistic basis for null models.* Global Change Biology, 2018. **24**(5): p. 1817-1826.

63. Grooten, M. and R.E. Almond, *Living planet report-2018: aiming higher.* Living planet report-2018: aiming higher., 2018.

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| --- |
| **Part C – Additional information** |

1. **Research areas**

Environmental science (40%)

Toxicology (plants, invertebrates) (35%)

Ecology (25%)

1. **Main applicant – additional information**

|  |  |
| --- | --- |
| Name: | Chong Zhang |
| Affiliation: | Wageningen University & Research  Aquatic Ecology and Water Quality Management Group |
| Position: | Master student |
| Paid position: | no |
| Full time or part time: | full time |
| End date of contract: | N/A |

1. **Other grant applications**

|  |  |
| --- | --- |
| Title proposal: | N/A |
| Applicant(s): | N/A |
| Funding agency and call: | N/A |
| Budget applied for: | N/A |
| Date of submission: | N/A |
| Estimated date of decision: | N/A |
| Difference with this proposal: | N/A |
| Describe difference: | N/A |

1. **Data management plan**
2. Will you collect or generate data that is suitable for reuse?

Yes

1. Where will the data be stored during the research?

Data will be stored by the applicants and the AEW chair group before the publication by a journal and/or when the conclusion is made that the data will not be included in a peer reviewed publication.

1. After the project has been completed, how will the data be stored for the long-term and made available for use by third parties? To whom will the data be accessible?

After the project being completed, the mechanistic models and the meta-model developed in the project will be stored in GitHub with an open access. The toxicity experiment data will be uploaded to the dataset GUTS and Add-my-Pet collection with open access for future use.

1. Which facilities (ICT, glove box, refrigerator, etc.) do you expect you need for the storage of data during and after the research? Are these available?

The data will be stored in GIS formats, in spreadsheet .csv or .xls files or in .doc word files or any other standard format or url links will be provided for existing databases.

1. **Public summaries**

Understanding how multiple stressors affect freshwater ecosystem

The chemical and ecological status of freshwater in Europe is widely affected by stressors associated with anthropogenic activities. Although assessment of single stressors like droughts and chemicals are well developed by regulators, the combined effects are not included in the evaluation process. In the ecosystem, the multiple stressors are intertwined with each other and their combined effects on ecosystems are usually non-linear. In the proposed research, we will develop a mechanistic model supporting by experimental results to furtherly understand the effects of multiple stressor interactions.

Begrijpen hoe meerdere stressoren het zoetwaterecosysteem beïnvloeden

De chemische en ecologische toestand van zoet water in Europa wordt sterk beïnvloed door stressfactoren die verband houden met antropogene activiteiten. Hoewel de beoordeling van afzonderlijke stressfactoren zoals droogte en chemicaliën goed is ontwikkeld door regelgevende instanties, worden de gecombineerde effecten niet meegenomen in het evaluatieproces. In het ecosysteem zijn de meerdere stressoren met elkaar verweven en zijn hun gecombineerde effecten op ecosystemen meestal niet-lineair. In het voorgestelde onderzoek zullen we een mechanistisch model ontwikkelen dat wordt ondersteund door experimentele resultaten om de effecten van meerdere stressorinteracties beter te begrijpen.

1. **Budget table**

