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Analysis and assessment of cumulative effects of anthropogenic pressures on ecosystem components

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Summary

Effects of stressors on organisms are most frequently analyzed in short-term experiments focusing on one stressor only. However, in their natural environment species are usually exposed to multiple stressors for a long time with varying stressor intensities and under fluctuating environmental conditions resulting in cumulative effects. Hence, there is a gap between experimental science and field conditions, which is relevant for nature conservation. For an effective management it is important to understand the effects of multiple stressors to adjust measures to meet the actual needs of ecosystem components. An underestimation of actual impacts might result in less ambitious programmes of measures or managing actions than necessary. In a worst-case, this would lead to the extinction of affected species. Therefore, the topic gained national and international attention in science and in legal frameworks. The Marine Strategy Framework Directive (MSFD) requests for example cumulative effects assessments to assess the environmental status adequately, which is the basis for the development of national programmes of measures.

In chapter one, I review published methods for cumulative effects assessments and discuss their applicability in the context of nature conservation and implementation of the MSFD. The reviewed methods comprise the indicator concept, cross-impact analyses, ecological network analyses, causal analyses using flow diagrams, the threshold approach, toxicokinetic chemical interaction models, Dynamic Energy Budget Moodels (DEB-models), geographical analyses, and expert judgement. I conclude that none of the methods is suitable alone and that a combined approach is needed to assess cumulative effects adequately.

The aim of my thesis was to develop a concept for cumulative effects assessment comprising literature data based, transparent, and reproducible methods to produce quantifiable results for cumulative effects assessments.

In chapter three, I present the overall concept. The first important component of the concept is the systematic organization, analysis, and visualization of literature data. Secondly, I developed new methods by adjusting and combining already existing methods to analyze cumulative effects on organism level, on habitat level and for a spatial perspective. Third, I developed a scheme for the realization of an online tool for cumulative effects assessment by identifying the modules and links between them needed to provide a suitable data flow and traceability of information. The online tool is realized with the Literature based Cumulative Assessment Tool (LiACAT), available on the biodiversity data platform 'mybiOSis'. It comprises modules for the extraction of literature data, for the organization and visualization of literature data, for filtering literature data, for the analysis of monitoring data, for geographical analyses and it comprises several modules for cumulative effects analyses. Moreover, species names are linked to a big online species database. Literature data serve as the basis for the construction of a network of links between pressures and effects, which are visualized in flow diagrams. The data extracted from literature are further used as inputs for a matrix analyzing interaction effects between stressors, for a model analyzing temporal dynamic cumulative effects on organisms, and for a network model for the analysis of cumulative effects on habitats. Lastly, impact maps can be created to identify hotspots where human pressures accumulate spatially and to identify where one can expect a high cumulative impact on the natural environment.

Next, I describe two of the proposed methods for cumulative effects assessment more in detail and apply test data to assess their applicability.

In chapter four, I analyze the cumulative effects of several anthropogenic and potential environmental stressors on blue mussels (*Mytilus edulis*) by combining a matrix model and an adjusted Dynamic Energy Budget Model (DEB model). First, I calculate the interaction effects between stressors with a cross impact matrix. Cross impact matrices assume that a complex system can be explained by the analysis of all pairwise-interactions. Those interactions are organized in a tabular structure. I applied this scheme and calculated the 'net effect' of all stressors on the effects of each of the stressors by calculating the corresponding row sums. Moreover, I analyze temporal dynamics related to the stressor response of the organism with a mathematical model. Here, I consider the uptake processes of heavy metals, acclimatization processes and potential temporal delays. I integrated these two modules into an established Dynamic Energy Budget Model (DEB-model) designed for *Mytilus edulis* (Saraiva et al. 2012) and calculated the cumulative effects of a given pressure situation for a geographical spot close to the East Frisian Island Norderney. The results show the impact of the multiple stressors throughout the life span of the mussel on reserve biomass, maturity, reproduction and growth in comparison to a stress-free control scenario. Moreover, I compared the method described above with an alternative method neglecting interaction effects to figure out if interactions matter. Indeed, there was a difference between the control scenario and the stress scenario, as well as between the stress-scenario analyzed with the consideration of interaction effects and without these. Thus, I conclude that the study of cumulative interaction effects as well as the consideration of temporal dynamics is relevant for the assessment of realistic pressure situations as those represented by the test data.

In chapter five, I present a modeling tool (Automatized cumulative interaction model - ACIM) to analyze cumulative effects of multiple stressors with a network model. The tool allows analyzing cumulative effects on higher biological levels and I tested it with data relevant for seagrass meadows as an example for a habitat. The results of the modeling tool indicate what type of interaction effects dominate in the studied system. Thereby, it differentiates between additive and multiplicative interactions and calculates the corresponding weight of each of the relevant stressors and the interactions. The modeling tool is directly linked to LiACAT, which I used for literature organization. In the modeling tool, a set of mathematical models, which are frequent within the field of biology (base models), are applied to dose-response datasets from scientific literature. If several variables influence the response, the modeling tool uses not only the base models but creates also all possible combinations of base models. Next, the parameters are optimized and for each dataset and best models identified. A filter was applied to ensure that the best models fulfill pre-defined minimum requirements. Only those were used for an automatic construction of a network model. To understand the relevance of composite models for the network model and for the study system, additionally one network model was constructed with single models only and thus excluding potential cumulative effects. I tested the modeling tool for a reference scenario and for two scenarios of increased anthropogenic stress. Moreover, I analyzed the model behavior under gradually increasing anthropogenic stress. The results revealed that the hyperbola, which the modeling tool identified most often as a best model alone or in combination with other models, is a characteristic model for the study system. Furthermore, the tool identified additive as well as multiplicative interactions indicating that solely additive approaches are not sufficient to analyze cumulative effects in seagrass meadows. Finally, there was a clear difference between the results when cumulative effects where included compared to the exclusion of cumulative effects. Thereby, none of the methods predicted generally more severe impacts than the other one. Instead, the pressure intensity determined which method predicted more severe impacts.

In the general discussion, I conclude that the results of the two models (chapter 4 and 5) indicate that the consideration of cumulative effects matters for the model outcomes. Hence, they should be considered in environmental assessments and for the development of measures for nature conservation. Both proposed methods aim for a realistic quantification of cumulative effects and they represent alternatives to assessments mainly based on expert knowledge for future assessments. However, many potential interactions haven not been studied yet and thus, big data gaps exist with a concomitant uncertainty in the model results. Therefore, the models should be applied carefully and additional methods to assess an environmental status are indispensable. Both methods are generally applicable for the cumulative assessment of the MSFD Descriptor 1 (biodiversity). In the future, a combination of the adjusted DEB-model and the ACIM modeling tool could be advantageous to address all aspects of cumulative effects. Moreover, the integration of these methods into a spatial ecosystem based analysis would be a next logical step, as soon as the methods have been applied to more species and habitats.

Zusammenfassung

Effekte von Stressoren auf Organismen werden am häufigsten in Kurzzeitexperimenten untersucht, die sich auf die Effekte eines einzigen Stressors konzentrieren. In ihrer natürlichen Umgebung sind Arten normalerweise jedoch über einen langen Zeitraum multiplen Stressoren mit unterschiedlicher Stärke und schwankenden Umweltbedingungen ausgesetzt, was zu kumulativen Effekten führen kann. Folglich gibt es einen Unterschied zwischen experimenteller Wissenschaft und Freilandbedingungen, was für den praktischen Naturschutz relevant ist. Für ein effektives Management ist es wichtig die Effekte multipler Stressoren zu verstehen, um Naturschutzmaßnahmen so anzupassen, dass sie den tatsächlichen Bedürfnissen der Ökosystemkomponenten entsprechen. Eine Unterschätzung der tatsächlichen Auswirkungen könnte weniger ambitionierte Maßnahmenprogramme oder Managementaktionen zur Folge haben als notwendig und im schlimmsten Fall zum Aussterben der betroffenen Arten führen. Daher erhielt dieses Thema nationale und internationale Aufmerksamkeit sowohl in der Wissenschaft als auch in rechtlichen Rahmenbedingungen. In der Meeresstrategie Rahmenrichtlinie (MSRL) ist beispielsweise eine kumulative Bewertung vorgeschrieben, um den Umweltzustand, der die Basis für die Entwicklung von nationalen Maßnahmenprogrammen ist, adäquat zu bewerten.

In Kapitel zwei gebe ich einen Überblick über publizierte Methoden zur kumulativen Bewertung und diskutiere ihre Anwendbarkeit im Kontext von Naturschutz und der Umsetzung der MSRL. Diese Methoden umfassen das Indikatorenkonzept, Cross-Impact Analysen, ökologische Netzwerkanalysen, kausale Wirkungsketten mit Fließdiagrammen, Grenzwertansätze, toxikokinetische chemische Interaktionsmodele, Dynamische Energiebilanz Modelle (DEB-Modelle), geographische Analysen und Konsultation von Experten. Schließlich komme ich zu dem Ergebnis, dass keine dieser Methoden für sich genommen geeignet ist und dass ein kombinierter Ansatz notwendig ist, um kumulative Effekte adäquat zu bewerten.

Das Ziel meiner Doktorarbeit ist es ein Konzept für die Bewertung kumulativer Effekte zu entwickeln, das literaturbasierte, transparente und reproduzierbare Methoden umfasst, um quantifizierbare Ergebnisse zu produzieren.

In Kapitel drei stelle ich das übergreifende Konzept vor. Die erste wichtige Komponente des Konzeptes ist die systematische Organisation, Analyse und Visualisierung von Literaturdaten. Zweitens entwickle ich durch die Anpassung und Kombination bereits existierender Methoden neue Methoden um kumulative Effekte auf Organsimenebene, auf Habitatebene und auf räumlicher Ebene zu analysieren. Drittens entwickle ich ein Schema für die Realisierung eines Online-Tools für kumulative Bewertungen, indem ich benötigte Module identifiziere und erarbeite, wo Links zwischen diesen gesetzt werden sollten, um einen geeigneten Datenfluss und eine Rückverfolgbarkeit von Informationen zu gewährleisten. Das Online Tool wird mit dem Literature based Cumulative Assessment Tool (LiACAT) realisiert, welches auf der Biodiversitätsplattform ‚mybiOSIs' verfügbar ist. Es enthält Module für die Extraktion von Literaturdaten, für die Organisation und Visualisierung von Literaturdaten, für die Filterung von Literaturdaten, für die Analyse von Monitoringdaten, für geographische Analysen und umfasst mehrere Module für die Analyse kumulativer Effekte. Darüber hinaus sind Artnamen mit einer großen Online Artendatenbank verknüpft. Literaturdaten bilden die Grundlage für die Konstruktion eines Netzwerkes zwischen Belastungen und Effekten, die in Flussdiagrammen visualisiert werden. Die aus der Literatur extrahierten Daten werden als Input für eine Matrix

zur Analyse kumulativer Interaktionseffekte zwischen Stressoren, für ein Modell für die Analyse zeitlich dynamischer kumulativer Effekte und für ein Netzwerkmodell zur Analyse kumulativer Effekte auf Habitate genutzt. Schließlich können Karten erstellt werden, die kumulative Auswirkungen zeigen und auf denen deutlich wird, wo menschliche Belastungen räumlich akkumulieren und wo folglich eine hohe kumulative Auswirkung auf die natürliche Umwelt zu erwarten ist.

In den nächsten beiden Kapiteln beschreibe ich zwei der vorgeschlagenen Methoden im Detail und wende jeweils Testdatensätze auf die Modelle an, um ihre Anwendbarkeit zu evaluieren.

In Kapitel vier analysiere ich kumulative Effekte mehrerer anthropogener Stressoren und potentieller Umweltstressoren auf Miesmuscheln (*Mytilus edulis*) indem ich ein Matrixmodell und ein angepasstes DEB-Modell miteinander kombiniere. Zunächst berechne ich die Interaktionseffekte zwischen den Stressoren mit einer Cross Impact Matrix. Für Cross Impact Matrices wird angenommen, dass ein komplexes System mit der Analyse von Zweierbeziehungen erklärt werden kann. Diese Interaktionen werden in einer Tabelle dargestellt. Ich wende dieses Schema an und berechne den sogenannte ‚Nettoeffekt', der den Effekt aller Stressoren auf jeden der Stressoren erfasst, in dem die entsprechenden Zeilen summiert werden. Darüber hinaus analysiere ich die zeitlichen Dynamiken, die die Antwort des Organismus auf Stress betreffen, mit einem mathematischen Modell. Dabei berücksichtige ich Aufnahmeprozesse von Schwermetallen, Akklimatisierungsprozesse und potenzielle zeitliche Verzögerungen. Diese zwei Module integriere ich in ein etabliertes DEB-Modell, das für die Miesmuschel entwickelt wurde (Saraiva et al. 2012) und berechne die kumulativen Effekte für einen geographischen Punkt in der Nähe der Ostfriesischen Insel Norderney. Die Ergebnisse zeigen die Auswirkungen von multiplen Stressoren auf Reservebiomasse, Reife, Reproduktion und Wachstum im Vergleich zu einem stressfreien Kontrollszenario über die gesamte Lebensspanne der Muschel. Außerdem vergleiche ich die oben beschriebene Methode mit einer alternativen Methode, bei der die Interaktionseffekte vernachlässigt werden, um herauszufinden, ob Interaktionen eine Rolle spielen. Tatsächlich gibt es einen Unterschied zwischen dem Kontrollszenario und dem Stressszenario sowie zwischen dem Stressszenario unter der Berücksichtigung von Interaktionen und dem Stressszenario ohne Berücksichtigung von Interaktionen. Daher schlussfolgere ich, dass die Untersuchung kumulativer Effekte sowie die Berücksichtigung zeitlicher Dynamiken für die Bewertung realistischer Belastungssituationen wie die des Testdatensatzes relevant sind.

In Kapitel fünf präsentiere ich ein Modelltool (Automatized cumulative interaction model - ACIM), mit dem die Analyse kumulativer Effekte multipler Stressoren mit einem Netzwerkmodell möglich ist. Das Tool erlaubt es kumulative Effekte auf höherer biologischer Ebene zu analysieren und ich teste es mit Daten, die für Seegräser relevant sind. Die Ergebnisse des Modell-Tools zeigen an, was für eine Art von Interaktionseffekte im untersuchten System dominieren. Dabei unterscheidet es zwischen additiven und multiplikativen Interaktionen und berechnet die entsprechenden Gewichtungen der jeweiligen Interaktionen und der relevanten Stressoren. Das Modell-Tool ist direkt mit LiACAT, das ich für die Organisation von Literaturdaten verwendet habe, verbunden. Im Modell-Tool wird eine Auswahl von mathematischen Modellen, die häufig in der Biologie vorkommen (Basismodelle) auf Dosis-Wirkungs-Datensätze aus der wissenschaftlichen Literatur angewandt. Wenn mehrere Variablen eine Wirkung beeinflussen, werden nicht nur die Basismodelle genutzt, sondern auch sämtliche mögliche Kombinationen von Basismodellen vom Tool erstellt. Als nächstes werden die Parameter optimiert und zu jedem

Datensatz werden die besten Modelle identifiziert. Ein Filter wird verwendet, um sicherzustellen, dass die besten Modelle zuvor definierte Minimalanforderungen erfüllen. Nur diese werden zur die automatische Erstellung eines Netzwerkmodells genutzt. Um die Relevanz der zusammengesetzten Modelle für das Netzwerkmodell sowie für das Studiensystem zu verstehen, wird ein zusätzliches Netzwerkmodell erstellt, das nur auf einfachen Basismodellen basiert und mögliche kumulative Effekte ausschließt. Das Modell-Tool teste ich für ein Referenzszenario sowie für zwei Belastungsszenarien mit erhöhtem anthropogenen Stress. Darüber hinaus untersuche ich das Modellverhalten unter sich graduell erhöhendem anthropogenen Stress. Die Ergebnisse zeigen, dass die Hyperbel, welche durch das Modell-Tool am häufigsten als bestes Einzelmodell oder als Teilkomponente zusammen mit anderen Modellen ausgewählt wurde, ein charakteristisches Modell für das Studiensystem ist. Außerdem identifiziert das Modell-Tool sowohl additive als auch multiplikative Interaktionen. Dies legt nahe, dass ein Ansatz, der ein rein additives Verhalten annimmt, nicht ausreichend ist, um kumulative Effekte in Seegraswiesen zu analysieren. Schließlich gibt es einen klaren Unterschied zwischen dem Ergebnis unter Berücksichtigung der kumulativen Effekte und dem auf Einzelmodellen basierenden Ergebnis. Dabei sagt keine dieser beiden Methoden generell höhere Auswirkungen voraus. Stattdessen hängt es von der Belastungsstärke ab, welche Methode stärkere Auswirkungen vorhersagt.

In der allgemeinen Diskussion schlussfolgere ich, dass die Ergebnisse der zwei näher dargestellten Methoden nahelegen, dass die Berücksichtigung der kumulativen Effekte für die Modellergebnisse eine Rolle spielen. Folglich sollten sie in Umweltbewertungen und bei der Entwicklung von Maßnahmen für den Naturschutz berücksichtigt werden. Beide vorgeschlagenen Methoden zielen auf eine realistische Quantifizierung kumulativer Effekte ab und repräsentieren Alternativen zu hauptsächlich auf Experteneinschätzungen basierenden Bewertungen. Jedoch sind viele potentielle Interaktionen noch nicht untersucht und daher existieren große Datenlücken mit einer damit einhergehenden Unsicherheit in den Modellergebnissen. Daher sollten die Modelle vorsichtig angewendet werden. Zusätzliche Methoden zur Bewertung des Umweltzustandes sind unerlässlich. Beide Methoden sind grundsätzlich für die kumulative Bewertung des MSRL Deskriptors 1 (Biodiversität) anwendbar. In der Zukunft könnte eine Kombination des angepassten DEB-Modells und des ACIM Modell-Tools vorteilhaft sein, um sämtliche Aspekte kumulativer Effekte zu adressieren. Außerdem wäre die Integration dieser Methoden in eine räumliche, Ökosystem-basierte Analyse ein nächster logischer Schritt, sobald die Methoden auf mehr Arten und Habitate angewandt wurden.

Figures

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1 General introduction

Marine ecosystems are exposed to an increasing number of different anthropogenic stressors with varying intensities, due to industrialization, the increasing exploitation of marine resources and the continuous spatial utilization of marine areas for human needs. The continuous development of new products leads to new chemical substances, ending up in the environment. Anthropogenic chemicals and pollutants are found all over the ocean owing to atmospheric or oceanic transport, and long-distance fishery fleets are able to reach even the most remote regions of the ocean. As these developments accelerated during the 20th century, scientists observed a serious decline of biodiversity and environmental health with high impacts on environmental processes and ecosystem functions (Sala and Knowlton 2006, Clausen and York 2008, Hooper et al. 2012). Lab experiments and field studies showed that these observations are caused by anthropogenic pressures linked to different human activities (Maxim and Spangenberg 2009, McKinney et al. 2010, Nordlund and Gullström 2013, Johnston et al. 2015). The need to improve this situation led to a range of different legislatives and international efforts (e.g. HELCOM 2009, OSPAR 2010, WFD 2000, REACH (EC 1907/2006))

In June 2008, the guideline for establishing a framework for community action in the field of the marine environment (Marine Strategy Framework Directive - MSFD, 2008/56/EG) was published focusing particularly on marine areas including the open sea with a special emphasis on an ecosystem perspective. The overall objective of this guideline is to achieve a good status of the marine environment in all marine regions by the year 2020. All member states are required to establish a national action plan for their marine waters to reach this. The good environmental status relates to qualitative Descriptors as listed in Annex I (MSFD, 2008/56/EG) comprising the following aims:

- "Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions.
- Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems.
- Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock.
- All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.
- Human-induced eutrophication is minimized, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters.
- Sea-floor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected.
- Permanent alteration of hydrographical conditions does not adversely affect marine ecosystems.

- Concentrations of contaminants are at levels not giving rise to pollution effects.
- Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards.
- Properties and quantities of marine litter do not cause harm to the coastal and marine environment.
- Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment.”

These descriptors are specified through respective Criteria and Indicators given by the European commission (Commission Decision 2017). For the definition of the good environmental status, the ecosystem approach has to be taken into account. Further, the MSFD requires an assessment of cumulative effects (MFSD 2008, Article 8bii), which can occur when several anthropogenic pressures accumulate spatially.

Multiple anthropogenic stressors in the marine environment interact in a complex way and evoke special impact and response patterns for marine organisms and community structures, which cannot be explained by the simple addition of the effects of the single stressors alone (Crain et al. 2008, Hooper et al. 2012, Holmstrup et al. 2010, Moe et al. 2013). Furthermore, the magnitude of cumulative effects depends on the intensities of the stressors in the environment, their temporal and spatial pattern of appearance, and their affinity to interact with other stressors and with the organism itself. The analysis of cumulative effects could help to better understand and predict the effects of anthropogenic pressures and to provide a more holistic perspective of the environmental health of ecosystems. A cumulative assessment would further allow to join different aims of the MSFD and to conduct overarching analyses.

However, the MSFD does not define the term cumulative effects explicitly and interpretations of the term differ greatly from each other depending on the context and the focus of interest. Moreover, despite the need and the legal requirement to assess cumulative effects, there is still no regionally agreed method for cumulative effects assessment in the North Sea and the Baltic Sea, which meets the challenge of addressing all different aspects of cumulative effects. Moreover, there is a lack of integration of experimental results conducted to understand cumulative effects and resulting scientific insights in currently applied methods for assessments.

The objective of the thesis is to develop a concept for the assessment of cumulative effects, which incorporates the current state of knowledge, which is scientifically sound and which can cover different kinds of cumulative effects. To clarify the major aspects of cumulative effects and to cover the whole range of perspectives upon it, I elucidate those in chapter 2.1. In chapter 2.3 I review and discuss already existing methods and approaches for cumulative effects assessment. Based on this review, I developed an overall concept, presented in chapter three. Moreover, the aim of my thesis was to consider different points of focus of cumulative effect assessments: Cumulative effects were analyzed with a focus on species level (chapter four) as well as with a focus on habitat level (chapter five). The chapters three, four, and five aim to answer different main questions and to fulfill specific tasks:

In chapter three, I address the following questions:

- How could reproducibility, comparability and transparency be provided in a concept for cumulative effects assessment?
- How can scientific insights and data be harmonized with applied monitoring programs and requirements for practical assessments?
- How can the results of cumulative effects assessments be visualized in an unifying scheme?
- How can practitioners get a quick overview of the state of knowledge about cumulative effects?

The task of chapter four is to conduct an analysis of cumulative effects of anthropogenic pressures on species level and to test a method with a given dataset from a monitoring station at the East Frisian island Norderney with the model species M*ytilus edulis*. The main questions of this chapter are:

- Is there a difference between model results for blue mussels of a reference scenario and a scenario including anthropogenic pressures?
- Which kind of cumulative effects likely occurred during the study period?
- How did the mussel likely respond to the pressure situation throughout its life cycle?
- Which stressors contributed most to the cumulative effect?
- Is there a difference in the results of the model when only additive effects are assumed compared to the inclusion of interaction effects?

Chapter five addresses cumulative effects assessment at habitat level. I developed a special methodological approach to integrate the „lessons learned“ from chapter four with regard to the nature of cumulative effects. This is realized in a larger network model consisting of several sub-models describing relationships between influences and influenced variables (e.g. influences of stressors on an ecosystem components). I collected literature about anthropogenic pressures affecting seagrass meadows and analyzed cumulative effects for different stress scenarios. The major questions of this chapter are:

- How does the model applied for seagrass meadows respond to increasing pressure scenarios?
- Can typical cumulative effects on habitat level, such as indirect effects, be simulated with the model?
- Is there a major difference in the model outcome applying only single models and the model outcome integrating also composite models?
- Are additive or multiplicative cumulative effects likely more frequently occurring in seagrass meadows?

2 Review of cumulative effects assessments

2.1 Overview of different kinds of cumulative effects

2.1.1 Temporal cumulative effects

Molinos and Donohue (2010) focused on interactions among temporal patterns of multiple stressors. They could show for the first time that organisms react differently to stressor combinations depending on the temporal pattern of the stressors. As this is a special effect, which is evoked by the combination of the stressors, one can interpreted it as a cumulative effect. Multifactorial experiments with periphyton and benthic invertebrates in freshwater were conducted to investigate the effects of regular and temporal variable pulses of addition of two stressors (in this case sediment addition and nutrient enrichment) (Molinos and Donohue 2010). Interestingly, nutrient enrichment only had an effect on stream biota in the experimental setups where regular added pulses of nutrients and variable pulses of sediment addition were combined. Furthermore, the results indicated that the simultaneous addition of stressors does not necessarily lead to synergistic effects and that instead asynchronous temporal patterns can in some cases lead to more severe ecosystem effects (Molinos and Donohue 2010).

The influence of temporal patterns was also shown for effects of single stressors. Benedetti-Cecchi et al. (2006) studied the temporal effect pattern of one stressor (aerial exposure) on algae and invertebrates. They further observed that different species responded differently to temporal patterns. While barnacles and coralline algae reacted more sensitive to variable temporal patterns, filamentous algae and coarsely branched algae were negatively affected by regular intervals of aerial exposure and temporal variance mitigated the effects (Benedetti-Cecchi et al. 2006).

Reum et al. (2011) emphasized the importance of the simultaneous appearance of certain environmental conditions, such as seasonal characteristics, coastal upwelling events for individuals of e.g. fish species at a certain age. A disturbance of such temporal synchronization could also be interpreted as a cumulative effect if many of such temporal patterns change (Reum et al. 2011).

Another aspect of temporal cumulative effects is the relevance of the exposure time itself, which may lead in combination of the occurrence of multiple stressors to special effects. Such effects can arise when organisms are exposed to different stressors for a long time period (Vethaak and Matínez-Gómez 2011). The prevalence of ulcers in fish is e.g. partly explained by the long-term effects of chemical pollution in sluices into the sea (Vethaak and Matínez-Gómez 2011). If the fishes would have been exposed to the stressor combination for only a very short time period, these effects might not have been observed and the simultaneous exposure to the different chemical might have had not such a significant effect.

2.1.2 Spatial cumulative effects

Siedentop (2005) described the spatial accumulation of single pressures. Even though each single pressure might not lead to severe environmental effects, their combined effect might be high because they occur close to each other. Siedentop (2005) defines this kind of cumulative effect as „space crowding“. Space crowding is usually visualized with a geographical analysis and can comprise crowding of the same kind of pressure as well as the cumulative effect of different kinds of pressures (Siedentop 2005). Space crowding has gained a lot of attention in cumulative effects assessment during the last years (e.g. Halpern et al. 2008, Selkoe et al. 2009).

2.1.3 Direct interactions

Direct interactions can occur between two stressors, between stressors and environmental variables or between stressors and organisms, in the water column, the sediment, the air or between these compartments.

One example for an interaction between stressors and environmental variables is the transformation of toxins through altered environmental conditions. The results of a study by Schipper et al. (2009) indicated e.g. a changed bioavailability of contaminants such as metals along a salinity gradient. The toxic form, i.e. of free metal ions, is more common at lower salinity (Hall and Andersson 1995). Furthermore, oxygen depletion can result in altered redox conditions and this way influence the remobilization of heavy metals (Borchard et al. 1988, Zwolsman et al. 1997). In addition, the sulfide content in sediments influences the binding of metals to the sediment and thus its concentration in the water column (Griscom et al. 2002).

An example for an interaction between different pressures is the interaction between marine litter and invasive species. Marine litter can transport species and facilitate in this way the spread of invasive species (Aliani and Molcard 2003). Moreover, if plastics sink down and mix with the sediment, bottom properties are altered: The sediment can become more permeable and temperature retention can be increased (Carson et al .2011).

Direct interactions were also observed between species and stressors. The retention of contaminants can be influenced by species activity because they can be transported by them. Through bioturbation and feeding activity of benthic species, chemicals can e.g. be buried into the sediment (Hedman et al. 2008). Not only in the environment, but also inside the body of organisms, interactions can influence the cumulative effects. Depending on the physiological characteristics such as capability to minimize osmotic stress, the toxicity of various substances varies between species (reviewed in Hall and Anderson 1995). Toxicity of chemical substances for euryhaline species for example is often lower compared to other species (reviewed in Hall and Anderson 1995). Within the body, substances can react with each other or compete for the same molecular target, which can alter the observed toxic effect as well (Kooijman 2010). In addition, lipid content and chemical composition influence the toxicokinetics (Kooijman 2010).

2.1.4 Indirect effects

The European Commission (2011) stresses that synergism can also happen at the level of ecological communities because changes of community structure are a result of different sensitivities of different taxonomic groups and changed prey-predator relationships when multiple stressors occur. Indirect effects can comprise altered behavior, such as migration behavior (Veethak and Matínez-Gómez 2011). The effect of multiple stressors on individuals can exaggerate to population level and effects such as population growth, persistence, possible extinction, or life-cycle closure as observed e.g. for the effects of pollution and climate change can be observed (Billoir et al. 2009, Bergek et al. 2012 and Rijnsdorp et al. 2009).

Furthermore, changes in the trophic structure are indirect effects. The increase of biomass or number of individuals in one trophic level, caused by a stressor, can influence other trophic levels and favor certain species groups. Wikner and Andersson (2012) could for example show that decreased phytoplankton biomass production due to decreased salinity and increased organic carbon discharge can alter the carbon flows in the coastal food web. Bioaccumulation is another important indirect effect (Zhang et al. 2011, Falandysz et al. 2002 and Zaldivar et al. 2011). All kind of other interactions between organisms such as competition, symbiosis, or synthrophy can also convey and possibly increase cumulative effects.

2.1.5 Synergism, antagonism and addition

In many articles, cumulative effects are divided into three categories: synergistic, additive, and antagonistic (e.g. Crain et al. 2008, Warne and Hawker 1995, Crofton et al. 2005), whereas the definition of these terms can be dissimilar. Folt et al. (1999) distinguishes three different concepts of interpretation of cumulative effects, which are described here in more detail.

2.1.6 Additive model

Many publications refer to the additive concept, where the single effects of each stressor are added up (Crain et al. 2008, Warne and Hawker 1995, Crofton et al. 2005). This concept is similar to the model of Concentration Addition (CA) from chemical mixtures (see 0, Loewe 1926, Bliss 1939). However, the additive model simply adds up the effects or stressors and the sum can theoretically be infinite high, whereas CA calculates the relative contribution of each stressor to the overall effect such that the sum always equals 1 (Kortenkamp et al. 2009, personal communication Backhaus 2012) (see 0).

In case the observed effect is more intense as expected by this assumption, the cumulative effect is designated as synergistic. In contrast, the effect would be termed antagonistic, if the reaction would be less than the sum of each single effect (Folt et al. 1999, Crain et al. 2008, Christensen et al. 2006, O´Gorman et al. 2012).

The investigation of multiple stressors is a relatively new focus of research, which might be the reason that most studies consider just two stressors and seldom more (reviewed in Crain et al. 2008). For this kind of experimental setup, the additive model might give a good orientation about the experimental outcome. However, for complex chemical mixture toxicity, the expected theoretical effects might become unrealistic because errors in the estimation sum up, which might lead to overestimation of toxicity and physiological or ecological predictions (c.f. e.g. Kortenkamp and Altenburger 1998, Pennings 1996).

It has been discussed why combinations react synergistic, additive or antagonistic and a widely used hypothesis is that „stressors acting through similar mechanisms may be additive, while those acting through alternative but dependent pathways may be synergistic.“ (Crain et al. 2008). Following a suggestion of Christensen et al. (2006) and Blanck (2002) similar stressor effects or the same mechanisms can lead to stress-induction and tolerance causing antagonism (Figure 1).

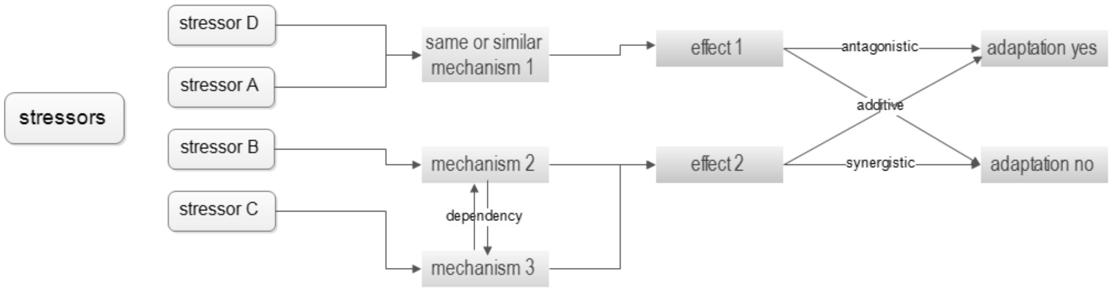

Figure 1 Theoretical explanations for antagonistic, additive and synergistic effects according to Crain et al. (2008), Christensen et al. (2006) and Blanck (2002)

This issue complicates the predictions of expected results as adaptation processes are hard to quantify and foresee with state of the art (Bijlsma and Loeschcke 2005 and Chapman 2000). Some species might be able to adapt to the new conditions, whereas others, which might even belong to the same taxonomic group, might not be able to. In some cases, it is possible that the successful adaptation of one stressor might facilitate the adaptation to another stressor. This might just be the case for certain substances or pressures, combinations of stressors or species.

It has been shown by toxicological lab experiments that the nature of the effect (synergism or antagonism) is not only dependent on the mechanism, but also on the concentration of the chemical substance (e.g. Jonker et al. 2005). In the following, I will use the term „stressor intensity“ as a general description of stressor manifestation such as concentration, intensity or magnitude across different stressor types except the context refers explicitly to one particular stressor type.

Crofton et al. (2005) could show that a mixture of thyroid-hormone disrupting chemicals with a wide range of effective doses acted additively at low doses and contrarily to that synergistically in high doses. Crain et al. (2008) reviewed more than 200 experiments investigating cumulative effects. In more than half of the pressure combinations, the outcome of the analyzed cumulative effects was contradictory: the same combination of two stressors led in some +cases to synergistic and in other cases to antagonistic effects. This was even the case if the same model organism was studied (Crain et al. 2008).

2.1.7 Multiplicative model

The multiplicative model constitutes an alternative to the additive model. It is applied in the investigation of cumulative effects, especially for competitive interactions (Folt et al. 1999). Soluk (1993) could show that an additive model modified for prey-predator interactions (Mendenhall 1979), did not provide good predictions for combined predator effects with functional responses and complex behavior interactions for stream fish and invertebrate predators, whereas a multiplicative model could better predict these effects. Both equations are based on probability estimates of the occurrence of a certain event: the additive model is defined as: $Cfs = Np\ (P\ f + Ps)$; the multiplicative model is defined as: $Cfs = Np\ (Pf + Ps - Pf\ Ps)$, where Cfs is the predicted combined consumption for a particular initial prey density (Np) and Pf and Ps are the probabilities of being consumed by predator f and s (Soluk 1993).

Pennings (1996) suggested to use the multiplicative model to describe the response towards consumers to describe the interaction effects between the chemical and mineral defense observed in a feeding experiment because he observed that the additive model predicted unrealistic results, when the individual defenses were highly effective. Similar problems could also arise when the individual effects of anthropogenic stressors on an organism are strong.

Folt at al. (1999) suggests that the multiplicative model can be applied when „stress from one source can be operated probabilistically by another source.“ Corresponding to the same structure of the additive model, effects will be categorized as synergistic, if the cumulative effect is more severe as predicted by the multiplicative model. An effect is classified as antagonistic if the cumulative effect is less severe than expected (Folt et al. 1999).

2.1.8 Simple comparative effects

The concept of the comparative model applies to an ecological system, where one stressor dominates in its stressor intensity or by the magnitude of the effect it causes while also other stressors are present. The effects of the other stressors might be more negligible through the magnitude of effect of the dominant stressor on the one hand, and on the other hand their combined effects do not exceed the level of this single worst stressor due to their cumulative interactions, which are partly antagonistic and partly synergistic (Bruland 1991, Folt et al. 1999). Studies on the toxicity of metals showed that the magnitude of the toxicity could be changed by an altered metal composition and concentrations, which might be caused by a shift in metal ion ratios and changed speciation (Bruland 1991). Moreover, the dominant metal could outcompete other metals, when they compete for the same molecular target (Bruland et al. 1991). According to their framework, Bruland et al. (1991) expected that the toxic effect of a metal was more severe in uncontaminated water samples with low concentrations of other metals than in water samples containing a mixture of high concentrations of other metals and high organic complexity. Furthermore, the interactions with other substances in the water column and in the body of the organism can relate to its importance or ecological function. Thus, Bruland et al. (1991) concluded that one substance could be a factor, which controls a biological process depending on its concentration and the chemical composition in its environment. A prominent example is the ecological function of iron in marine environments. Martin et al. (1988) revealed the limiting role of iron for phytoplankton growth in nutrient rich waters. For a categorization of antagonistic and synergistic effects, the combined effect when several stressors are present is compared to the effect due to a single stressor. If the combined effect is higher, the effect is categorized as synergistic, if it is lower than the effect of the single stressor; the effect is categorized as antagonistic.

2.2 Definition of cumulative effects

The European Commission (European Commission 1999) defines cumulative effects as „impacts result from incremental changes caused by other past, present or reasonably foreseeable actions together with the project“. Furthermore, the European Commission emphasizes that also indirect impacts, which are defined as „impacts on the environment, which are not a direct result of the project, often produced away from or as a result of a complex pathway“. Also impact interactions, which describe „the reactions between impacts whether between the impacts of just one project or between the impacts of other projects in the areas“ are regarded as very relevant for cumulative effects assessment by the European Commission (1999). It is expected that these three aspects are analyzed in an integrated way.

Given how differently the term „cumulative“ is defined across diverse scientific disciplines, I clarify here the different aspects of cumulative effects, which are relevant for the marine biota and in the context of environmental assessment:

Long-term exposures to stressors can lead to effects, which might not have occurred in that extent if the organisms had the chance to recover earlier. The prevalence of ulcers in fish for example could partly only be explained by the long-term effects of chemical pollution in outlet sluices (Vethaak and Matínez-Gómez 2011). Furthermore, simultaneous appearance of certain environmental conditions such as seasonal dependent temperature fluctuations, coastal upwelling events, often correlate with certain live stages or reproduction events (Reum et al. 2011). Thus, temporal cumulative effects can occur if temporal synchronized patterns are disturbed leading to an enhanced effect compared to the theoretical situation without temporal synchronization (Reum et al. 2011). Additionally, multifactorial experiments revealed that the temporal frequency pattern of stressors (e.g. regular pulses versus temporal variable pulses) influence the effects on organisms (Molinos and Donohue 2010). The term „time crowding“ describes the appearance of stressors in short time intervals (Siedentop 2005).

Siedentop (2005) emphasized also the role of spatial cumulative effects, space crowding, where stressors accumulate in a certain area and the impact of the stressors is altered due to their spatial distribution pattern.

Direct interactions between stressors or pressures such as transformation of toxins in line with an altered bioavailability in certain salinity regimes (Schipper et al. 2009, Hall and Andersson 1995) or the facilitated transportation of substances by invasive species (Aliani and Molcard 2003) can alter the environmental impact of a stressor as well. Furthermore, interactions can occur between species activity such as by bioturbation altering the spatial distribution of substances deposited in the sediment or by defence mechanisms of the organism against a stressor (reviewed in Hall and Anderson 1995).

Moreover, the effect of multiple stressors on individuals can exaggerate to population level effects such as population growth, persistence, possible extinction, or life-cycle closure as observed e.g. for the effects of pollution and climate change (Billoir et al. 2009, Bergek et al. 2012 and Rijnsdorp et al. 2009). Different sensitivities of species to stressors can further induce an advantage for certain species and thus alter the community structure eventually leading e.g. to increased predation pressure for other species as stressed by the European Commission (2011), which means a pressure on top to the anthropogenic pressure for those species. Bioaccumulation increases the effect of stressors for the top predators (Zhang et al.2011, Falandysz et al. 2002) and carbon flows in the food web can be disturbed e.g. due to the negative effect of anthropogenic pressures on phytoplankton biomass production (Wikner and Andersson 2012).

The nature of cumulative effects is usually specified with regard to a certain reference value (Folt et al. 1999, Crain et al. 2008, Warne and Hawker 1995, Crofton et al. 2005). Depending on the concept applied, this reference value represents the sum of the single effects of the stressors (additive model), the product of the single effects of the stressors (multiplicative model) or the effect of one of the stressors (simple comparative concept) (Folt et al. 1999, Bruland 1991, Soluk 1993). If the effect is greater than the reference value, the effect is termed synergistic; in case of a smaller effect it is antagonistic (Folt et al. 1999).

To summarize I used the following definition for „cumulative effects“:

One major characteristic of cumulative effects is the alteration of the impact due to the number, the intensity and the spatial or temporal pattern(s) of the pressure(s) or their sources.

Equally important are influences owing to characteristics or processes of the ecosystem components, habitats or ecosystems as well as influences due to interaction effects with regard to environmental parameters.

The nature of cumulative effects is classified as synergistic, antagonistic or additive indicating the difference of the actual effect in comparison to a certain model only based on the single effects alone ignoring any appearance of cumulative effects.

Cumulative effects assessment was defined by Judd et al. 2015: „Cumulative effects assessment is a systematic procedure for identifying and evaluating the significance of effects from multiple pressures and/or activities. The analysis of the causes, pathways and consequences of these effects is an essential part of the process.“1

For the present study, the reference for cumulative effects was defined according to the simple comparative model because this way much more literature data could be applied (for this model only one control value for one stressor is required). However, in the DEB-model the additive model is followed in the way that the strength of the single stressors are added up.

Transition threshold are here defined as the stressor intensity and - where appropriate - the exposure time at which a first reaction of the species could be determined as a response to the stressor. It should not be confused with legislative thresholds.

2.3 Methods and concepts for the assessment of cumulative effects

2.3.1 Indicators

Overview

0There are different kinds of definitions for indicators being discussed and refined intensively (De Wolf 1983, Schroevers 1983, Slooff and Zwart 1983, Zonneveld 1983, Kennedy and Jacoby 1999 and Rees et al 2008). Beanlands and Duinker (1983) denoted an indicator as „(i) a

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[1] http://sesss09.setac.eu/embed/sesss09/Adrian_Judd_Cumulative_effects_assessment_in_practice.pdf.

biophysical component or variable, which is monitored to detect change in that component or variable or (ii) a calculated index of the condition of all or part of an ecosystem".

Furthermore, Beanlands and Duinker (1983) defined special requirements for usable indicators, supplemented by Kennedy and Jacoby (1999):

- A biological indicator should be „representative of the performance of a valued ecosystem component"
- „It should be made clear what aspect of ecological structure and function the indicators represent and how they will show that the system has changed"
- „Biological indicators should be measurable"
- „Biological indicators should respond quickly and unambiguously to inputs"
- „They should integrate the effects of multiple pollution inputs without confounding identification of their source"
- „They should be distributed over a spatial scale that includes undisturbed areas"
- They „should have been previously studied"

ICES (2005) defined five main criteria for the selection of indicators: they should be specific, measurable, attainable, realistic, and time bound (SMART). Furthermore, they should reflect status and trends (ICES 2005). Frameworks for monitoring indicators have been developed and it has been intensively discussed, which species groups are most suitable for what type of environmental assessment and what conclusions can be drawn from it (e.g. Slooff and Zwart 1983, Zonneveld 1983, Rees et al 2008, Heink and Kowarik 2010). Kennedy and Jacoby (1999) applied the framework described above and tested the suitability of meiofauna as biological indicators by checking each of these requirements and concluded that investigating the meiofauna would indeed be suitable for indicating overall ecosystem heath and that it deserves more attention in environmental assessment as it does now.

A comprehensive user-oriented framework for the selection and implementation of indicators for fisheries management was developed by Rice and Rochet (2005), which could also be transferred for other purposes and indicator groups. In contrast to many other frameworks, it considers the different point of views from technical experts and advisors, decision-makers and managers and the general audience. It also takes into account their expertise and possible contributions to the success of the indicator concept (Rice and Rochet, 2005).

There are three further major methods for environmental assessments employing indicators: Either specific characteristics for a certain level of ecosystem health are predefined or the indicator system is species based or a combination of both (e.g. HELCOM 2009, OSPAR 2010, WFD 2000). The latter approach is often combined with measurements of chemical and physical parameters (e.g. WFD 2000). If the approach is species based, there are different ways of structuring the species composition to derive information about cumulative effects of anthropogenic pressures.

Taxonomic distinctness/ relatedness

Warwick and Clarke (1998) found a significant relationship between taxonomic distinctness and anthropogenic pollution for nematodes as they tested sites in the UK with different types and levels of pollution ranging from sludge dumping grounds to heavily industrialized and sewage-polluted areas. The method was also tested for other species groups (e.g. Xu et al. 2001). The indicator for taxonomic distinctness was developed as an alternative index for biodiversity (Warwick and Clarke 1998). However, biodiversity can also be seen as an „ultimate measure of ecosystem health“ as stated by Leonard et al. (2006).

Xu et al (2011) proposed an indicator system for anthropogenic pressures based on the taxonomic relatedness of ciliated protozoa. They grouped ciliated protozoa with multivariate statistics by environmental variables and the spatial pattern (Xu et al. 2011). They showed that the taxonomic distinctness was well correlated with eutrophication and anthropogenic stress in a semi-enclosed bay in the western part of the Yellow Sea in China (Xu et al. 2011).

Indicator species

Indicator species, which are suitable for environmental gradients, can be identified by multivariate statistics: for example, principal component analysis (PCA) of environmental parameters and canonical correspondence analysis (CCA). Thus, phytoplankton species related parameters with the strongest explanatory power for the environmental parameters can be determined (e.g. Pan et al. 1996, Sagert et al. 2008). Sagert et al. (2008) furthermore defined eutrophication classes based on cluster analysis of phytoplankton parameters, so that groups for certain stages of eutrophication were formed. PCA and the B4-broken stick method can be used to identify the most relevant pressures of an ecosystem (King and Jackson 1990, Chu et al. 2003).

The WFD focused on biological quality elements (BQE) and certain species groups, which play an important ecological role and are suitable for monitoring and assessment, fulfilling e.g. the criteria of representing a large variation of ecological niches within the group, and are easily identified (WFD 2000). However, some species groups are not applied as indicators in the WFD (2000) despite their applicability and relevance in ecosystem dynamics such as zooplankton (Jeppesen et al. 2011). The WFD requires consideration of indicators for phytoplankton, macroalgae, angiosperms and benthic invertebrate fauna by monitoring, as well as analyses of taxonomic composition and abundance, biomass, cover frequency and intensity of phytoplankton blooms for transitional and coastal waters (WFD 2000). For the benthic invertebrate fauna, disturbance-sensitive taxa have to be taken into consideration, following the Saprobic Index (Pantle and Buck 1955). The Saprobic Index was originally developed by Pantle and Buck (1955) and extended and refined thereafter (e.g. Sladéćek and Tućek 1975, Rolauffs et al. 2003, Friedrich and Herbst 2004, DIN-NAW I 3 UA AK 6). It classifies benthic invertebrate species into categories, which reflect a certain pollution level of the place of finding. The indicator organisms reflecting a high pollution status have certain characteristics for being able to cope with different environmental conditions and/ or have certain general sensitivities (e.g. Pantle and Buck 1955, WFD 2000, Rolauffs et al. 2003). Reference conditions are mainly derived from historical data and recent data about habitats with very little human influence (Rolauffs et al. 2003). Furthermore in the WFD takes into account different natural ecological types and complements the biological measurements with physic-chemical and hydromorphological quality elements (WFD 2000). Fish fauna is considered only in transitional waters but not in coastal waters. For this species group pressure sensitivities are considered as well (WFD 2000). It has also been argued that phytoplankton would be a good indicator for water quality in transitional waters and concepts for coastal areas were developed and applied in monitoring programmes (e.g. Dürselen et al. 2006, Facca and Sfriso 2009).

A species-based indicator system is not necessarily based on abundance data; it can also consist of surrogates, which are more suitable to describe the status for a particular species group. Sagert et al. (2008) developed for example an indicator system for phytoplankton in the Baltic Sea to assess eutrophication. They based their identification of indicators on historical data in order to take into account natural variation, which can be quite high for phytoplankton. Eutrophication was best explained by phytoplankton biomass, bio-volumes of certain phytoplankton groups/ size classes of diatoms as well as the percentage of certain phytoplankton groups (Sagert et al. 2008).

Historical data is commonly used for indicator-based assessment and serve many purposes. Amour and Lobry (2009) could include a temporal dimension in a species group based indicator method by taking into account temporal trends for all indicators based on historical data. They focused exclusively on fish-based indicators reflecting general ecological status (Amour and Lobry 2009).

To identify and choose a suitable species group as indicator is a challenging task. Some species groups with a short life span and fast reproduction rate might reflect acute environmental stress quite well (Carignan and Villard 2001). On the other hand, species groups with a complex life cycle, which include different environments and behavior might give a more comprehensive overall picture of environmental health and mirror long-term pressures and cumulative temporal effects (Carignan and Villard 2001). Carignan and Villard (2001) distinguished between different kinds of indicator species, which could be suitable for environmental assessment: keystone species with a large influence on the environment, umbrella species with big area requirements, dispersal-limited species and process-limited species sensitive to ecological processes.

An interesting approach was presented by Koop et al. (2011): They realized the need of early warning systems to achieve effective conservation strategies in time. As a practical solution, they proposed to measure physiological fitness indicators of benthic invertebrate fauna by standard field and lab measurements (Koop et al. 2011). Fitness in this context is defined as „the physiological capabilities of the organism“ (Willmer et al. 2006) […] „and depends essentially on its ability to cope with abiotic and biotic changes in the environment and on the physiological and biochemical strategies it uses in response“ (Koop et al. 2011). They suggest measuring energy storage components and metabolic rate or investment in growth determined by the indicators adenylate energy charge for acute impacts, triglycerides and glycogen for chronic impacts and RNA/ DNA ratio for growth rate (Koop et al. 2011).

Holistic approaches

Indicators can include environmental as well as human-related indicators and indicators for anthropogenic pressures. Recently environmental indicators are often completed with economic and social indicators incorporating human actions as part of the ecological system (e.g. Esty et al. 2005, Wiegland et al. 2010). The internationally used indicator environmental sustainability index (ESI) applies e.g. such indices such as environmental health, air quality, biodiversity, and private sector responsiveness (Esty et al. 2005). The indicators are specified by variables and metrics and summed up (Esty et al. 2005). Additionally they are weighted according to their relevance with principal component analysis (Esty et al. 2005).

Halpern et al. (2012) developed an index that considers ten public goals, including indices such as biodiversity as well as food provision or coastal livelihoods and economics. Each of these descriptors reflects the current condition, trend, pressures, and resilience (Halpern et al. 2012). This index does not correspond to natural boundaries, such as ecosystem type. Instead, it is calculated per country (Halpern et al. 2012).

Applicability of the indicator concept for assessments

Monitoring programmes for indicators are widely used and proposed in directives and international agreements and studies (WFD 2000, ICES 2005, Dürselen et al. 2006,Sagert et al. 2008, OSPAR 2010, HELCOM 2010) and thus, there is in-depth experience and expertise available. Proposals and concepts could be transferred from the WFD for the coastal habitats and extended to the areas, which are not covered by the WFD but by the MSFD. Indicators systems are applied worldwide and combined with different methods and baselines. Chu et al. (2003) used e.g. more than 200 fish species as indicators for water quality in watersheds in whole Canada, whereas historical data were used for defining reference conditions. The implementation of a regional scale for the GIS data showed that the natural variability shaped eco-districts. The assessment included very different kinds of pressures and environmental parameters such as climate change, waste, or road density (Chu et al. 2003). Such applications underline the practicability of this method.

However, just a few studies deal with cumulative effects of very different kinds of stressors (but see e.g. Muniz et al. 2011) as required by the MSFD, which comprises such dissimilar stressors as plastic pollution, fisheries, and changes in siltation.

Another weakness of the indicator method is that it only describes the state of the art but cannot serve as an early warning system. The species might be extinct already in a specific area and too much damage might have happened before the cumulative effect is observed. Once an ecosystem is destroyed, a recovery is uncertain as mentioned and underlined with a hypothetical example by Koop et al. (2011).

Some indicator methods can be rather descriptive and difficult to quantify (see examples in review by Canter and Atkinson) 2011, which was a request for the development of the concept of cumulative effect assessment for the MSFD.

Compared to other indicator systems based on species composition indices, the method of physiological indicators (Koop et al. 2011) reflects environmental changes quite early, which might be a big advantage. Furthermore, the physiological indicators are general indicators for ecosystem health, leaving out the problem of finding a common unit of quite different stressors and effects. One major disadvantage is that it is not possible to detect the most severe stressor with this method. The method could be complemented with other concepts such as a threshold approach if applied for the MSFD as other indicator concepts are in many assessments (see 2.3.5). If the method would be implemented, it also needs to be considered that different groups of organisms have different sensitivities to environmental stress and that e.g. benthic invertebrates cannot reflect the health and status for some groups with different behavior such as migrating fishes etc. Some species groups might be more vulnerable to a particular stressor as another group of organism. Therefore, a careful selection of indicator species is critical for the success of this concept.

Warwick and Clarke (1998) argued that trophic composition and species composition varies depending on habitat type. Therefore, it is important to consider these aspects in cumulative environmental effect assessment. Habitats or ecosystem types are also implemented in the final assessment in the WFD (WFD 2000).

To sum up, the indicator method is a sound and approved method for environmental assessment and it provides a very good and comprehensive picture of the state of the art being able to reflect the stability of the ecological quality. Temporal and spatial patterns can be reflected by the method based on the growing availability of long-term data, even though seasonal effects will not be covered. One difficulty might be that it is hard to select species, which reflect the cumulative aspect of very dissimilar kinds of pressures due to the different vulnerabilities. Furthermore, this method does hardly offer any possibility to integrate genetic considerations. Direct interactions between stressors cannot be investigated with the indicator method alone.

In combination with other methods, a sound selection of indicator species could be very useful for the implementation of the MSFD to mitigate the data amount necessary to conduct some analyses. It would e.g. be possible to refer to an indicator concept and to choose for example very sensitive species, key species or umbrella species (Carignan and Villared 2002).

2.3.2 Cross-impact analysis

Overview

The cross-impact analysis was originally developed by Hayward and Gordon when they developed a game (Gordon and Hayward 1968). It was first applied to understand the impact of major technological and environmental developments and their holistic effect on an overall sector (Gordon and Hayward 1968). One major aim of cross-impact analysis was to structure forecasts and to understand the interactions of different developments. Gordon and Hayward (1968) also proposed that cross-impact analysis could be used as a decision tool as potential effects can be easier understood and be made transparent. The visualizing tool is a table displaying the probabilities of e.g. certain inventions or developments in rows and columns (Table 1). For cumulative effects assessment the concept can be transferred to the effects of multiple stressors and environmental effects and e.g. „development" replaced by „stressor" in contrast to the original table. Each cell can now be filled with a particular relationship either showing that an effect of one stressor would be made either more or less likely by the occurrence of another stressor. Alternatively, there is no connection between the two issues.

In their example, Gordon and Hayward (1968) referred to the logical relationships of altered probabilities of developments, environmental changes and effects.

If the probability of feasibility of limited weather control would be e.g. 20 %, this would change the probability of crop damage from adverse weather eliminated (Gordon and Hayward (1968).

Gordon and Hayward (1968) distinguished three different linkages between events: unrelated, enhancing and inhibiting, whereas an enhancing relationship is subdivided by an „enabling" linkage and a „provoking" linkage. Inhibitory linkages are divided following the same logic structure into „denigrating" and „antagonistic" linkages. These linkages are in a first step shown by an arrow pointing upwards or downwards drawn in the cell connecting the two events (Gordon and Hayward 1968). The strength of the connection is indicated by the thickness of the arrow (see Table 1, Gordon and Hayward 1968).

Cross-impact analysis can also be quantified by application of the equation (Gordon and Hayward 1968):

$$P'_n = f\ (P_n, M, S, t_{m,}, t)$$

where Pn is the probability of Dn (representing a development, impact or stressor) alone, P´n the probability after occurrence of Dm, M is a function of the connection mode, S is a measure of the strength of connection, tm is the time in the future of the occurrence of Dm and t is the time in the future for which the probabilities are being estimated (c.f. Gordon and Hayward 1968). Linear relationships were considered and used for the first tests (Gordon and Hayward 1968). The calculation of estimates was based on expert judgment but tested and adjusted afterwards with a computer programme (Gordon and Hayward 1968).

Table 1 Cross-impact matrix adapted from Gordon and Hayward (1968) upward arrows indicate an increased probability of an effect, a minus- sign indicates a decreased probability of an effect

	then probability of an effect of			
if this stressor would occur:	Stressor 1	Stressor 2	Stressor 3	Stressor 4
Stressor 1		↑	–	↑
Stressor 2	↑		–	↑
Stressor 3	–	–		–
Stressor 4	–	–	–	

Enzer (1972) established a thought experiment where he showed the logical limitations of dependencies in the concept. He investigated the nature of dependencies, described different categories for them, and defined the limitations of possible probabilities. Enzer (1972) summarised four different kinds of dependencies of events, which can be assigned to effects related to the background of cumulative effects:

- The effect does only occur if another certain effect occurs and thus the probability of occurrence strongly increases if this effect occurs.
- The effect just occurs, if another certain effect does not occur or a reaction takes place just in case of a certain event.
- Complete independency of e.g. two effects and mechanism of action. The probability is in this case 0.5.
- One stressor or effect enhances or inhibits the occurrence of another stressor or effect. In this example, the probability is either higher or lower than 0.5 depending on the nature of cumulation (synergistic or antagonistic).

Depending on the probability of occurrence of one single event and the nature of interaction, only a certain value for the probability is mathematically possible (Enzer 1972). These relationships constrict the probabilities to certain values (Enzer 1972). Enzer (1972) identified these logical laws and implemented them into an equation for binary relationships:

$$P_A = (P_B)(P_{A/B}) + (1 - P_B)(P_{A|\bar{B}}),$$

with PA being the expected probability of event A for the interval specified, PB being the expected probability of event B for the interval specified, PA/B being the changed probability of event A given that event B occurs within the interval specified and PA|$\bar{B}$ being the changed probability of event A given that event B does not occur within the interval specified. (Enzer 1972). A computer program can test these assumptions (Gordon 1994). The program is also used to adjust and test the expert judgments by testing the probabilities of randomly occurring events against the estimates of probabilities defined by experts (c.f. Gordon 1994). Gordon (1994) proposed that experts should additionally correct for inconsistencies of the estimated probabilities and he underlines the positive side-effect of cross matrix analysis due to the improvement of knowledge about the nature of interactions and feedback systems. Sensitivity testing should be performed by running the calculations again with an altered probability of a factor. It is tested how much the system would change (Gordon 1994). For policy or management actions probabilities could be changed, new events added or type of interaction could be altered and the matrix could be run again to see the possible result. Enzer (1980) established a computer program (INTERAX), which applies cross-impact analysis on a scenario-based approach.

Kane (1972) developed the cross-impact analysis further and showed a way to integrate mixed data in the model, so that in case precise data is available, it could be applied and on the other hand, expert judgments could fill knowledge gaps. Instead of entering numerical data in the matrix, he recommends plus and minus signs (four categories of plus and four categories of minus). These categories ale scaled and the interactions are weighted according to the strength of interaction (Kane 1972). A Matrix Model can also be used in a way that the cells are connected with equations (Dixon and Montz 1995). Consequently, concrete relationships can be defined. Dixon and Montz (1995) used additionally different layers of matrices to investigate indirect effects.

Weimer-Jehle (2008) proposed a modification of the cross-impact analysis where expert judgment is categorized in different levels ranging from „strongly promoting direct influence“ to „strongly restricting direct influence“ (Weimer-Jehle 2008). The cumulative effect of different impacts is calculated by adding up the expert judgments (Weimer-Jehle 2008). The method includes an indication of strength and nature of interaction (positive or negative) and it considers to the same time the impact of each single subject by subdividing the impacts in additional categories (0) (Weimer-Jehle 2008). This method can be tested for consistency by simply balancing the impacts: Both possible positive and negative effects are noted for each variable and it is assumed that the actual answer always equals the maximum impact sore (Table 2, Weimer-Jehle 2008). Consistency can be checked with an equation where different scenarios are checked for consistency and all possible solutions can be found (c.f. Weimer-Jehle 2008). This extension of cross-impact analysis is called cross-impact balance analysis (CIB analysis).

Table 2 Cross-impact matrix, positive numbers indicate positive dependency, negative number a negative one, the number indicate the strength of the relationship, table adapted from Weimer Jehle (2008)

		Stressor 1			Stressor 2			Stressor 3			Stressor 4			Stressor 5		
		+	0	-	+	0	-	+	0	-	+	0	-	+	0	-
Stressor 1	+				0	0	0	0	0	0	0	0	0	1	0	-1
	0				0	0	0	0	0	0	0	0	0	0	0	0
	-				0	0	0	0	0	0	0	0	0	-1	0	1
Stressor 2	+	0	0	0				0	0	0	0	0	0	2	0	-2
	0	0	0	0				0	0	0	0	0	0	0	0	0
	-	0	0	0				0	0	0	0	0	0	-2	0	2
Stressor 3	+	0	0	0	0	0	0				2	0	-2	0	0	0
	0	0	0	0	0	0	0				0	0	0	0	0	0
	-	0	0	0	0	0	0				-2	0	2	0	0	0
Stressor 4	+	3	0	-3	2	0	-2	2	0	-2				0	0	0
	0	0	0	0	0	0	0	0	0	0				0	0	0
	-	-3	0	3	0	0	0	-2	0	2				0	0	0
Stressor 5	+	2	0	-2	1	0	-1	-3	0	3	0	0	0			
	0	0	0	0	0	0	0	0	0	0	0	0	0			
	-	-2	0	2	-1	0	1	3	0	-3	0	0	0			
			↓				↓	↓			↓					↓
Balance		1	0	-1	1	0	-1	5	0	-5	2	0	-2	-2	0	2

This kind of matrix allows the following relationships (c.f. Weimer-Jehle 2008):

- Direct interactions with a certain strength
- Unidirectional influence
- Bidirectional influence with different strengths
- Influence under a certain condition, for example if a certain effect just occurs if another stressor has a „positive“ effect
- Differentiation of the different strengths of several stressors on one stressor
- Reinforcing interactions
- Conflictive interactions

The CIB analysis could be also applied for checking symmetry of a system, an analysis known from theoretical physics of complex systems (Weisskopf 1969, Lane 1986). This theory assumes that natural systems always show general symmetry and a tiny dissymmetry disrupting the perfect picture (Weisskoff 1969). Lane (1986) transferred this concept to biological systems for food web analyses. Parallel to this idea, also in cumulative effect assessment unexpected effects can occur, interrupting the symmetry of the consistency of the matrix. However, the general assumptions of the matrix should lead to a picture, which reflects the symmetry of the natural situation.

CIB analysis can also be applied in combination with automata network analysis, which is often applied in complexity research (Kauffman 1993). This kind of network analysis links each unit of the network with a positive, neutral, or negative input and output in line with the matrix, as discussed by (Weimer-Jehle 2008). If the units represent species in an ecosystem, „genetic regulatory circuits“ and effects on the whole ecosystem can be analyzed (Kauffman 1993).

Applicability of matrices for the assessment of cumulative effects

The Cross-impact analysis was first tested empirically (Gordon and Hayward 1968) on the historical decision to deploy the Minuteman missile and included nearly 30 events with potential linkages and transportations related events (Gordon and Hayward 1968). Since them, cross-impact matrices are widely used and discussed in a broad range of research fields, e.g. in economy (Schuler et al. 1991, Vickers 1992), political science (Gordon et al. 1970), social sciences (Jackson and Lawton 1976), planning (Murphy 1989, Makridakis 1990) and technology (Choi et al. 2007).

The cross-matrix method is an effective way of visualizing interactions between issues and provides a quick overview to binary cumulative effects of a complex topic for e.g. the interested public or politicians. The applicability of existing data for this kind of analysis is high, because research has focused on binary relationships (Gordon and Hayward 1968). Another advantage is the possibility to change equations for updating the matrix regularly to new findings.

A problem is the choice of method used for revision of the marginal probabilities: Fontela and Gabus (1974) as well as Duperrin and Godet (1975) criticized the mathematical methods applied to correct the probabilities because they lack logic and consistency with probability theory. Duperrin and Godet (1975) propose another kind of mathematical solution to reach consistency. This solution produces a ranking of the probabilities providing a kind of index of reliability and merged cross-impact analysis with scenario development. This method however has been criticized as well, because of the high number of possible probability sets (Mitchell and Tydeman 1974). Mphahlele et al. (2011) conducted a comparative study and concluded that the Monte Carlo technique compared it with the difference equation technique: both resulted in opposite general tendencies of the result. Thus, the development of reliable statistical methods for the cross-impact analysis is still an issue.

Matrix Models might be a good way to model cumulative effects if the focus is on the nature of interactions (synergistic, antagonistic). The CIB method could be applied for the MSFD, because it can model the overall cumulative effect of anthropogenic pressures based on binary interactions.

However, it seems impossible to present all interactions of all pressures and all ecosystem components in one matrix. Different species groups are affected in a different way by certain stressors and have certain vulnerabilities. One example is the strong interaction of temperature and acidifiaction on mussels comprising even a feedback system through the carbonate cycle (Melzner et al. 2011).

The method comprises interactions on a binary basis, which fits well to the kind of data, which are available for cumulative interactions in the marine habitat. To the same time, it reflects the complexity and can show with how many other stressors a particular stressor interacts. There are examples when two stressors interact in one way if they are the only main drivers but in another way when a third stressor occurs (reviewed in Crain et al. 2008). In matrix analyses applied on a spatial scale, such dependencies could be incorporated by integrating just those pressures, which are actually present on a spatial spot and by calculating the overall balance of influences.

Linkages between issues are probably much more complex as represented in cross-impact matrices and comprise a whole set of different functions. For example, the nature of an interaction might change after a certain exposure time. Even after more than four decades research about multiple stressors such relationships are still hardly known and a lot of effort is needed for these investigations. However, such kind of analysis can give a hint about the magnitude of the cumulative nature on different species groups and might give indications for vulnerabilities related to cumulative effects.

2.3.3 Ecological Network Analysis

Overview

Ecological network analysis was derived from general system theory by Patten (1978). The environment of each component is divided into at least two components, called environs: One input and one output environ (Patten 1978, Fath and Patten 1999). A connection between two components displays an output as well as an input. Such a network can consist of many different components. Therefore, the approach becomes holistic and ecosystem system characteristics can become visible (Fath and Patten 1999). Network analysis can be linked to the mathematical concept of state-space theory, which describes the transfer of one component into a new state by a certain input and the corresponding output, which makes it possible to quantify system changes of the network (Zadeh and Desoer 1963 in Fath and Patten 1999). The number of pathways can be calculated by pathway analysis (Fath and Patten, 1999). Fath and Patten (1999) summarized three different ways for more detailed interpretation: flow analysis, which analyses the flow intensities (Hannon 1973), storage analysis, which provides information about storage intensities (Patten and Higashi 1995) and by utility analysis, which reveals the utility intensities of the system (Fath and Patten 1998). The three latter analyses refer to indirect pathways and non-dimensional characteristics (Fath and Patten 1999). A temporal dimension can be integrated in network analysis (e.g. Fath and Borett 2006). Overall systems characteristics are tested mathematically, such as homogenization or the magnitude of the effect of indirect effects (Fath and Patten 1999). A test can be performed for possible amplifications in the system, which occurs e.g. in case „the summed total amount of flow through a compartment is greater than the input into the network“ (Fath and Patten 1999). Furthermore it can be tested, if the system is characterised by a network synergism, meaning that the number of positive net flows is higher than the negative net flows („integral utilities“) (Fath and Patten 1999). Fath and Borrett (2006) developed a MATLAB function for network analysis comprising these analyses. This MATLAB function offers a flexible basis for sound interpretation of environmental data (Fath and Borrett 2006).

The work of Fath and Patten builds upon earlier attempts to analyse networks: Finn (1976) introduced three commonly applied measures for the interpretation of network analysis: the „sum of all compartmental throughflows or the total system throughflow (TST) […], the average path length of an inflow (APLi) […] and the cycling index (CI), which quantifies the importance of cycling in the system“. Several software tools have been developed to) facilitate the application of network analysis such as Ecopath with Ecosim (EwE)[2] (Christensen and Walters 2004).

Network analysis in its traditional application describes often changes in food web structure. Thus, the nature of transactions is usually mass-energy related (Wulff 1989). Changes in such structures can however also give indications of ecosystem changes, loss of species, simplifications, or unevenness, which can be caused by anthropogenic pressures. The analysis with Ecopath with Ecosim allows to draw conclusions about the total system throughput (TST) indicating the magnitude of the ecosystem development (Christensen and Walters 2004, Patricio et al. 2006). By calculating the magnitude of flows, it is possible to interpret information about system development and maturity, the diversity of flows and the ascendency as a measure of overall ecosystem growth and development (Christensen and Pauly 1992, Christensen and Walters 2004, Patricio et al. 2006).

Applicability of ENA for the assessment of cumulative effects

Ecological Network Analysis is often applied for investigating energy flows in food web models (e.g. Fath and Patten 1999, Christensen and Walters 2004, Wulff 1989) but also for the purpose of characterization of the environmental status of a region in combination with other models (Christensen and Walters 2004). Its generality allows applying this framework for a broad range of purposes. Whole ecosystem structures, species interactions, and bioaccumulation processes can be modeled. Interactions between stressors can be modeled and as a consequence, direct, indirect effects and feedback loops can be visualised in an easy understandable way.

Furthermore, equations can be added to describe the nature of interaction and interactions can be quantified and calculated (Fath and Patten 1999). Indirect effects are taken into account by the fact that all equations of the nodes are connected with each other. Therefore, if there is e.g. a connection between I and j and I and k, the intensity of these flows influence the indirect relationship between k and j (Fath and Patten 1999). The structure of this framework offers a variable level of complexity, which can be adapted depending on the type of question. On the other hand, this complexity might also be a challenge because not all modes of interactions are investigated and known.

Ecological Network Models can be connected with spatial models (Christensen and Walters 2004), such that they can cover a wide range of cumulative aspects. ERSEM is a network model for the ecosystem dynamics in the North Sea and has a temporal and spatial dimension (Lenhart 2001). Burkhard et al. (2010) combines this ecosystem model with a transportation model (via waves, tides, wind etc.), a hydrodynamic model, a food web model (Ecopath), and GIS maps for the evaluation of cumulative effects demonstrating the flexibility of network models. For an

[2] www.ecopath.org

application on MSFD objectives, the method can also be combined with other methods in order to cover all key issues of cumulative effects assessment.

2.3.4 Causal analyses using flow diagrams

Overview

A causal analysis is often presented in a flow diagram, where pressures, their effects, and pathways are visualized and relationships between pressures and effects can be presented. Feedback systems can be adhered by integrating loop elements. Flow diagrams can be used both as visualization tool, as well as model elements to calculate flows. However, in this case a common unit is necessary (European Commission 1999). The statistical analysis of flow diagrams can be performed e.g. with structural equation modeling (SEM) (Trevino et al. 2007).

The method resembles network analysis. Even though there is some overlap to some degree, in contrast to network analysis the purpose of flow diagrams is rather to focuses on origin and effect of an issue in order to answer specific questions (European Commission 1999).

Applicability of causal analyses using flow diagrams

Causal analysis using flow diagrams is done sometimes as a preparation for project approval/ disproval. For example, the effects of the channel widening of the Keil Canal were analysed with causal analysis and flow diagrams (Bundesanstalt für Gewässerkunde in European Commission 1999). Another example of an application is a study about threats to the avifauna on oceanic islands (Trevino et al. 2007). The authors analyzed the causes and revealed the most severe threats, which led to extinction of several bird species. They considered not only the direct effects of the pressures on the avifauna but also how the different pressures influenced each other (Trevino et al. 2007). This study is also an example for a quantitative application of the method (Trevino et al. 2007). Generally, flow diagrams provide a quick overview and can contribute to a basic understanding of processes, which might be relevant for cumulative effects assesment.

2.3.5 Threshold approach

Overview

A threshold approach for cumulative effect assessment from a planning perspective was suggested by Dickert and Tuttle (1985) for defining a carrying capacity of the environment for land use as a practical decision tool for project approvals. Thresholds, which should not be exceeded, can be defined based on extrapolation of historical data and can integrate a spatial and temporal perspective. The authors considered a geographical area or ecosystem as a whole in contrast to individual assessment of single projects, because they claimed that those project-related investigations cannot reflect the entire ecological status sufficiently (Dickert and Tuttle 1985). They searched for episodic events in the historic record and separated natural from human-induced impacts as good as possible. Moreover, they analyzed the hydrology, the upland erosion and deposition and geomorphological characteristics. These data was used to evaluate the erosion susceptibility and derived the extent of land disturbance (Dickert and Tuttle 1985).

With regard to organisms, the threshold value is currently defined as the situation, when no effect is observed, even after prolonged exposure time and the probability of death is does not exceed control conditions (Jager et al. 2011). Population survival can be reflected by the model by the multinomial distribution likelihood function, which includes the killing rate and the selected dose metric (scaled damage, internal concentration, scaled internal concentration, or external concentration).

For example, a threshold method was applied in the WFD (2000), where limits for the categories of environmental status were e.g. defined for priority substances (Lepper 2005). EQS („Environmental Quality Standards“) can describe not only survival but also sublethal effects such as endocrine regulation in animals or carcinogenicity for man (Lepper et al. 2005). The derivation of EQS takes the different behavior of chemicals in the water, the sediment and in biota into account and additionally integrates a bio-concentration- and bio-magnification factor (Lepper 2005). The limits for EQS are mainly derived from toxicological tests of indicators, whereas the annual average quality standards are based on chronic data, the maximum acceptable concentrations (MACs) relate to short term toxicological tests (Lepper 2005). In most cases the EQS and corresponding environmental quality criteria (EQC) refer to results of species sensitivity distributions from toxicological tests (Posthuma et al. 2002, Lepper 2005) and the safety factor method (Lepper 2005). Predefined safety factors are applied as a precautionary method in order to account for variability and possible lack of data (WFD 2000). This factor should be related to threshold values and general guides in the technical guidance document for risk assessment of substances (European Chemical Bureau 2003). The value of the assessment factor depends on the kind of method used (long-term/ short-term, NOEC/ L(E)C50 etc.) (Lepper 2005) These assessment factors equal those discussed on a workshop of the OECD (1992).

Environmental quality criteria (EQC) can also be derived from the results of the SSD data e.g. of certain indicator species (Posthuma et al. 2002), which are frequently applied in conceptual frameworks and monitoring programmes (e.g. WFD 2000, Swedish EPA 2000).

QSARs (see 0)) will often be applied for the derivation of the Predicted No Effect Concentration (PNEC) if no data or no plausible data is available (Cronin et al. 2003). In this case, the test with QSARs can help to decide about the further procedure for possible tests (Cronin et al. 2003). The European chemicals legislation REACH (2006) requests three comprehensive datasets to calculate the PNEC, which should also account indirectly for ecosystem functioning and stability of ecosystem structure: It is assumed that thresholds for the most sensitive trophic level also would protect all the other, less sensitive trophic levels. Additionally an appropriate assessment factor is applied „which accounts for intra- and inter-laboratory variation of the data, biological, short-term to long-term extrapolation and laboratory to field extrapolation“ (Backhaus and Faust 2012).

The OSPAR Commission also uses QSARs for the identification of the most severe chemicals, which should be prioritized in the management (Cronin et al. 2003). JAMP guidelines for the integrated monitoring assessment (OSPAR 2012) it is suggested to use threshold levels for the biological effects leading to a color code of red, blue, and green. These classifications are based on Background Assessment Concentration or Criteria and Environment Assessment Criteria, which are analyzed on several physiological levels in indicator species and in the water column as well as in the sediment (OSPAR 2012).

In many assessment methods, the threshold- and indicator method are merged in a way that indicators define the affiliation to a certain class of ecological status, whereas a threshold approach defines the acceptable limits and possibly an acceptable deviation. Such an approach was for example applied by HELCOMs Holistic Assessment of Ecosystem Health status (HOLAS) (HELCOM 2010). In this approach three different assessments, HEAT (HELCOM Eutrophication Assessment Tool), BEAT (HELCOM Biodiversity Tool) and CHASE (Hazardous Substances Status Assessment Tool) are combined to the holistic approach. This was possible because they use these two similar basic methods of cumulative effect assessment. This is also in line with the WFD and some of the threshold values are derived from this Directive (WFD 2000).

A threshold approach is also applied in the General Unified Threshold Model of Survival (GUTS) (Jager et al. 2011). This model is a toxicokinetic- toxicodynamic model, which comprises several sub-models (Jager et al. 2011). It calculates the uptake of the toxic substance over time, estimates the expected damage with stochastic death models and defines a threshold distribution with individual tolerance models. The expected „damage“ is a measure of the effect of a specific compound without any specification of the kind of damage and integrates a recovery rate. The GUTS model can integrate also other kinds of stressors than toxins (Jager et al. 2011). Depending on the best fit or the availability of data, the survival probability is derived based on toxicokinetics or on the scaled damage.

The General Unified Threshold Model of Survival (GUTS) equals in many ways the DEB model (Kooijman 2010), introduced in 2.3.8. However, GUTS exclusively relates to survival, and growth and reproduction are no output variables (Jager et al. 2011).

The death of an individual is assumed to be a stochastic death, whereas pressures can be modeled as pulses and a linear time elapsed is additionally complemented into the equation. In general, only one stressor is modeled (Jager et al. 2011).

Applicability of the threshold approach for the assessment of cumulative effects

The threshold approach is a well-approved method applied in many international agreements and regulations (WFD 2000, CEMP assessment OSPAR 2009, HELCOM 2010,). This might be due to its good practicability. Once decided and agreed on by all contributing parties, thresholds are clearly defined, hardly questionable, easy to control, and it is hard to camouflage the actual environmental status. The transferability of thresholds to a color-code indicating the environmental status is straightforward and easy understandable for all kinds of users. The threshold approach is a very transparent method and due to its simplicity, different bio-geographical regions and countries can easily be compared. In many cases, corrections according to the baseline conditions are integrated. For example, the descriptors of the environmental status in the WFD are recommended to be adapted to the specific type of water body (WFD 2000).

The threshold approach aims at protecting all species (often based on SSDs). However, the species number as well as the number of chemicals and stressors is high and ecotoxicological tests cannot provide data for all species. Therefore, OSPAR and ICES agreed on a minimum of three species for the definition of an EAC (OSPAR/ ICES 2004) and in some cases, the chosen species might not be the most vulnerable ones leading to an underestimation of an effect (ICES 2012). Furthermore, if SSDs are the foundation for the designation of threshold levels, often a 5 % percentile is accepted as proposed by Posthuma et al. (2002). This is risky because it ignores local adaptations of species to certain environmental conditions. For example, some species in the Baltic and not able to tolerate certain environmental conditions due to a low genetic diversity and local adaptations, whereas the same species occurring in the North Sea can indeed tolerate the same conditions (Magnusson and Norén 2012). Moreover, the precautionary range of accepted stressor levels might not be sufficient for some very vulnerable species with a narrow tolerance window.

In general, a threshold value related to carrying capacity is very difficult to estimate. This will be especially the case, if sufficient data on a topic does not exist. Depending on e.g. the data availability extrapolation, different factors are included in the calculation of threshold values in several concepts (WFD 2000, OSPAR/ ICES 2004, Lepper 2005). However, to our best knowledge, the numbers the scientific derivation of the values of these factors are not clear (OECD 1992, WFD 2000, Lepper 2005) and it seems uncertain if this precautionary factor accounts sufficiently for possible synergistic effects.

Temporal effects cannot directly be considered in the threshold approach. Thus, the prolonged exposure to a stressor, which might either lead to more severe effects or the induction of adaptation processes reducing the effect are not integrated.

The QSAR approach, which is often used in combination with a threshold approach, is very useful to fill data gaps in toxicity data. Toxicological tests are avoided and the approach is rather a mechanistic one following a structural logic (for details of grouping see van Leeuwen et al. 2009). QSARs are applied in very many international decision-making frameworks, especially in the USA and Europe for the prediction of ecological effects and the behavior of chemicals in the environment (Cronin et al. 2003). Despite the broad application knowledge about the behavior of substances is unevenly distributed to the research fields: whereas there is relatively much knowledge about carcinogenic chemicals available, less data on other environmentally relevant substances can be found (Cronin et al. 2003). Moreover, predictions of effects with QSARs do not yet work well in complex mixtures (c.f. Cronin et al. 2003). Therefore, more research is needed in this area.

The threshold approach is hard to combine with whole community effects or indirect effects via interactions between species. It might be possible to derive thresholds for all pressures mentioned in the MSFD. However, combination effects cannot be integrated in this approach due to its structure.

2.3.6 Classical methods for the analysis of chemical mixture toxicity

Overview

Concentration addition

The theory of the concept on concentration addition (CA) assumes that the chemicals analyzed act in a similar way and have a similar mechanism of toxicity or have the same molecular target site (review and equations (see Drescher and Boedeker 1995). Originally, the concept was developed by Loewe (1926) and Bliss (1939). The concentrations of each substance are added up taking into account the response probabilities of each substance

$$\frac{z_1}{P_1^{-1}\{P_{CA}(z_{1,}z_2)\}} + \frac{z_2}{P_2^{-1}\{P_{CA}(z_{1,}z_2)\}} = 1,$$

where zi is the concentration of a substance and Pi -1 is the inverse of the response probability of (zi). Here, z1, z2 give the concentration of either substance in an equieffective combination (z1 + z2) (c.f. Drescher and Boedeker 1995). The response probabilities can be derived from dose response curves (Boedeker et al. 1993). Boedeker et al. (1993) stressed that „for concentration addition the combined effect will always exceed that of the weakest substance alone and be less than the effect which could be achieved from the most potent substance by applying the amount of concentrations present in the combination".

In case it is assumed that the toxicity or nonlethal effects of each substance have the same strength, CA and IA can be compared by the equation

$$P_1(z_1 + pz_2) \underset{\geq}{\leq} P_{1A}(z_1, z_2) \quad ,$$

published by Drescher and Boedeker (1995).

Independent action

The calculation mechanism of the model of independent action or also called response addition model is based on dose response curves of single compounds (Boedeker et al. 1993). The independent action is applied for substances, which are expected to act independent from each other, have different modes of action and different molecular targets (Boedeker et al. 1993).

It is assumed that the response probability is equal to the sum of the response probabilities of each of the substances subtracted by the product of the substances (Boedeker et al. 1993). Boedeker et al (1993) stated that in case of independent action „the combined effect will always be weaker than or equal to what is achieved by simple summation of effects" (see 2.3.6)

The EU Commission (2011) published an equation for independent action:

$$E(C_{mix}) = 1 - \prod_{i=1}^{n}(1 - E(C_i)),$$

where $E(C_{mix})$ is effect of the chemical mixture (C_{mix}), and $E(C_i)$ is the effect of the „individual mixuture component i at the concentration c_i. „Effects are expressed as fractions of a maximum possible effect (0% ≤ E ≤ 100%) „ (c.f. EU Commission 2011).

Applicability of the application of the concepts „concentration addition" and „independent action" for the assessment of cumulative effects

The outcome of many experiments supported the thesis that the cumulative effect of different compounds can be greater than the effect of the individual substances and the methods of CA and IA are widely applied (e.g. Feron and Groten 2002, Walker et al. 2005, Wolansky et al. 2009). Several computer programmes have been developed to facilitate the application of these models (e.g. CombiTool, BioMol, Reaction Network Modeling).

The models of IA and CA have one major shortcoming, they require detailed toxicological tests. The European Commission (2011) criticizes that sometimes, when such tests were not performed, calculations or assumptions were made which led to over-interpretation and arguable conclusions. Furthermore, there is a variation of the results in toxicological tests over time which is not considered with the method CA (Baas et al. 2007, Baas et al. 2010) and might be the cause for unexpected and contradictory results (Cedergreen et al. 2007 and Baas et al. 2010). In some cases, CA underestimated the toxic effects (Crofton et al. 2005, Coors et al. 2012, sublethal effects of cadmium, carbendazim and hypoxia on Daphnia by both CA and IA), but correct prediction of lethal effects by IA). However, Backhaus and Faust (2012) conclude that these seem to be rare cases. Overestimation of CA was often hypothesised theoretically and specifically investigated for algae by Faust et al. (2003) with both, chemicals having similar and dissimilar mechanisms, representing a realistic mixture of 16 different biocides. They could indeed show an overestimation of CA whereas the analysis with IA gave realistic results. However, CA predicted an effect, which was not more than 3.2x higher than the observed outcome (Faust et al. 2003). Concentration addition and independent actions do both not include interactions between the substance in their theoretical concept (e.g. EU Commission 2011, Backhaus and Faust 2012).

Boedeker et al. (1993) specified the differences of CA and IA models: the outcomes of the models depend on the function of the dose response curve, differences between the distribution functions Weibull distribution, logistic distribution, and normal distributions. These factors determine, if the predictions of experimental results of the IA would be higher or lower as the predictions of CA (Drescher and Boedeker 1995). Crofton et al. (2005) argues that in some cases the reasons for a wrong outcome might lie in the duration of exposure, which was too short to reach a steady state.

Both concepts do not take direct interactions between chemicals into account, which might be a factor with increasing importance as the number of hazards in the environment rises and enhances the risk of a wrong prediction (Rider and LeBlanc 2005). The European Commission differentiates between three different interactions: toxicokinetic interaction, toxicodynamic interaction and metabolic interaction. These interactions might explain why a mixture effect is higher or lower as predicted by CA or IA (European Commission 2011). Another problem with the application of the model of concentration addition and independent action is the lack of knowledge about the exact mode of action of specific chemicals (Backhaus and Faust 2012). Therefore, predictions must be made according to certain characteristics of the chemicals, which adds some uncertainty (Boedeker et al. 1993).

Cedergreen et al. (2008) tested and reviewed applications of the models for CA and IA in more than 150 data sets with nearly 100 different mixtures and seven different test systems comprising gram-negative bacteria, activated sludge microorganisms, zooplankton, microalgae, a duckweed, and two angiosperms. The models could just predict 10 % of the outcome of the experiments by using CA and 20 % by IA with no significant different accuracy between the two methods (Cedergreen et al. 2008). Other authors argue for the reliability of CA (Altenburger et al. 1996, Faust et al. 2003, Kortenkamp et al. 2009) or IA (e.g. Faust et al. 2003).

Some Scientists even transfer the two models to higher levels of physiological organization as a reference value (Folt et al. 1999, O´Gorman et al. 2012, Crain et al. 2008). This transfer could be risky in practice, as many authors have stressed that there is a huge lack of information of mode of action and the targets of many chemicals (e.g. Cedergreen et al. 2008, Backhaus and Faust 2012).

The European Commission (2011) proposes in general to use CA instead of IA and to fill knowledge gaps of possible interactions by expert judgment.

Boedeker et al. (1993) propose to use the model of concentration addition as a worst-case estimation and to use it even for mixtures, which act independently because of its good predictions of all kinds of mixtures. Similar to the point of view of Boedeker et al. (1993), recently Faust et al. (2003) proposed to use concentration addition as a precautionary approach but argue that both methods, CA and IA, require theoretical assumptions, which are unlikely to occur under natural conditions.

When considering the application of CA and IA as a module for a model assessing the cumulative environmental status of marine habitats in relation to the MSFD, one needs to be aware that most of the applications of these concepts are applied for experimental setups. In experimental setups components are artificially mixed for the purpose of answering a specific scientific question and thus do provide little information about the applicability for realistic mixtures as pointed out by Backhaus and Faust (2012). Moreover, most of the literature is founded on freshwater environments (e.g. Backhaus and Faust 2012) and some conclusions might not easily be transferred to saltwater conditions due to the differences in the chemical composition of the water and thus different behavior of the chemicals therein.

In contrast to natural mixtures just two to three components are used quite often, which also raises the question how accurate these concepts model the real environment, where many different stressors occur (Backhaus and Faust.2012).

Backhaus and Faust (2012) developed a decision tree for finding the best solution for risk assessment. They propose to estimate the risk first with a CA approach integrating „predicted no effect concentrations“ (if available) and thresholds. In contrast to common procedures, relevant species groups representing trophic levels and sensitive groups are considered (Backhaus and Faust 2012). In case a threshold of these groups is exceeded, it is checked if IA should be applied depending on the natures of mechanisms and the probability of a relevant difference of CA and IA analyses. If this is the case and additional studies confirm the exceedance of limit values, risk management and further studies are recommended (c.f. Backhaus and Faust 2012). An interesting aspect of this concept is that an ecosystem level is integrated in the risk assessment. A risk in the application of this method is however that other kinds of pressures such as fisheries can hardly be integrated in the concept, which might act cumulatively to the different species groups as well. The outcome of the decision three is due to its structure likely to be rather related to IA than to CA (Backhaus and Faust 2012). However, both models ignore some biological and chemical knowledge as that there are indeed interactions between chemicals, interactions with the chemical composition of the species group and adaptation processes, which interfere with the effect of the toxins.

CA and IA could be integrated in other concepts such as species sensitivity distributions (SSD) and provide an estimate for a Potentially Affected Fraction as a Measure of Ecological Risk (Posthuma et al. 2002). This opens the possibility of a wider use of these concepts for ecological risk assessment (Posthuma et al. 2002). The concepts are also included in DEB models under very special circumstances (Kooijman 2010). However, the problems described above need to be solved for an adequate application in cumulative effects assessment.

2.3.7 Toxicokinetic chemical interaction models

Overview

In order to circumvent data gaps of the various effects of an increasing number of hazards, which is a major problem in eco-toxicological assessments, a method was developed to predict the effects, behavior, and interactions of chemicals using an approach based on the quantitative structure-activity relationships (QSARs) (OECD 2004). A software tool can simplify this process:

the OECD QSAR Application toolbox (OECD 2009) can group compounds e.g. according to their chemical structure, a common metabolite or the mechanisms or mode of action depending on the kind of question and data availability. Data are derived by comparison of data from experimental tests and other already existing descriptions (OECD 2009). It is assumed that chemicals with similar features act in a similar way and that a group of chemicals follows a certain pattern. The software also provides a database on regulatory inventories (OECD 2009). The application of the QSAR methods spreads and is often used by regulatory agencies (reviewed in Cronin et al. 2003, European Commission 2011). A comprehensive review of the applications of QSARs is given elsewhere (Cronin et al. 2003).

The basic idea of physiologically based toxicokinetic models is to reflect the behavior of various chemicals in a specifically described organism including mathematical functions and anatomic structure (Haddad and Krishnan 1998). Physiologically based toxicokinetics (PBTK) models distinguish between the different organs and their chemical characteristics as well as circulation systems, cycles, uptake- and eliminations routes. Consequently, the disposition of the toxin can be followed, defined and calculated with mass balance differential equations if sufficient information about the target organism and the stressor is known (Haddad and Krishnan 1998). Concerning the interactions of different chemicals, Haddad and Krishnan (1998) distinguish two major interferences during the uptake process: „the interference with an active uptake process and the modulation of the critical biological determinant of uptake“. Other interaction processes are chemical interactions, where several chemicals compete for the same binding side of a molecule in the body or can bind to a molecule if another toxin did before. Direct interactions between toxins and modifications are also considered (Haddad and Krishnan 1998). The focus of PBTK models is on binary mixtures. However, there are possibilities to model complex mixtures. One option is to model each chemical separately but including all interactions with other chemicals or molecules in the body; the chemicals are thereby connected with these interactions building a network of dependencies (Haddad and Krishnan 1998). This method was also tested experimentally with a mixture of alkyl benzenes in rats and humans (Tardif et al. 1997). Moreover, the method was tested in environmental assessment in combination with the biological hazard index (BHI) (Haddad et al. 1999). It was revealed that the consideration of mixture effects indeed resulted in different outcomes, both higher and lower than the predictions of CA, which could be explained by certain interferences with the metabolism (Haddad et al. 1999). A few years later, Lee and Landrum (2006) focused on time-dependent effects as well as biotransformation processes and proposed a method based on the Damage Assessment Model (DAM), which refers to a critical cumulative level when 50 % mortality is observed (LC50). In contrast to Haddad et al. (1999) they proposed to account for cumulative effects by damage addition, which relates to the model of „concentration addition“, and sums up the effects assuming no interactions between the toxins (see 2.1.6) (Lee and Landrum 2006). Bioinformatics tools have been developed to analyze toxicokinetic processes based on PBTK models e.g. with SBML (System Biology Markup Language) models (Cheng and Bois 2011). A special tool provides merging of different smaller of SBML sub-models for investigation of complex mixtures and interactions (Krause et al. 2010).

Applicability of toxicokinetic chemical interaction models for the assessment of cumulative effects

There are very different kinds of toxicokinetic chemical interaction models (see 0). Therefore, it is difficult to draw general conclusions. The different focusses of the models reflect the complexity of chemical interactions and depending on the type of cumulative effect studied, the method should be carefully chosen. In cumulative effect assessment, also direct interactions should be considered (European Commission 1999). Toxicokinetic chemical interactions are furthermore important for the analysis of the effects of e.g. hazardous substances, related to descriptor 8 in the MSFD (MSFD 2008).

2.3.8 DEB model

Overview

Dynamic Energy Models (DEB models) are based on Dynamic Energy budget theory, which delineates the metabolic organization of organisms and allows understanding, quantifying, and foreseeing physiological effects under certain circumstances (Koojmann 2010).

The development of DEB models started in the 1980s (e.g. Kooijman and Metz 1984, Zonneveld and Kooijman 1989). The main model organism for testing the hypotheses was Daphnia magna. By now, a wide range of species groups are investigated and the model can be modified for special physiological, behavioral or life cycle dependent characteristics for certain species groups (e.g. Jager et al. 2005).

The standard DEB model assumes that food intake is dependent on surface area or body volume and that uptake rate is dependent on food density (Kooijman and Metz 1984, Kooijman 2010). The type of measure for the effect investigated depends on the type of organism studied, practical reasons, or ecological importance. For large marine mammals, for instance length measurements are analyzed, whereas for other species groups other kinds of measurements are taken; e.g. biovolume is more suitable to measure for phytoplankton (Kooijman 2010). Furthermore, the energy gained from food is divided into fixed fraction, soma κ, which is used for somatic maintenance, somatic work, and growth; the remaining energy (1- κ) is mobilized into reproduction and maturity maintenance or maturation in case of juveniles (Figure 2) (Kooijman 2010).

Based on these assumptions, detailed physiological processes, anthropogenic pressures, defense mechanisms, and environmental conditions can be incorporated in the model (Kooijman 2010). The whole model is dynamic and reserve mobilization is dependent on several factors such as overheads of assimilation and mobilization. It reflects also an order of priority: maintenance for example is usually prioritized to investment in growth (Kooijman 2010). It is possible that the metabolic structure is organized in more than one reserve depending on the physiological structure of the organism (Kooijman 2010). The mobilized energy (κ) might be influenced by some environmental factors as proposed for presence of parasites (Hall et al. 2007) or day-length (Zonneveld and Kooijman 1989).

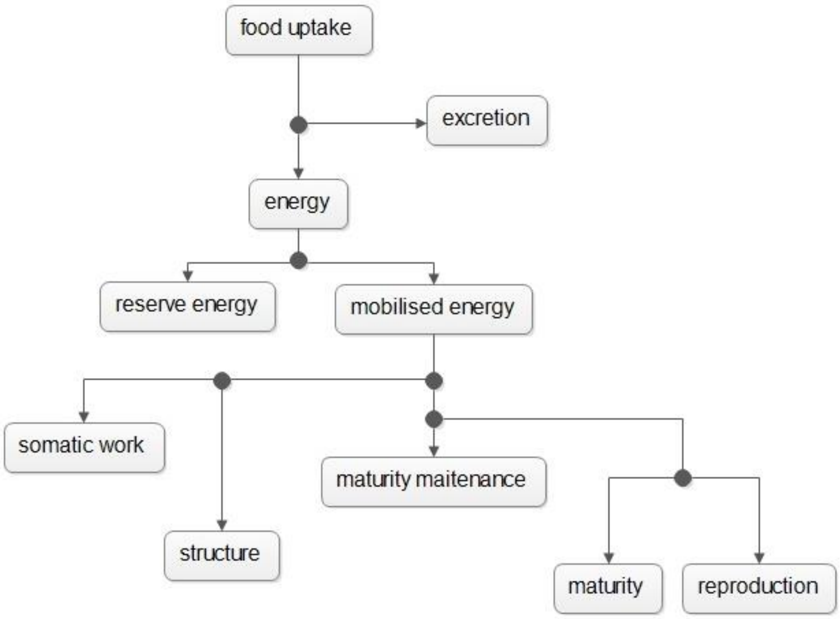

Figure 2 Energy fluxes in the standard DEB model, after Kooijman (2010).

The principles of homeostasis are an important part of DEB models: It is assumed that the chemical composition of the body can roughly be described in five homeostasis concepts in order to cope with the high complexity of the body (Kooijman 2010).

Kooijman (2010) distinguishes between strong, weak, structural, thermal and acquisition homeostasis. Strong homeostasis describes „the strict constancy of the chemical composition of pools (Kooijman 2010)“. Weak homeostasis is the „constancy of the chemical composition of the individual as a whole as long as substrate availability in the environment remains constant, even when growth continues“ (Kooijman 2010). Structural homeostasis „is the constancy of the shape of the individual during growth“: Thermal homeostasis reflects the „constancy of the body temperature“ depending on the heating system of the organism and acquisition homeostasis describes the „constancy of the feeding rate, independent of food availability“ (Kooijman 2010). Another basic principle of DEB models is the balance between demand and supply systems (Kooijman 2010). All metabolic rates in the DEB model are linked to the Arrhenius relationship, which is species specific and is therefore an input variable for the model (Kooijman 2010). Life cycle peculiarities of species can be considered in DEB models: embryo stage can for example be treated as energy reserve without a feeding and reproduction mode for egg laying animals, whereas juveniles feed and grow but do not invest energy in reproduction. A metabolic switching is also incorporated in the model, which is modeled rather as a smooth or scattered transition dependent on food density (Kooijman 2010). Even other ways of reproduction can be expressed by the DEB model such as cell division for phytoplankton (Muller et al. 2011). Aging can also be expressed in the model by considering ROS, even though there are few species groups where this is a large part of the life cycle (Kooijman 2010).

For the standard DEB model the following data is needed with ranked importance (c.f. Lika et al. 2011):

- „Maximum length and body weight; weight as function of length“
- „Age, length and weight at birth and puberty for one food level; mean life span (due to ageing)“

- „Growth (curve) at one food level: length and weight as functions of age at constant (or abundant) food level“
- „Reproduction and feeding as functions of age, length and/or weight at one food level“
- „Growth (curve) at several (N1) food levels; age, length and weight at birth and puberty at several food levels“
- „Reproduction and feeding as functions of age, length and/or weight at several (N1) food levels“
- „Respiration as function of length or weight and life span at several (N1) food levels“
- „Elemental composition at one food level, survival due to ageing as function of age“
- „Elemental compositions at several (N1) food levels, including composition of food“
- „Elemental balances for C, H, O and N at several body sizes and several food levels“
- „Energy balance at several body sizes and several food levels (including heat)“

The DEBtox model and software (http://www.debtox.info/home.php) was developed in order to better understand and predict interactions of toxics as alternatives to commonly used NOEC/ EC50 analyses of toxicological tests (Kooijman and Bedaux 1996). It considers uptake mechanisms as well as toxicokinetics and physiological interactions in the combination with the standard DEB model (described above) (Jager et al. 2010). The output of the model gives estimates of time-dependent effects on endpoints in time such as survival, reproduction, and growth (Jager et al. 2010).

In contrast to other methods, the basic equation of the hazard rate expresses the probability to survive depending on time increment and environmental conditions, interpreting death in general as a random event. (Kooijman 2010). Uptake rates of substances through the water as well as via food chain are integrated in the model and kinetics are considered. If sufficient data is available, it will be possible to correct the compartment kinetics for dilution by growth, changes in lipid content and metabolic transformations changing e.g. the lipophilicity of the original compound. Substances in DEB theory are generally classified as „too little“, „enough“ and „too much“. Thus, toxins are considered in DEB models if they exceed the No Effect concentration (NEC) separating the level „enough“ and „too much“. If several toxins act on the organism, the cumulative effect is usually incorporated in the DEB model automatically via the Energy budget if the stressors have different physiological targets. However, if the toxins have the same physiological target * the interaction of the substances is considered by a Taylor approximation:

$$„s = \frac{c_e^A}{c_*^A} + \frac{c_e^B}{c_*^B} + B_*^{AB} c_e^A\, c_e^B,$$

where c_e^A and c_e^B are the scaled tissue concentration above the NEC, $c_*^A$and $c_*^B$are the tolerance concentrations and the interaction parameter B_*^{AB}can be positive, in case of synergism and negative, in case of antagonism“.

If there is no direct interaction between the compounds, the equation equals the concepts of CA and IA (see 2.1.6 and Kooijman 2010). Moreover, a competition model as described in Kooijman (2010) can be implemented for special cases.

Complex interactions of compounds with enzymes are simplified by the introduction of „Synthesizing Units“ (SU) representing generalized enzymes, which transform compounds chemically. In contrast to usual enzyme kinetics, transformations hinge on fluxes instead of substrate concentrations and backward fluxes are neglected. Moreover, the model allows for interactions between SU, which reflects the natural conditions in the body (c.f. Kooijman 2010).

For the DEBtox model, survival data from toxicological tests (raw data) and hazard concentrations are necessary additional to the data required by the DEB standard model (Kooijman and Bedaux 1996). Furthermore, data about changes of the distributions of energy mobilization into growth, reproduction, and somatic maintenance can substantially improve the model output (Kooijman 2010).

During the last years, there has been put a lot of effort to improve, refine and extent the DEB model. The DEBtox model was generalised and applied for different kinds of species and concrete toxicants (e.g. Muller et al. 2010). Further, Sokolova et al. (2012) proposed to combine DEB models with the oxygen- and capacity-limited thermal tolerance concept for assessing stress tolerance in aquatic invertebrates utilizing ATP as a link between both models. Population models such as Leslie or matrix population models were used for further application of the outputs of DEB models (Lopes et al. 2005, Billoir et al. 2007, Billoir et al. 2009, Jager and Klok 2010). Some studies even integrated interactions between different species groups (e.g. Billoir et al. 2009) and the DEB model was applied to model evolutionary processes (Alunno-Bruscia et al. 2009). Furthermore, 3D models, which can describe concentrations of pollutants in the water column as well as in sediments and show biomass distributions of different species groups, were combined with Environmental Quality Standards (EQS) and DEB models. It was proposed to use these approaches for the WFD and the MSFD (Zaldivar 2008, Zaldívar et al. 2011).

Applicability of the DEB model for the assessment

The DEB standard and DEBtox model (0) were originally developed for environmental assessment and are applied so far for toxicological tests in science (e.g. Arzul et al. 2006, c.f. online bibliography[3]). The OECD (Musset 2006) describes the DEBtox model as a biology-based approach in detail indicating that the method is frequently used. However, it was not applied in the WFD and at least in Germany toxicological data are expressed as concentrations of observed effects (LC50, EC50, LOEC[4]). One reason for this might be that to the time of implementation of the WFD DEBtox models were not tested sufficiently enough, still relatively new and major features, which makes the combination with other models possible, were not developed yet in a practical and suitable way. Therefore, this method is not approved as much as other methods such as the indicator or threshold method (see 2.3.1 and 2.3.5). However, developments towards a possible application in the MSFD have been made and the method is rapidly developed further to comprise a broad range of ecological effects (Zaldívar 2011, Alunno-Bruscia et al. 2009). General obstacles of this method are its complexity, which requires sound background knowledge for being able to consider all the relevant mechanisms concerning the biology of organisms such as life cycles, uptake mechanisms, or energy storages (Kooijman 2010). Some of the required data are not easily accessible and need to be determined with

[3] www.bio.vu.nl/thb/deb/DEB_papers.pdf

[4] http://webetox.uba.de/webETOX/

experiments. Alternatively, they can be derived from similar species or bioaccumulation equations (Lika et al. 2011, Zaldívar 2011). On the other hand, the model profits from its generality in its basic assumptions and the possibility to apply the general concepts and ideas to any organism providing detailed predictions and data input can be reduced to a minimum (Kooijman 2010, Lika et al. 2011). However, if data input is too sparse, there is a risk that the model could give wrong predictions. Experimental tests support the reliability of the method since so far predictability of the results was high (Baas et al. 2009 and Baas et al. 2010). This was even the case for complex mixtures with up to 80 different components (92 % correct) (Baas et al. 2009 and Baas et al. 2010). In ecotoxicology, the method was tested for a broad range of organism groups ranging from phytoplankton (Muller et al. 2011) to marine mammals (Klanjscek et al. 2007). A software tool was developed facilitating the use of the complex framework[5], important model organisms have been studied, and the default function of the software now comprises more than 100 species[6].

The strength of the method lies in its comprehensiveness combining findings and knowledge of physics, mathematics, and biology and by the consideration of a wide range of laws and models for kinetics, growth, or homeostasis (Kooijman 2010). Many ecological processes and biological interactions and peculiarities can be incorporated, which allows adaption of the model to certain purposes and cases (Kooijman 2010). Furthermore, the output variables growth, survival, and reproduction allow for the derivation of the model to higher ecological levels and ecosystem perspectives (Kooijman 2010, Alunno-Bruscia et al. 2009).

However, when considering using the DEB model for the implementation of cumulative effects assessment of the MSFD it became obvious that modeling all occurring species with all possible interactions and effects would be time-consuming. One possibility for an application may be to focus on relevant species, to modify the DEB model in a way that it allows generalizations for species groups or to use indicator species or model organisms for the derivation of possible effects on a species group as it has been done partly already (Klanjscek et al. 2007). The DEB approach comprises most of the aspects of cumulative effects but will not cover all aspects of assessment requirements mentioned in the MSFD if not combined with other models and methods. For example, effects of fisheries cannot be addressed properly with the standard DEB model.

2.3.9 Geographical Analyses

Overview

Geographical analyses are used to analyze the spatial extent of different pressures and to estimate where many pressures overlap. Several layers presenting issues such as marine reserves, shipping routes etc. can be visualized and the cumulative impact of different pressures can be calculated per grid cell, which gives an easily understandable result of the analysis (e.g. Coll et al. 2012). Animal movements can also be integrated in the spatial analysis and be related to spatial features. However, for this, special methods are needed (Desrochers et al. 2011). Spatial analysis is usually conducted with the Geographical Information System Software (GIS)

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[5] http://www.debtox.info/home.php

[6] http://www.bio.vu.nl/thb/users/bas/lectures/oslo2012a.txt

and it is possible now to analyze 3D and temporal data if needed[7]. GIS analysis is further often combined with other methods. In many studies, the threats are weighted by factors derived from expert opinion (see 0), so that the relative impact and intensity of pressures can be considered in the analysis. Coll et al. (2012) investigated the overlap between marine biodiversity, marine reserves, and different weighted impacts for the Mediterranean Sea to identify areas priority areas for nature conservation. Halpern et al. (2008) analyzed cumulative threats worldwide based on a GIS analysis (see details below). The HELCOM Baltic Sea Impact index adapts this method according to the special conditions in the Baltic Sea and it was directly related to the pressures listed in the MSFD (HELCOM 2010, HELCOM 2018). Furthermore, the pressures were refined and quantified (HELCOM 2010, HELCOM 2018).

Wildlife vulnerability maps

These kinds of methods are usually on the one hand based on the sensitivity of an ecosystem or species/ species groups and on the other hand, they consider the spatial, intensity dependent and temporal cumulation of pressures. The output of the analysis is a map, where areas of special concern are visualized (e.g. Selkoe et al. 2009, Coll et al. 2012).

While many studies fail to differentiate clearly between sensitivity and vulnerability (e.g. van Bernem et al. 2000, Coll et al. 2012), MacDonald et al. (1996) highlight the distinction between vulnerability and sensitivity. They define vulnerability as the actual exposure of an organism to a stressor, whereas sensitivity describes the fragility and ability to recover from a threat (MacDonald et al. 1996). Fragility and recovery ability are categorized in classes of different strength based on expert judgment (MacDonald et al. 1996). Intensity of the pressure is incorporated in the proposed equation to determine the cumulative effect (MacDonald et al. 1996). The method is applied for benthic species threatened by different fishing techniques: MacDonald et al. (1996) suggest an indicator- or key species concept for the investigation of the cumulative effect of fishing, and proposed a list of suitable species (MacDonald et al. 1996).

Van Bernem et al. (2000) focused specifically on the cumulative effect of oil spills in the Wadden Sea. In order to define particular sensitive areas they developed an environmental sensitivity index (ESI). Besides the main pressure of oil, they consider also other interfering factors, which can hamper the recovery, such as oxygen deficiency and rank different habitat types relating to vulnerability. For evaluation of the vulnerability, an index value was calculated for the different species groups and habitats (ecological compartments) considering the critical properties, which matter in case of an oil spill. In the compartment „Benthos–Sediment“ for example the physiological sensitivity, the ecological sensitivity, its importance as food, its metabolic importance, its capability of dispersal and the duration of reproductive period ranging from one to three are taken into account (Van Bernem et al. 2000). The index values are based on expert knowledge. The results are averaged and corrected for abundance class and sediment conditions Van Bernem et al. 2000). The results of the different compartments are summed up, and they provide the final index for the wildlife vulnerability map. This map contains a spatial and temporal dimension (c.f. Van Bernem et al. 2000). Later, this classification method was automated based on the results of the first study using Auto associative Networks, which can detect abnormal situation by e.g. identification of outliers, filtering of noisy data and data compression (Schiller et al. 2005).

[7] http://training.esri.com

Another approach was developed for potential oil spills at the Lithuanian coast, which comprises besides a biological based index also one for coastal features as a measure of recovery potential and an index for socio-economic resources (Depellegrin et al. 2010). However, this method has a more refined scale for the weighting factors but does on the other hand not define biological characteristics as detailed as in the study by Van Bernem et al. (2000).

Coll et al. (2012) investigated the cumulative effect by producing wildlife vulnerability maps with a special emphasis on biodiversity and protected areas in the Mediterranean. They defined areas, where the cumulative threat is particularly high, and where these areas overlap with areas of high conservation status. Thereby, also areas could be found, which are of interest for studying the effects of cumulative interactions of stressors sue to their complexity of pressures and do not belong to an area of conservation concern. Biodiversity was modeled at a spatial scale of 0.1°x 0.1° grid cells and expressed by percentage of species of a certain species group occurring in a certain grid cell (Coll et al. 2012). The impact of various anthropogenic threats was weighted according to their impact on each of the species groups, whereas each pressure was ranked separately Coll et al. 2012). This weighting was based on expert judgment (Coll et al. 2012). The cumulative threat was calculated by adding up the weighted impact of the pressures (categories between 1 and 5) so that a GIS map could be created for each species group and each pressure category. In a second step, a GIS map was created, where all occurring species groups and all threats were summed up for each grid cell representing areas of concern where high biodiversity and high cumulative threat match (c.f. Coll et al. 2012).

Halpern et al. (2008) suggested a method comprising 17 different anthropogenic threats, most of them mentioned in the MSFD as well. Following their method, in a GIS map the ecological status of each grid cell is calculated by

$$I_c = \sum_{i=1}^{n} \sum_{j=1}^{m} D_i * E_j * \mu_{ij} ,$$

where Di is the log-transformed and normalized value (scaled between 0 and 1) of an anthropogenic driver at location I, Ej is the presence or absence of ecosystem j (either 1 or 0 respectively), and μij is the impact weight for the anthropogenic driver I and ecosystem j (range 0 to 4) (Halpern et al. 2008). One basic assumption in this study is that pressures add up, and although the authors are aware of possible synergistic effects, these are not taken into account in their analysis (Halpern et al. 2008). Vulnerabilities of 20 ecosystem types worldwide were ranked by experts and an overall weighted average score for each ecosystem was defined for the anthropogenic threats, respectively and presented in a matrix table (Halpern et al. 2007). Expert knowledge will be needed to rank the scale, the frequency, functional impact, resistance, recovery time, but also for ranking the uncertainty of the knowledge the authors referred to in their evaluation (Halpern et al. 2007). The method was applied on a regional scale as well (Selkoe et al. 2009). It turned out that the application of the global model hardly reflects the actual environmental status as it was appraised by regional experts (Selkoe et al. 2009). Large errors could be explained by the authors by the lack of crucial threats, which were not considered in the global assessment such as the influence of alien species and wrong habitat classification by the global model (Selkoe et al. 2009).

Another interesting approach was presented by De Lange et al. (2010), who focused on traits, which determine the vulnerability of species. Thereby, they distinguished between internal and external exposure and effects on individual and on population level for each of the six chemical stressors. The traits comprise e.g. habitat preference, behavior and life history, as well as effects within the body and represent the probability and extent of exposure, the different kinds of effects and recovery. Whenever possible, literature data has been used and in case of data gaps been supplemented with expert knowledge (De Lange et al. 2010). With this comprehensive method, a wide range of ecological questions can be explained. The driving forces behind vulnerabilities can be extracted, vulnerability maps can be made based on these data, and the vulnerabilities of species groups can be compared (c.f. De Lange et al. 2010). Life-history characteristics are also used by Stelzenmüller et al. (2010) to derive a sensitivity index for the effects of aggregate extraction. Sensitivity indices of fish and shellfish are estimated and a map was produced based on long-term distribution data and indicator kriging showing the most vulnerable areas to this pressure (Stelzenmüller et al. 2010).

Breue et al. (2002) integrated the bioavailability, uptake, and biodegradation of the toxins, which fluctuate a lot depending on present environmental chemical and physical conditions as well as organism-related processes in an environmental compartment model (Breure et al. 2002). The mass flows are estimated by mass balance equations with the „SimpleBox model“ (Breure et al. 2002), where the movement and transformation of substance is divided into different compartments („boxes“): the regional scale, continental scale and global scale, which can be subdivided (Breure et al. 2002). They analyzed not only the multiple effects of different chemical compounds but also the effect of chemicals in combination with non- chemical stress (Breure et al. 2002).

Geographical Analysis combined with species sensitivity distributions

Some of the geographical assessments are based on species sensitivity distributions (SSDs) as for example the study of sensitive areas for amphibians by Fedorenkova et al. (2012). Fedorenkova e al. (2012) applied species sensitivity distributions for revealing the most severe threats for amphibians by a rank-based approach as described by Postuhuma et al. (2002) and Aldenberg et al. (2002). The results of their study were in line with other studies and could determine the most important causes for the decline of amphibians in the study area (Fedorenkova et al. 2002).

SSDs are derived from laboratory tests, where the reaction of one stressor on a species is observed (Posthuma et al. 2002). The outputs of such tests are often mortality data, e.g. LC50 data. However, also sublethal effects can be interpreted in SSDs. It is assumed that individuals of species react differently to a stressor due to intraspecific variation, assuming that data is normally distributed. The ranges of tolerances of the species to a specific substance can be determined by using these SSDs. The SSDs are widely used in ecological risk assessment, mainly in North America and Europe (Posthuma et al. 2002).

The results of SSD analyses could directly be transferred to maps with environmental data, but Aldenberg et al. (2002) argue that one should be careful to apply toxicological data directly to field data and implement them in maps. The reasons for that are the quite variable field conditions the organisms are exposed to, which are not directly comparable with the stable artificial lab conditions (Aldenberg et al. 2002). In a first step, exposure concentrations are corrected for bioavailability under natural conditions and all measured and estimated concentrations of a certain spot are mixed to reflect natural variability (Aldenberg et al. 2002). In a second step, the ecological risk of exposure is calculated by the probability density function of the field concentrations and the probability that a random individual of a species pool would encounter conditions causing a negative effect corresponding to the SSD (Aldenberg et al. 2002).

Applicability of geographical analyses for the assessment of cumulative effects

Geographical Analysis is a popular method for impact mapping (e.g. Halpern et al. 2008, Depellegrin et al. 2010, Stelzenmüller et al. 2010, Coll et al. 2012). Implementation of geographical analyses for cumulative effects assessment includes the implementation in contingency plans and risk assessment. The index method for constructing vulnerability maps for sensitivity for oil spills was e.g. applied in the German Oil Spill Contingency Plan (Van Bernem et al. 2000). However, for other stressors such as marine litter, a more comprehensive monitoring program is needed for actual applicability (Van Bernem et al. 2000). Wildlife vulnerability maps based on a trait-based method as described by De Lange et al. (2010) were applied in risk assessment of soil pollutants in Denmark (Lahr et al. 2010). Geographical Analyses are very illustrative and at the same time, comprise detailed and complex information, which can be interpreted quickly. For politics and for conservation management, such mapping methods can be very helpful decision tools for e.g. prioritizing areas of concern or high value, defining marine reserves or for localizing areas suitable for restoration projects (Ban et al. 2010). Furthermore, they might help to mark out areas for projects such as offshore wind parks, where the cumulative impact of the project on the ecosystem is predicted to be comparable low. An interesting aspect is the possibility to integrate habitat related issues into the assessment. For this, data may be quantitative on one hand and comprise e.g. the spatial extent, or it may be qualitative and can be combined with SSDs on the other hand (Fedorenkova et al. 2012). If such components are not integrated when analyzing impacts on certain species though, the apparent clear picture of effects can be deceptive and result in misleading interpretations (de Vries et al. 2012). By application of geographical analyses, a huge range of questions can be answered such as: What are the most severe pressures in a defined region? Where do pressures overlap with marine protected areas or the occurrence of red listed species?

The method can be combined with many other methods and can comprise very different kinds of pressures and environmental conditions and movements (e.g. Breure et al. 2002, Selkoe et al. 2009, Fedorenkova et al. 2012). However, in many cases, some questionable assumptions are made to simplify this approach: the effects of stressors are assumed to act additively and pressures are assumed to decay linearly (e.g. Ban et al. 2010). Synergistic, antagonistic effects are usually not considered (Ban et al. 2010) and the method does not account for interactive effects in the water column, within the body of the organism or indirect effects due to interactions with other individuals or environmental conditions. Furthermore, in some cases, a geographical analysis might not reflect the nature of certain threats to the environment and rather divert from it because the geographical distribution of the threat is not as relevant as other aspects.

2.3.10 Expert judgement

Overview

Since cumulative effects assessment is a relatively new field of research and thus, the data base is often very small with regard to a certain problem, experts are often consulted to estimate relative effects of certain pressures on organism groups, on a whole ecosystem, or on general severity of a certain threat. The number of experts consulted varies. Sometimes exclusively experts from one university are asked (Depellegrin et al. 2010). In some cases the authors themselves take the task for ranking of pressures according to their assumed impact based on their expertise (Halpern et al. 2012). Experts can also be chosen in a systematic and replicable way (Halpern et al. 2007). Halpern et al. (2007) searched with key words in databases for authors, who published about a specific topic, a method. The method applied in Halpern et al. (2008) was a foundation for several other surveys (HELCOM 2010, Breen et al. 2012, Andersen and Stock 2012 for the HARMONY project). For an evaluation of the method, they compared the results from the expert consultation with a quantitative assessment (Halpern et al. 2007). Expert consultations are not only used to rank impacts but also to define thresholds (Livingston et al. 2005).

Expert judgment for ranking pressures is done on scales of different refinements, and guideline criteria to find an appropriate estimate are often provided (Halpern et al. 2007). Experts were asked for the holistic assessment HOLAS (HELCOM 2010) to define a weighting score for impacts on biological ecosystem components by considering the three criteria in four increments respectively (functional impact, which is broken down by the number of species/ trophic levels affected by the impact, recovery according to the expected recovery time, and the assumed resistance of the component against the pressure ranging from „no impact“ to the level „vulnerable“) (HELCOM 2010, method based on Halpern 2007 and 2008). Four countries and the HELCOM secretariat proposed weighting score, which were averaged for a final weighting score. Breen et al. (2012) consulted 30 experts from 16 European countries who classified the descriptors of the MSFD in three levels for five different ecosystem component parts according to five criteria for each area of the four seas covered by the MSFD (Breen et al. 2012). These include besides the main criteria mentioned above also e.g. „pressure persistence beyond activity cessation“ and „frequency of occurrence of the pressure“ but lacking a functional criterion (Breen et al. 2012). A similar ranking method is also applied by Andersen and Stock (2012) via an online survey, whereas in this study also relatively detailed bio-geographical information and partially species distribution data is considered to reflect community impacts.

The European Commission (1999) describes „Expert opinion“ in their guidelines besides consultations, questionnaires, and checklists„“. Expert opinion relates to a general project structure meaning that one project coordinator should gather different experts and organize regular meetings for the exchange of views. The project structure should facilitate communication between the experts since cumulative effects are complex and the project members should represent different disciplines reflecting the nature of cumulative effects (European Commission 1999).

Consultations addresses rather people involved in the process of implementation, scientific background, or persons who are concerned with the cumulative effects and participants of such meetings are often authorities, experts, businessmen, and people from the local community (European Commission 1999). The main purpose of such meetings is to gather information and to integrate the people concerned with the project. Other methods for achieving information are questionnaires, which can be answered in written form or during an interview (European Commission 1999). Questionnaires help to structure information in advance and can be evaluated in a quantitative way. Questionnaires can integrate scenarios and detect especially socio-economic effects (European Commission 1999). Checklists are tables, which have a predefined structure for specific information (European Commission 1999). They are used to collect e.g. information about potential effects of impacts in different periods, geographical areas or on different organism groups (European Commission 1999). The experts can fill the cells either simply with checkmarks or with key words describing the effect or kind of interaction (European Commission 1999).

Varis and Kuikka (1997) described a statistical method for the analysis and evaluation of expert judgment based on belief networks and probability distributions in impact matrices. The method is deduced from artificial intelligence research and it includes an uncertainty analysis (Varis and Kuikka 1997). This example shows how expert judgements can be combined with other methods.

Applicability of expert consultations for the assessment of cumulative effects

Expert consultations are very frequently used (e.g. Halpern et al. 2007, 2008, Depellegrin et al. 2010, Livingston et al. 2005) and can be very productive for the overall working progress, providing the possibility for exchanging different views and aspects about cumulative effect assessment (European Commission 1999). The EU Commission (1999) highlights the importance of expert meetings and argues that for smaller projects expert opinion alone will be sufficient. However, projects that are more complex require expert opinion rather as a starting point for the application of other methods (c.f. European Commission 1999).

Moreover, the involvement of project members, persons concerned with the project and scientists of different disciplines in the identification and evaluation of cumulative impacts can expand the understanding of cumulative interactions. Another advantage is that this method can theoretically cover all aspects of cumulative aspects. However, the judgments can be very subjective and very often depend on personal experiences, intuition and worldviews (Halpern et al. 2007, European Commission 1999). Sometimes literature data and expert evaluation are mixed, whereas it is not always transparent in which cases literature data are used and in which experts were consulted (e.g. Halpern et al. 2012, Coll et al. 2012). Uncertainties arise if experts are asked to judge about cumulative effects over a wide geographical range or if their answers are extrapolated to a huge area as it was done by Halpern et al. (2008), because their expertise often rather lies on a regional level. Regionally, some special threats might be important, which are negligible in such a global analysis. Therefore, a transfer of assessments of a larger area to a regional scale can be problematic. In the worst case, this can happen in very valuable ecosystems, such as a „pristine" coral reef ecosystems (Selkoe et al. 2009). Thus, it is important to investigate the regional important threats Selkoe et al. (2009) and to base an assessment on the judgment of experts familiar with the region of interest.

Sometimes rankings derived by expert judgment can also result in logical inconsistent conclusions. In a study by Halpern et al. (2007) destructive fishing was ranked as a least severe threat than non-destructive fishing. The authors realized that this unexpected result emerged and stated the reason that the experts did not experience this threat in their regions of expertise and that the frequency of threat was one of the criteria for the scoring of impacts (Halpern et al. 2007). In some cases, such errors of the method might not be detected. Therefore, a validation of the outcome of such a method as done by Halpern et al. (2007) is critical. Furthermore, choice of criterions, questions in questionnaires and ranking procedures need to be phased.

2.4 Literature

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3 Overall concept for cumulative effects assessment

3.1 Rationale for the choice of methods and proposal for an overall concept for cumulative effects assessment

As a preparation for the development of an appropriate concept for cumulative effects assessment, we compared various methods for cumulative effects assessment with regard to the relevant aspects of the Marine Strategy Framework Directive (MSFD) they cover. Furthermore, we scrutinized the scientific quality of the methods considering reproducibility, quantification, and subjectivity. Another criterion was the comprehensiveness and practicability of the methods. None of the methods we reviewed could cover all relevant aspects of cumulative effects alone. Hence, a combination of different methods would be the best option to represent cumulative effects as they likely occur in a natural situation as good as possible without hampering perspicuity.

The literature research showed that a systematic organization and visualization of literature data is necessary to promote the incorporation of scientific insights into practical assessments. To promote a continuous integration of new literature and to allow the flexibility for the integration of new methodological improvements as well as a high level of transparency throughout the different steps of the cumulative effects assessment, methods from computer science are useful. The application of an online database to structure and organize literature data combined with different assessment tools turned out to be an appropriate approach to provide a suitable frame to ensure these aspects. Via links to aggregated information as well as to the original data source, the online tool assures traceability. The online tool allowed the visualization of information derived from literature in flow diagrams to provide an overview of the relationships between human activities, anthropogenic pressures, other influencing variables and effects on ecosystem components.

Moreover, it was necessary to adapt methods for cumulative effects assessment so that they were applicable to different levels of quality of information as well as to different levels of biological organization. For a suitable integration of monitoring data and for the coverage of the various aspects of cumulative effects it was necessary to adjust some of the methods reviewed to fulfill the requirements stated in the introduction. Keeping this in mind, we adjusted the matrix method (see 4.3.2) for a generalized method in cumulative effects assessment. Further, we combined a special matrix type with a modified DEB model and developed a specialized structure for a network model for cumulative effects assessment.

Matrices and cross-impact analyses allow the combination of very different kinds of data such as qualitative and quantitative data. This way it is possible to utilize a maximum of available information, which helps to mitigate the problem of the lack of data in this research area. Furthermore, due to their simple structure, matrices facilitate the analysis of very different kinds of interactions, such as interactions between different stressors as well as interactions between different ecosystem components. Thus, the analysis of many different anthropogenic impacts on a certain indicator species as well as on the ecosystem is possible. Because matrices allow so many different kinds of cumulative effects assessments, we integrated them into the 'framing tool' (LiACAT) as a central element and visualized the corresponding literature data in flow diagrams.

For the analysis of cumulative effects with a focus on the species level, a combined approach of matrix model and DEB-model turned out to be the most suitable approach. DEB models focus on one model species. However, the general structure of the DEB models is universal, so that it is possible to adjust the basic model structure for the analysis of cumulative effects of other species with only a few modifications. We tested this by transferring the DEB model for *Mytilus edulis* into a model for *Crassostrea gigas*. A further advantage of DEB models is that they simulate temporal dynamics considering both the life cycle of a species as well as uptake mechanisms of toxins. Therefore, they can potentially cope well with the complexity of temporal dynamic cumulative effects and are likely able to reflect a scenario more realistically than static models. As DEB models predict certain endpoints in time such as growth, the model is verifiable and it is possible to compare the results with laboratory tests or field data. However, interaction effects between stressors were not yet integrated at the start of my thesis into DEB models to predict cumulative effects based on literature data. However, they served for derivation of interaction factors based on conducted experiments (Baas et al. 2009). Hence, we needed to add a special module for assessing interaction effects.

The combination of the DEB model with a matrix model solved this problem. We adjusted the method proposed by Weimer-Jehle (2008) for calculating the overall influence of each of the relevant stressors by taking account of the influences of other stressors on its effect. Thereby, we derived information of the influences from literature data. In turn, we used the results of the matrix model as input variables for the DEB model to calculate cumulative effects throughout the life cycle an organism. We tested this method with data for blue mussels (*Mytilus edulis*) to assess the cumulative effects of the heavy metals cadmium, copper, zinc and lead in combination with changes of pH-values, temperature and oxygen depletion (chapter 4).

For cumulative effects assessment on habitats, another kind of modeling approach is required. On the one hand, the influences on habitats are diverse and range from anthropogenic pressures to environmental variables and influences by changes in species composition or even species functional traits. Changes of traits of the characterizing species of a habitat such as a significantly reduced growth of seagrass leaves might affect other species living in this habitat, who need shelter. Therefore, the effects which need to be observed in habitats are various and range from shifts of abundances of certain species to traits of the characterizing species and to affected functions of the habitat.

Network models can generally handle such complex interaction networks to describe e.g. structural changes in food webs. However, the special behavior of interaction effects between stressors or between stressors and other influences resulting in cumulative effects need special emphasis. Furthermore, for practitioners it would be useful to be able to select, which anthropogenic pressures should be included in the analysis. To facilitate the integration of literature data about cumulative effects, to allow a continuous update of the model based on new scientific insights and to generate flexible outputs depending on the data available, we propose a new structural framework for the analysis of cumulative effects of anthropogenic pressures on habitats. It is a network model, which constructs itself based on the data input, called Automated Cumulative Impact Model (ACIM). The results of this modeling tool provide indications for cumulative effects in the habitat of interest. We tested this method for the evaluation of cumulative effects of anthropogenic pressures on seagrass meadows (chapter 5).

The spatial perspective of cumulative effects is highly relevant when it comes to the development of programs of measures and management plans. We propose to apply geographical analyses additionally to the methods described above to identify areas of concern with regard to cumulative effects. Our aim was to develop a tool, which allows the calculation of cumulative indices for species and habitats based on monitoring data with regard to geographical spots or raster cells. We propose to calculate a matrix model for each of the raster cells with a focus on species level as well as habitat level to provide information about the spatial distribution of cumulative interaction effects. To provide input data for each raster cell, data from monitoring stations need to be interpolated spatially. The results of the interpolation for each of the raster cells are then presented in a map. For a cumulative geographical analysis, an aggregation of the results for species and habitats to a single index is necessary. This index should reflect the overall health of the ecosystem with regard to the spatial distribution of anthropogenic pressures and ecosystem components. For such an aggregation, we propose to use an additive approach as e.g. applied in Halpern et al. (2008) or in HELCOM (2018). Additionally, maps should be produced for the outputs of interest (e.g. for the filtering activity of mussels) to support an adequate interpretation of the map.

Moreover, it would be important to understand the spatial distribution of the anthropogenic pressures without the consideration of special responses of the occurring ecosystem components. HELCOM realized this for the assessment of the Baltic Sea with the Baltic Sea Pressure Index (HELCOM 2010, HELCOM 2018). However, the results of these two HELCOM reports are not directly comparable with each other as they integrated a mean value for an impact score based on score of expert judgements for the effects on different ecosystem components and the values differed between the assessments. To solve this problem, we propose to apply a combined approach of fixed threshold values to normalize the strength of the pressures with a geographical analysis. This would provide reproducibility and comparability between different years. We tested the application of thresholds for anthropogenic pressures together with a geographical analysis to identify spatial hot spots of anthropogenic pressures. However, we did not yet include species and habitat related effects due to time-limitations[8]. Nevertheless, the combination of the results of the models focusing on different ecosystem components with the spatial distribution of anthropogenic pressures is an important part of the overall concept and should be a long-term aim. Therefore, geographical tools are also included in the framing tool LiACAT.

To summarize, we propose to combine data from monitoring programs and literature data in models and to track the data flow in an organized structure by an online database as a framing tool to provide a high transparency and flexibility. Moreover, we suggest the visualization of literature data in flow diagrams to provide quick overviews of the state of the art. For the cumulative effect assessment, we propose to consider at least three levels of organization: the species level, the habitat level, and the spatial perspective reflecting an ecosystem perspective. For the species level, we propose to apply a combination of the Matrix method and DEB model, for the habitat level, a network analysis (ACIM), and for the spatial perspective, a geographical analysis for assessing the ecosystem health combined with a threshold approach focusing on the spatial distribution of anthropogenic pressures (Figure 3).

[8] This analysis is therefore not part of the thesis but will be published online by the UBA (Eilers et al. 2021).

3.2 Realization

In the following, we describe the structure of the framing tool combining a literature database and integrating different kinds of assessment tools. Thereby, we elucidate already implemented features as well as planned features. The text should give an overview of the overall concept, describe data flows and show links between the different modules of the concept. In chapter 4 and 5 of this thesis, I describe two of the proposed methods for cumulative effects assessment more in detail.

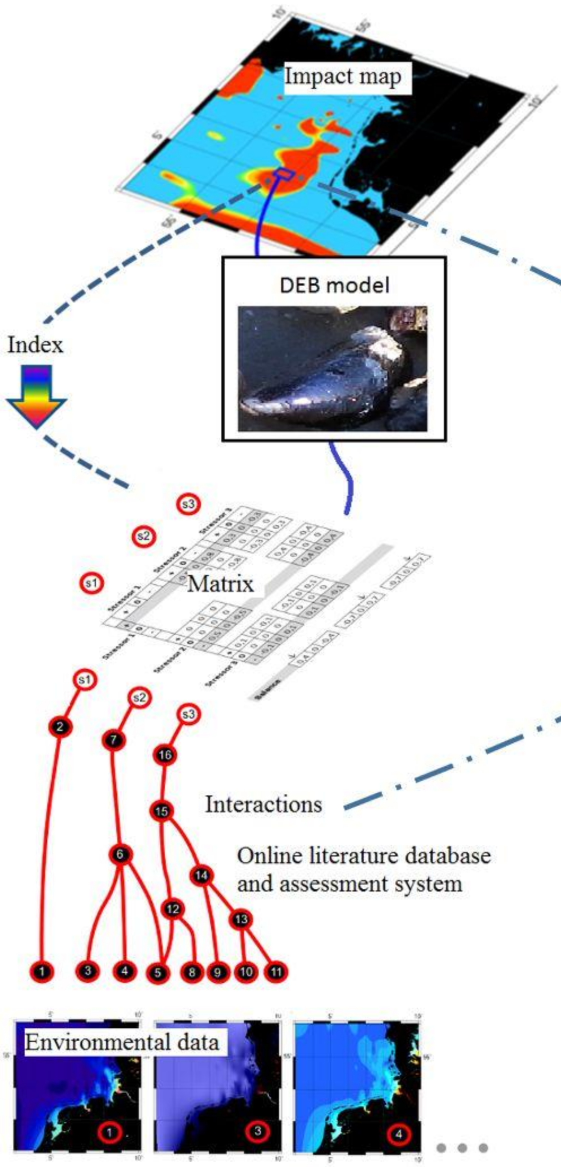

Figure 3 Overview of the main methods and data flows proposed for the overall concept. The DEB model focuses on species level, ACIM focuses on habitats, and the cumulative index provides a general value for interaction effects applicable for different kinds of focuses

The framing tool is called '**Li**terature based **A**nalysis and **C**umulative **A**ssessment **T**ool' (LiACAT) and is hosted on the biodiversity data platform 'mybiOSis'[9]. LiACAT consists of different single modules for the organization, structuration and visualization of literature data, for the download tools, for geographical modules and for assessments (Eilers et al. 2014, Jong et al. 2015, Eilers et al. 2020).

The user enters first general literature information about a publication in LiACAT in a special window, the literature input form. Additionally, the user enters information about the relationships between pressures and ecosystem components in the 'relationships editor', a special input form for information. This information is accessible by links to the relationship throughout the LiACAT tool. In a further module, the user extracts and digitizes literature data from graphs. This module is based on the freeware tool 'WebPlotDigitizer'[10]. We integrated it into LiACAT and connected it to other modules of the tool. Moreover, we implemented two modules for the geographical visualization of data. One of them serves for the visualization and calculation of spatial cumulative effects, the other one serves for general geographical analysis such as area calculations and is applicable to calculate the area of spots with particularly high-pressure intensity or to calculate the overall burden in an area. In the following, we describe the most important building blocks of the overall concept for the analysis of cumulative effects in relation to LiACAT (Figure 4).

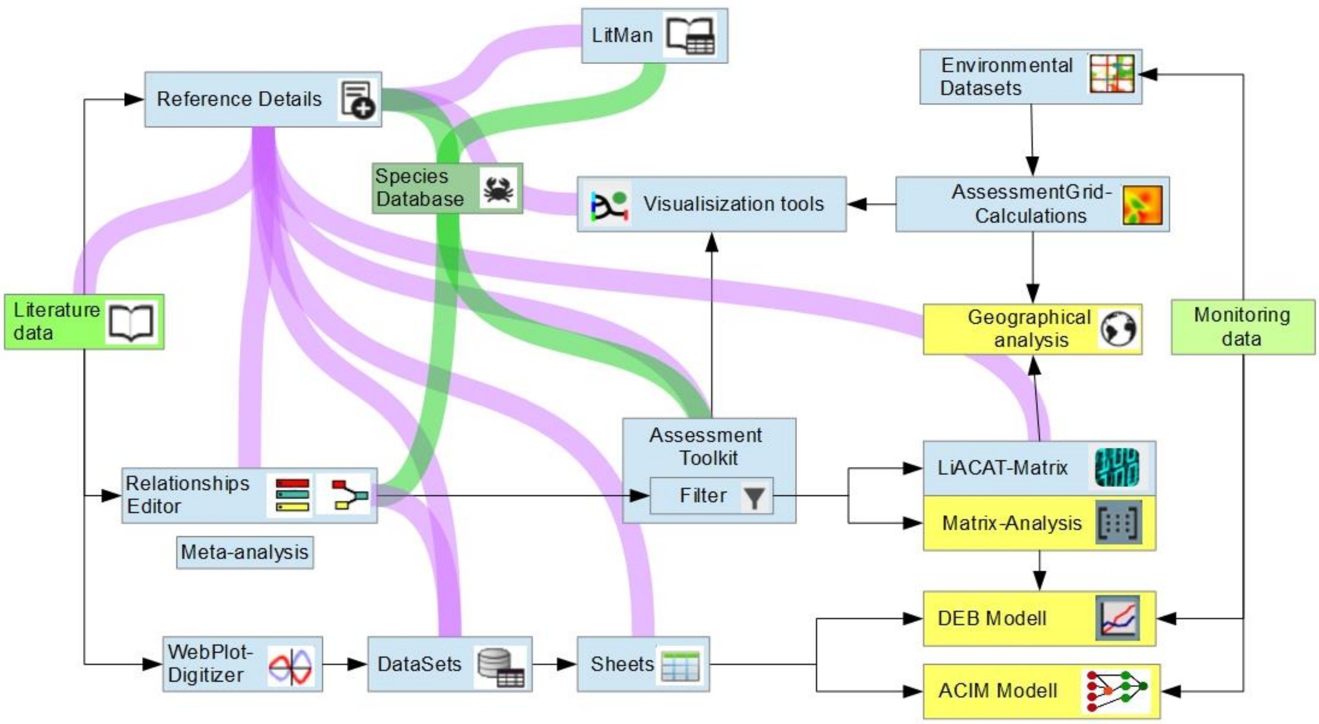

Figure 4 Links between the most important modules in LiACAT and relationships to conducted analyses for cumulative effects assessment

[9] https://kladia.info/klados/
[10] http://arohatgi.info/WebPlotDigitizer/

3.2.1 Entering literature data

LiACAT enters basic bibliographic data automatically into the literature data input form in LiACAT when the user drops links of standard literature software such as EndNote into the corresponding entry field. However, the user needs to enter further information manually. LiACAT saves these data in a synoptic file (called 'ReferenceDetails'). Links to the 'ReferenceDetails' as well as the most important information of the file is compiled additionally in a tabular structure, in a module called 'LitMan'. Here one can get an overview of all literature belonging to one project and it is possible to search in all columns for keywords of interest for filter the data correspondingly.

In the 'RelationshipsEditor', the user enters data with regard to individual relationships between human activities, pressures and effects on ecosystem components. To initiate a relationship, it is necessary to define at least one source (e.g. a pressure) and one target (e.g. an effect) leading to several different effects (targets). Usually one relationship represents one experiment. The user adds further information about the relationship such as the context of the investigation, results of the experiment, and the framework conditions as related information. Such information comprises for example data about the environmental conditions, the experimental conditions, information about the species observed, and the magnitude of the effect as well as statistical data, which are useful for meta-analyses. LiACAT aligns the further information the user enters about cumulative effects to relationships. A text field provides the option to enter any additional general important information about the relationship. A special function further allows entering information about the relationship graphically to show the effects of increases or decreases of sources. Additionally, the user can assign datasets describing the response relationship to the relationship data pool. The user can invoke any information about relationships from the 'assessment toolkit'. Here, one can select data from the 'AssessmentToolkit' for the application for a cumulative analysis.

Additionally to entering the data directly in the data input form, the user can assign the content of a publication to predefined topics. Most of them concern the MSFD. Furthermore, we added additional aspects, which matter with regard to the topic 'effects of anthropogenic pressures on the marine environment', to the list of topics. For the categorization of the topics the publications deals with, the user needs to set checkmarks for the relevant topics of the publication. We organized this list of topics in a hierarchical form with main topics and subtopics, to find the topics easily. LiACAT links the categorization to the assessment tool, so that the user can later use this information to restrict the assessment to the predefined topics.

The user further extracts data from figures of the publication (unless they are provided in a table) with the freeware program 'WePLotDigitizer'11, which we integrated in LiACAT and connected to other modules, so that these data are directly transferred and can be used in the other modules. This linkage made it possible to partly automatically extract data from literature for later analyses. LiACAT saves these semi-automatically extracted data in a fixed table structure ('DataSets'). If datasets from different publications deal with the same topics, the user can combine different datasets in another module with the same table structure ('Sheets'). In this module, one can also download the data as csv files.

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[11] http://arohatgi.info/WebPlotDigitizer/

These files serve as the basis for further analyses. The modeling tool for cumulative analysis ACIM, which we integrated in LiACAT, calls these data. This modeling tool sets the single datasets in relation to each other und describes the data with mathematical functions to analyze the combined effect of different anthropogenic pressures on a habitat. Further, we used some of the extracted data for an adjusted DEB model, which allows the investigation of temporal dynamic cumulative effects on a species. For both of these models, we integrated monitoring data and tested different scenarios.

3.2.2 Cumulative analyses and assessment tools

Both of the models mentioned above (ACIM and cumulative DEB model) belong to a set of methods, which we developed particularly for the analysis of cumulative effects of anthropogenic pressures. These models allow the assessment of the combined effects of various pressures on an ecosystem component. Moreover, we developed a method to analyze spatial cumulative effects of various anthropogenic pressures based on existing approaches (Halpern et al. 2008, HELCOM 2010, HELCOM 2018). By applying this method, we could identify geographical spots, which indicate on a map where pressures accumulate spatially (Eilers et al. 2020).

For the species based approach, we first conducted a literature research to derive information on the interaction effects between pressures and for setting up a matrix. We entered these data into a table using the names of the stressors as column and row names. Thereby, the row names represented the influences of the stressors and the column names represented the stressors, which are potentially influenced. In each cell, we entered information about the interaction between the stressors expressing in which way one stressor influences the effect of another stressor on the organism. We stated this information is stated in form of a certain value, a formula, or a description. If possible we also included information about the magnitude of influence. In a second step, we selected literature data, which are relevant for the question of interest and which fitted best to the conditions of the study area. Next, we summed up the values of each of the cells in one column resulting in a value for a 'netto effect' of each stressor under the consideration of the influences of other stressors on the effect. These values slipped into further calculations. The matrix-method was based on a method proposed by Weimer-Jehle (2008).

We sorted the values for the 'net effects' of the single pressures afterwards based on the kind of effects and aligned them to the intensities of the pressures in the marine environment. A modeling tool considering the uptake of a substance, possible adaptations of the organism to the stressor as well as temporal effects calculated a normalized intensity of the stressors. The results were integrated into a DEB model emulating the life cycle of the organism and in which the influences of the stressors under the consideration of their temporal dynamic intensity are analyzed. The outputs of the model are quantified cumulative effects due to the pressure situation on the development, the reproduction and on the growth of the organism throughout its life cycle.

To analyze cumulative effects on habitats, we developed the modeling tool ACIM. According to this method, first literature data, which describe effects in dependency of stressor intensity and exposure time, need to be extracted with the WebPlotDigitizer from publications and saved as a sheet in LiACAT. ACIM then calls these data, which serve as a basis for the construction of a network of relationships. For each of the datasets – describing for example the relationship between a pressure and an ecosystem component – the modeling tool determined a mathematical function from a pool of predefined functions (base models), representing the model statistically most suitable to describe the data. Thereby, it optimized parameters for the functions, so that the characterized model represented the data as good as possible. If data are available describing the influences of two or more aspects, it optimized all possible base models and combinations of base models and compared them to each other based on statistical values to find the best model to describe the observed effect. Observed effects, which are possibly influenced by the exposure time, were also treated this way. This way it was possible to model temporal dynamic interaction effects. Next, the tool checked if the determined best functions to describe the datasets fulfilled predefined minimum statistical requirements and sorted out those not fulfilling these. Afterwards, the modeling tool visualizes the model results together with the extracted data.

In a next step, the modeling tool combined the identified functions with each other in an additive approach to construct the network of cumulative effects. This network consists of any influences and the corresponding effects, which are relevant in the habitat and is based on the available literature data available for these relationships. As a practical test of the method, we analyzed the effects of anthropogenic pressures on seagrass meadows.

For the analysis of spatial cumulative effects of various anthropogenic pressures and human activities in a larger geographic area, we developed a method inspired by Halpern et al. (2008) and HELCOM (2018). For a practical test of the proposed method, the selection of the type of data followed the selection of data used for the HELCOM report on the Baltic Sea (HELCOM 2018) as good as possible. However, we tested the method with data from the North Sea to evaluate if a similar method as applied for the Baltic Sea could be also applied for the North Sea region.

Generally, in the method described by Halpern et al. (2008), the magnitude of the pressure effect on ecosystem components is expressed as an impact score summed up for a defined geographical area (a raster cell). This way a map is created, which provides an overview of the spatial distribution of the impact situation for the area in question. Thereby, the intensity of a certain anthropogenic pressure or human activity as well as the abundance data of ecosystem components are taken into account.

In contrast to the method applied for HELCOM and the method presented by Halpern et al. (2008), we did not apply sensitivity scores with regard to different ecosystem components and did not consider their spatial distribution. Instead, we used threshold values for stressors of the pressure topics ‘eutrophication’ and ‘hazardous substances’, which are used by OSPAR and which should reflect the sensitivity of the marine environment to these stressors in general. First, we interpolated data from monitoring stations with regard to stressor intensities spatially. This way we could produce impact maps covering the whole study area and then combine them with other spatial data. For the interpolation of hazardous substances, we used not only data of the concentrations of these but also sediment data for improving the spatial modeling, as the binding of many substances depends on sediment characteristics. For physical disturbances and physical losses due to human activities and anthropogenic pressures, we calculated the percent of the spatial area affected. If corresponding data were available, we also considered the intensity of a pressure. With regard to HELCOM, we applied weighting factors for different kinds of physical disturbances to reflect the relevance of these in comparison to each other. Finally, we produced a map showing the overall pressure situation. Further, we created maps showing the impact due to certain pressure topics. The available data as well as the conditions in the North Sea differ from the Baltic Sea. Therefore, partly we needed to apply other data and to simplify the method. It is possible to view the results of this analysis and to further analyze them in one of the geographical modules of LiACAT ('MapExplorer').

3.2.3 Assessment Tool

Different tools for the analysis of cumulative effects as well as visualization tools are accessible through the module 'assessment toolkit' in LiACAT. One important part of this module is the filter, which one can use to select topics, single elements and relationships, species, the time period, and the geographical area the analysis should be based on. This way even a huge amount of literature data is manageable. Special directly integrated tools in the assessment toolkit are visualization tools for literature data as well as the ACIM-program, and a matrix analysis for the evaluation of interaction effects. Moreover, links for further tools such as a special assessment matrix based on tolerance values and for the cumulative DEB model for the analysis of cumulative effects of anthropogenic pressures on a species are prepared. In the assessment tool, it is possible to integrate further programs for cumulative analyses. The visualization tools in the assessment toolkit comprise two different kinds of Sankey diagrams, which show the network of relationships between influences of pressures and effects. These diagrams further show how many literature data are extractable for a certain relationship.

Further, the assessment toolkit is linked to the geographical module 'mapExplorer' for the visualization of spatial data. The user can also apply the 'mapExplorer' to show the spatial distribution of a cumulative index and to calculate how the overall anthropogenic pressure situation is in an area of interest.

3.2.4 Calculation of a cumulative index value

This matrix analysis evaluates the cumulative interaction effects on one ecosystem component. After a selection of topics for the analyses, LiACAT creates the structure of the matrix automatically based on the different elements of the relationships belonging to the selected topics. Further, information previously entered in the 'RelationshipsEditor' is accessible from the matrix. When clicking into single cells of the matrix LiACAT provides the corresponding literature data and the source with a link to the literature. Further, it is possible to calculate a value for each matrix cell based on defined intensities of the influences of the stressors. For this, the user needs to provide tolerance values (or transition thresholds) and the optimum values of the ecosystem component with regard to the stressors as well as an interaction factor. Based on these values a normalized weighted interaction value is calculated. The user can use data for the intensities of the stressors derived from monitoring data saved in LiACAT, apply mean values of a certain area or test a scenario of interest. The interaction factors represents the relative influence of one stressor on the effect of another stressor.

The single weighted interaction values are summed up per stressor or element being influenced. Finally, LiACAT calculates the sum of these partial results to calculate the overall cumulative index. This overall cumulative index value indicates for a given pressure scenario if the cumulative effect of the particular combination and the intensities of the stressors rather lead to a synergistic or to an antagonistic overall effect.

In a further extension of this module, the GIS-matrix module, instead of manual inputs for the intensities of the influencing elements, spatial data – e.g. from monitoring programs – are applicable as input data. The user first needs to enter these spatial data in LiACAT. Then, one needs to define the time period and the geographic area. Further, the user needs to specify the desired size for the raster cells, the preferred interpolation method for the spatial representation of the data and the type of the cumulative model suitable for the application. As a result, a map shows in which areas cumulative interaction effects lead to higher and where to lower index values due to the combination of the pressures and influences and due to the combinations of the intensities of the different influences. This result should always be interpreted together with assessments of single pressures and ecosystem components, as the cumulative overall index provides in this form only an additional information about the cumulative effects known so far and is not a standalone assessment. It is also possible to use it as complementary information to assessments based on methods with regard to the method applied in Halpern et al. (2008) because these methods do not comprise interaction effects.

3.2.5 Visualization tools

Results of such analyses as well as monitoring data are visualized with the module 'MapExplorer'. In this module, some spatial analyses such as area calculations and geometric measurements are conducted as well. Moreover, it is possible to integrate monitoring stations in these maps to give an impression of the uncertainty with regard to the spatial interpolation.

Based on the literature information of the relationships the user can create 'Sankey diagrams' showing the network of relationships of a certain scene. The visualization reflects the cause-effect chain derived from the data entered in the 'RelationshipsEditor'. The user can choose between a simple form of visualization and a visualization, in which single elements of the relationship network are summarized to larger topics. When choosing the second method for the visualization, it is possible to access all information of the single relationships and it is possible to zoom into the diagram and expand the single relationships of one topic to get a higher degree of detail. This way, transparency is provided also in the visualization tools.

3.3 Literature

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4 Analysis of cumulative effects caused by anthropogenic pressures – applying cross impact matrix analysis and a DEB model

4.1 Abstract

Human activities affect marine environmental conditions in multiple ways, thus creating a number of potential stressors for specific ecosystem components. This implies that ecosystem components are exposed to a large number of stressors. To estimate the overall effect of these, it may not be sufficient to analyze the effects of the single stressors one by one but require also an analysis of cumulative interaction effects.

The objective of the study was to develop a model for cumulative effects assessment based on literature data that can be applied to the Marine Strategy Framework Directive (MSFD).

I propose a modular approach combining a model for intensity-response relationships of single stressors with consideration of exposure time, acclimation response and time delay of the onset of an observed effect, a cross impact matrix to model interaction effects between different stressors, and a dynamic energy budget model to simulate interactions with the organism during its life cycle. I applied the approach to model the impact of heavy metal concentrations, acidification, oxygen depletion and temperature increase on the blue mussel (*Mytilus edulis*).

A literature research showed that most of the interactions between anthropogenic stressors for this topic were synergistic (33.3%). However, also antagonistic effects (19.0%) and complex interactions between stressors with both antagonistic and synergistic relationships were reported (16.7%).

Applied to data from a monitoring station at Norderney, the stressor model predicts a decrease in reserve biomass, delayed maturity, impaired reproduction, and decreased growth in *M. edulis* compared to a stress-free control scenario. Reproduction was affected most severely: less gametes were produced and fewer spawning events occurred in the stress scenario. Compared to a model assuming only additive effects, the inclusion of interaction effects led to higher overall impact strength. Moreover, the difference between the additive model and the complete interaction model increased with increasing stress intensities. With regard to the evaluation of the ecological status of an ecosystem component, the study revealed that an analysis of cumulative effects is essential to get a comprehensive and more realistic picture of the potential anthropogenic impact.

4.2 Introduction

Blue mussels are exposed to various anthropogenic pressures, as they live in coastal habitats where many of pressures accumulate spatially, see, e.g., Halpern et al. 2008, HELCOM 2018, Andersen et al. 2013. Multiple anthropogenic stressors in the marine environment interact in a complex way and evoke special impact and response patterns for marine organisms and community structures, which cannot be explained by additive effects alone (Crain et al. 2008, Hooper et al. 2012, Holmstrup et al. 2010, Moe et al. 2013). Thus, cumulative effects may modify the impact of human pressures on the environment. To enhance our ability to predict the consequences of human impact, it is crucial to gain an understanding of such cumulative effects.

Accordingly, the Marine Strategy Framework Directive (European Commission 2008) requires the analysis of such cumulative effects. However, there is still no common understanding of the term „cumulative“ and no common agreement on the assessment of cumulative effects has been achieved yet. In order to give an overview of the various aspects related to this term, the general introduction of the thesis provides a detailed description and a proposal for a definition. This proposal is based on definitions from scientific publications, on compilations by an OSPAR working group focusing on cumulative effects assessment (ICG-C) (Judd et al. 2015), and a publication by the European Commission with regard to environmental assessments of cumulative effects (European Commission 1999). For the analysis and assessment of cumulative effects with regard to the implementation of international directives and for regional assessments, the most commonly used methods calculate indices and are based on various approaches including:

- scoring systems (Halpern et al. 2012),
- expert judgements (MacDonald et al. 1996, Bernem et al. 2000, Coll et al. 2012, Halpern et al. 2008),
- threshold approaches (Dickert and Tuttle 1985, WFD 2000, Lepper 2005), and
- geographical analyses (Halpern et al. 2008, Coll et al. 2012, HELCOM 2018)

Moreover, specialized models have been developed for assessing cumulative effects with respect to pressures or ecosystem components, for example for non-indigenous species (Leidenberger et al. 2015), food webs (Chaalali et al. 2015), hydrodynamics and pollution (Zalesny et al. 2014), rare fish species (Zhou et al. 2012), radioactivity (Batlle et al. 2008), as well as mammals and sound (Siderius and Porter 2006). However, only very few approaches consider the mechanisms leading to specific combination effects (but see Lokke et al. 2013, Segner et al. 2014, Cosme et al. 2015). Even though research of the effects of multiple stressors has gained increasing attention and scientific observations revealed new relevant insights (Crain et al. 2008, Holmstrup et al. 2010, Moe et al. 2013, Hooper et al. 2012), this knowledge has not been integrated into practical assessments sufficiently. To conduct more comprehensive cumulative analyses based on experimental and field data rather than on expert judgements, the development of holistic concepts and models as well as the parallel development of corresponding software tools is crucial.

The main objective of this study was to develop a model for the assessment of cumulative effects of anthropogenic pressures on a species level. The model should be based only on literature data instead of the application of expert judgements to assess the magnitude of anthropogenic impacts. The approach should also allow to integrate new available scientific data continuously. Further, new scientific findings and models with traditionally applied methods in environmental assessments should be harmonized with existing monitoring programs and corresponding data.

4.3 Methods

For this purpose, I combined and adapted a matrix analysis (Weimer-Jehle 2008) with a dynamic energy budget model (DEB) model (Koojiman 2010) and a model representing the response of the organism to single stressors. The matrix analysis was adapted to cope with the more general information on qualitative and quantitative aspects of interactions between stressors. The DEB model was refined to simulate temporal dynamic effects caused e.g. by varying environmental conditions during its life cycle and the model for single stressors analyzed and predicted interactions between the stressors and the organism. As a proof of concept, I applied the model for the assessment of the effects of heavy metals, acidification, oxygen depletion and temperature fluctuations on blue mussels (*Mytilus edulis*) and compared it to real data from a monitoring station at the east Frisian island Norderney. To get an overview about the state of knowledge of cumulative effects for this pressure combination as well as for the visualization of the literature information, an online tool (LiACAT) was used for literature analysis, which was particularly designed for this purpose (https://kladia.info/klados/, Eilers et al. 2017, HELCOM 2016).

4.3.1 Literature review and literature database

For the literature review, I used the search engines „Web of Science", „Aquatic Sciences and Fisheries Abstracts" and „Google Scholar". Search terms were „multiple stressor*", „cumulative*", a search term of the pressure of interest (here: Zn OR zinc or Cd OR Cadmium, Cu Or copper or Pb OR lead or pH OR acidi*, temp*" for the title) and a search term for the species of interest (*Mytilus* OR "blue mussel*" as a topic). Additionally, I screened important reviews and critical papers for relevant literature references therein. I included only literature data dealing with effects on *Mytilus edulis*, fulfilling the quality standards described in Supplement 1. Further, only studies evaluating the effects of single stressors and their combinations against a stressor-free control were applied for the analysis. Preferably, I applied data from experiments, where the experimental conditions resembled the environmental conditions near Norderney.

To organized and visualize literature data, I used the portal software LiACAT („Literature Analysis and Cumulative effects Assessment Tool"), an online tool located at the biodiversity online portal mybiOSis[12]. This tool facilitates the organization, extraction, visualization and provides some tools for the analysis of literature information with regard to cumulative effects (Eilers et al. 2017). However, the model focusing on the analysis of cumulative on species level is not implemented yet.

As most literature about cumulative effects of anthropogenic pressures on blue mussels comprised information on interactions between the heavy metals Pb, Cd, Cu Zn and, pH, temperature, oxygen concentration and salinity, the focus of this study lies on these stressors and environmental variables to test the methodological approach.

4.3.2 Cross impact matrix

Cross-impact matrices originate from game theory and were applied first in economics and social sciences (Weimer-Jehle 2008, Gordon and Hayward 1968). I modified a special version of a cross-impact matrix (Weimer-Jehle 2008) to calculate interaction effects. The method facilitates the analysis of a complex network of interactions in a simple and clear way by focusing on binary relationships. Information of these binary relationships is entered in a table: The influencing variables are listed in rows and the variables being influenced are organized in columns (Gordon and Hayward 1968). Gordon and Haward (1968) focused on the probabilities of events and innovations in the context of forecasting developments. Weimer-Jehle (2008)

[12] https://kladia.info/docs/index.php?title=project_details&projectid=2

further developed the method for the application in social science to analyze the process of opinion building. He differentiated between positive, negative and neutral opinions, and analyzed all possible interactions between each of these subdivisions. Moreover, he calculated a balance indicating how the opinion of a single person is influenced by all the other opinions, taking into account the influences of the others by summing them up for the row corresponding to this person. I adapted the method for the interactions between anthropogenic pressures affecting ecosystem components using the categories „increase“, „decrease“ and „complex relationship“ analogous to positive, negative and neutral opinions of the model by Weimer-Jehle 2008 to describe how the stressors interact with each other. Furthermore, the inputs for the interaction values were not derived by questionnaires but from literature. Whenever possible, the relationship between stressor intensity of the influencing stressor and observed effect was expressed in an equation.

Further, I considered whether the observed effect was an adverse or a positive one. For example, growth as a variable for an observation is a positive effect whereas the observation of mortality is an adverse effect. Before calculating the interaction factor, I ensured that deteriorated conditions always were associated with the same sign, independent of whether the adverse effect was measured by an increase or decrease in the response variable. The interaction factor was calculated with the following formula:

$$\boldsymbol{Intfact} = (\boldsymbol{CombEff} - \boldsymbol{SinglEff})/\boldsymbol{SinglEff} \quad (1)$$

where Intfact represents the interaction factor of the influencing stressor on the effect of the other stressor, combEff is the value of the observed effect when both stressors are present, and SinglEff is the value of the observed effect when only the stressor of interest (influenced stressor) was present.

Based on the assumption that the direction of the influence matters, I considered for example not only the influence of Cd on the effect of Cu but also the influence of Cu on a Cd effect. The reason for this procedure lies in the possible element-specific effects on the molecular level. Many interactions occur during the uptake process (e.g. Elliot et al. 1986), where direct interaction effects may be very relevant. However, the uptake routes can differ between metals and trace metals are found in different parts of the body (Soto et al. 1996), indicating that interactions between metals might at least partly also occur indirectly. Further, metallothioneins bind different metals to a different degree depending on its chemical characteristics (Voets et al. 2009). Thus, the production of metallothioneins might be triggered by one metal (Viarengo et al. 1981) and can be seen as an influence of one metal on the other. Consequently, also the influence on a process (here triggering the metallothionein productions) might be element specific. This supports the hypothesis of the relevance of directivity. Thus, influences of both directions were considered in the matrix and the row sums of all influences on one stressor added up to calculate an overall cumulative effect with regard to this specific stressor.

However, to my best knowledge, there is no experimental study, which specifically tested directivity of cumulative effects. Therefore, I tested an alternative approach assuming that the direction of the influence does not matter and observed differences are random. Based on this assumption, I divided the interaction factors by two, when interactions occurred bidirectional (see Figure 71 - Figure 78).

The first approach - assuming directivity - should best describe the reality when the interactions have a different mode of action, whereas the second approach would be more adequate when the mode of actions would be the same or be caused by direct interactions between both stressors. However, a stressor has likely several modes of action and some of them may be equal to other stressors, whereas others are rather unique. Currently, the percentage a certain mode of action occurs is still unclear. This might even be dependent on the intensity and differ between different species e.g. due to differences in the chemical composition of the body.

To capture the influence of intensity, I calculated an interaction factor for each of the tested intensities of the stressor, if data were available for different intensities of the influencing stressor. Based on these data, a model was derived to describe the relationship between the interaction factor and the intensity of the influencing stressor. I tested linear as well as non-linear models for a good fit with the data and for a plausible description of the relationship. This equation was completed by a term for the multiplication of the effect level of the influenced stressor and this final equation served as input for the matrix (see equation (**1**) and 7.1). This implies the assumption of a linear relationship between the effect level and the interaction factor. If, for instance, only a few molecules of cadmium enter the body of an organism, the chance to interact with another heavy metal is low, whereas the chance is higher, the more cadmium molecules are taken up.

If a publication did not provide sufficient data to derive an equation to describe the relationship between the intensity of the influencing stressor and the interaction, I assumed that the interaction factor between the two stressors is fixed and that the stress intensity of the influencing variable did not matter. Even though this assumption might not be true in reality, I made this assumption due to the lack of information as a preliminary solution until better data are available. The interaction factor was calculated as described above based on the data for the combined effect and the effect caused by the influenced stressor alone (7.1.2)(3) for the single value of the tested concentrations. As reasoned above, I assumed also here a linear relationship between the influenced stressor and the interaction factor. Therefore, following the same procedure, the interaction factor was multiplied by the effect level of the influenced stressor.

In some cases, different stressor intensities of the influencing stressor and even different stressor intensities of the influenced stressor were available but it was not possible to derive a clear pattern for a relationship between the intensities and the interaction due to the sparse data set. In these cases, I used those data sets of experiments which represented the conditions of the test-scenario as good as possible, i.e. a comparable stressor intensity or a comparable ratio of stressor intensities to each other. This procedure resulted also in a single value for the interaction factor and was also complemented by a multiplicative term. This way the matrix was filled in and further calculations could be done.

The row sums of the matrix represented the „net-effects“ of the corresponding stressors under consideration of all interaction effects of the other influencing stressors and served as input values in a DEB model.

$$\boldsymbol{NetEffStr} = \boldsymbol{Str} + \sum_{\boldsymbol{i=1}}^{\boldsymbol{i}} (\boldsymbol{Intfact_i} * \boldsymbol{Str}) \qquad (2)$$

where NetEffStr reflects the effect of one influenced stressor under consideration of all influences of other present stressors, Str is the effect of this stressor alone without any influence, i are the other stressors present, and Intfact are the interaction factors of the other stressors, representing their influence on this stressor.

In most cases, publications did not reveal if the interaction between stressors occurred outside the organism in the water body or inside the body of the organism. Therefore, I could not differentiate between these cases as well. Based on the reviewed literature, it is likely that interactions occurred during the uptake process as well as due to interactions on molecular level within the organism. I assumed that it does not matter for the outcome of the model where the interaction took place and aligned the interactions with processes happening within the body of the mussel.

To realize this, I first calculated the biological relevance of the intensity of the influenced stressor for the effect on the organism in a special module of the DEB model (single-stressor-model, see section below). Subsequently, I incorporated the interaction effects: the result of the balance calculated with the matrix for the corresponding stressor determined if the overall effect of one stressor increased or decreased a physiological process in the organism.

In contrast to the DEB-model approach of Bedaux and Kooijman (1994), I did not calculate an interaction factor based on lab experiments with DEB modeling but derived the information about the interaction from literature data instead. By applying the matrix method described above, it was also possible to integrate the directionality of influences and complex interactions (of non-linear nature), which were not included in their approach.

4.3.3 Application of DEB models for the analysis of cumulative temporal effects of anthropogenic pressures and environmental factors

A detailed description of DEB models can be found elsewhere (Kooijman 2010). Briefly, DEB models are based on the Dynamic Energy Budget theory, which delineates the metabolic organization of organisms and allows quantifying, and foreseeing physiological effects as well as endpoints in time such as reproduction, growth and death (Kooijman 2010). The standard DEB model assumes that food intake is dependent on surface area or body volume and that uptake rate of food is dependent on food density (Kooijman and Metz 1984, Kooijman 2010). The energy gained from food is divided into a fixed fraction, soma κ, which is used for somatic maintenance, somatic work, and growth and the remaining energy (1- κ), which is mobilized for reproduction and maturity maintenance (or maturation in case of juveniles) (Kooijman 2010). The model consists of a system of differential equations describing the relevant physiological processes of the organism during its life span.

Environmental stress and anthropogenic pressures influence metabolic performance and can be integrated in DEB modeling (Kooijman and Bedaux 1996, Baas et al. 2007, Jager et al. 2010). Baas et al. 2007 developed a method for deriving information about interaction effects between binary mixtures of metals by applying a DEB model combined with the model of concentration addition and independent action for the analysis of survival data of experiments. Jager et al. 2010 analyzed sublethal effects of more complex toxic mixtures by applying a DEB model for experimental data. They considered different modes of action for each of the compounds. In this way, they applied a biology-based approach. However, the authors did not consider cumulative interactions between the substances. Instead, they focused on differentiating between substances affecting a process through the same targets and substances affecting it through independent targets (Jager et al. 2010). Lastly, they fitted the model to the experimental data for deriving interaction factors.

In contrast, I applied a DEB model to predict the effects of a combination of stressors with different intensities based on experimental results derived from literature data without knowing the actual impact the scenario has on the ecosystem component. The core model applied is based on a DEB model specification for *Mytilus edulis,* validated with data from the North Sea (Saraiva et al. 2012). I used the parameters and basic equations of this publication, adapted the model to the type of data I used as input, and integrated the results of the matrix analysis to account for the influence of anthropogenic pressures. The further developed model for cumulative effects of the present study incorporates a biology-based approach by assigning the intensity of the stressors to the affected targets, similar to the approach described by Jager et al. (2010).

The effects caused by anthropogenic pressures are additional factors to the equations of the DEB model describing physiological processes and the life cycle of the organism. Thus, the affected processes increased or decreased resulting in an altered behavior of the model due to the direct links as well as due to the connections between the differential equations representing different processes. Depending on the stress intensity, this can lead e.g. to decreased growth or to a disturbance in the development of maturity.

Already existing scripts of different DEB models inspired me in the programming (software packages developed by Kooijman and others[13]) and I wrote the scripts for modeling cumulative effects on blue mussels in Matlab (https://de.mathworks.com). In general, the meta-model consists of several sub-scripts (Figure 5). For each stressor, I created one file to analyze its effects and the change of the effect depending on exposure time and concentration based on a theoretical model. Literature data served for parameter estimation of this model simulating temporal dynamic effects. Compared to the original model (Kooijman and Bedaux 1996), I put special emphasis on possible temporal delays of the onset of effects, but also calculated parameters to characterize acclimation and „normalized stress intensity“.

This „normalized stress intensity“ depends on the intensity of the stressor, the exposure time and species-specific characteristics such as uptake- and elimination rates and tolerance values.

These data serve as input data for the core DEB model reflecting the life history of an organism in the main program (Saraiva et al. 2012). Here, additionally, interaction effects between the stressors and *Mytilus edulis* are considered.

[13] http://www.debtox.info

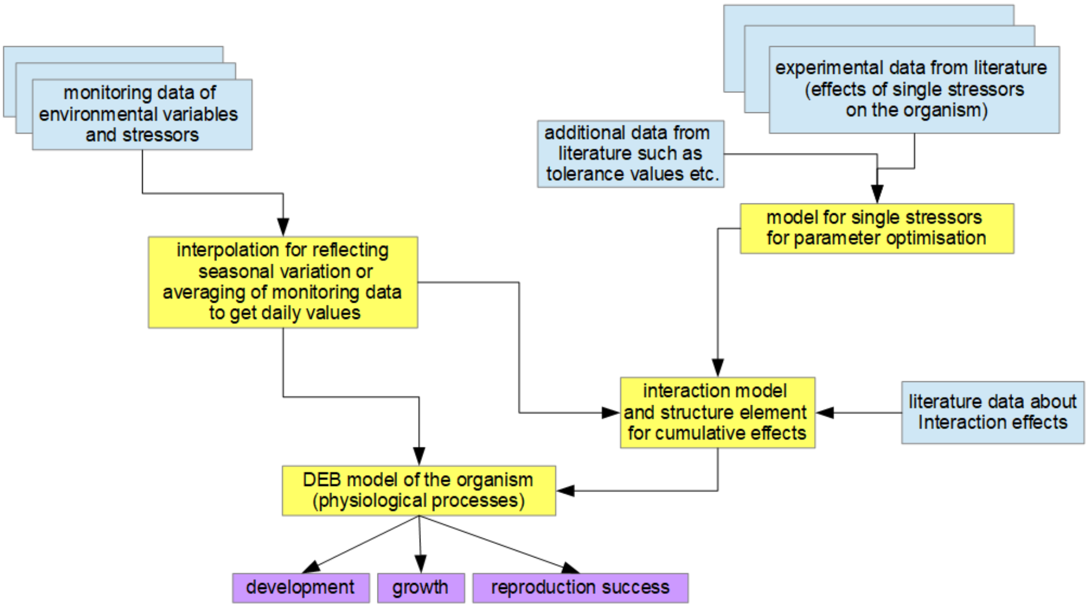

Figure 5 Structure of the DEB model for analyzing cumulative effects on Mytilus edulis. In the red boxes are the parts, which are additionally used for analyzing the effects of anthropogenic pressures. The red arrow marks a connection specific for this model. The other parts are used for the reference model without anthropogenic pressures and Temperature extremes.

Analyses of the effects of single stressors

For each stressor, I modeled the interaction of the stressor with the organism separately before the integration of interaction effects. To set up the model, I considered the pattern of dose-response curves (e.g. Baas et al. 2009) and patterns of defense mechanisms, such as production of metallothioneins (Han Zhao-Xiang et al. 2013). Interactions between the stressor and the organism could occur between the aspects of effect strength, time delay, acclimation, and species-specific tolerance limits. The aim was to model the change of an effect over time and to optimize the parameter values assigned to these aspects based on literature data from experiments. The basic idea for the model was derived from Kooijman and Bedaux (1996) and adapted to the aspects described above.

For the module simulating the change of the effect of a stressor on the organism over time, the following theoretical assumptions were made:

- The effect of a stressor depends on the stressor intensity as well as on the exposure time.
- The response of an organism to a stressor can change over time.
- These processes can be dependent on each other.
- The pattern of the change of an effect over time is universal and can thus be transferred from one target to another, e.g. the pattern of the effect of a stressor on mortality data was transferred to a physiological process such as respiration by applying the same time-dependent percentage of change of the process.

A normalization procedure of the intensities of the stressors based on the „transition threshold“ of the organism made the data comparable and prevented bias, caused by the application of different scales and ranges in the dataset. The transition threshold was defined as the onset of an altered response due to an external forcing. Tolerance and optimum values of the model species derived from literature. In practice, I used the lowest value of stressor intensity where an adverse effect was observed being aware that responses at lower stressor intensities cannot be excluded. For example, a transition threshold could be the upper limit of tolerance as described in Pörtner 2010. The values for the parameters are fixed in the model and were not optimized. The normalized stress intensity was calculated as follows:

$$s = |(env - opt)|/|(tol - opt)| \tag{3}$$

where s is the normalized stress intensity, env is the intensity of the stressor in the environment, tol is the transition threshold and opt is the optimum value. A value greater than 1 indicates that the tolerance limit of the species is exceeded for the environmental data set applied (Figure 6). The smaller the value is the more beneficial are the environmental conditions for the species of interest.

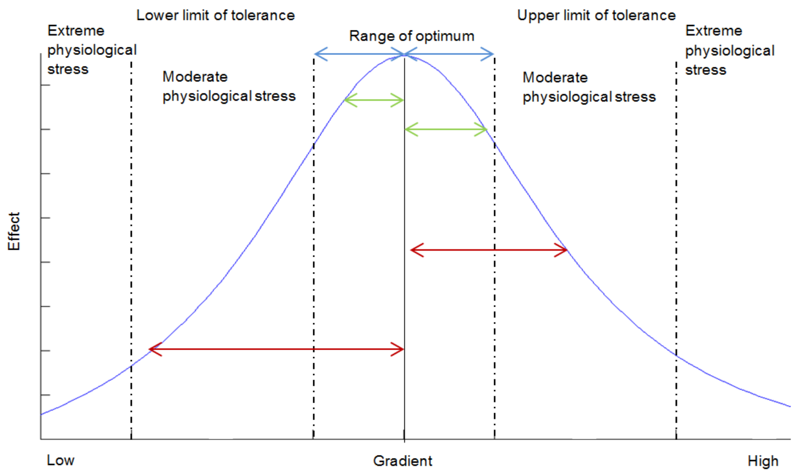

Figure 6 Example for a tolerance curve for an environmental stressor. Red arrows indicate situations, where the environmental stressor exceeds the transition threshold; green arrows indicate examples where environmental data are within the tolerable range. Figure modified after Pörtner (2010), based on Shelford"s law of tolerance (Shelford 1931). Note that the shape of the curve does not need to be symmetrical and only represents an example

Based on the framework of DEB theory (Kooijman 2010), I considered the uptake and elimination rate as well as growth dilution for the estimation of the internal concentration in the organism. For other stressors such as acidification I deleted these functions in the corresponding scripts and replaced the term for concentration by the direct intensity of the stressor.

The stress effect, depending on the stressor intensity, is described by the function

$$\mathbf{e}_{stress} = ß * \mathbf{s} * (\mathbf{1} - \mathbf{a}) \tag{4}$$

where ß is the „effect rate" with the dimension 1/stressor intensity * time and s is the stress intensity (see above). The variable „a" describes the acclimation of the organism to the altered environmental conditions and can reach values between zero and one. The stress effect does not necessarily represent the observed reduction of a response variable because further aspects such as a possible time delay also influence the effect (see equation (5) and (6)).

The dynamics of the acclimation „a" depends on the acclimation rate „alpha", which is relative to the effect „e" and is dimensionless:

$$\frac{\mathbf{da}}{\mathbf{dt}} = \propto * \, \mathbf{e} * (\mathbf{1} - \mathbf{a}) \tag{5}$$

In case of a time delay, there is a difference between the expected onset and the development of the effect. Thus, the change of the effect depending on exposure time is expressed as:

$$\frac{\boldsymbol{de}}{\boldsymbol{dt}} = \boldsymbol{\gamma} * (\boldsymbol{e_{stress}} - \boldsymbol{e}) \tag{6}$$

where γ is 1/ time delay (the higher the value for γ the smaller the time delay) and e_{stress} reflects the expected effect without time-delay (see equation (4)). If no time delay occurs, e_{stress} equals e and de/dt equals zero.

The simulated alteration of the response and thus the response itself is recalculated as follows:

$$\frac{\mathbf{dR}}{\mathbf{dt}} = -\mathbf{e} * \mathbf{R} \tag{7}$$

where e here represents the effect as described in equation (6), and R is the value for the observation (response variable).

The parameters „∝", „γ „ and „ß" of the model were determined by an optimization process. To achieve this, I applied the Matlab function „fmincon" to minimize the difference between experimental data from literature sources and the model data (see list of applied literature data in 7.1). To test the performance of the model with the derived parameter values, the relative error and the relative standard deviation were calculated. The model calculated the internal concentration, the acclimation, the effect, and simulated the response variable. The script visualized the results in graphs.

The optimized parameter values (alpha, beta and gamma) shaped then the modeling of the study period of the test scenario: For each stressor I applied monitoring data representing intensities of the stressor in the water column serving as input data for the model of single stressor effects and integrated the results into the DEB core model. Further, I aligned the effect „e" to the physiological processes affected by the corresponding stressor in the core model (see 7.1).

Monitoring data and core model

The DEB model specification for the blue mussel *Mytilus edulis* by Saraiva et al. (2012) is driven by environmental parameters such as the availability of food and the fluctuations in temperature. This model is a stand-alone model of a control scenario. In a further step, I combined the model with the matrix analysis and the stressors of interest resulting in an additional module to simulate a stress scenario. Third, the model simulated the impact on *Mytilus edulis* without any interaction effects to represent a scenario assuming that no interaction effects occurred applying a purely additive approach. In contrast to Saraiva et al. (2012), in all of these three core DEB models (control scenario, stress-scenario, additive scenario), I used phytoplankton monitoring data instead of Chl-a data were used and the corresponding calculated carbon content based on the species-specific biovolume (Hillebrand et al. 1999). I interpolated the weekly measurements of phytoplankton data and the corresponding carbon contents, which showed seasonal fluctuations. Additional carbon sources or sources of nitrogen and phosphorous were not considered.

Due to these changes, the DEB-parameter „algal binding probability“ needed to be adjusted to generate realistic outputs for the control scenario (full list of parameter values in 7.1, Table 14Table 14 DEB-parameter values used for the main DEB-model). I chose this value manually and did not perform a modeling procedure because corresponding data to run an optimization process were not available.

To test the cumulative impact of anthropogenic pressures on *Mytilus edulis* under realistic conditions, I used data from a monitoring station at Norderney as input variables. The stressors cadmium, copper, lead, zinc, acidification, elevated temperature, and oxygen depletion represented some of the most relevant stressors for the blue mussel and therefore I focused on these. Furthermore, suitable literature data for these stressors had been available for the model.

The salinity conditions reported in the literature data about interactions with other stressors resembled those of the monitoring station. Therefore, these response data did not have to be corrected for unfavorable salinity conditions. Further, the literature search revealed that the salinity conditions at the monitoring station were suitable for *Mytilus edulis*. Therefore, salinity stress was not included in the model for the Norderney data. However, in the results section the theoretical interactions observed are shown.

Data for the metal concentrations, pH values and oxygen concentrations originated from the regular monitoring program of a sampling station at Norderney (data provided by NLWKN). Data used for daily water temperatures are results from the HAMSOM model (Pohlmann 1996).

The data values from the monitoring program for heavy metals and pH were interpolated to daily data in Matlab with the piecewise cubic hermite interpolating polynomial method (Figure 54 and Figure 55). Oxygen concentrations were measured with a higher frequency between 1999 and 2002 than between the years 2005 and 2010 in the monitoring. Therefore, I decided to analyze the available data from the years 1999 to 2002 as well to get an impression of the seasonal pattern of the oxygen concentration at the monitoring station. However, the winter 1999/2000 was characterized by unusual storms and a historic flooding. Therefore, these data were excluded from the modeling and only data from June 2000 to December 2002 were used to generate the typical seasonal pattern of the oxygen concentrations. To derive seasonal dependent daily oxygen concentrations, a model was determined with the curve fitting tool of Matlab. Inspired by the work by Fidino and Magle 2017, I selected a sinus curve with a fixed seasonal cycle to describe the data for these years. In a second step the same model type was applied to model the data for the years 2005-2010 (study period). Thereby, the parameter for a shift along the x-axis, which defines the peak of the sinus curve was derived from the model for June 2000 to Dec 2002. Further, the optional start points were also derived from this previous model run. As there are less data between 2005 and 2010, uncertainty remains high for the oxygen concentrations, in particular for the summer months (see Figure 53 and Figure 54). However, it is likely that the seasonal pattern was better reflected by applying this method than a simple interpolation between the measured data points. The data for temperature and pH showed a seasonal pattern, too (see Figure 55 and Figure 56). However, for pH values, more data were available and thus I conducted an interpolation as for the heavy metals.

Metal concentrations were only measured twice a year in February and November for the monitoring program. Even though seasonal patterns or seasonal differences of metal concentrations for Zn and Cd were observed in some studies (e.g. Scholten et al. 1998, Loewe et al. 2013, Baeynes et al. 1998, Burton et al. 1993, Kremling and Pohl 1989, Lourino-Cabana et al. 2014, Zwolwsman et al. 1997), these data refer to lower concentrations, mostly measured in the open sea. The observed seasonal variations might be driven by phytoplankton uptake (Burton et al. 1993, Dixon et al. 2006). However, the uptake is species-dependent and thus highly depends on the species composition (Rick and Dürselen 1995). In coastal areas, where blue mussel habitats occur, metal concentrations are typically higher and vary more due to the land-based inputs (Loewe et al. 2013). The fluctuations of metal concentrations between years at the station at Norderney were so high during the study period (Zn concentration e.g. 3.8-17 µg/L), that a reasonable seasonal pattern, which was observed in the range of approximately 2 µg/L for coastal areas (Loewe et al. 2013) could not be modeled for this monitoring station realistically. The reason might be that the monitoring station at Norderney is situated closer to land than the stations analyzed by Loewe et al. (2013) and that the harbor in the nearer surroundings had an influence.

For the metals Cu and Pb, no clear seasonal patterns could be established as either such effects were not observed in previous studies in the North Sea or results from different studies were contradictory (Lourino-Cabana et al. 2014, Baeynes et al. 1998, Zwolwsman et al. 1997, Kremling and Pohl 1989). To capture the variation between years, in a first scenario I interpolated the metal concentrations with the piecewise cubic hermite interpolating polynomial method acknowledging that we do not know anything about the concentrations between the measurement events. Further, to compare this stress scenario with a continuous pressure situation, I calculated the mean of the metal concentrations throughout the years.

For the control-scenario, I used input values for temperature from the HAMSOM model, but eliminated temperature values above the transition threshold of 25°C (Zittier et al. 2015) and replaced them by this threshold value to avoid critical temperature values with adverse effects on the organism in this scenario.

All data refer to a station west of Norderney (Nney_W_1: latitude: 53° 42' 5,975'', longitude 53° 42' 5,975'') and cover the time span of 28^{th} of April 2005- 11^{th} of August 2010.

Integration of the interaction effects

The cumulative interaction effect for each stressor was calculated using the principles of the matrix method. As input data for the matrix, equations as well as interaction values derived from literature were used (see Table 17). The effect level, which is then aligned to a process, is calculated as follows:

$$\boldsymbol{effect\ level} = \boldsymbol{eStressor} + \sum_{\boldsymbol{i=1}}^{\boldsymbol{n}} (\boldsymbol{eStressor_i} * \boldsymbol{Interact_i}) \tag{8}$$

where eStressor is the effect dependent on several factors such as acclimation. For each time step, a value for eStressor was calculated depending on exposure time and further variables, based on the literature data with the equations described above with the single stressor model („e"). The term „interact" represents the balance calculated with the matrix method (see method section 4.3). If sufficient data were available, I estimated the balance of the interaction effect also for each time-step, depending on the intensities of the environmental variables and stressors.

The interaction was temporally dynamic because daily stressor intensities and corresponding values for „e“ and „interact“ were used: for each time step, a value for eStressor was calculated depending on exposure time and further variables, based on the literature data with the equations described above, and served as input variable for the interaction matrix. The interaction indices for each stressor were linked to stressors acting on the same target by addition. The cumulative effect of different stressors acting on the same process were then integrated in an equation describing the corresponding process: the sum of the effects of the stressors, reflecting a „net - increase or -decrease“ of this physiological process, was assigned as a factor to the process.

The output of the model provides information on the theoretically predicted values for processes relevant for population size such as altered growth and reproduction considering all relevant stressors and estimate how the organism is affected considering its life cycle (Kooijman 2010).

4.4 Results

Matrix

Overall, I searched for literature information on 84 theoretically possible interactions of the stressors, namely: the influence of pH, oxygen concentration, temperature, and salinity on the effect of each of them on *Mytilus edulis.* The influences were divided into the categories „increase“, „decrease“ and „any other alteration or overarching description of the influence“. If acidification for example led to decreased growth and increased temperature enhanced this effect, I marked the corresponding cell linking „decreased pH“ and „increased temperature“ with the label „synergistic“. For the stressors, which could be influenced, only the unfavorable direction for the organism was considered (decreased pH, decreased oxygen, decreased salinity and increased temperature). Too low temperatures or too high salinities can theoretically also represent stress for *Mytilus edulis*. However, at the monitoring station at Norderney, these were no relevant stressors during the study period and therefore I did not consider them here.

Synergistic effects were most abundant with 28 (33.3%) (marked as red in Table 3), 15 (19.0%) were antagonistic (blue) and 14 (16.7%) were complex, contradictory or unclear. „Complex“ means that the interaction can be antagonistic or synergistic dependent on the environmental conditions or the stressor intensities (violet color in Table 3). For 24 interactions, the interaction could be quantified. No information could be found for 39 theoretically possible interactions. The references for the interactions as well as some more information about the experiments are provided in together with justifications for the in- or exclusion of the data for the model. A description about the data treatment and the derivation of the interaction factor is provided in 7.1, Table 17.

For some interactions, I found divergent information in the literature. In this case, I used studies with experimental conditions close to the conditions measured at the monitoring station at Norderney from 2005 to 2010. All concentrations refer to concentrations in the water column, because the uptake of chemical substances was modeled in the DEB model based on the uptake from the water column (see below).

Table 3 Interactive effects of different stressors on blue mussels. Violet: synergistic and antagonistic both reported in literature and/ or complex relationship observed, red: synergistic interaction(s), blue: antagonistic interaction(s), grey: no interaction effect observed, white: no information, beige: filled in to either describe an effect comprising high and low values or to mark, that the interaction is characterized by the information about increased and decreased values. The plus-sign „+“ means an increase of the stressor, the minus-sign „ – „ means a decrease of the stressor. The line in the middle can mean any kind of alteration of the stressor such as increased fluctuation or gradual increase or decrease in a broad range of values below and above the optimum values of the species. For heavy metals only increases are shown as the optimum is assumed to be zero or relatively close to zero. The word „formula“ means here, that the interaction could be quantified and a formula could be applied to describe the influence.

	Cd (+)	Cu (+)	Pb (+)	Zn (+)	pH (-)	O2 (-)	Temp (+)	salinity (-)
Cd (+)		formula	formula	formula				
Cu (+)	formula			formula				
Pb (+)								
Zn (+)	formula	formula					formula	
+								
pH	formula	formula	formula	formula				
-								
+								
O2								
- / anoxia								
+		formula		formula				
Temp	formula		formula					
-								
+								
salinity								
-								

Cadmium (Cd)

The literature analysis revealed that the effect of Cd on blue mussels is influenced by increased concentrations of Cu, Pb, Zn, as well as by the environmental variables pH, oxygen concentration, temperature and salinity. Copper may act synergistic as well as antagonistic, depending on the concentrations of both metals (Cu and Cd): At a high concentration of Cd (20µg/L), Cu concentrations of 10 and 20 µg/L depressed the uptake of Cd in mussel tissue. In contrast, at lower concentrations of Cd of 10 µg/L, elevated Cu concentrations increased the uptake of Cd (Elliot et al. 1986). Therefore, the interaction was categorized as complex (violet). Because the concentration of copper at the monitoring station at Norderney was higher in comparison to the cadmium concentration (Figure 63), the interaction corresponding to a high Cu concentration and a lower Cd concentration of the publication was applied in the DEB model. To calculate the relative increase of the uptake due to the presence of copper, I calculated the difference between the cadmium concentration in the mussel without copper at a cadmium concentration of 10 µg/L in the water and the cadmium concentration in the mussel at the same cadmium concentration in the water and at a copper concentration of 20 µg/L in the water and then divided the difference by the cadmium concentration in the mussel without the presence of copper ($(Cdmussel_{\text{Cu and Cd comb}} - Cdmussel_{\text{Cd\_alone}})/ Cdmussel_{\text{Cd\_alone}})$. The sparse data did not allow for describing mathematically the dependency of the interaction on the copper and cadmium concentration. Therefore, I assumed that the uptake of cadmium is always elevated when copper is present at a higher concentration than cadmium. Moreover, I assumed that an increased uptake of cadmium can be equated to an increased Cd effect in the organism. The concentrations in the experiment of Elliot et al. (1986) were higher that concentrations measured at the monitoring station of Norderney (7.1 ,Figure 49). On the other hand, the exposure time in the experiment was only 10 days. I assumed that the interaction under natural conditions in the environment with lower concentrations in the water but longer exposure time is similar to the interaction observed during the experiment of Elliot et al.1986. As described in the methods section, the cadmium effect was later multiplied by the predicted increase of the effect due to copper and then added to the cadmium effect.

Pre-exposure to a polluted site with increased concentrations of Fe and Pb led to synergistic effects as shown by Sheir and Handy (2013). The authors observed an inhibited phagocytosis activity and increased the neural red uptake as a response of Cd exposure in mussels exposed to increased Fe and Pb concentrations compared to the reaction of mussels from a reference site exposed to Cd.

Synergistic as well as antagonistic influences of Zn on the effect of Cd were revealed by Elliot et al. (1986): like the influence of Cu on the effect of Cd, the nature of the interaction depended not only on the metal concentrations but also on the ratio of the concentrations. The zinc concentration was five to ten times higher than the cadmium concentration in the wastewater. At very high zinc concentrations of 200 µg/L and at a bit lower concentration of zinc of 100 µg/L and a cadmium concentration of 20 µg/L the effect of zinc was antagonistic, whereas at 100 µg/L zinc concentration and a cadmium concentration of 10 µg/L, the effect was synergistic. The concentration of zinc at the monitoring station of Norderney was much higher than the concentration of cadmium, but in general much lower than the concentrations tested in the experiment. Therefore, I calculated the increase of the effect with the data of 100 µg/L zinc and 10 µg/L cadmium as described for the influence of copper on the cadmium effect.

Vercauteren and Blust (1999) showed that the presence of Zn led to antagonistic effects for the uptake of Cd in soft tissues, gills and the digestive system, but synergistic effects for the uptake of Cd in the hemolymph. In contrast, only synergistic interaction effects between pH and Cd were reported under reduced pH conditions: George (1983) showed that the percentage of metals bound to metallothioneins and granules continuously increased with increased pH. Thus, mussels in water with higher pH were more effective in their defense mechanisms against the toxic effects of Cd than mussels in water with lower pH. A synergistic interaction was also shown by Han et al. 2013, who reported that decreased pH values exacerbate the effect of increased Cd as indicated by an increased mortality rate, increased uptake rates, decreased phagocytosis, increased percentage of eosinophilic hemocytes, and altered defensive response (metallothionein concentration).

Low oxygen concentrations in the water led to synergistic effects concerning the effect of Cd on the condition index (Fischer 1986). However, low oxygen conditions also led to antagonistic effects indicated by a decreased tissue concentration and a decreased Cd/ shell weight index (Fischer 1986). Increased temperatures led to synergistic effects for the effect of Cd on the condition index as well as for the uptake of Cd (Fischer 1986, Mubiana et al. 2007). Mubiana et al. (2007) showed that the uptake rate of Cd increases with elevated temperature. Moreover, the influence of temperature on the Cd effect was also characterized by a complex interaction: Fischer (1986) showed that the condition index (CI) of *Mytilus edulis* at increased Cd concentration was highest at 7.3 °C and decreased towards warmer as well as towards colder temperatures in the temperature range between 5 and 25°C, indicating a Gaussian relationship.

Generally, decreased salinity led to synergistic effects as observed by Struck et al. (1997), Mubiana et al. (2007), and Wang et al. (1997), who investigated uptake rates and accumulation of Cd under different salinity regimes. In contrast, Fischer (1986) observed inconsistent effects and could not detect a significance when he tested the effect of salinity on soft tissue Cd concentration, condition index and Cd/cell weight index at different temperatures. As Lehnberg and Theede (1979) revealed by growth and survival experiments with mussel larvae, the effect of salinity on the impact Cd is interlinked with the effect of temperature. Therefore, the interaction was also categorized as „complex".

Copper (Cu)

Interestingly, the nature of the interaction between Cd and Cu was asymmetrical at the concentration ratios of relevance for the test data set: whereas Elliot et al. (1986) observed a synergistic effect of Cu on the uptake of Cd for a concentration of 20 µg/L copper and 10 µg/L cadmium, the presence of Cd influenced the uptake of Cu antagonistically. Moreover, the nature of the influence of Cd on the Cu effect depends on the concentrations and the ratio of the concentrations of both metals: at equal concentrations of the two metals, the effect of Cu was synergistic (Elliot et al. 1986). Corresponding to the conditions observed at Norderney, the increase of the Cu effect was calculated based on the results observed for concentrations of 20 µg/L copper and 10 µg/L cadmium equivalently as described above for the influence of copper on the cadmium effect. No suitable literature data were available on an influence of Pb on effects of Cu, or the data were not applicable. Zn led to synergistic effects in the experiments by Elliot et al. (1986), indicated by an increased uptake of Cu.

Synergistic effects also characterized the influence of decreased pH values on the effect of Cu: under increased Cu concentrations, *Mytilus edulis* increased respiration in unfertilized eggs (Akberali et al. 1985); acidification further reduced the egg respiration and increased the uptake rate of Cu. Thereby sperm cells had generally a three-fold higher uptake rate of Cu than eggs and the respiration was inhibited when exposed to Cu (Akberali et al. 1985). In contrast, the influence of pH on fertilization seems to be relatively low: only at a pH being as low as 6.5, an adverse effect on the effect of Cu in combination with increased other metals (Zn, Pb, Cr, Ni and As) on egg fertilization of *Mytilus edulis* could be shown (Riba et al. 2016). Data from a copper mine in a coastal environment showed that the concentration of Cu in the water column and the pH level were inversely correlated (Koski et al. 2008). Moreover, Han et al. (2013) quantified observations about adverse interaction effects regarding defense mechanisms (phagocytosis and metallothionein production), uptake rates and mortality rates.

Anoxia slightly decreased the mean lethal time of *Mytilus* (the time span of survival after stress exposure started) at Cu exposure under salinity conditions of 32 (Weber et al. 1992). The influence was therefore categorized as „synergistic effect". Moreover, Giarratano et al. (2011) showed that the concentrations of Cu were lower in summer when oxygen concentrations in the water were higher than in winter (in Argentinia). Phillips (1976) also studied the effect of temperatures on uptake rates of Cu, but no clear pattern could be observed.

In contrast to other metals (Cd and Pb), elevated temperature had an antagonistic effect and decreased the uptake rate of Cu at temperatures between 6 and 26°C (Mubiana et al. 2007).

For salinity, an opposite pattern was observed: increased salinities led to a decreased concentration of Cu in blue mussels (Struck et al. 1997, Mubiana et al. 2005).

Lead (Pb)

Increased Cd concentrations in the water led to antagonistic effects as indicated by increased concentrations of Pb in digestive glands and gills and decreased necrosis, inflammation and neoplasmia (Sheir et al. 2013). No suitable data were available for the influence of Cu and Cd on Pb specific effects. Acidification led to synergistic effects as shown by Han et al. 2013 indicated by increased concentrations of Pb and increased mortality. As a response of the organism, the percentage of esoinophilic hemocytes increases (Han et al. 2013). The mortality decreased by about 35% when pH was reduced from 8.2 to 7.7. A defense mechanism against metals, the zymosan phagocytosis, was disturbed under low pH conditions compared to high pH conditions during Pb exposure (Han et al. 2013). No relevant literature data were available for the influence of oxygen concentration on Pb effects on *Mytilus edulis*. Elevated temperature influenced the effect of Pb synergistically, as data about uptake rates of Pb presented by Mubiana et al. (2007) showed. In lab experiments antagonistic effects were shown for high salinity conditions as indicated by lower Pb uptake at high salinities than at lower salinities (Phillips 1976). However, field studies could not confirm an influence of salinity on Pb uptake in analysis of different influences (Struck et al. 1997).

Zink (Zn)

Vercauteren and Blust (1999) showed antagonistic as well as synergistic effects in the presence of Cd for the uptake of Zn: At Cd concentrations of 10ug/L, the uptake rate of Zn in soft tissues, gills and in the digestive system decreased (by about 25%, 25% and 40% respectively), whereas the Zn uptake in the hemolymph increased 3 fold (+200%). The authors suggested that at high metal concentrations the transport of Zn from the hemolymph to other body compartments is inhibited (Vercauteren and Blust 1999). In compliance with that, Sheir et al. (2013) observed higher internal concentrations of Zn in digestive glands and gonads under the influence of Cd in mussels having experienced a pre-exposure of Zn in the field and afterwards being exposed to increased Cd concentrations in the lab.

Synergistic effects were observed for the influence of Cu on the accumulation of Zn in blue mussels: Elliot et al. 1986 showed an increased uptake rate with increased Cu concentrations. The nature of the effect was consistent for different experiments of the same publication for different concentrations and the synergistic effect increased with increasing Cu concentrations. Moreover, a factorial experiment and measurements of the atom absorption spectrometry showed an increase of 25% of the Zn concentration when Cu was present (Kaitala 1988). No literature data were available about the influence of Pb on Zn effects.

Acidification led to synergistic effects on the effect of Zn: Riba et al. (2016) observed that the fertilization of eggs was impacted more severely at low pH values of 6.5. Moreover, the pH value plays an important role in the detoxification process in blue mussels: the higher the pH value, the more Zn was bound to granules (George 1983), which also indicated an exacerbating effect by decreased pH.

Data from Giarratano et al. (2011) suggested that increased oxygen concentrations resulted in antagonistic effects, because the Zn concentrations in *Mytilus edulis* were lower in summer, when the oxygen concentrations were higher than in winter (in Argentinia). Increased temperature led to synergistic effects for the impact of Zn: Cotter et al. (1982) observed a decreased mean lethal time. However, in contrast to the other metals described above, temperature did not have an influence on Zn accumulation in *Mytilus edulis* (Cotter et al. 1982). Synergistic effects of decreased salinity and antagonistic effects of increased salinity were observed for Zn effects on *Mytilus edulis*: At lower salinities, uptake rates of Zn (Wang et al. 1996) and internal Zn concentrations (Cotter et al. 1982, Struck et al. 1997, Mubiana et al. 2005) increased. In contrast, the bodymass (Cotter et al. 1982) and the condition index (Cotter et al. 1982) decreased at lower salinities. Increased salinities had antagonistic effects.

pH

No data were available about the influence of increased Cd concentrations on effects caused by decreased pH. However, the pH value in the water was reduced by the occurrence of high Cu and Zn concentrations (Akberali et al. 1985). Therefore, the interaction was categorized as „synergistic“ for both metals. Moreover, Melzner et al. (2013) revealed a correlation between hypoxia and decreased pH levels in the water (categorized as synergistic interaction) and reasoned that respiration and dynamics of the carbonate chemistry could be important drivers of this interaction. Salinity also influences the pH in the water and is related to the carbonate chemistry: with increasing salinity, the pH level increases (Melzner et al. 2013) (here categorized as synergistic interaction). Even though, all these interactions occur directly in the water, corresponding effects on organisms can be expected.

Oxygen/ anoxia

Pre-exposed to Cd, *Mytilus edulis* was more sensitive towards anoxic conditions and the mean lethal time (time until death) decreased significantly, indicating a synergistic effect (Veldhuizen-Tsoerkan et al. 1991). Similarly, Weber et al. (1992) observed a synergistic effect indicated by decreased survival time, when they exposed blue mussels to Cu and bubbled the water with N_2 to generate anoxic conditions. As described above, there is an interaction in the water between pH and oxygen concentration of synergistic nature, which occurs in the water column (Melzner et al. 2013). Decreased salinity led to antagonistic effects indicated by an increased survival time at anoxic conditions (Babarro and Zwaan 2002) and at seawater bubbled with N_2, Weber et al. 1992).

Temperature

No data were avaliable for the influence of increased Cd, Cu and Pb concentrations on effects of temperature. However, a synergistic interaction for the influence of temperature was reported for Zinc, which had a significant influence on the thermal tolerance of *Mytilus* (Cotter et al. 1982). Moreover, the lethal temperature was reduced by approximately 1°C at 1.0 mg/L Zn (Cotter et al. 1982). The response pattern of the condition index to increased temperatures depended also on the Zn concentration (Cotter et al. 1982). Acidification exacerbated the negative effect of an elevated temperature of 25°C significantly (Hiebenthal et al. 2013) and the influence was therefore categorized as „synergistic“. No literature data about an influence of oxygen depletion on temperature effects on *Mytilus edulis* were found.

Both very high as well as very low salinity conditions led to synergistic effects: The growth, relative fluorescence intensity, condition index and mortality are affected (Hiebenthal et al. 2012) as well as the development of larvae (Lehnberg and Theede 1972). Overall, the interaction between temperature and salinity is complex and dependent on the temperature values. Moreover, the pattern differed between the different types of observations (between the observation of the development of larvae: Lehnberg and Theede 1972 and growth of bivalves Hiebenthal et al. 2012).

Salinity

No literature data were available about influences of metals, acidification, or oxygen depletion on the effect of salinity on *Mytilus edulis*. However, information on the influence of temperature was available, indicating synergistic effects for temperature extremes and a complex interaction depending on the intensities of the two stressors temperature and salinity. As *Mytilus* is adapted to high salinities, low salinities represent a negative impact and thus, a pressure. At those conditions, increased temperatures enhance the negative effect on the blue mussels as shown by decreased growth (Brenko and Calabrese 1969) and increased mortality (Cotter et al. 1982). Lehnberg and Thede (1979) showed the complexity of the nature of the interaction between salinity and temperature, implying that the optimum values for both environmental variables form an ecological niche, whose pattern slightly variates depending on the age, additional factors, and the type of observation studied. This pattern was also supported by data from Brenko and Calabrese (1969), who showed that increased and decreased temperatures decreased the survival and the growth at high and low salinities, whereby particularly for survival, high temperatures had a worse effect than low temperatures (Brenko and Calabrese 1969).

4.4.1 DEB Literature model for temporal dynamic effects of single stressors

The alteration of parameter values had consequences for the response variable as well as for the behavior of aspects describing the model, such as effect strength and the indicator for acclimation. In contrast to the response variable, the effect strength describes the magnitude of the effect for a given moment, whereas the response variable indicates the effect on an observation assuming that the impacts sum up over time. However, the organism can also recover and possibly compensate a previous impact caused by anthropogenic stressors if the stressor intensity decreases or if the organism can successfully adapt to the pressure situation. The indicator for acclimation shows how much the organism is able to respond to a given pressure situation. The greater the acclimation indicator, the better the ability to adapt to the conditions as indicated by the response variable.

Acclimation

The parameter α (see equation (**5**)(3)) represents the ability of the organism to adapt to the presence of the stressor and it shapes the pattern of acclimation (Figure 7). Overall, the greater the parameter α, the faster the maximum of acclimation is reached. Consequently, the effect strength is mitigated by a high value of α. The fast reaction of the body (high α-value) prevents a strong increase of effect strength along with an increasing internal concentration). The indicator for acclimation is closely interwoven with the effect strength and flattens the curve of the effect strength. Consequently, the influence of the acclimation can also be seen in the response variable, which can be compared with the observational data of the experiment.

Effect rate

The value for the effect rate determines how strong the effect is per time step and per stressor intensity and has therefore a strong influence on the effect strength (equation (**3**) and (**4**)). The higher the value for the effect rate, the stronger is the adverse effect on the organism. A high value for the effect rate results in a higher effect strength and a steeper curve of the rise of the effect strength. Consequently, the response variable is affected more severely when the effect rate is high and adverse impacts on the response variable are observed earlier. However, a stronger effect also leads to a stronger acclimation reaction of the affected organism. In contrast, a lower effect rate leads to a less steep effect strength in the first time period of exposure to the stressor and the acclimation response starts slower and less steep (Figure 8).

Time delay

The parameter γ determines the time delay of the onset of the effect (equation (**6**)): The smaller γ is, the greater is the time delay. A high value of γ leads to a sudden onset of an adverse effect on the response variable, whereas a low value for γ results in a postponed and rather smooth course of an adverse effect on the response variable with a less severe effect during the first time period. However, after a while, the response variable is adversely affected as strongly as for a high value for γ. The influence of γ becomes most obvious in the behavior of the effect strength. The pattern of the effect strength over time is altered: the hump-shaped curve of the effect strength becomes more stretched. Thus, the peak of the effect strength is delayed. This entails that the effect strength influenced by a small γ-value is not permanently smaller that the effect strength influenced by a greater γ-value. Instead, the curves overlap each other.

The acclimation is influenced by the effect strength and thus indirectly by γ: The smaller γ, the more slowly and less steep the acclimation starts until it reaches its maximum (Figure 9).

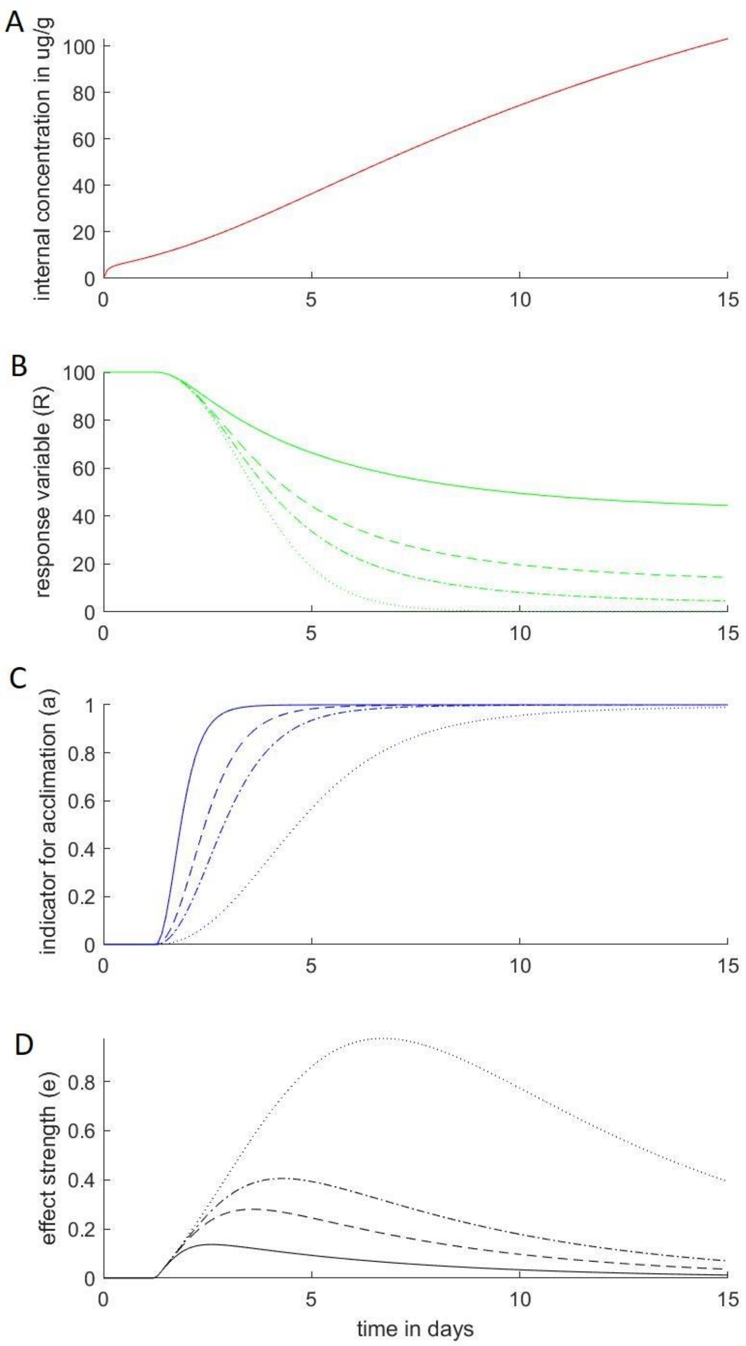

Figure 7 Model behavior with altered values for the parameter alpha (acclimation), values: 0.5 (dotted line), 2.5 (-.), 5 (--) and 20 (solid line). Example: the uptake of a heavy metal by the blue mussel and the response of the organism. A: internal concentration of a chemical substance, B: response variable (here survival data as an example), C: indicator for acclimation (a low value represents a strong acclimation), D: effect strength due to the stressor, depending on acclimation, time delay stressor intensity and transition threshold

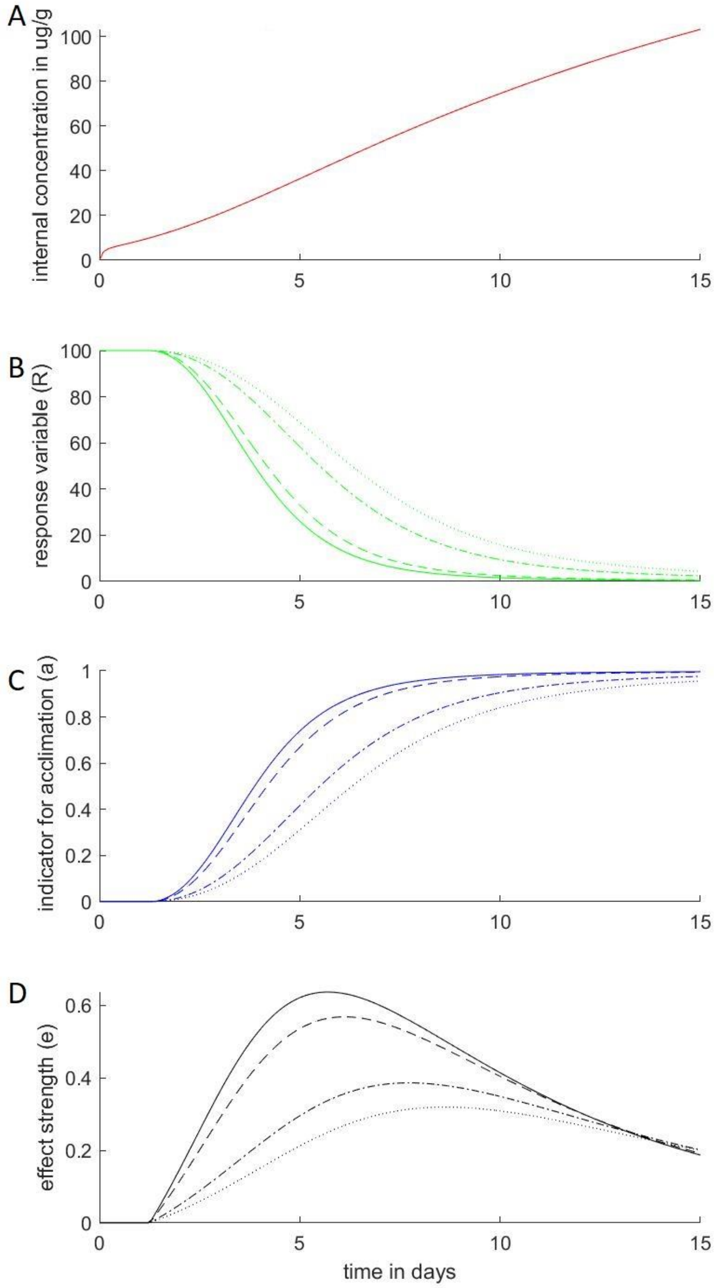

Figure 8 Model behavior with altered values for the effect rate, values: 0.2 (dotted line), 0.3 (-.), 0.7 (--), and 0.9 (solid line). Example: the uptake of a heavy metal by the blue mussel and the response of the organism. A: internal concentration of a chemical substance, B: response variable (here survival data as an example), C: indicator for acclimation (a low value represents a strong acclimation), D: effect strength due to the stressor, depending on acclimation, time delay stressor intensity and transition threshold

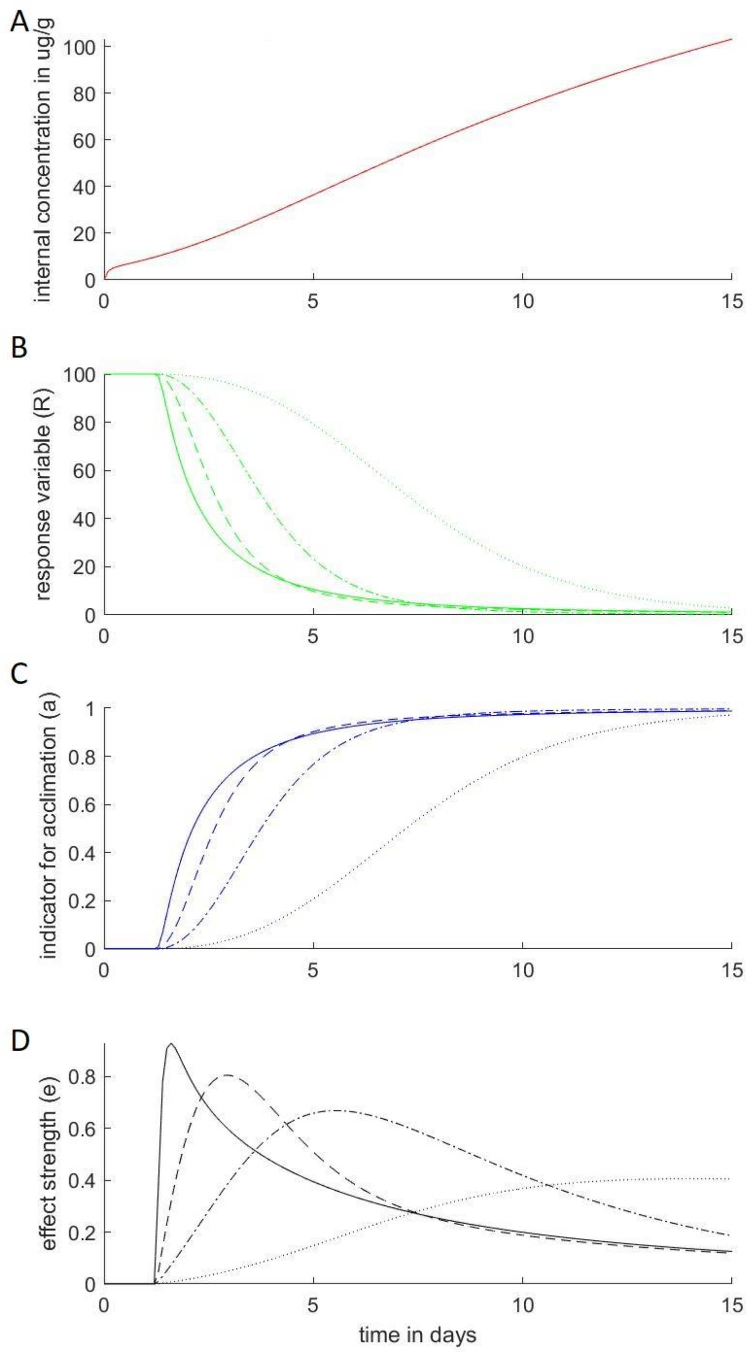

Figure 9 Model behavior with altered values for the parameter γ indicating time delay, values: 0.02 (dotted line), 0.2 (-.), 1 (--), and 10 (solid line). Example: the uptake of a heavy metal by the blue mussel and the response of the organism. A: internal concentration of a chemical substance, B: response variable (here survival data as an example), C: indicator for acclimation (a low value represents a strong acclimation), D: effect strength due to the stressor, depending on acclimation, time delay stressor intensity and transition threshold

4.4.2 Test of the model with literature data

The model output showed the magnitude of the observed effects of different stressors derived from literature depending on the intensity of the stressor and the exposure time in a 3-dimensional space (Figure 10 and Figure 42 - Figure 48 in 7.1). The fit of the model with the literature data for the observations differed depending on the uniformity of the literature data and resulted in different values for the relative standard error and standard deviation (7.1).Regarding heavy metals, the shapes of the model outcome for Cd, Cu and Zn resembled each other, whereas the shape for Pb was different (Figure 42 - Figure 45 in 7.1). The modeled surface for the response to Pb did not level off but decreased linearly at high concentrations and long exposure times. After a short time delay of the onset of an effect, the response variable decreased rapidly first and then more gradually over time (Figure 10 in the main text and Figure 42 - Figure 45 in 7.1).

In contrast to the models for these metals, models for oxygen depletion as well as for decreased pH were flatter and the response variable decreased with decreased oxygen concentration or pH value and with increasing exposure time (Figure 47 and Figure 48 in 7.1).Major differences in the shape of the model in contrast to the ones described above could be seen for the simulation of the influence of temperature: Performance was best at the optimum value and decreased at increasing and decreasing temperatures (Figure 46 in 7.1). Even though the general pattern of the data could be reproduced by the model for the different stressors, some data points were not represented well (Figure 10 and Figure 42 - Figure 48 in 7.1). E.g., the model results based on data by Strömgren et al. 1982 showing the effect of Cd on the growth of *Mytilus edulis* performed more inadequate at the highest tested concentration of 3µg/L Cd and at exposure times less than 20 days than at the lower concentrations (Figure 10).

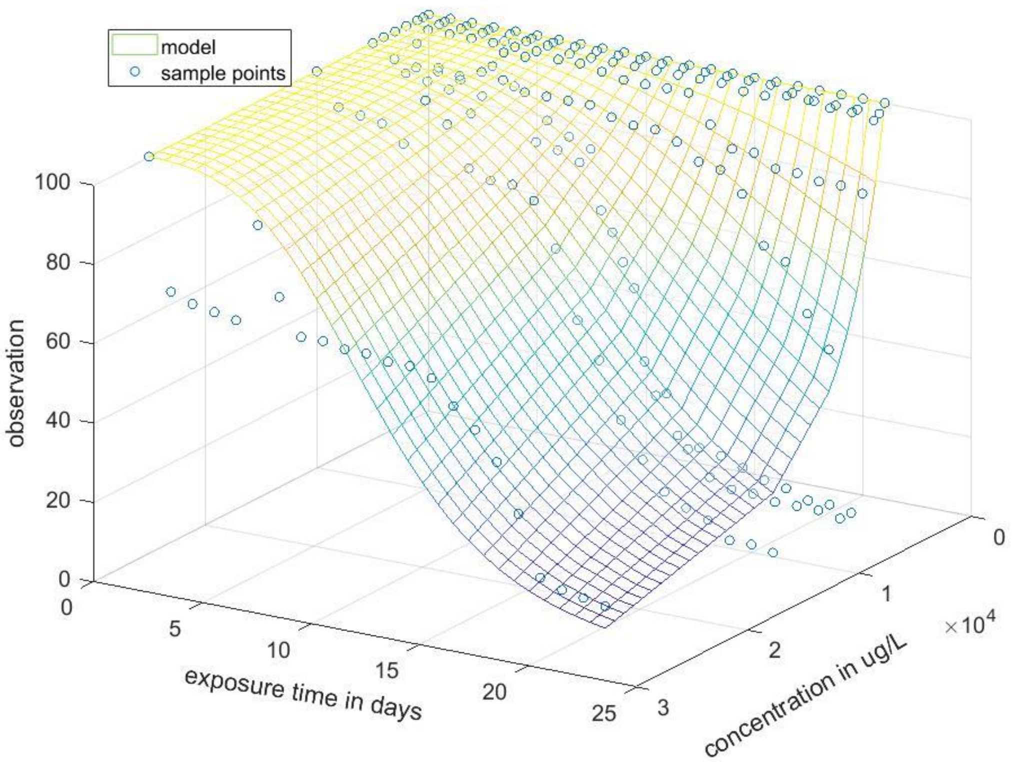

Figure 10 Comparison between model results and observations from Strömgren et al. 1982

The optimized parameter values differed at maximum by eight orders of magnitude between the different stressors tested (Table 4): Whereas for Cd and for Pb a value close to one for alpha was determined, the alpha-value for the response towards Cu, Zn and pH was five to seven orders of magnitude lower. The alpha value for the response towards oxygen depletion was about 0.5. The optimized value for the parameter beta was highest for the response for a decreased pH, whereas the corresponding value for Zn was lowest. The analysis of the response to Zn and to increased temperature conditions both revealed a value above 40 for the parameter „gamma". This indicates the absence of a time delay for the onset of a measureable observation for these two stressors. In comparison to the parameter values for Zn and temperature, the values for „gamma" were lower for the response to the stressors Cd, Cu, Pb, pH and oxygen, indicating a time delay.

Table 4 Optimized values for the parameters beta (effect rate), gamma (time delay) and alpha (acclimation) as well as results from the statistical analysis (relative standard error RSE and relative standard deviation RSD)

Parameters	**Cd**	**Cu**	**Pb**	**Zn**	**Temp**	**pH**	**Oxygen**
beta	0.0016	0.0520	0.0001	$1.309e^{-5}$	0.0443	0.1952	0.0210
gamma	0.0051	0.0006	0.0009	49.9763	45.6804	0.0019	0.0196
alpha	0.9999	$1.733e^{-5}$	0.9999	$9.986e^{-7}$	0.9966	$8.544e^{-6}$	0.5083
RSE	0.1631	0.1143	0.0454	0.0682	0.0747	0.1651	0.0109
RSD	0.2712	0.6529	0.0549	0.1095	0.0937	0.2627	0.0209

4.4.3 DEB main model

Based on the data from Norderney between 2005 and 2010 the inclusion of interaction effects resulted in generally higher effects and strong seasonal fluctuation compared to the model with additive effects only (Figure 11). In both models, the effect level increased with time. The differences between reference model and stress model results cannot be explained by the stressor intensities alone. Instead, they were substantially characterized by uptake and accumulation processes as well as interaction effects (Figure 11 to Figure 16). The results from the DEB-model provided a lot of information on processes that are not easily observed in the field, such as reduced filtration rate, reduced energy reserve, growth inhibition and increased costs for maturity maintenance.

The fluctuation of the reserve biomass could be explained by food conditions and favorable temperature (Figure 13) as well as by the utilization of the reserves during food scarcity, e.g. during winter. Likewise, the simulation indicates a seasonal growth pattern (Figure 16). Growth periods were clearly visible and were in line with the environmental conditions which prevailed at Norderney during that time period. The stress scenario disturbed maturation and led to a time delay for the first spawning event. Moreover, the number of produced gamets and the number of spawning events decreased under the stress-scenario. Compared to the control scenario, it took longer to build up the reproduction buffer in the stress scenario and thus, the organism could not make use of favorable conditions in terms of temperature and food conditions for spawning.

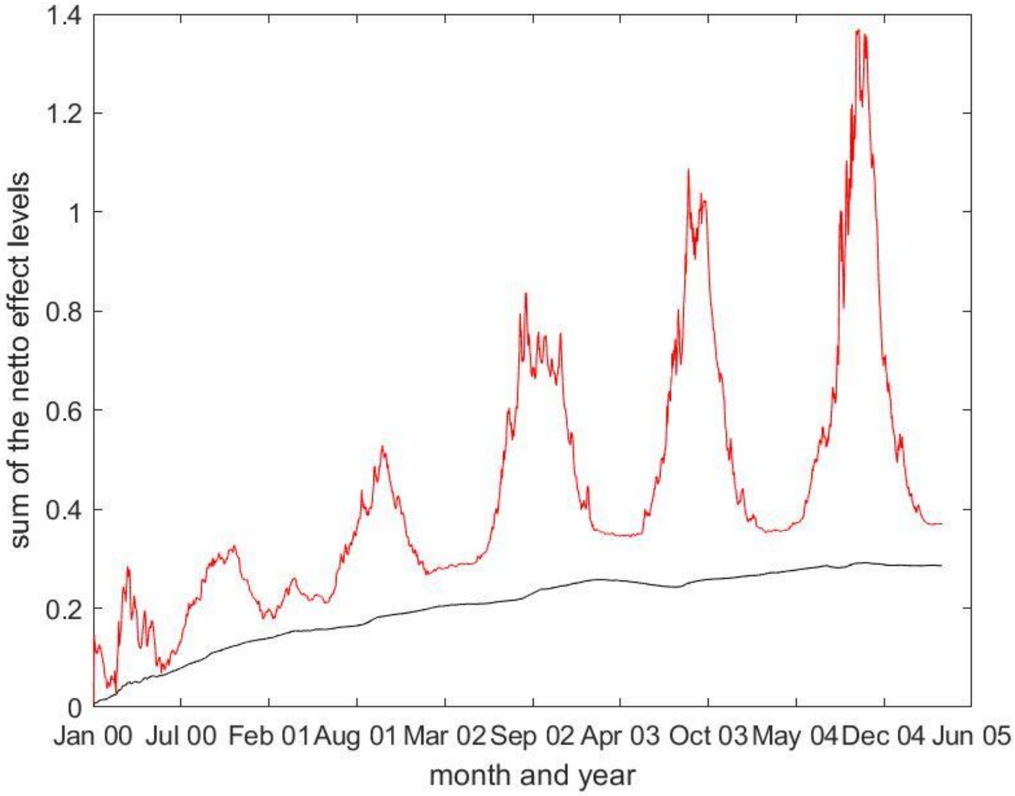

Figure 11 Comparison between predicted effect strengths assuming only additive effects (black line) and assuming interaction effects between the stressors (red line)

The structure biomass was build up periodically and the amount that was build up increased with time (Figure 12). The reserve biomass increased but also decreased periodically following a seasonal pattern (Figure 13). In comparison to the control scenario, cumulative effects of anthropogenic stressors led to an overall decrease of reserve biomass for the tested data set (by 84%) over the complete life span. Compared to the pure additive model, the effect on the reserve biomass was 60% percent stronger in the cumulative model.

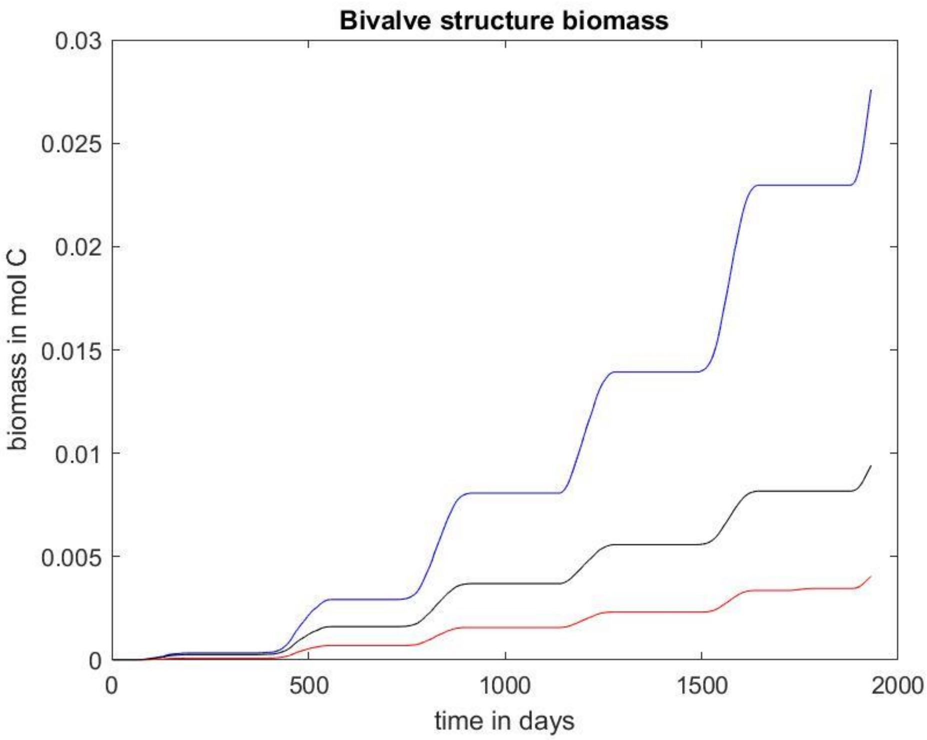

Figure 12 The change of structure biomass during the simulated life span of *Mytilus edulis*. The blue line represents the results for the control scenario, the red one the stress-scenario and the black one the scenario assuming only additive effects.

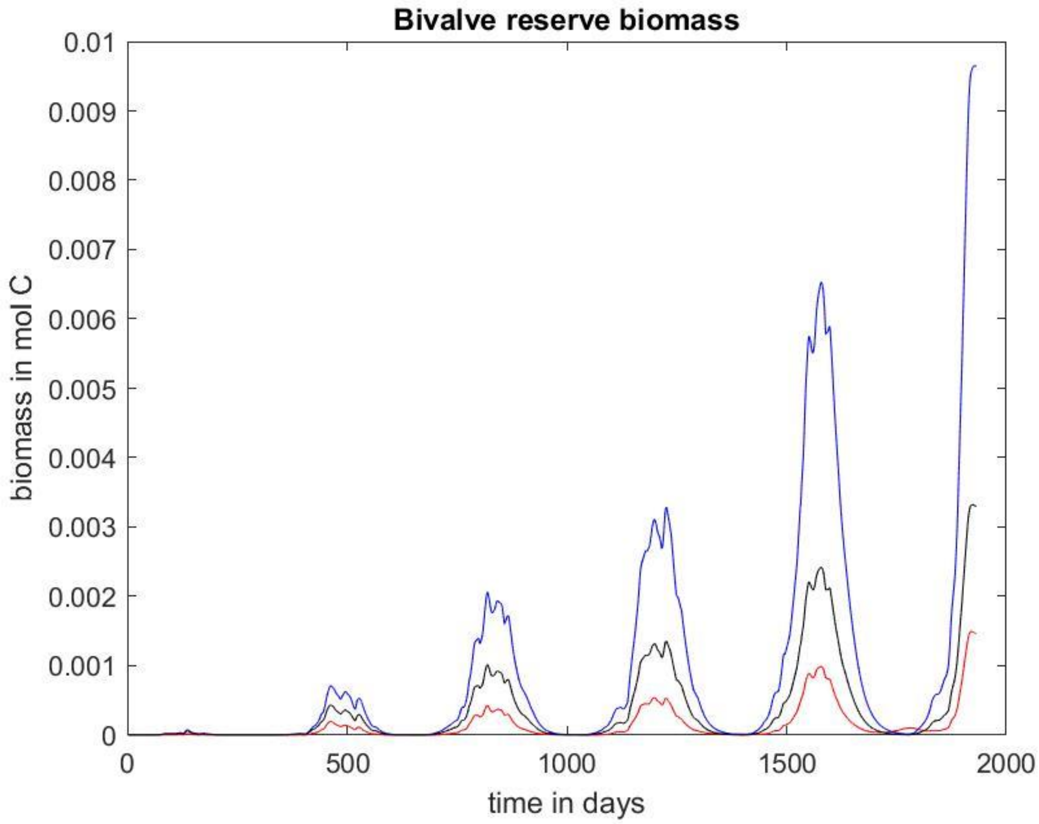

Figure 13 The change of reserve biomass during the simulated life span of *Mytilus edulis*. The blue line represents the results for the control scenario, the red one the stress-scenario and the black one the scenario assuming only additive effects.

The energy invested into maturity strongly increased during two periods before maturation: The first period started about 70 days after the development of the ability to feed (topophore larvae) and the second one about 400 days after. The investment in maturity was generally smaller in the stress-scenario than in the control scenario (-15 % in the additive model and -58% in the cumulative model). Moreover, the blue mussel reached maturity later in the stress scenario (at day 470) than in the control scenario (at day 427) and in the additive stress-scenario (at day 441) (Figure 14). The calculated effect based on the pure additive model was less pronounced than the calculated effect based on the cumulative model with the integration of interaction effects (difference of 52 %).

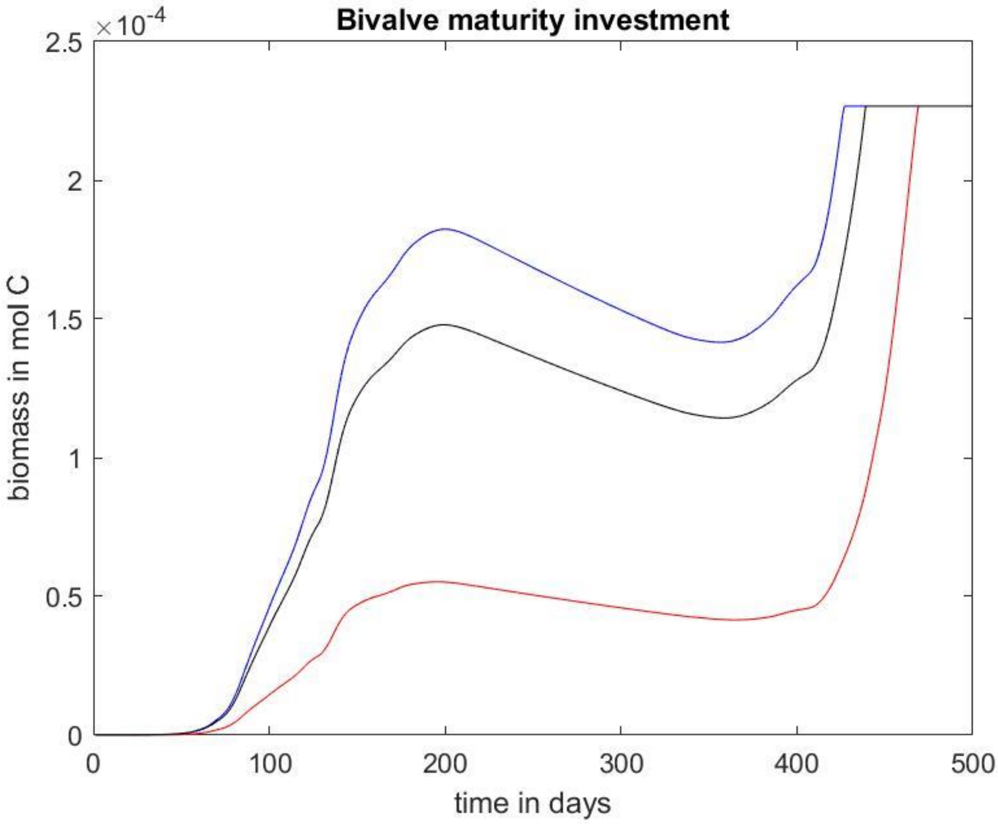

Figure 14 Energy invested into maturity expressed as biomass C (representing the development of reproductive organs). The blue line represents the results for the control scenario, the red one the stress-scenario and the black one the scenario assuming only additive effects.

The mussels allocated energy for reproduction periodically (Figure 15). The biomass of the reproduction buffer increased after maturation until the environmental conditions were favorable for spawning (defined by a minimum temperature of 9.6°C and a gonado-somatic ratio of 0.2). Then the buffer emptied almost completely, indicating the release of gametes into the environment. In the control scenario, the reproduction buffer was emptied six times during the time period tested. In some years, the reproduction buffer was emptied twice. In contrast, in the cumulative scenario, the release of gametes occurred only three times during the time period and in none of the years gametes were released two times. When interaction effects were neglected in the model, gametes were released at five events during the time period and in one year gametes were released twice (additive model). The peaks of the biomass of the reproduction buffer were smaller in the stress- scenario than in the control- scenario and thus, the number of gametes was smaller and the timing of gamet release differed (later spawning in the stress scenario) (Table 5).

Table 5 Spawning events in the control and in the cumulative stress scenario

	Number of gametes released			**Days after birth**		
	Control scenario	**Additive model**	**Cumulative model**	**Control scenario**	**Additive model**	**Cumulative model**
1st spawning event	794	$2.73*10^6$	$1.201*10^6$	426	484	540
2nd spawning event	$3.368*10^6$	$4.550*10^6$	$2.857*10^6$	470	798	865
3rd spawning event	$5.171*10^6$	$6.443*10^6$	$3.951*10^6$	532	889	1273
4th spawning event	$1.167*10^7$	$1.001*10^7$		823	1260	
5th spawning event	$1.360*10^7$	$1.461*10^7$		917	1629	
6th spawning event	$2.5385*10^7$			1236		
7th spawning event	$4.1973*10^7$			1585		

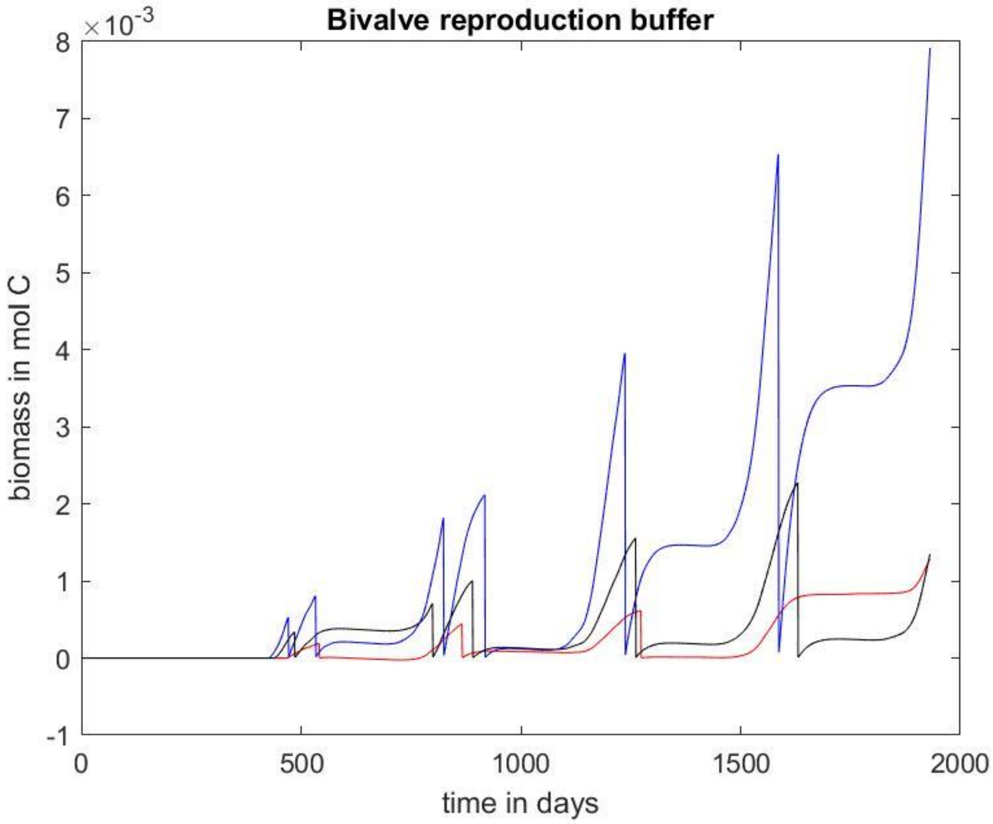

Figure 15 Biomass of the reproduction buffer in mol C. The blue line represents the results for the control scenario, the red one the stress-scenario and the black one the scenario assuming only additive effects.

Model growth of *Mytilus edulis* varies seasonally (Figure 16). In the stress-scenario, the growth of *Mytilus edulis* was depressed (by 23 % in the additive model and by 43% in the cumulative model). During the study period, the gap between the size of the mussels modeled for the control-scenario and of the mussels modeled for the stress scenario increased gradually over time. In the control- scenario, mussels attained a size of 5.1 cm whereas mussels grew to a size of 2.7 cm and 3.6 cm in the stress-scenarios with integrated interaction effects and additive effects, respectively (Figure 16). The magnitude of the effect was less pronounced in the additive model (difference of 23 % to the control) compared to the cumulative model (difference of 43% to the control).

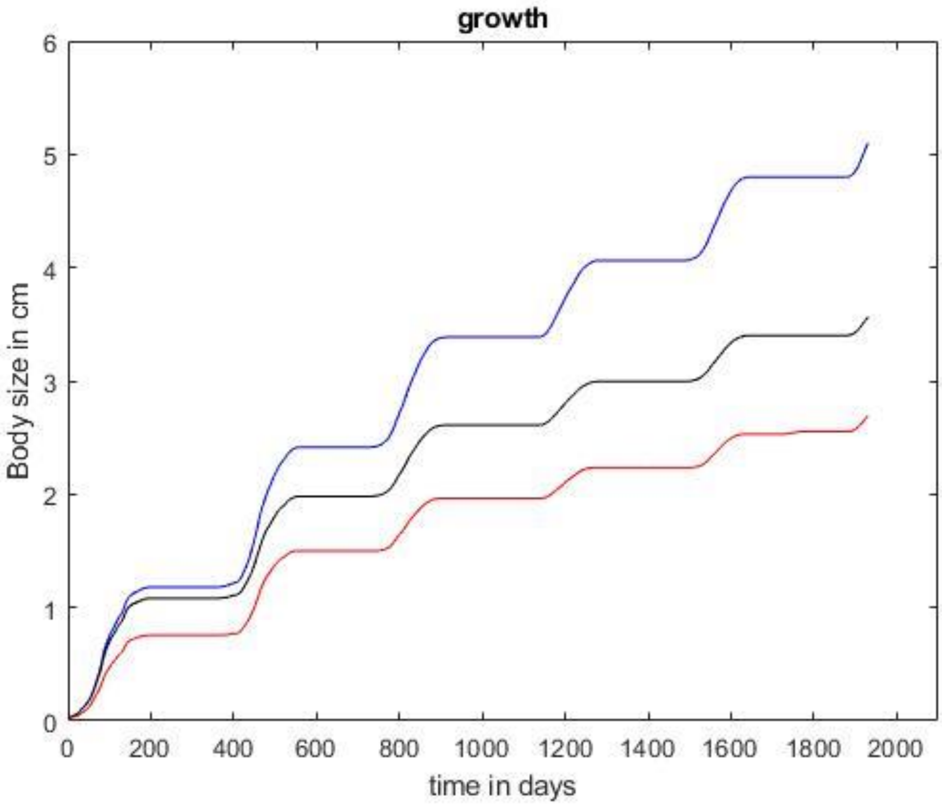

Figure 16 Growth of Mytilus edulis. The blue line represents the results for the control scenario, the red one the stress-scenario and the black one the scenario assuming only additive effects.

To summarize, the model predicted a clear difference between the control scenario and the stress-scenarios (Table 6 and 7.1). Moreover, there was a difference between the additive scenario and the cumulative scenario (Table 16 in 7.1).

Table 6 Relative differences between models and scenarios

Relative difference	**Additive model compared to the control**	**Cumulative model compared to the control**	**Cumulative model compared to the additive model**
structure biomass	-0.59946	-0.83191	-0.58034
reserve biomass	-0.59976	-0.84334	-0.60858
maturity	-0.1485	-0.58872	-0.517
reproduction	-0.74781	-0.82996	-0.32574
Body size	-0.23806	-0.4319	-0.25441
spawning	-0.62659	-0.92083	-0.78797
effect level			1.1015

Comparison of the contribution of each of the stressors

To reveal the relative importance of the different stressors I ran the model with all stressors except the focal one and compared the outcome to the full model. This test showed that the pressure due to decreased pH values contributed most to the model outcome for the structure biomass, the reserve biomass, the reproduction and the growth in the cumulative model as well as in the additive model. Further, in the cumulative model, stress due the temperature, zinc concentration and copper concentration had major influences on growth (Figure 17). In contrast, in the additive model, only copper had a clear influence on growth in the later life stage of the mussel (Figure 18). Thus, the integration of the interactions in the model had an influence on the predicted contribution of the stressors to the overall effect.

Increased temperature and zinc concentration and to a smaller degree by decreased pH values influenced the cumulative effects on the maturity investment in the cumulative model (Figure 19). Hence, the contribution of the stressors differed in the different life stages of the mussel and it mattered which processes the stressors targeted. In the additive model, the contribution of decreased pH values was higher compared to the cumulative model and temperature had a much smaller effect (Figure 20). This indicates again that the choice of model influences the predicted contributions of the stressors.

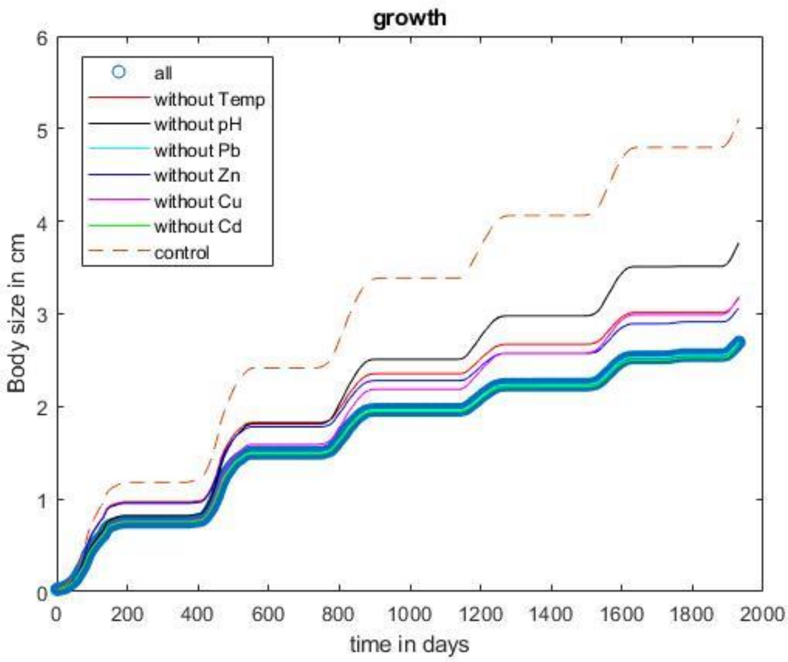

Figure 17 Contribution of each of the stressors to the cumulative impact on growth - cumulative model

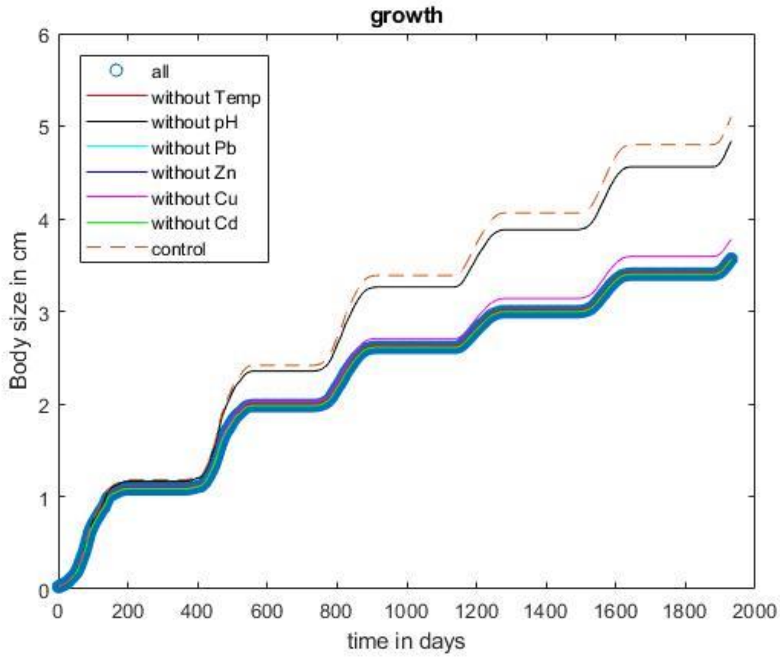

Figure 18 Contribution of the stressors to the cumulative impact on growth - additive model

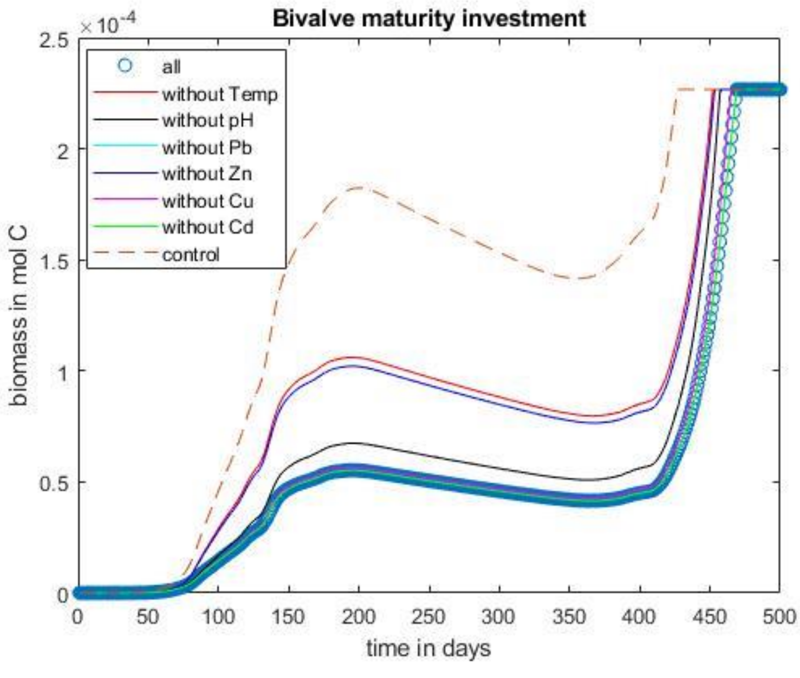

Figure 19 Contribution of each of the stressors to the cumulative impact on maturity investment- cumulative model

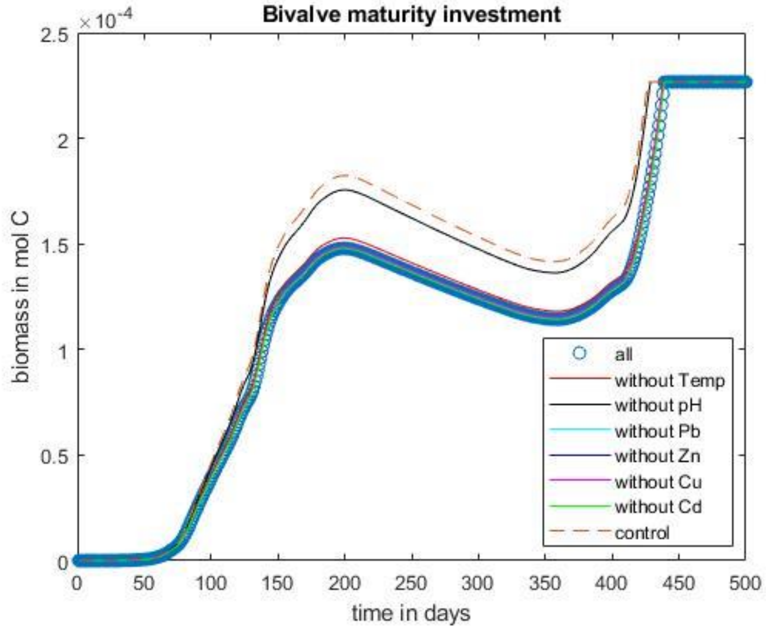

Figure 20 Contribution of each of the stressors to the cumulative impact on maturity investment- additive model

Comparison of methods

In four cases, bidirectional interactions were included in the cumulative model. The corresponding interactions were between Cd and Cu, between Cd and Zn, between Cu and Zn and between Zn and temperature. Models runs without these bidirectional interactions predicted slightly smaller impact on the blue mussel in general. This became most obvious in the modeling of the maturity investment. There were no distinct contradictions between simulations with and without bidirectional interactions (7.1). The comparison between the model run with interpolated heavy metal concentrations based on the measured data of the monitoring station at Norderney and the application of a single mean value for the whole study period showed that the continuous pressure situation resulted in slightly more severe effects on the blue mussel. However, this effect was rather marginal (7.1).

4.5 Discussion

The described model approach proved useful to analyze cumulative effects of anthropogenic stressors on blue mussels. Time-dependent effects such as caused by accumulation of substances are reflected as well as interactions of the substances with the target organism, which could e.g. symbolize defense mechanisms. However, due to lack of information the nature of some interactions remains enigmatic so far. Further, an experimental validation of the proposed model is needed to quantify the uncertainties and to obtain information on the accuracy of the model. Therefore, we should interpret the predicted effects as indications for the magnitude of cumulative effects only, keeping in mind the limited knowledge base and the known uncertainties. However, the model could be used to explore relative differences in cumulative stressor effects between different environmental settings, e.g., between different monitoring stations. To refine the outcomes, more literature data as well as higher frequencies of measurements at the monitoring stations are needed. The following discussion will interpret the results and describe the uncertainty related to the data and the model. Moreover, I discuss possible applications. In the second part, I draw conclusions and elucidate an outlook.

4.5.1 Review of the matrix method and DEB model

Discussion of the matrix model

The matrix provided a quick overview over binary cumulative effects of a complex network of interactions and influencing variables. The colors in the cells of the matrix (Table 3) indicated the nature of nature of interaction in a qualitative way, which provided a first impression of the ratio between synergistic and antagonistic interactions for a given scenario. The applicability of existing data for this kind of analysis is high, because research has focused on binary relationships between anthropogenic stressors as well as between environmental parameters and stressors on ecosystem components (reviewed in Crain et al. 2008). Moreover, it has been advantageous that matrix analyses generally allow the combination of qualitative and quantitative data assessment.

Uncertainty of the matrix

For the combined model (matrix and DEB model), only the quantified interactions of the matrix could be considered. Therefore, some information from literature needed to be disregarded. Concerning the equations filled into the cells of the matrix, it has to be kept in mind that the nature of those interactions could depend on the stressor intensities in a non-linear way or on the ratio between the stressors (see e.g. data by Elliot et al. 1986). To consider these aspects comprehensively, more data will be needed comprising more different stressor intensities and exposure times. With additional data, other mathematical models could be tested in order to identify the optimal simulation of the interaction processes.

The integration of environmental processes affecting the interactions could further help to improve model structures. The research by Sheehan and Power 1999, who investigated the influence of seasonality on defense mechanisms, indicates this as well. It is also possible that interactions change beyond a threshold of stress- intensity. This aspect should also be integrated as soon as such information becomes available. So far, these are only some possible explanations for different interaction types which do not follow a linearity and depend on the intensities of both stressors.

For some stressor pairs, literature data about both possible directions of influences had been found and integrated into the model. In these cases, theoretically it may be unclear if these two datasets describe the same interaction or if they describe different effects e.g. due to distinct mechanisms. If both datasets would describe the same interaction effect, it would be considered twice in the matrix calculations, although actually only one interaction effect occurs - resulting in double counting and thus potential bias of the result of the matrix calculations. However, double counting showed no significant effect for the test data set. This can be explained by the difference of the corresponding interaction data of the double pairs with regard to the algebraic signs and by the values itself. The data derived from results by Elliot et al. (1986) comprise some of such. One likely explanation lies in the experimental setup: The sequence of addition of metals differed. This indicates that the data reflect distinct kinds of interactions. Thus, a differentiation of directionality is appropriate based on the current state of knowledge.

However, this might not be true for all interactions of the test scene. Some differences between the interaction data may also be explained by natural variability and bias due to sparse data sets. Further, it remains unclear if the conclusions stated above are valid for other species and other stressor combinations. Moreover, overlaps between the influences of both directions due to partly same mechanisms are conceivable and knowledge about these should be included into the model as soon as we know more about the exact molecular processes and the ratio of overlap of the interaction. Future research may show indeed that two stressors do not contribute equally to an overall interaction. Overall, I would emphasize again that the results obtained here represent the current state of art. The more data will be available, the more complex the model setup can be and the more precise it can be.

4.5.2 Discussion DEB- model

Single- stressor model

The test of the single-stressor model with data on the effect of metals on *Mytilus edulis* suggested that the response mechanisms differ substantially between the selected stressors with regard to time delay of the effect, effect rate, and the acclimation process.

In terms of acclimation, the results indicated that the organisms are able to adapt more easily to stress caused by non-essential metals (Cd and Pb) than by essential metals (Cu and Zn). Some indications for possible differences in acclimation processes have been observed in experiments: Neuberger-Cywiak et al. 2005 identified different acclimation processes in mussels for Cd and Zn: Increased Cd concentrations led to less oxygen consumption than treatment with Zn. Within this context, oxygen consumption could be seen as an adaptive response as mussels can close their shells to reduce metal uptake. Similarly, ammonium excretion differed between Zn and Cd exposed mussels after 48 h. Furthermore, results by Bengtsson et al. 1992 showed that the cysteine proportion of metal binding proteins was adjusted for a Cd binding protein in earth worms and the results further indicated that Cd was likely to be bound at several different iso-metallothioneins. On the other hand, the authors did not find any indications for specific Cu- and Zn-specific binding proteins. Possible different detoxification processes may also explain differences in the degree of acclimation between the different metals tested. Such differences have been revealed in a fungus, in which the expression of P-type ATPase AfCrpA and metallothionein AfzrcA differed between the exposure to the metals Cu and Zn (Cai et al. 2018). To confirm the hypothesis of different response patterns for acclimation processes with regard to essential and non-essential metals, additional experimental data are needed.

Differences in the parameters for acclimation, time delay of response and effect rate, which characterize the responses of the blue mussel to different metals, may be explained by their varying degree of connection to different proteins (Thompson et al. 2011). In their study, they only identified only four of 25 protein spots, which responded to more than one metal. The specific proteins, the metals are linked to, have different expression profiles and may have different cellular pathways (Thompson et al. 2011), which would explain the differences of the parameters in the cumulative DEB model. The expression of proteins with a biological function linked to an essential element may differ fundamentally from the expression of proteins involved in stress response. In the following, I exemplify this principle on the basis of the biological functions of copper linked to different proteins.

Copper is an essential metal and e.g. a constituent of cytomchrome oxidase, which transfers electrons from cytochrome c to oxygen and is important for cell respiration (García-Esquivel et al. 2002, Scott 1995). It is also a constituent of the enzyme tyrosina (Aguilera et al. 2014), which is important during the early development of *Mytilus* (*galloprovincialis*) for the shell formation (Migliolo et al. 2019, Ramesh et al. 2019) as well as for byssal adhesive proteins in mussels (Zhang et al. 2019, Numata and Baker 2014, Horsch et al. 2017). Further, copper - as an element of lysine oxygenase - is needed for the synthesis of collagen, which is found in the byssus of *Mytilus* spec. (Rodriguez et al. 2017). In the heamolymph, copper has an important role for some hemolymph proteins (Davenport and Redpath 1984, Thompson et al. 2011), for example for the functioning of Cu,Zn-superoxide dismutase, which reduces the number of superoxide molecules and thus the number of free radicals (Nedd et al. 2016). A prominent role of copper in bivalves heamolymph is further its function as a constituent of hemocyanin, where it binds oxygen and thus facilitates its transport (Terwilliger et al. 1988).

Because of the biological functions of copper, many adverse effects of copper are linked to proteins functions associated with the byssus, haemolymph functions and respiration, but the literature indicates that adverse effects can also be observed at higher levels of organization. In contrast to copper, an exposure to increased zinc concentrations lead to a response of many proteins involved in shell adhesion and shell calcification (Thompson et al. 2011). As these proteins decrease in response to increased Zn concentrations, blue mussels are likely more fragile to acidification, which further affects the shell. This is in line with literature data collected for the interaction matrix (interaction effect observed by George 1983). A biological explanation for the simulated direct onset of a response towards the exposure to Zn as simulated by the cumulative DEB model requires further examination as no corresponding information was available in the literature.

However, there are indications for the simulated differences between essential and non-essential metals. For the essential metals Zn and Cu, a different response pattern was observed in contrast to Pb experimentally: Thompson et al. (2011) showed that the average intensity of proteins decreased when the concentration of the two essential metals increased whereas the average intensity of proteins decreased when mussels where exposed to the non-essential metal Pb.

For oxygen, the acclimation parameter was optimized to an intermediate value compared to the other stressors. A regulatory response to hypoxia stress was shown experimentally e.g. by Giannetto et al. (2015), who figured out a prompt gene expression for the hypoxia-inducible factor-alpha (HIF-alpha) in response to air exposure and showed a modulation of the HIF-alpha as well as HIF-prolyl hydroxylases proteins with a time dependency. Moreover, also other regulatory processes were observed in bivalves as response to hypoxia in the lab described by different authors, e.g. transcriptional regulation of pyruvate kinase and phosphoenolpyruvate carboxykinase by Le Moullac et al. 2007, metabolic adaptations by Isani et al. 1995 or phosphorylation regulations by 6-phosphofructo-1-kinase by Michaelidis and Storey 1991. Acclimation processes were also observed in the field (Altieri 2006).

The alpha was low for pH-stress, even though regulatory processes such as calcification (Berge et al. 2006, Michaelidis 2005) or regulations in the acid-base and ion status seem to exist (reviewed in Pörtner 2010). Possibly, the acclimation processes could not compensate the effect of pH reduction for the selected data set from literature; regulation was maybe only minimal as observed for *Crasssostrea gigas* (Lannig et al. 2010). Zittier et al. (2018) recently could not find a regulatory response for the pH level in the haemolymph and no accumulation of bicarbonate in blue mussels at control temperatures. These results fit well in with the result of the parameter estimation for acclimation processes in the single- stressor model.

Uncertainty of the single- stressor model

Uncertainty in the single-stressor model was mainly related to data availability, model formulation, model characterization and the underlying assumptions (see methods part). The highest uncertainty lies in the assumption that a response pattern to a stressor is universal and can be transferred from one observation to another. Heavy metals accumulate to different degrees in the different parts of the body (Soto. et al. 1996) and in the different parts of the body defense mechanisms might differ. Therefore, different response patterns seem likely. Hence, as soon as research discovers a pattern for different types of responses for different observations, this aspect should be considered in the model and the pathway integrated when the effects are linked to physiological processes.

For the selection of stressors in the present study, the data availability differed for the different stressors and literature sources: For lead, altered temperatures, different pH values and decreased oxygen conditions, less data had been available as for Cu, Cd and Zn. This could explain the differences in the error values for the models with regard to oxygen depletion and acidification as well as the difference in the pattern of the modeled surface of Pb effects compared to the modeled effects of Cu, Cd and Zn.

More data on single stressor response patterns, covering wider temporal and stressor intensity ranges, could also allow for further refining of the model formulation. The results indicated that the response pattern might be a bit more complex than shown by the model applied in the present study (Figure 42 - Figure 48 in 7.1). The introduction of a second threshold level might improve the model. This second threshold level could e.g. symbolize the „critical threshold" opposed to the „pejus threshold" in line with the thermal tolerance window (Pörtner 2010). However, with the amount of data available for the present study, the identification of second thresholds was not possible and assumptions about these would have led to further uncertainties in the parameter definition. Furthermore, the introduction of further parameters would have been in misbalance to the available data.

The effect of the parameters for acclimation and for time delay showed some similarities. To analyze whether the model could differentiate between a pattern caused by a time delay of an effect and a pattern caused by acclimation in the optimization process, I conducted a start-value analysis. I tested the optimization with 900 different combinations of start values for the three parameters gamma, alpha and beta with the dataset for Cd as an influencing stressor. Whereas the variation for the best value for beta was comparably low, the variation of the best parameter values for alpha and mu was higher and thus, the best parameter values were split for alpha between low and high values. This was possibly an indication for an interference of the estimation of the best parameter of alpha and mu. The problem might particularly occur for sparse data sets and therefore more tests of the optimization process are needed. Another option to improve the parameter optimization would be to define thresholds for realistic values and this way restrict possible outcomes for parameter values, e.g. for the parameter indicating time delay (gamma). Such a method of restrictions for allowed parameter values was tested by Lika et al. 2011 for the optimization of DEB parameter values. However, data about time delays are not accessible yet and could currently be only be based on assumptions by experts.

In the current model, some assumptions led to uncertainties. For example, the assumption that any whole organism response would be affected in a comparable way should be further investigated by a systematic literature review focused on this issue and experiments should be performed if needed. The literature search conducted for this thesis showed that some interaction effects of stressors differed in relation to the type of response being investigated (Lehnberg and Theede, 1979, Cotter et al.1982). This might be the case for other stressors and responses as well. To mitigate the possible bias caused by different response patterns, I tried to focus on observations on a higher hierarchal level such as survival or growth that are likely to be a result of many different responses. As it would be almost impossible and not justifiable from an ethical point of view to test all kinds of responses and potential differences for every single stressor, a mechanistic understanding and the identification of general patterns to explain interaction effects are crucial (Hopper et al. 2013, Segner et al. 2014).

Uncertainty related to transition effect data/ tolerance data

Since most of the publications I found did not focus on precise transition thresholds, testing conditions in the studies varied with regard to the time steps and the number of stressor intensities being investigated. This led to uncertainties in threshold value estimates. Nevertheless, these estimates were used to normalize the data and for this purpose, the preciseness might have been sufficient. An alternative would have been to model data to derive no-effect concentrations as proposed by Kooijman 1996 and tested by Baas et al. 2009 for survival data. I did not apply this approach in the final model to reduce possible errors caused by uncertainty from an imbalance between parameters to be optimized and available data points from literature. However, for comprehensive datasets this alternative method could improve the output of the model.

Main DEB- model

The results for the control scenario are comparable with the study from Saraiva et al. (2012). This is not surprising as I derived the core DEB model from this publication and the substantial addition of the model was the integration of cumulative effects. Smaller differences between the model results could be explained by the use of different input data (see 4.3). For example, the use of phytoplankton biomass data instead of chlorophyll-a data could have also led to some differences in the model output. Nevertheless, the simulated growth of *Mytilus edulis* resembled field observations (e.g. Antsulevich et al 1999, Munch-Petersen and Kristensen 2001).

In comparison with the stressor scenario, the results of the DEB model showed that cumulative effects could influence the impact of the metals Zn, Cd, Cu and Pb on *Mytilus edulis* in combination with elevated temperatures and decreased pH. It was possible to use the DEB model to simulate time-dependent effects of anthropogenic stressors and to integrate these in the context of the life history of an organism. The simulation describes growth and reproduction of a single blue mussel specimen that represents the average individual of one age cohort. For a further development of the model, several model runs could be started with slightly different characteristics and starting times and be combined to an individual based model simulating the response for several individuals that differ from each other. For example, it is possible to introduce inter-individual differences in basic characteristics such as growth rate.

Moreover, the model run with different sensitivities and abilities would simulate possible differences in responses between individuals, which are very likely to occur in nature. Regarding the timespan the DEB model covered in the test, there was a risk that weaknesses in the parameter estimation for the single model refinement added up over time and created a bias. This might e.g. be the case for the single model for oxygen depletion because the generated model does not reflect the response of *Mytulis edulis* to very low oxygen concentrations very well. Here, more data about responses in the lower range would help to improve the model. Possibly, also an additional parameter could be added to improve the model. However, one aim of the present study was to test a generalized method applicable to any stressor without the need for stressor specific adjustments.

There was also a risk that the model overestimated the cumulative effects due to potential double counting: this might potentially occur when stressors were reported to affect several physiological processes but when these were in fact the result of a cascading effect caused by just one mechanism instead of actual independent effects with different modes of actions. On the other hand, it should be kept in mind that many interactions are not yet understood and that for the interactions, for which information was available, synergistic effects occurred more frequently than antagonistic effects. This circumstance could potentially rather lead to an underestimation of the cumulative effects.

Another possible overestimation of the effects resulted from the assumption that an interaction effect observed for one endpoint represents the interaction for further endpoints as well and thus, could be aligned to all DEB processes the stressor affects. However, data on interactions were scarce and by far not all processes were covered. Consequently, the alignment of interactions only to those processes that were investigated would likely underestimate the effects to a large extend.

Moreover, some relevant aspects were not integrated in the model, which could be of relevance in some scenarios. Potentially, a reduced clearance rate could reduce the uptake of metals. I did not integrate a feedback link to cover this aspect. However, the lack of a feedback link between the clearance rate of the main model and the uptake model to calculate the internal concentration of metals might not lead to an overestimation of effects, because such a feedback would only occur at a very drastically decreased clearance rate, as shown by experiments of Vercauteren and Blust (1999).

4.5.3 Discussion of model results for the test scenario

For the species *Mytilus edulis*, the combination of the matrix method and the DEB model was suitable for the analyses as a test with experimental data from the literature and monitoring data showed. The data availability for DEB parameters and for experimental data was good in comparison to other species occurring in the North Sea. The tests showed that relevant aspects of cumulative effects as described in the introduction could be addressed with the combination of the matrix method and the DEB model. The model allowed differentiating between different combinations and intensities of stressors. Further, it was possible to consider interaction effects. The model can be used to evaluate whether the stressor combination at a monitoring station rather mitigated or reinforced the impact on an organism.

Moreover, the model could cover the cumulative effects of some relevant anthropogenic stressors for blue mussels, which are not easily observable in the field such as effects on the number of produced gametes. However, the model did not comprise all relevant anthropogenic pressures blue mussels are exposed to. Impacts such as fisheries had not been covered. To integrate such effects, a population model would be needed. Other anthropogenic stressors could directly be integrated into the model. For example, the model could be easily extended to cover more chemical substances or other environmental variables affecting physiological processes. A combination of the cumulative DEB model with a population model could be a further next step.

Influence of each of the stressors on the main model output

The comparison of stressor relevance showed that the pH value and corresponding interaction effects exerted a major influence on *Mytilus edulis* in the model. The sensitivity of *Mytilus edulis* and other mussels for acidification is well known (George 1983, Zittier et al. 2018). A comparison of these influences including the consideration of interaction effects for the monitoring station at Norderney has not been done before. Further, also temperature had a major effect on the model outcome. Reason for this was not only the influence of increased temperature values on the mussel, but the interactions between heavy metals and temperature, which had a major influence on the magnitude on the effect of the heavy metals.

In addition, copper and zinc influenced the model output clearly. The relevance of zinc in the model can be explained because its concentrations at the monitoring station at Norderney exceeded for some samplings the tolerance values for *Mytilus edulis* (Figure 52 in 7.1). The influence of copper for the overall model outcome can also be explained by an exceedance of tolerance values (Figure 50 in 7.1). Additionally, the interactions with other stressors strongly influenced to results. However, these interactions are actually only based on a few data points and associated with a high uncertainty. Other stressors, such as the oxygen concentrations had little influence on the overall effect on *Mytilus edulis.* In case of this stressor, it needs to be considered that the data availability was sparse and that the single model for oxygen depletion did not well reflect the species response at low concentrations. Indeed, the modeling of the oxygen concentration for Norderney indicated, that the oxygen concentrations were possibly not ideal throughout the study period and that blue mussels might have been affected by oxygen depletion during some periods (Figure 53 and Figure 54 in 7.1). A reduction of the stressors contributing most to the adverse impact on *Mytilus edulis* in the simulation would likely improve the performance of the mussel and stabilize the population.

Uncertainty in the data/ modeling of environmental parameters

The heavy metal concentrations used for the model study were derived from the regular monitoring program and only measured twice a year. Therefore, it was not possible to model seasonal fluctuations. Consequently, a higher frequency of samplings is needed to improve and refine the model output. Regarding heavy metals, the integration of contaminant concentrations in the sediment could be integrated in the model as an advanced feature in future versions. Influence on the release of metal release such as stormy weather and wave dynamics or human activities like dredging might further be integrated. These aspects are so far not covered by the model and thus represent some uncertainty. Uncertainty lies also in the literature data used for the modeling: Since the publication rates are usually not evenly distributed over the various research topics, there could be a bias in the results for specific issues that had been reported only by a small number of authors. Some effects might even be overlooked.

Method comparisons

The method comparison showed that the special treatment for those interactions with bidirectionality did not have a major influence on the model outcome for the test data set. However, this might be different if the model would be tested with another data set, for example with higher heavy metal concentrations. However, so far, it remains unclear, which method would be more appropriate as discussed above in the discussion of the matrix method. The comparison between the integration of interpolated heavy metal concentrations versus and integration of mean values for all stressors throughout the study period showed only marginal differences. One reason for the similarity might be uptake processes leading to similar internal concentrations in both scenarios. One explanation for the slightly stronger effect of mean concentrations could be the process of excretion: When the concentration of heavy metals was smaller, the mussels could excrete a certain amount of heavy metals and this way the mussel might recover a bit.

4.5.4 Relevance for the MSFD and regional assessments

The combination of the matrix method with the DEB model generates explanatory power for observations at monitoring stations and may be instrumental as complementary information for the development of management plans. For the latter application, the model should be run for different monitoring stations and the results should be visualized to identify areas where the stressor combination may be particularly adverse for the species of interest. By overlaying such maps with species distributions, areas of concern can be identified. This technique has been thoroughly tested and applied with other indices and species distribution models (Halpern et al. 2008, Coll et al. 2012, Fedorenkova et al. 2012). I tested the cumulative DEB model presented here exemplary for a dataset at a second monitoring station in Schleswig-Holstein. The test showed that differences between the monitoring stations with regard to the simulated cumulative effects on *Mytilus edulis* were observable in the model results. This indicates that the model can reflect differences in the conditions and would be suitable to compare different locations.

The basic structure of the cumulative DEB model could also be used for other species. Only with a few changes in the parameter values, the input data as well as with small model adjustments, the model will also be applicable for other species. DEB model parameters have been developed by now for many species[14]. To exemplify this, I transferred the cumulative DEB model for *Mytilus edulis* into a model for the oyster *Crassostrea gigas* with the corresponding parameters and conducted a small literature analysis to cover the most relevant sensitivities towards anthropogenic stressors. Differences in the response pattern between the two species emphasized that the model is also applicable to compare different species with each other with regard to cumulative effects.

As an extension of the model, the results for different species could further be combined in an ecosystem model. Even though complex models generally bear the risk of statistical and systematic errors (e.g. Bernem et al. 2000), the negligence of cumulative effects in environmental assessments would also lead to errors in the judgment of risks for ecosystem components and whole ecosystems. Underestimations of impacts caused by cumulative effects

[14] https://www.bio.vu.nl/thb/deb/deblab/add_my_pet/index.html

could prevent governmental stakeholders from setting up respective management plans and legislation and thus, have severe consequences for the survival of the affected species. Furthermore, researchers have put major effort in the development of modeling complex systems and statistical methods for testing the reliability and robustness of these systems. Publications show major improvements in this field of research (e.g. Barrat et al. 2004, Kooijman 2010, Majda and Gershgorin 2011). Consequently, it will be possible to improve the predictability of the present model with increasing data availability and further work on model refinements and extensions.

Many previous ecological assessments applied in the context of the MSFD were based on expert judgements only (see introduction). Most of the time, the simple additive approach had been preferred (e.g. Halpern et al. 2008, HELCOM 2018). However, there is an urgent need to base such assessments on scientific evidence and real data rather than on expert judgement, in order to provide more clarity and transparency for the assessment process. In the context of the Marine Strategy Framework Directive, the model presented in this study will be applicable to evaluate cumulative effects with a special focus on descriptor 1 (biodiversity) focusing on the assessment of the status of species. It could further well serve as method to implement Article 8 (“Assessment”) 1 b ii of the MSFD, which requires a cumulative analysis (“[…] an analysis of the predominant pressures and impacts, including human activity, on the environmental status of those waters which: […] covers the main cumulative and synergetic effects; […]”).

Even though the proposed method offered the possibility to conduct a quantitative analysis of cumulative effects, the literature search showed that still many relationships and mechanistic pathways are not yet understood. Therefore, expert knowledge is still required to estimate e.g. which species are likely to resemble in their response to different stressors. Further, it is necessary to identify what kind of possible adjustments would need to be made if data from one species should be transferred to another species. More research is necessary to understand the impact of cumulative effects on the environment from a holistic point of view, reflecting the ecosystem level as a whole (but see Moe et al. 2013).

4.5.5 Conclusions

The magnitude of cumulative effects depends on the intensities of the stressors in the environment, their temporal and spatial pattern of appearance, and their affinity to interact with each other as well as with the organism itself. The strength of the proposed model for cumulative effects assessment lies within its modular structure, which allows the integration of temporal dynamic responses of the organism to single stressors, interaction effects between stressors, and indirect interaction effects. Metabolic and energetic processes link these interaction effects during the life cycle of an organism. The consideration of complex cumulative interaction effects in contrast to a solely addition of effects resulted in a much better output in terms of plausibility and transparency. The fact that cumulative effects occur cannot be neglected as such effects have been observed for many stressor combinations (see Table 17 in 7.1).

The online tool LiACAT facilitated the visualization of large datasets, while still providing a sufficient degree of detail. The matrix method offered a very flexible approach, because literature data of very different quality and detail could be used: Simple color codes indicated the nature of the cumulative effect and an equation described the interaction being either dependent or independent of the stressor intensities. In future, expert estimations could help to handle cases where literature data from another species give hints to the likely reaction of the species of interest.

The strength of the DEB model is its capability to model temporal dynamic processes and effects as well as of cascading effects and life cycle dependent effects. Furthermore, the model system can predict selected effects comprising both the population relevant effects such as growth and reproduction, and effects on physiological processes. Moreover, the method is very flexible, so that new scientific findings can easily be integrated. The results provided a valuable overview over knowledge gaps and pointed to the kind of data needed in order to get better and more realistic results for cumulative effects assessment. An expansion of the current monitoring programmes to cover seasonal effects would be important. Furthermore, understanding the universal patterns of stressor interactions from a mechanistic point of view is important.

The application of the DEB model allowed analyses of temporal dynamic effects over a longer time span. With regard to the impacts of heavy metals at the study region at Norderney, the model results indicated that cumulative effects reduced the reproduction success and impaired the development of juveniles as well as the growth of a cohort of *Mytilus edulis* from the year 2005.

4.5.6 Further development and outlook

As an extension of the currently implemented DEB model for cumulative effects, further setups of the model could be implemented for different species. The outcome in form of reproduction data, mortality rates etc. could be transferred to a population model (Saraiva et al. 2014). The model results should also serve as data input for a geographical analysis.

An approach to combine DEB models with a network model to simulate trophic interactions, competition for food and predatory mortality and starvation was conducted for primary producers and consumers in the marine habitat by Maury et al. (2007). Interactions with other species groups should be analyzed in the network model, such as changes in food web structure etc. The elements of the network model should be divided further if needed at this level. The division of a species or species group in different size classes and age classes may be important for reflecting vulnerabilities and for food web structure refinement. By applying a network model, not only direct but also indirect cumulative interactions between the stressors could be described.

The method presented in this study refers only to a small selection of stressors. However, more anthropogenic pressures should be integrated into the model: As litter is often ingested during feeding and affects the maximum feeding rate, this pressure should also be integrated in the equation of ingestion rate (e.g. for birds): The litter would accumulate in the body and therefore, the maximum feeding rate is expected to decrease respectively.

Another example for an important impact is the influence of siltation on the feeding success of fish. This hampering effect should also be regarded in the model equation in order to analyze the consequences for the feeding success. However, it needs to be considered that the impairment of feeding activity does not add up cumulatively like litter in the other example. Instead, the impairment is dependent of the present conditions, which might change over time. Other processes of the DEB model can also be altered: Diseases caused by pathogens or elongated migration routes might increase the energy demand for e.g. somatic maintenance leading to a reduced growth or reduced reproduction success. Further types of impacts should be integrated into future versions of the model for new anthropogenic stressors as well as for the ones already integrated, as soon as corresponding data and information are available.

The literature review indicted that the nature of an interaction might not only be determined by the identity of two stressors but also on the intensity and the ratio of the intensity of the stressors to each other and the exposure time. These aspects should also be considered in future analyses of cumulative effects.

4.6 Literature

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5 Automated cumulative impact model for analyzing effects of anthropogenic pressures on habitats – tested for impacts on seagrass meadows

5.1 Abstract

Cumulative interaction effects are likely to be dependent on the intensity of the stressors and their timing. However, magnitude and direction of cumulative effects are often not known or if so, only for a limited range of exposure times and intensities. To utilize the best available knowledge, we developed a modeling tool forming a mathematical network model based on available literature datasets. It can easily update predicted response patterns when new research results become available due to its structure and automatisms. The focus of the model development was to facilitate the integration of cumulative effects with a pre-defined ruleset into a network model based on a set of common mathematical models. The modeling tool applies a ranking of models to select the best models and constructs the network model based on these.

We tested the modeling tool with data about anthropogenic impacts on seagrass meadows and compared a network model comprising cumulative effects with one excluding cumulative effects. For seagrass meadows, some interaction effects had a major influence on the outcome of the network model. Moreover, compared to the model including single stressor datasets only, more datasets could be included.

Therefore, it is likely that the network model including cumulative effects would provide a better fit with field observations. We conclude that a prediction the response of an ecosystem or a species to multiple pressures should not be based on its reaction to single stressors alone, but needs an estimation of interaction effects. A next step would be an exhaustive experimental test of the results in a multi factorial experiment.

5.2 Introduction

Seagrass meadows are of high ecological importance. They provide valuable habitat structure and support a very high biodiversity (Whippo et al. 2018, Lin et al. 2018), with many species listed as threatened (Hughes et al. 2009). Epiphytes on seagrasses serve as food sources for many invertebrates (Kharlamenko et al. 2018, Schanz et al. 2002). Seagrass meadows also improve the water quality by reducing the number of bacterial pathogens (Lamb et al. 2017).

The role of seagrasses for the ecosystem is connected to its traits (Paul et al. 2012, Vergés et al. 2007). The shape and size of seagrasses create optical features providing shelter for many species from predators. Therefore, seagrass meadows serve as nursery areas for fish. Some of these fish species are particularly associated to seagrasses (Polte and Asmus 2006).

Most seagrass species have a high above ground biomass. Therefore they take up a substantial amount of nutrients and have a high impact as a carbon sink (Duarte et al. 2005, Duarte et al. 2013). Seagrasses further mitigate effects of storm surges (Reusch and Chapman 1995) and reduce erosion in dependence of the density of its ramet and its above – and belowground biomass.

While providing many ecosystem functions on the one hand, seagrasses are on the other hand sensitive to anthropogenic pressures and require specific environmental conditions. For example, they prefer rather sheltered areas with low velocity to establish new habitat patches (Schanz and Asmus 2003).

The extent of seagrass meadows declined worldwide by 110 km² per year from 1980 to 2009 due to anthropogenic impacts (Waycott et al., 2009). Approximately every fifth seagrass species was at risk to extinction already in 2011 (Short et al. 2011).

Seagrasses do not form a taxonomic but an ecological group (Hemminga and Duarte 2000). Taxonomically they belong to the angiosperms and the order Alismatales, which comprises many aquatic and wetland plants. Seagrasses are species of this order living submerged in the marine environment and have a grass-like morphology (Hartog and Kuo 2007, Daru et al. 2016). Information on DNA sequences of eight different seagrass families is available (Daru et al 2016) in GenBank indicating the taxonomic diversity of this group. Worldwide there are approximately 70 seagrass species reported (Hemminga and Duarte 2000), which could be grouped to different 'phyloregions' describing their different geographic and phylogenetic origins (Daru et al. 2016). Despite their different origins, seagrass meadows are threatened worldwide by similar threats such as eutrophication, changes in hydrodynamics and fisheries (Duarte et al. 2002, Burkholder et al. 2007, Orth et al. 2006). Nevertheless also some species-specific sensitivities exist, e.g. to water depth (Boscutti et al. 2015).

Because of their high ecosystem value and their endangerment by human activities and anthropogenic pressures[15], the protection of seagrass meadows has been put on the agenda by several legislatives and conventions (OSPAR 2009, OSPAR 2011, OSPAR 2012, HELCOM 2013). In Europe, seagrass meadows are relevant in the Habitats Directive (92/43/EEC) and considered as a 'special habitat' in the Marine Strategy Framework Directive (MSFD 2008, COM DE 2017/848/EU).

Some of the major threats to seagrass meadows are well known and a reduction of those might already improve the condition of seagrass meadows substantially (reviewed e.g. in Duarte 2002). However, a deep understanding of the system-related, cumulative effects could improve the effectiveness of management actions by focusing on the mitigation of potentially occurring positive feedback loops and synergistic effects resulting from different pressures and environmental conditions. This is particularly relevant, when it is unclear why management actions did not result in the intended improvement.

Human activities altered the environmental conditions in the sea and affect seagrasses simultaneously in several ways. Thereby, the effects of anthropogenic pressures are interwoven with each other. For example, the use of artificial fertilizers led to increased nutrient concentrations in the sea favouring the growth of epiphytes and macrophytes, which reduced the light availability for seagrasses (Cabaco et al. 2008, Burkholder et al. 2007). At the same time, sea level rise (Passeri et al. 2015), the construction of dikes (Thu et al. 2019), and offshore wind parks (Zhang et al. 2009) lead to alterations of hydrodynamics. Increased current speed can be unfavourable for seagrasses and additionally also detach snails from their leaves, which feed on epiphytes, exacerbating the adverse effects (Schanz et al. 2002). The resuspension of sediments due to dredging activities further leads to temporary light reduction, apart from the direct

[15] https://www.iucn.org/content/seagrass-habitat-declining-globally

mortality caused by the physical disturbance by hopper dredgers (Do et al. 2012). Reduced light availability can lead to reduced photosynthesis activity and reduced growth (Ralph et al. 2006). Antifouling paint additives used on ships can further reduce photosynthetic activity (Chesworth et al. 2004).

Some human activities cause complex responses. Climate change for example results e.g. in sea level rise as well as increased water temperature. Both of these aspects affect seagrasses. Increasing water temperatures further favour the spread and growth of the protist *Labyrinthula zosterae* causing the wasting disease, which led to a dramatic decline of the seagrass population in the Southern North Sea (Burkholder et al. 2007). A recurrence of this protist combined with exacerbated climate change effects in the future might result in an effect, which is greater than the effect caused by one of these pressures alone.

The complexity of such interactions require special tools to get an overview, to quantify and to assess the cumulative effect of all the anthropogenic pressures from a holistic perspective. The importance of cumulative effects has been acknowledged and the need of its assessment has been integrated in legislations and directives as well as regional conventions (MSFD 2008, COM DE 2017/848/EU,OSPAR 2017, HELCOM 2017). Testing methods for cumulative effects assessment with a focus on special ecosystem components can help to better evaluate these.

Many approaches for cumulative effects assessment conducted so far integrated expert judgement surveys (e.g. Halpern et al. 2008, HELCOM 2017, OSPAR 2017, Parravicini et al. 2012, Brodersen et al. 2018 for seagrass assessments) and most assume additive effects of anthropogenic pressures (e.g. Zacharias and Gregr 2005, Halpern et al. 2008). The idea of additive effects evolved in the context of the prediction of effects of toxic mixtures and was studied intensively. The assumption of additivity is widely applied but also questioned for its overall validity since then (Altenburger et al. 2013, Warne and Hawker 1994, Crofton et al. 2005). As an alternative to additive models, some scientists suggest to apply multiplicative models when the chemical substances are expected to affect the organism independently at different biological targets and use a different molecular pathway (Morse 1978, Abendroth et al. 2011). However, this method was neither always appropriate to explain observations adequately: a meta-study revealed that only 20% of the datasets could be explained by the multiplicative model and only 10% by the additive model (Cedergreen et al. 2008).

Synergistic and antagonistic effects of anthropogenic pressures have been observed for a broad range of organisms (Crain et al. 2008, Hooper et al. 2012, Holmstrup et al. 2010, Moe et al. 2013). Such cumulative effects were more frequently observed than additive effects in multi-factorial experiments on mortality as Darling and Cote (2008) concluded in their meta-analysis. For seagrasses, additive as well as more complex response patterns have been reported for the presence of multiple stressors (e.g. Ceccherelli et al. 2018, Moreno-Marín et al. 2018).

For the development of a method for cumulative effects assessment focusing on habitats, it is important to allow a comparison of assessment results between different ecosystem components. Further, to be able to aggregate different types of effects with different units, a normalization procedure is needed. For the normalisation of different effects, it is common in environmental assessments to calculate ecological quality ratios, where the actual condition or trait of an ecosystem component is compared to a reference condition (WFD 2000/60/EC, Gobert et al. 2009).

In contrast to many other species and ecological groups, for seagrasses some data and methodological approaches with regard to cumulative effects of anthropogenic pressures already exist. Parravicini et al. 2012 applied scores based on expert judgements to define the pressure intensity at the pressure sources and then added an additional variable to reduce the pressure intensity with the distance to the source. To assess the status of the seagrasses, following a common approach used in the WFD, they conducted field studies and compared their results to historical reference conditions reflecting a good environmental status. They applied random forest modeling to link the status of the seagrass to the categories to the anthropogenic pressures by applying regression trees and to predict a categorical status score. Their approach included the effects of anchoring, a commercial harbour, pipe outlets, coastal outfalls, urbanization, SCUBA diving activity, beach-nourishment, and fishery (Parravicini et al. 2012.). The application of random forest modeling for cumulative effects assessment has the advantage that the model does not assume additive effects per se and can also handle non-additive effects (Parravicini et al. 2012). On the other hand, the model did not consider effects of exposure time, provided only categorical outputs, and did not allow generating continuous data as results limiting the generation of a general understanding of relationships by the model. Moreover, due to the application of local expert knowledge, the model is very restricted to the investigated area and thus the model cannot be used for other areas.

Random forest modeling was also applied by Holon et al. 2018 together with spatial modeling to predict the status of seagrass meadows and to assess the impact of human activities. They based their analysis on different impact categories such as “industrial effluents”, “human made coastline” instead of analysing specific pressures like single hazardous substances or certain constructions at the coast. The intensities were normalised based on min and max pressures occurring in the study region limiting the scope of the study results to the study region. Moreover, they focused on a single effect (dead matte cover), which allowed them on the one hand a good model performance for predicting this aspect and to reveal tipping points, but on the other hand did not capture the effects on the variety of biodiversity relevant traits of seagrass meadows and effects on community composition.

In contrast, Kruusemae et al. (2016) included also biological and environmental processes in their model. They modeled the effects of hydrodynamics and eutrophication on the above ground biomass of seagrass under consideration of modeled influences of the associated fauna and flora on seagrass and on relevant environmental parameters with a process-based ecological 3D model to identify areas for potential recovery of seagrass meadows. The model handled nutrient cycles and hydrodynamics in detail but was restricted to these influences on seagrass meadows. Their model was purely data-driven and did not involve expert judgment survey results. Singer et al. 2017, who forecasted the seagrass distribution for different environmental scenarios, also considered species interactions.

A Bayesian network model for seagrass was developed by Maxwell et al. 2015 to calculate the likelihood of ‘high’ opposed to ‘low’ starting seagrass biomass and the additional category ‘absent’. Moreover, the graphical presentation of the network presents relevant feedback loops and important ecological responses. This network was applied to predict the anthropogenic impact under different input scenarios of fishing effort, water movement, sediment grain size and nutrient availability. Unfortunately, the field data they used for model validation were non-uniformly distributed with only a single location with absent seagrass, decreasing the trustworthiness and relevance of the stated accuracy of the model of 100% for predicting the absence of seagrass. Moreover, the suggested bistability may also be an artefact due to the model setup because the number of categories of the nodes of the network were pre-defined to two. This questions if the model can really capture the complexity of the anthropogenic impact pathways in seagrass meadows and would be suitable as a management tool.

Reum et al. 2015 chose to assess seagrass habitats with a qualitative network model. Instead of assessing one trait of the seagrass, they investigated the effects of different species and ecological guilds but focused on only one major pressure: ocean acidification. In contrast to the network model by Maxwell et al. (2015), the output of their qualitative network model was a community matrix containing results for the different elements of the network. Interactions between nodes were derived by literature research and expert consultations. State changes of network nodes were based on explicit plus- and minus signs to represent a decrease of increase dependent on the plus or minus signs of the influencing variables and were weighted by interaction strength. However, this implied that the qualitative network model was suitable for relationships with a clear directivity of response (Reum et al. 2015), but could not cope well with more complex response patterns or interaction patterns, which are dependent on one or several other variables or which were dependent on exposure time.

A geospatial approach to assess the impact of anthropogenic pressures on seagrasses with respect to the upper limit of their distribution was applied by Montefalcone et al. (2019). They applied a simple scoring system for the occurrence and strength of the anthropogenic pressures with only three categories (0-2) for the broader categories urbanization and urban waste, industrial activity, ports, tourism, sediment load, agricultural waste, anchoring, and rip currents. The pressure scores were added up to generate an index for each location. They further used a hydrodynamic model to identify the positioning of the breaking depth and to derive a theoretical natural position. Next, they compared it with the actual upper limit of the meadow they derived from aerial imageries and field studies. The distance between the modeled natural upper limit to the observed actual upper limit was then correlated to the anthropogenic pressure index.

The literature search showed that there is a lack of models predicting the effects of many anthropogenic pressures and human activities affecting seagrass meadows simultaneously, which are solely data-driven, quantitative, work with continuous data instead of categorical data and can handle multiple responses. Moreover, there is a lack of models with a holistic comprehensive view capturing the complexity of interactions and system effects such as feedback loops.

Our aim was to develop a flexible approach to assess cumulative effects on habitats allowing not only multiple drivers of the model but also a variety of model outputs to reflect multiple effects on the habitat. The development of the model was targeted to serve as a science-based tool for cumulative effects assessment with regard to environmental assessments. Moreover, the method should be transparent, repeatable and not be biased by subjectivity. Therefore, we developed a standard procedure to simplify the integration of new scientific insights based on literature data. Instead of a fixed system, we aimed at the development of a very flexible approach. A network of interactions between human activities, anthropogenic pressures and effects on habitats allowing also species interactions and influences of environmental variables should automatically be constructed based on available literature data. The output of the modeling tool should provide results, which can be compared to assessments of other ecosystem components.

5.3 Methods

The overall aim of the applied methodology was a quantification of the magnitude of a cumulative impact driven by a given pressure scenario. First, we conducted a literature search and then extracted and organized suitable data as well as relevant information in an online literature analysis and cumulative assessment tool (LiACAT) on the database mybiOSis (https://kladia.info). The aim was to allow the traceability of the data to the original data source and to provide a suitable structure to choose the data, which should be included in the model. A description of LiACAT can be found elsewhere (Eilers et al. 2014, Jong et al. 2015, Eilers et al. 2020). Therefore, in the following, we focus on the description of the modeling tool. We downloaded and integrated literature data from LiACAT into a modeling tool (ACIM – Automated Cumulative Impact Model), which we programmed for the analysis of cumulative effects. During the development of the modeling tool, we continued the literature search.

We designed the modeling tool to achieve the automatic construction of a network model based only on literature data without any pre-assumptions about structural links between its network elements (environs). The network model should further be easily expandable, when new data become available. The data analysis should be impartial and be based on the mathematical models, which are common in biology. Lastly, the method should be applicable especially in the context of the MSFD and serve for the analysis of different scenarios to test management options, as well as for the analysis of spatial data and chronological sequences.

The modeling tool ACIM used the literature datasets to train pre-defined 'base-models'. These 'base-models' described relationships between one input variable and an effect. They comprised a linear model, a quadratic model, a sigmodal model, a hyperbola, a bell curve and an exponential model. For datasets with several potential explanatory variables, the modeling tool tested the influence of each variable with a 'base-model'. Additionally, it analyzed all possible combinations of the different input variables with all possible combinations of 'base-models'. The modeling tool optimized the parameters of the models and characterized them. Next, the modeling tool identified the best models for each dataset out of the optimized models. Further, a filter with pre-defined criteria defined which datasets should be included for further analyses. The resulting explanatory variables and the response variables the formed the environ nodes of the network, whereas the corresponding models formed the edges. Finally, we defined scenarios and corresponding input values for the network to calculate the effects on the ecosystem component (Figure 21).

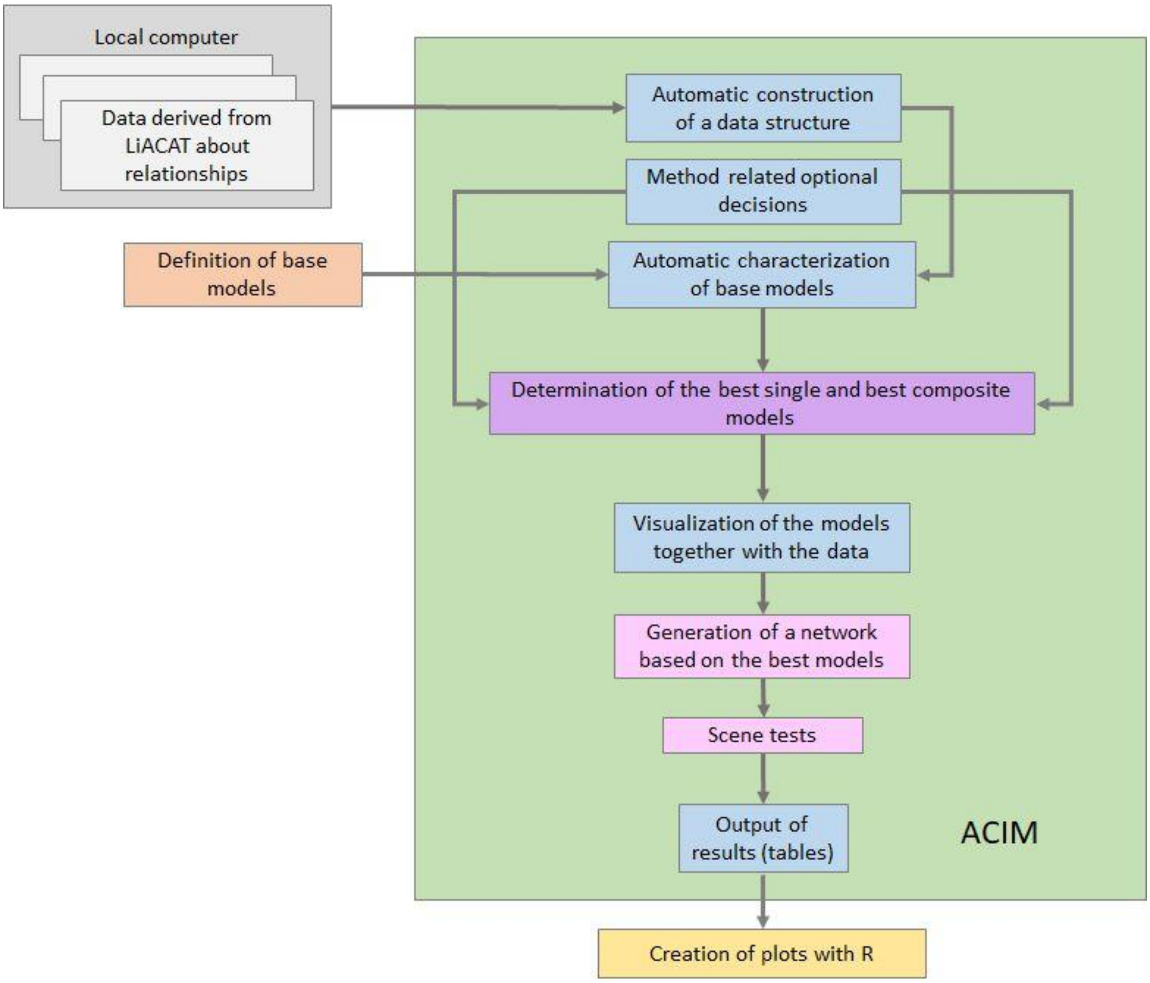

Figure 21 Major processes steps. Abbreviations: LiACAT: Literature based Analysis and Cumulative Assessment Tool – realized on the mybiOSis platform (https://kladia.info/klados/) , ACIM Automated Cumulative Impact Modeling (tool realized with Scilab)

5.3.1 Literature search and literature data handling

A selection of key words used in the MSFD for anthropogenic pressures served as search terms for the literature search (structure of table 2 in the attachment of the MSFD (MSFD 2008/56/EG) listing relevant pressures for the marine environments in the European marine regions). We used the corresponding terms for the pressures as well as the word 'seagrass' and the genus name '*Zostera*' were for the search boxes in Web of Science[16] and occasionally - when only few results were displayed- in Google Schoolar[17]. The literature search comprised topics about the effects of single stressors as well as the effects of interaction effects.

We further collected data about adverse effects driven by environmental parameters, which potentially influence the effects of the anthropogenic pressures or which have a substantial influence on the ecological status of seagrass meadows. We saved also influences of other species (for example the effect of the presence of *Mytilus edulis* on segrass meadows) when they appeared in the search results, but did not search for them explicitly.

[16] https://apps.webofknowledge.com/

[17] https://scholar.google.com

We applied the following criteria for the selection of useful data for the integration into the model:

- We used only data, which dealt with seagrass meadows. Thereby, we preferred data sets about effects on *Zostera noltii* and *Zostera marina* to data about effects on other seagrass species, which do not occur in the Southern North Sea region.
- We preferred data of experiments with various stress-intensities and exposure times. (We mixed data of different exposure times and stress intensities from different experiments when the experimental conditions resembled each other).
- The basic experimental conditions (apart from those, which were manipulated), were supposed to be similar to the conditions in the North Sea.
- The paper provided some basic statistical data and the description of the applied method was transparent and reproducible.

We mainly used publications available through the library of the Carl von Ossietzky University of Oldenburg and the University of Hamburg or freely available papers. However, in cases of access restrictions, we contacted the authors via the social networking site for scientists 'Research Gate' or email. We conducted the literature search to several time points between April 2016 and February 2019 for the different key word and entered the relevant information from the publications in LiACAT. This online tool (Literature Analysis and Cumulative Assessment Tool, LiACAT) was specially designed for the analysis and oraganizatin of literature with regard to cumulative effects assessment (Eilers et al. 2014, Jong et al. 2015).

Basic bibliographic data was either filled in automatically in LiACAT when an endnote file was provided, or entered together with basic information about the content (e.g. about the species investigated, the kind of effects observed and the stressors addressed in the study). We extracted data presented in figures, which showed the relationship between the stressor(s) and the ecosystem component with the free online tool 'WebPlotDigitizer'[18], which is integrated and combined with other features in LiACAT. If provided in the publication, we extracted all measured data points. In other cases, authors showed only means and error bars or standard deviations. In these cases, we extracted only the means from the figure. If data points were not clearly visible e.g. due to overlaps with other data points, we excluded those.

Apart from stress/ pressure-response data, we extracted data needed to simulate indirect effects or pathways the same way. If one publication dealt e.g. with the effect of a certain pressure, which affected the photosynthesis, which in turn affected the growth of a seagrass, we not only used the data describing the effect of the pressure on the photosynthesis but also data about the relationship between reduced photosynthesis and growth if available. In the following, the potential influencing factors are denoted as 'sources' and the influenced variables are denoted as 'targets' to indicate the direction of the effect. Observations can be sources and targets to the same time. This is the case for intermediated effects as described above for the case of photosynthesis.

We saved the extracted data in a tabular structure together with information about units, names of the variables, a link to the publication, species name etc. In LiACAT it is possible to combine

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[18] http://arohatgi.info/WebPlotDigitizer/

these tables from different publications dealing with the same relationship and we used this function where applicable. The structures of these tables is fixed, so that data can later be read column wise into local programs like ACIM. We then downloaded the tables with the data as csv files on a local computer. Before the application of the data to test scenarios, we checked for consistent unit use. In case of inconsistent units for the same variable, we performed unit conversions following standards. E.g. for the harmonization of units for oxygen concentrations in water, we applied the HELCOM-COMBINE standard method (HELCOM 2017). To provide consistency throughout the datasets, the response variables needed to represent an observation of a good environmental condition. If this viewpoint was not provided in the publication, we recalculated the data before application for the model if possible (e.g. we transformed mortality data to survival data).

5.3.2 Data analysis

First, we selected mathematical models, which are commonly observed within the field of environmental biology (see e.g. Otto and Day 2007 and equations 1 to 6) to model the literature data sets (in the following called 'base models'). All these base models allowed a shift along the x-and y- axis (parameters "a" and "b" in the equations below respectively) to fit the data by corresponding parameters. The equations should represent the relationship between potentially influencing factors (sources) and the influenced variable such as the response of an organism (target). Potentially explanatory variables comprised manipulated experimental conditions, environmental conditions as well as exposure time. If the literature data set comprised several potential explanatory variables for the response, the modeling tool optimized each base model for each variable separately. Further, it identified all possible combinations of explanatory variables and models and optimized the corresponding parameters (numbered consecutively p). The set of models comprise

1) a linear model ($y = p_1(x + a) + b$),

2) a quadratic model ($y = p_1(x + a)^2 + p_2(x + a) + b$),

3) a sigmodial model ($y = \frac{p_1 e^{p_2(x+\mathrm{a})}}{p_1 e^{p_2(x+\mathrm{a})} + (1-p_1)} + \mathrm{b}$)

4) a hyperbola ($y = \frac{p_1}{(x+a)} + b$),

5) a bell curve (Gauss-model) ($y = \mathrm{b} + p_1\, e^{\frac{(-(x-\mathrm{a})^2}{(2p_3)^2}}$), and.

6) an exponential model ($y = b + p_1 e^{p_2(x+a)}$)

Thereby, we split the exponential model in an exponential decay model and an exponential growth model by limiting the range of parameter values accordingly and suggesting corresponding initial parameter values (see attachment script 'models'). For the other models, we used ones as initial parameter values.

For the creation of the model combinations, the modeling tool added first additional parameters functioning as weighing factor for the influence of each of the potential influencing variables to each of the base models. It then combined these resulting model-parts for each variable by adding them up (assuming an independent influence) as well as by combining them with multiplication (assuming a dependent influence). In case of a combination of two models, this results e.g. in the equation:

$$model_1 * G_1 + model_2 * G_2 + (model_1 * model_2 * G_3)$$

Thus, the influencing variables will show cumulative behavior by either sharing the strength of the influence on the target indicated by different weighting factors (G_i) of the models of the variables, or by a direct interaction indicated by the multiplicative part of the model being unequal to zero or both. We restricted the possible values for the weighting factors to values between zero and one.

Once the modeling tool created all models, it trained them with literature data and optimized the parameters of the models by minimizing the square error in an iterative process.

Finally, it calculated and saved the number of explanatory variables, the initial parameter values, the optimized parameter values, the mean square error (MSE), the AIC value (Akaike information Criterion), the variance, the adjusted R^2 value, information about the linked sources, the definition of the mathematical model, and the corresponding error function for each model.

5.3.3 Filtering

The modeling tool disregarded all models with an adjusted R^2 threshold of at least 0.6 for further analysis and did not use them to construct the network. We chose this value for R^2 as a compromise to exclude models with a bad fit on the one hand and to include sufficient models to construct a network structure. However, this is just an exemplary value adjusted to the test data and should just show the basic functionality of the modeling tool. We did not apply a statistical test considering the number of data points because in many cases we could only extract the mean value of the raw data from the graphs of publications (see above) and thus a statistical test would not reflect the original dataset and be appropriate. If a variable in the original dataset did not vary in intensity or if the optimized best model had a R^2 value <0.6, we assumed that this variable had no influence of the ecosystem component and it was excluded from the network-model. Even though this assumption is likely to be wrong in some cases, we decided to follow this principle instead of applying expert knowledge to focus on the aim of a data-driven model and to avoid the mixture of assumed and literature based models in the analysis.

Next, the modeling tool categorized the models according to their type into the following categories

- models with one quantity of the stressor but different exposure times ("time models")
- models with one exposure times but different stressor intensities ("intensity models")
- and combined models.

For each type as well as for all models of a dataset, the modeling tool selected the most parsimonious model based on the AIC values. This allowed some flexibility for the construction of the network model and thus we could utilize more literature data compared to the application of only the best models regardless of its category. To review the models, the modeling tool created graphs for each of those models wherever possible (up to 3D graphs for models with two explanatory variables). For the visualization of the models, it applied equally spaced values ranging from the minimum to the maximum of the values provided in the corresponding literature data. Based on these most parsimonious models, the modeling tool constructed a network model representing the interactions between sources and targets.

5.3.4 Construction of the network-model

To construct the network model, the modeling tool combined the identified best models of the datasets from the different literature sources. These included models with several sources as well as models with one source only. Each source and each target represented an element of the network model (environ). Based on the names of the sources and targets of the best models, the modeling tool pooled the environs to a list of source- and target environs without duplicates. Starting with the target environs, the modeling tool created nodes to link source names and corresponding models to them. As a preparation for this, it checked the targets for sources belonging to the target.

In case of overlaps of sources by several models of different datasets (see example in Figure 22, red arrows), the modeling tool chose the model with the smallest AIC value. If this procedure led to the exclusion of a composite model, it used the remaining models derived by the same dataset representing only the influences of the sources one by one (single model). The aim was here to include the corresponding sources in the network and thus to utilize as much information as possible for the network model. For the theoretical case of equal AIC values, the modeling tool sorted the corresponding models for each target first by the number of sources applied for the model and secondly by the sample count of the corresponding dataset.

We then defined input values for all solely sources and compiled reference values for all targets based on a literature research (see 5.3.1). Further, we defined thematic groups for the solely targets. This should allow the provision of a more concise result, because the number of solely targets can potentially be high and thus lead to many individual results – possibly hard to interpret.

Next, the modeling tool calculated the network. It estimated values for all targets of the network model and normalized those between zero and one based on previously researched corresponding reference values of the targets for seagrass. If the target environ was an inner element of the network model, this calculated value served as input value for the targets it was connected with. The modeling tool followed this procedure until all value ratios for the solely targets were calculated. Based on the concept of effect addition (Folt et al. 1999, Backhaus and Faust 2012, Loewe et al. 1927), we assumed that effects impairing the same response parameter (biological endpoint[19]) add up if we didn´t have further information about an interaction.

[19] The term biological end point here not only relates to response on molecular level, but also on whole organism responses such as growth.

Therefore, the modeling tool calculated the sum for the aggregation of the results of the models at nodes of the network.

After the calculation of all target values, the modeling tool calculated mean values for each of the thematic target groups based on the results of the solely target values. We sorted the target names to the thematic groups “biological effects”, “chemical composition”, “growth”, “nutrients”, “photosynthesis”, “reproduction”, “survival”, “vitality” and “other effects”.

As a result of the whole procedure, the modeling tool produced tables with data defining the models and providing AIC-, R^2, adjusted R^2- an mean square error- values as well as a table showing the results of the target values together with the reference values and a table showing the results for the thematic groups.

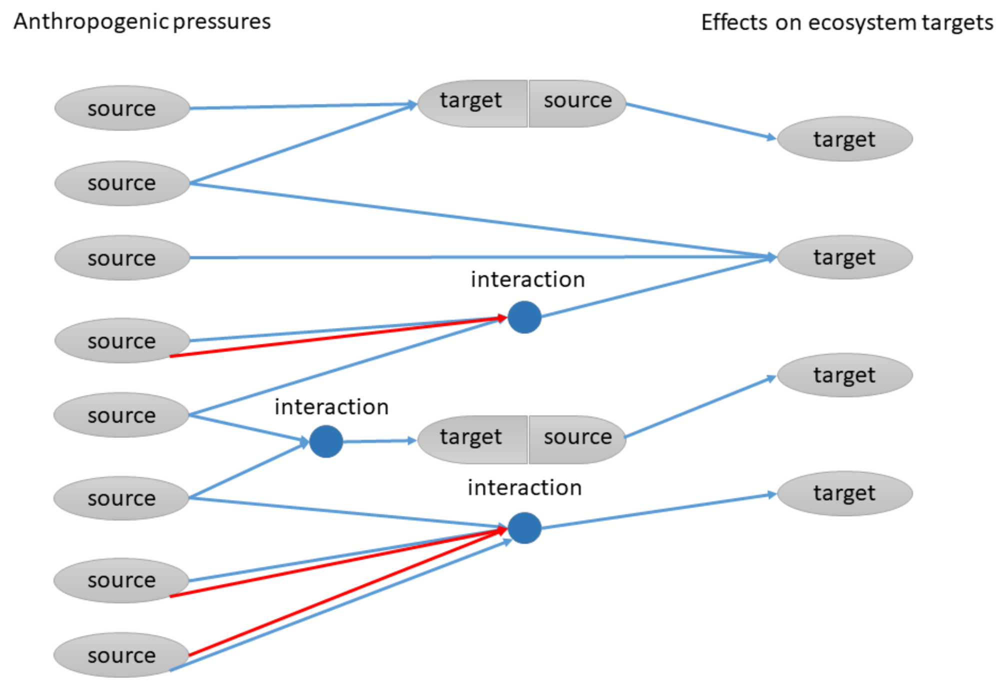


Figure 22 Schematic visualization of different kinds of literature data. Examples for overlaps between two models of different datasets highlighted in red

5.3.5 Analyses

One aim of the construction of the network model was to reveal the theoretical type of interaction between different stressors. We wanted to figure out if there is a special characteristic of the interactions for the study system. The modeling tool should help to figure out if the stressors act in combination predominantly additively or multiplicatively and if interaction factors are predominantly positive or negative. We used the information about the calculated parameter values indicating the weight for the models to check each of the models for a multiplicative and for an additive term.

Moreover, we wanted to figure out if the consideration of cumulative effects matters for the network structure. Therefore, we set up a network for only considering “single intensity models” neglecting cumulative effects and influences of exposure time and compared the results to the network model including cumulative effects. The modeling tool produced both types of results automatically. Further, we checked how much literature data was applicable for the network model by comparing the number of all potential explanatory variables (sources) to the number of sources actually used in the network model. Lastly, we counted how many times the modeling tool used each of the mathematical model types in the network model to figure out if some model types are characteristic for the study system. We produced the graphical presentations of these results with Scilab as well as with R-Studio based on the tables provided by the modeling tool.

5.3.6 Implementation

We wrote the scripts for the modeling tool with Scilab, an open source software for the numerical computation and high level programming language[20]. First, we designed the basic structure of the script. Based on this structure, we added further functions to the scripts and made changes concerning the data analysis and presentation of the outputs of the model. The program structure for the modeling tool consists of three different scripts: one main script, one script for the mathematical base models and the construction of model combinations, and one script with a focus on the links between network elements and the formation of the network model. Thereby, the main script called the two latter mentioned subscripts (see structure in Figure 23).

The modeling tool got the literature data from a local folder for model creation and parameter optimization. To optimize the computing power and corresponding time of the modeling tool, we included a special function so that the modeling tool saves the created models permanently. Thus, after the first time the model runs, it only creates models for new literature datasets. This way, one can use the tool to update already constructed network models.

For each of the target environs derived from the literature datasets, the modeling tool created nodes. It then created models based on the literature data and attached all relevant information to the environs by using indices. For each environ, the modeling tool aligned the following information from the literature data:

- information about the name of the source or the target,
- the unit related to the environ,
- indices values representing links to other environs,
- information about the status of the status of the environ indicating if it was processed in the model already,
- information about a calculated value in case of target environs,
- an aggregated value, which is particularly relevant, if the target environ is connected to several sources,
- a group name for the effects,
- and a reference value for the target (for sources this field stayed empty).

The modeling tool then attached indices for the model nodes to the target environ nodes to connect the environs to the corresponding model nodes. Lastly, it identified indices for

[20] https://www.scilab.org/

connecting the model nodes with the corresponding source nodes and attached them to the model nodes of the targets. The modeling tool calculated the network as described above and saved the results as csv data.

We tested the modeling tool with data concerning effects on seagrasses. Thereby, we pooled data from different seagrass species. We assumed that seagrasses are in general sensitive to the same set of pressures and resemble in their general response patterns. Nevertheless, the tool attaches species names from the literature data to the environs. Consequently, it is possible to entangle effects of different species if needed.

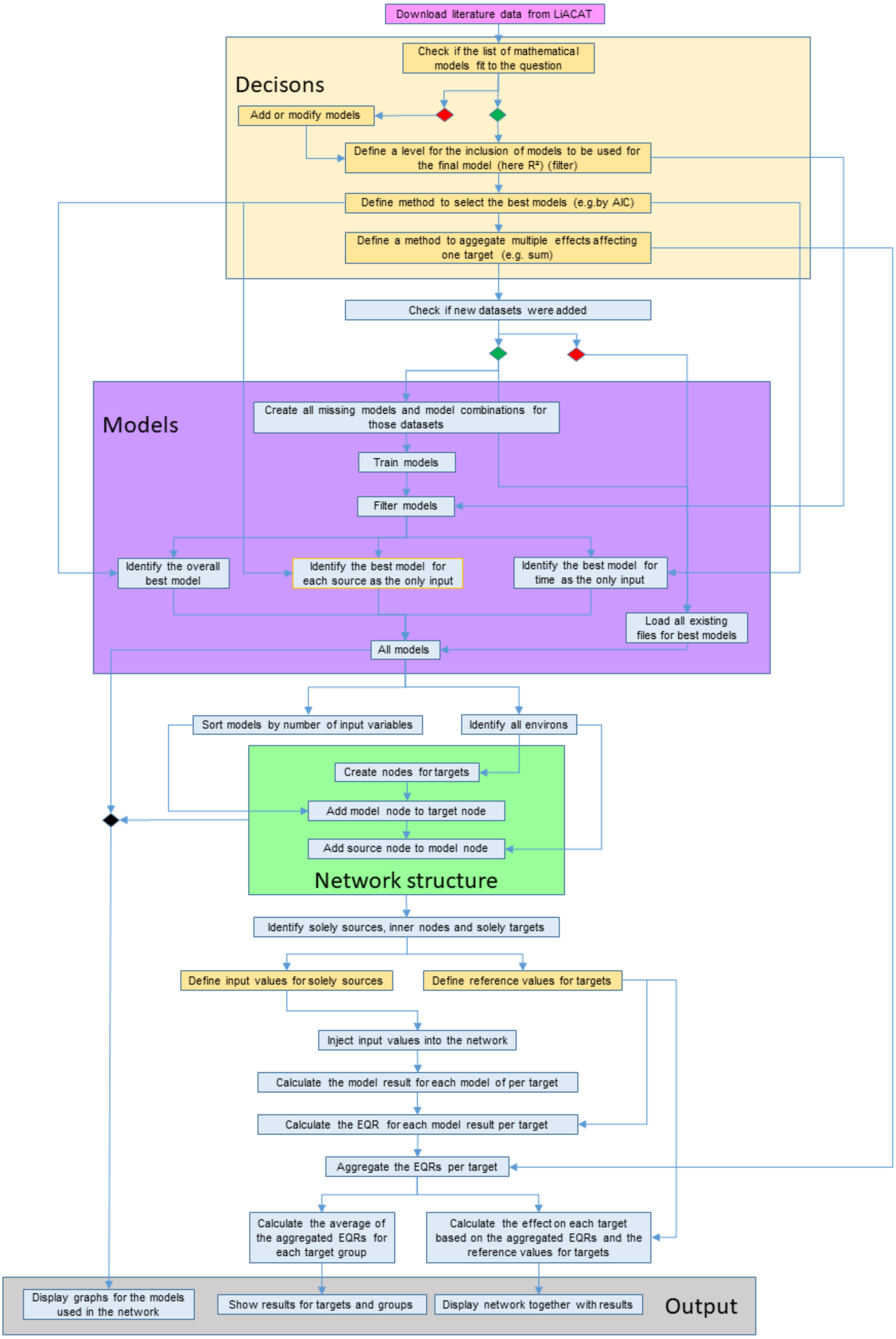


Figure 23 Structure of the model with the most important procedures

5.3.7 Evaluation

To test the functionality of the modeling tool and to evaluate if it provides reasonable outputs, we applied the modeling tool for data about seagrass meadows. We chose data about seagrass meadows due to their important ecological role and because comparably much literature data about effects due to anthropogenic pressures and environmental influences were available. The practical tests should give insights to the model behavior.

We used the names of the targets as search terms for a literature research to get data about corresponding suitable reference values. Additionally to these terms we added "seagrass*" and "Zostera" as search terms. We conducted the literature research in 2017, 2018 and 2019. 'Web of Science' served as the main literature database. However, if we did not find the required information, we used 'Google Scholar' complementary. Moreover, we screened the literature already collected for the construction of the network for suitable reference values. For the construction of scenarios we analogical used the names of the identified solely source names as search terms. Thereby we aimed at getting realistic values for the situation in the North Sea and suitable values for the development of scenarios. Therefore, we added the search term "North Sea" to the source names as search term for the literature research.

The reference values for targets with regard to seagrass meadows were characterized by high concentrations of chlorophyll, carotenoids and sucrose concentrations in seagrasses reflecting a good resource use capacity (chlorophyll, Zhao et al. 2016), protective properties (carotenoids, Zhao et al. 2016) and good health status (sucrose, Govers et al. 2015). The value for the photosynthetic rate should reflect measurements under control conditions (optimum conditions for seagrasses). Thereby, we defined the absence of an inhibition of photosynthesis as the optimum. We set reference values for growth- and vitality indicators according to the maximum mean values measured or the values measured at the control conditions (in most cases these were the same, see Table 18, 7.2). Regarding organism and cell-survival, we defined 100% survival and 100% green leaves (0 percent necrosis) as the optimum. Species composition data got individually reference values for each single target theme (Table 18, 7.2). We set the erosion indicators (bed shear stress and eroded sediment mass) to values at which erosion was unlikely to take place (Widdows et al. 2008).

We tested three different major scenarios of anthropogenic pressure situations: The first scenario reflected oligotrophic conditions with lower nutrient conditions, higher light availability and more oxygenated water. Moreover, the water contained no herbicides. The copper concentration comply with the optimum value for *Zostera marina* (Zhao et al. 2016) as copper is an essential metal. The pH values reflected mean values measured in the German Bight (based on monitoring data of a station at Norderney, Table 19, 7.2). For velocity, we used the maximum tested velocity of the literature datasets included in the model. This max value was in within the range of values measured in the North Sea, but clearly below average of a monitoring station at Norderney. No burial occurred in this scenario (for details see Table 19, 7.2). We set the time of the year to summer time and simulated the effects on potential targets for one month for the described conditions (for details see Table 19, 7.2).

The second scenario mirrored a substantially higher nutrient load, lower light availability and oxygen depletion. The concentration of herbicides and the copper concentration in the water represented maximum values measured in the North Sea if available. The other input values remained the same as in the first scenario (for details see Table 19, 7.2). This scenario should reflect eutrophication and chemical pollution.

In the third scenario, eutrophication and chemical pollution pressures mirrored a stronger pressure. Therefore, we doubled the value for nitrogen load and copper and reduced light availability and oxygen concentration by 50% with respect to the values used in the second scenario. We treated herbicides differently because the concentrations measured in the North Sea were clearly below the lowest concentration used in the experimental dataset and it would have not been possible to see an effect of the influence of increased herbicide concentration by only doubling the highest concentrations measured in the North Sea. Therefore, we multiplied the herbicide concentrations by 100. We set the sediment height to an increase of 50 cm to model effects of disposal of sediments as a further anthropogenic pressure. The environmental variables remained the same as in the second scenario (for details of the scenario definition see Table 19, 7.2).

Additionally to the three scenarios, we tested the model behavior by gradually increasing the anthropogenic pressures. For this, we used approximately the minimum and maximum values reported in the applied literature data and the modeling tool created equally spaced steps between these values (Table 20, 7.2).

5.4 Results

5.4.1 Literature analysis

Most papers dealing with pressures affecting seagrasses dealt with effects of eutrophication, altered hydrodynamics, and climate change. Mortality and growth were the most frequently studied effects. Moreover, the literature search revealed a network of interlinkages describing the pathways from pressures to effects (Figure 24). The LiACAT tool allowed the visualization of the results of the literature search in a concise way while still providing traceability to the original sources, data and extracted information.

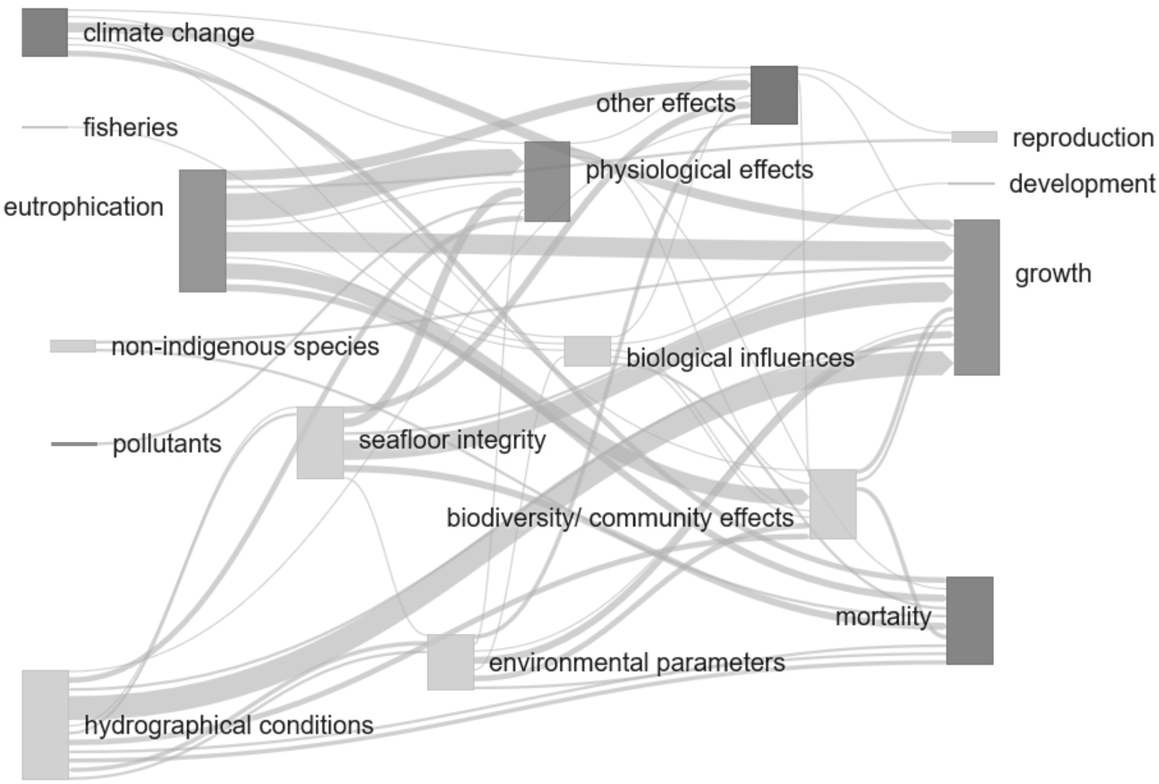


Figure 24 Graphical visualisation of the results of the literature search. The thickness of the lines indicate the number of relationships found in literature. The boxes represent groups and include in most cases several different elements as indicated by the number of lines linked to them. Data from publications showing that a relationship between two variables did not exist were not included in the graph.

From about a hundred papers selected in the literature search, we could use 14 papers comprising 80 relationships for the construction of the quantitative network to analyse effects on seagrass meadows. The pressures included eutrophication, chemical substances, physical disturbances, alterations of hydrodynamic conditions, and acidification (Figure 25). Thereby we considered season and exposure time as additional influencing variables. The modeling tool selected about 82.6 % of the potential explanatory variables after the filtering of the models for R^2 values above 0.6. The best models, selected by the smallest AIC value, comprised nine models with two input sources. The rest of the models for the relationships comprised only one input variable. The modeling tool identified cumulative models with more than one input variable with the involvement of the input variable velocity and burial as well as with the involvement of exposure time as explanatory variable particularly often. It identified 16 solely sources, 41 solely targets and three inner environs (bed shear stress, shoot density, and shoot density) (Figure 25). Some of the environs (e.g. effects on photosynthesis, the influence of copper and velocity) dominated the network due to the availability of corresponding literature data.

When the modeling tool constructed the network only with models with one input variable, the number of environs was less. The network then comprised 16 solely sources, 35 solely targets and only one inner environs (Figure 25), because some models got a smaller R^2 value than the ones comprising more than one variable and were therefore excluded from the network construction.

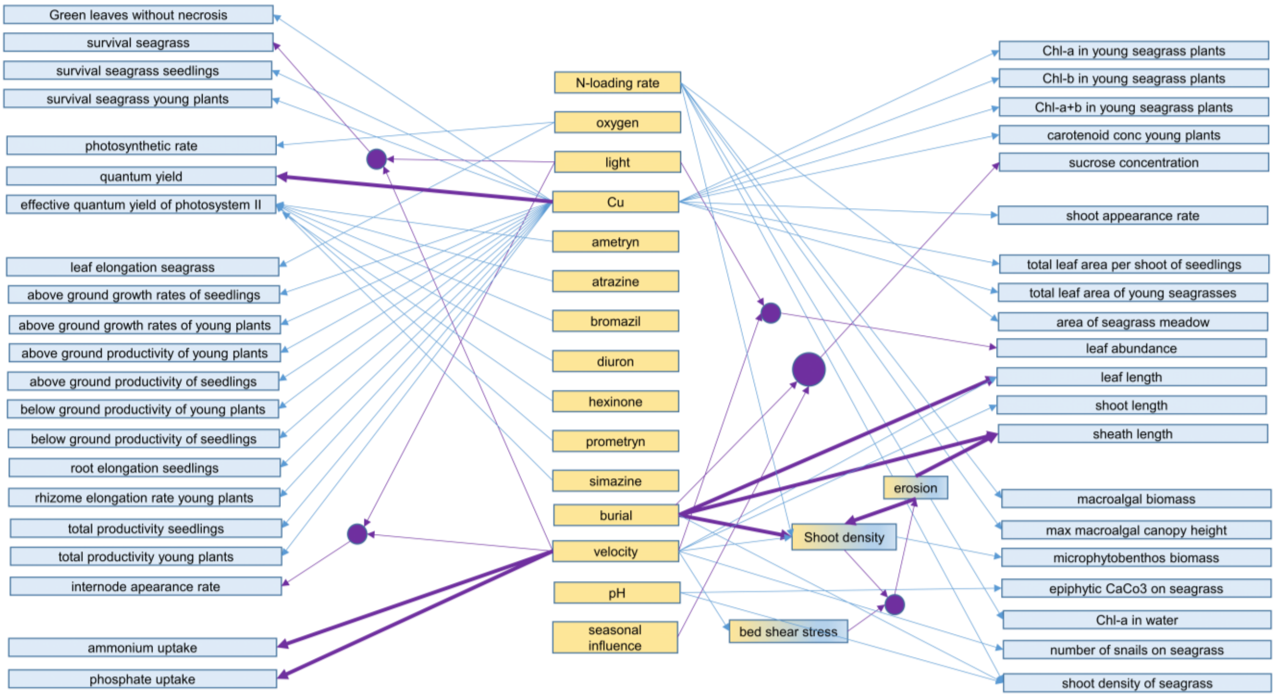


Figure 25 Visualization of the relations between sources (yellow) and targets (blue).Modeled interactions between stressors: violet dots, violet arrows: interactions with time, blue lines: single-variable relationships

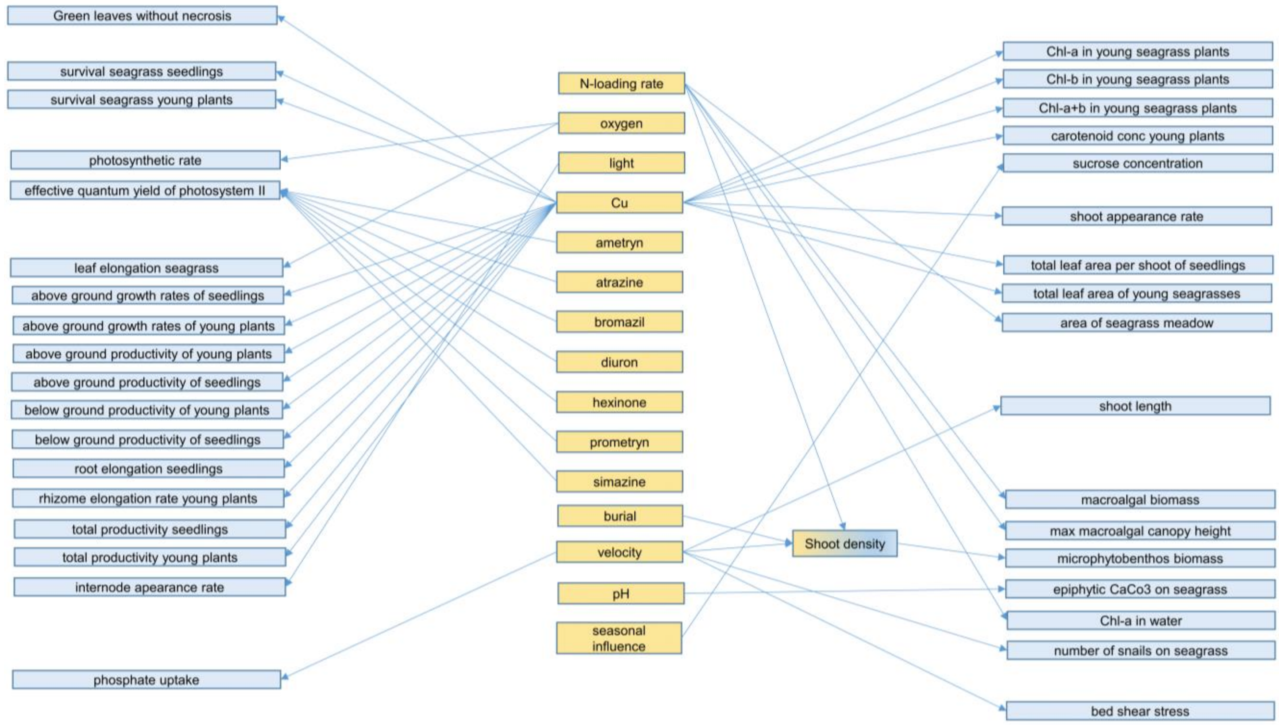


Figure 26 Visualization of the relations between sources (yellow) and targets (blue). Modeled interactions between stressors: violet dots, violet arrows: interactions with time, blue lines: single-variable relationships

5.4.2 Model selection

In the cumulative network, the modeling tool selected the hyperbolic, linear, and exponential decay models most frequently as one-variable models, whereas it selected the Gaussian and the quadratic model less frequently (Figure 28). The model for exponential increase as well as the sigmoidal model fitted to none of the datasets best. Among the composite models, it identified the hyperbolic model and the linear model most frequently for reflecting the influence of one of the explanatory variables. Even though the modeling tool never chose the sigmoidal model for a one-variable model, it could best explain the influence of one explanatory variable in one composite model. For the composite models, the modeling tool identified additive as well as multiplicative parts with different weights and no clear pattern (Table 9).

When we run the model with single-variables models only, the hyperbola, the linear model and exponential decay models were still the most frequently used models, but the difference to the other models was not so clearly pronounced (Figure 27). As for the cumulative network, the modeling tool never chose the sigmoidal and the model for exponential increase.

All of these observations referred to the best model selection based on the best AIC value. When we applied the least square error, the modeling tool chose the linear model less frequently. However, apart from this, the results were comparable.

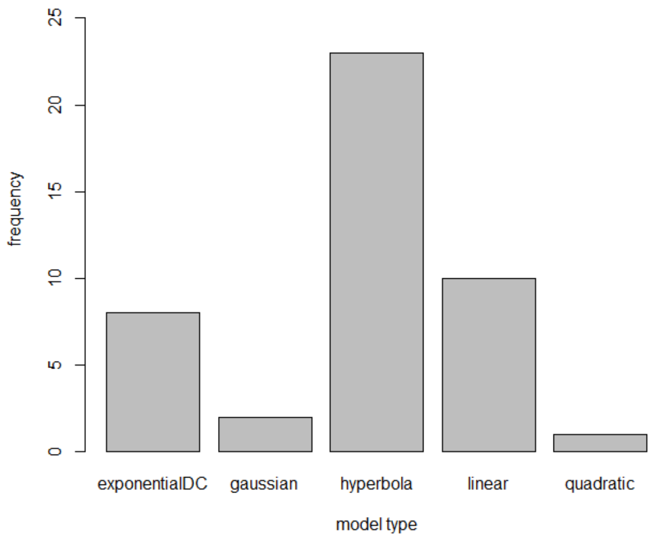


Figure 27 Frequency of the determined best fitting models (only including single models as potential models)

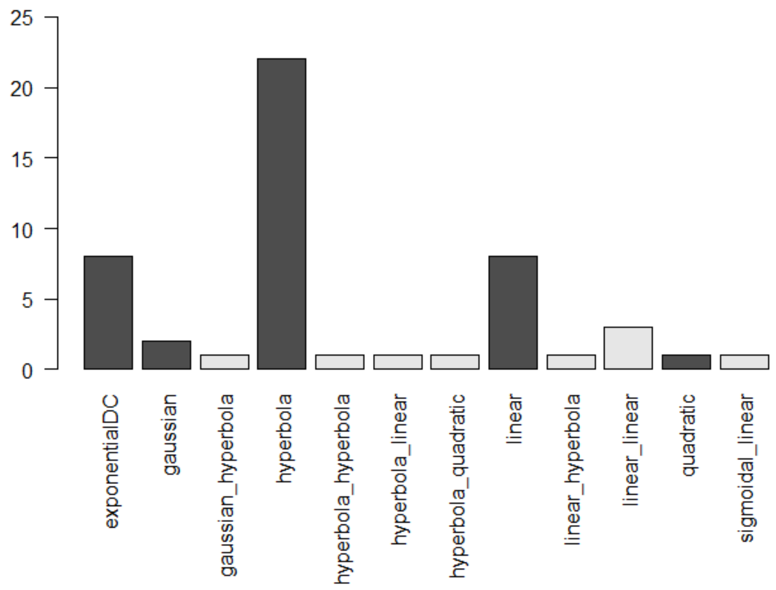


Figure 28 Frequency of the determined best fitting models including composite and single models

5.4.3 Method comparison - cumulative and single models

Overall, the modeling tool identified 43 targets for the network integrating cumulative effects, whereas it only identified 37 targets with the network based on one-variable models.

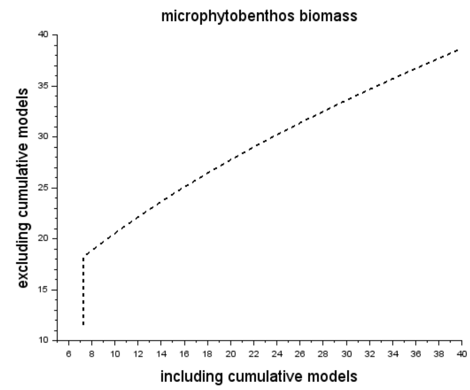
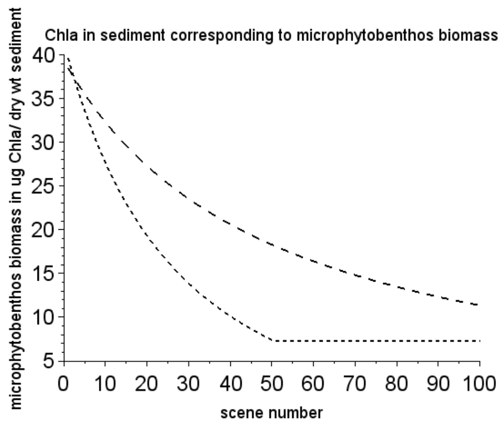
A comparison of the result of the first scenario (oligotrophic scenario) with the reference values set for the targets based on literature values showed that the estimated targets of the network integrating multi-variable models differed less from the reference values than the ones calculated with the network based only on single-variable models.A comparison between these two types of network with regard to the results testing 100 pressure scenarios showed that most results correlated with each other (see Table 7 for those cases where the results differed from each other). The modeling tool estimated generally higher values for the 'phosphate uptake' when the network model based on cumulative effects was run than for the network model based on single models only. The 'effective quantum yield of photosystem II' decreased very quickly with increasing stress intensity in both types of network models. For the targets 'microphytobenthos biomass', 'shoot density', and 'sucrose concentration in seagrass' we observed intersections of the model results of the both types of network models (Table 8). Thus, it depended on the strength of the pressure which type of network model predicted stronger effects.

We did not find a significant difference between the mean of the R^2 values of the two methods for constructing the network model (Figure 29).

Table 7 Comparison of input variables for the network model including multivariable models and one variables models affecting the same target and in the most right column Pearson correlation coefficient between the network models with regard to the target response for uniformly increasing pressure scenarios

Sources and models used in network model including multi-variable models	**Sources and models used in network model with one-variable models**	**Target name**	**Pearson correlation coefficient for uniformly increasing pressure scenarios**
shoot density (*Zostera noltii*) (linear)	shoot density (linear)	Chl-a in sediment corresponding to microphytobenthos biomass	0.9501
velocity (hyperbola)	velocity (hyperbola)	shoot density *Zostera noltii*	0.9501
Copper and time (hyperbola and hyperbola)	ametryn (exponential decay), atrazine (exponential decay), bromacil (exponential decay), diuron (exponential decay), hexazinone (Gaussian), prometryn (Gaussian), simazine (exponential decay)	Effective quantum yield of photosystem II	1
velocity and time (Gaussian and hyperbola)	velocity (hyperbola)	phosphate uptake seagrass	0.9999
burial and seasonal influence (linear and linear)	seasonal influence (hyperbola)	sucrose concentration in seagrass	0.9980

Table 8 Left column: Correlation between the cumulative model (including single and composite models) with model based on single models only. Right column: Simulation of model results with increasing pressure intensities (100 scenarios). dots: cumulative model, dashed line: model based on only single models

<table>
<tr><th>Correlation</th><th>Model behavior under uniformly increasing pressure situations</th></tr>
<tr><td>
</td><td>
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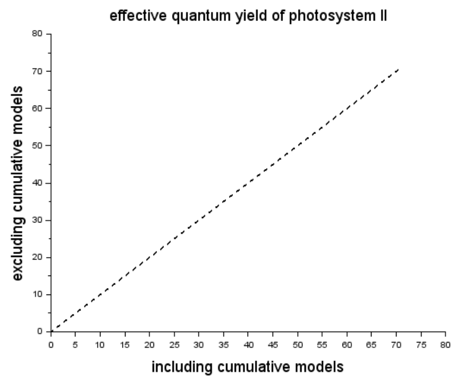
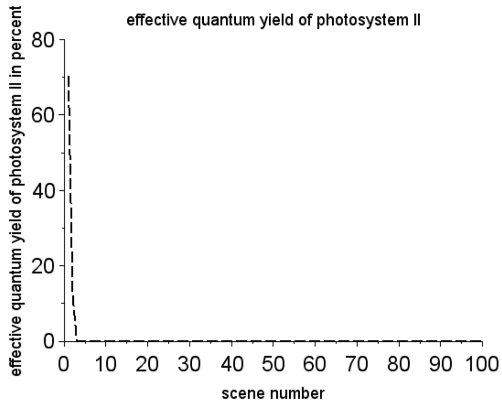
<tr><td>
</td><td>
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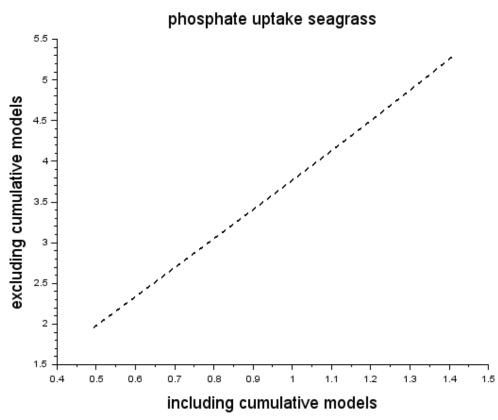
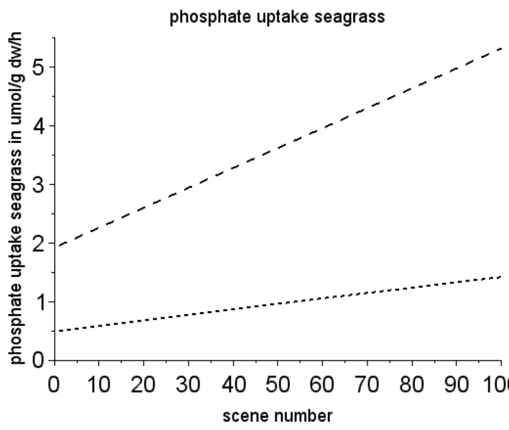
<tr><td>
</td><td>
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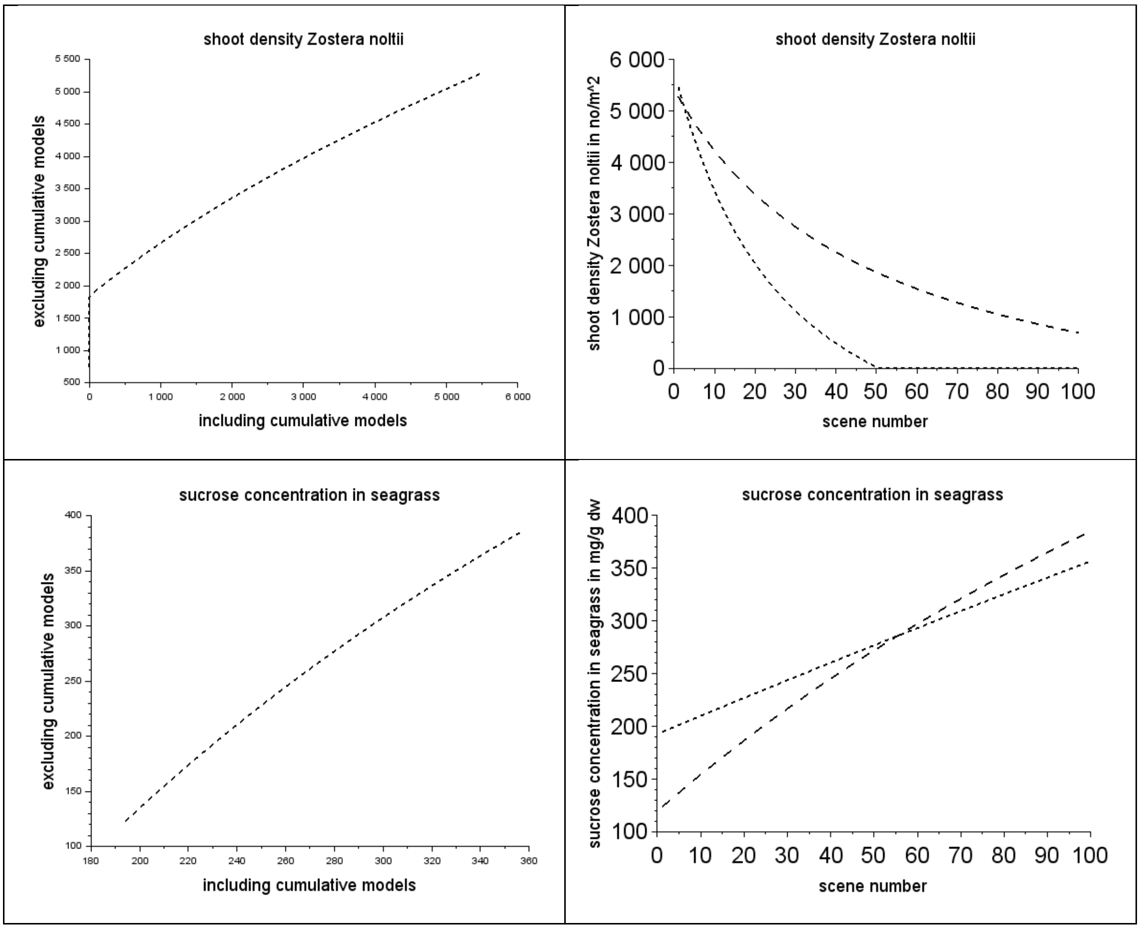

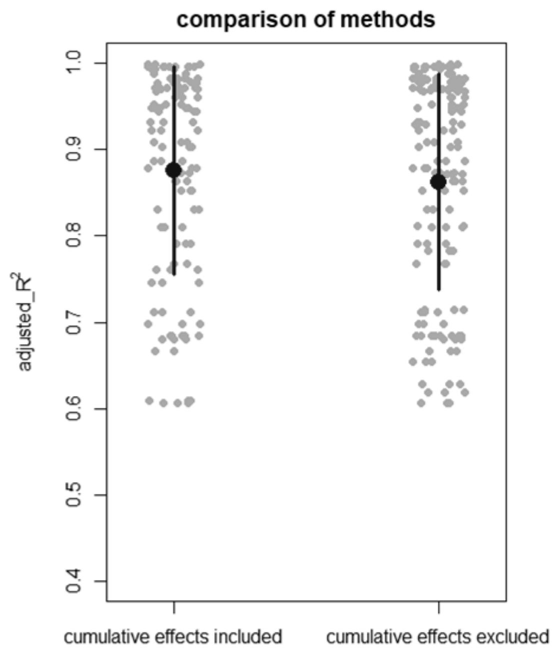

Figure 29 Comparison between the model based on single models only and the model based on all available models with regard to the adjusted R^2 values

The model outcome of the three scenarios reflecting a low-pressure scenario, an intermediate-pressure scenario, and a high-pressure scenario differed from each other (Figure 31, Figure 32, and Figure 33 - Figure 38). Moreover, we observed some differences between the model outcomes based on the network model including cumulative effects opposed to the network model excluding cumulative effects (Figure 31 and Figure 32). The mean of the normalized results of the target group ‘nutrient uptake’ was clearly lower in the model including cumulative effects in all three scenarios, compared to the model excluding cumulative effects. Moreover, the outcome with respect to photosynthesis differed between the methods, with a lower mean of the normalized results for the reference scenario of the cumulative network model compared to the model excluding cumulative effects. The mean of the values with regard to the chemical composition decreased with increased stress intensity in both method options. In the target group ‘vitality’, the mean values were generally higher in the model comprising cumulative effects. However, in the network model excluding cumulative effects the difference of the two stress scenarios to the reference scenarios was greater than in the model including cumulative effects.

Even though we screened the literature specifically for interaction effects, only few datasets were appropriate for the analysis and could be included into the cumulative network model. Thus, most models in the cumulative network model were single-variable models and less than a quarter of the models were composite models with two influencing variables (Figure 30).

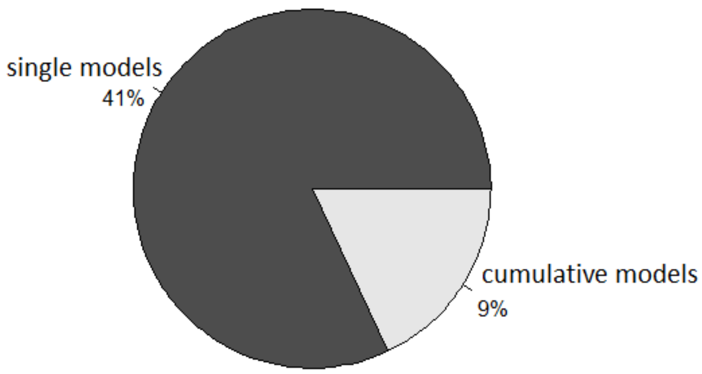

Figure 30 Frequency of model types

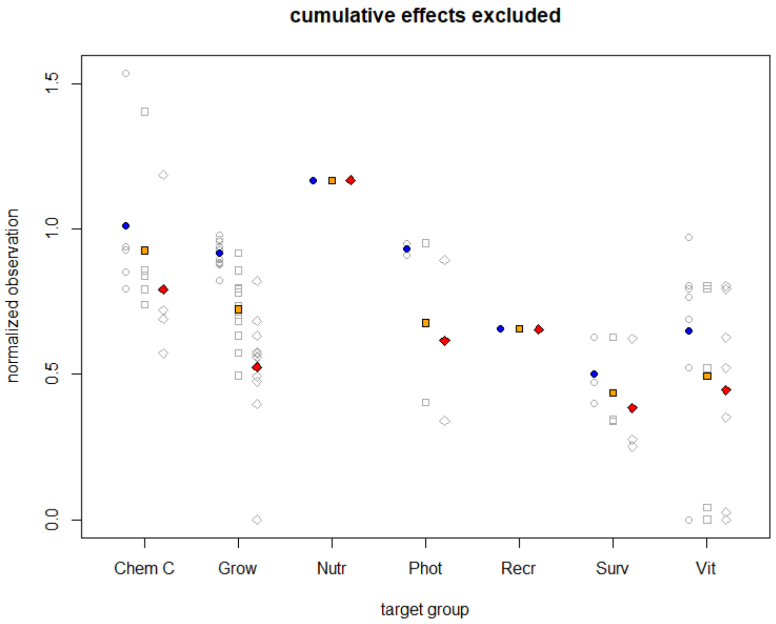

Figure 31 Calculated results of an intermediate scenario (yellow) and a high-pressure scenario (red) in comparison to a reference scenario with respect to the observation topics chemical composition, growth, nutrients, photosynthesis, recruitment, survival and vitality. Results of network model including cumulative effects

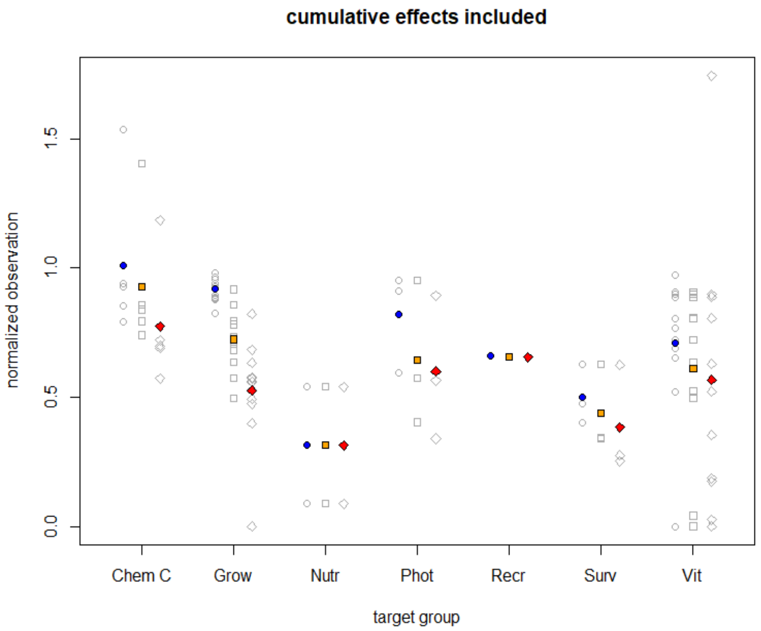

Figure 32 Calculated results of an intermediate scenario (yellow) and a high-pressure scenario (red) in comparison to a reference scenario with respect to the observation topics chemical composition, growth, nutrients, photosynthesis, recruitment, survival and vitality. Results of network model including cumulative effects

Table 9 Cumulative effects. Multiplicative versus additive weights

Target	Source 1	Model part for source 1	Weight for additive model part 1	Source 2 (and 3)	Model part for additive source 2	Weight model part 2	Weight for multiplicative combination of models
ammonium uptake seagrass	velocity	hyperbola	0.561	time	linear	0	1
Eroded sediment mass	Bed shear stress	linear	1	Shoot density seagrass	hyperbola	1	
Leaf abundance rate	light	hyperbola	0.975	velocity	quadratic	0	1
Leaf length *Z. marina*	burial	linear	0.993	time	linear	0.109	1
Leaf length *Z. noltii*	burial	sigmodial	1	time	linear	1	1
Phosphate uptake	velocity	gaussian	0	time	hyperbola	0	1
Quantum yield	copper	hyperbola	0.981	time	hyperbola	0.959	0
Sheath length	burial	linear	0.963	time	linear	0	
Sucrose concentration	burial	linear	0.789	Seasonal influence	linear		

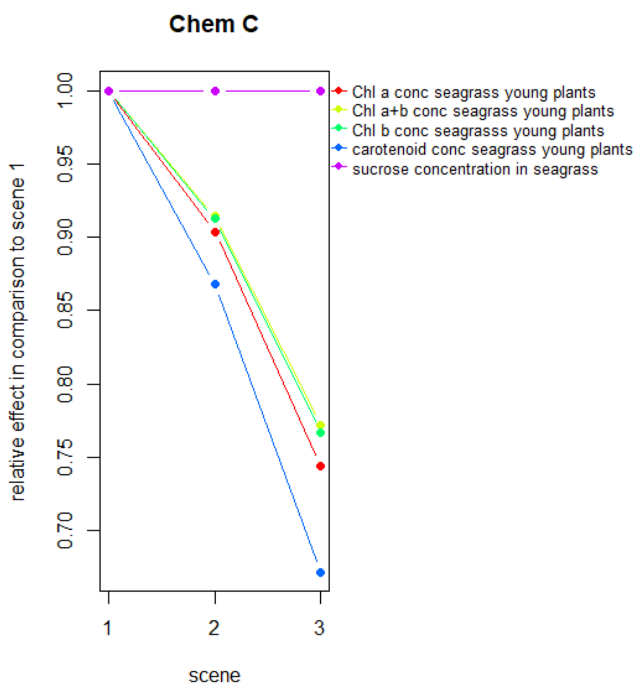

Figure 33 Results for effects on the chemical composition based on the intermediate scenario (scene 2) and the high-pressure scenario (scene 3) with respect to a reference scenario

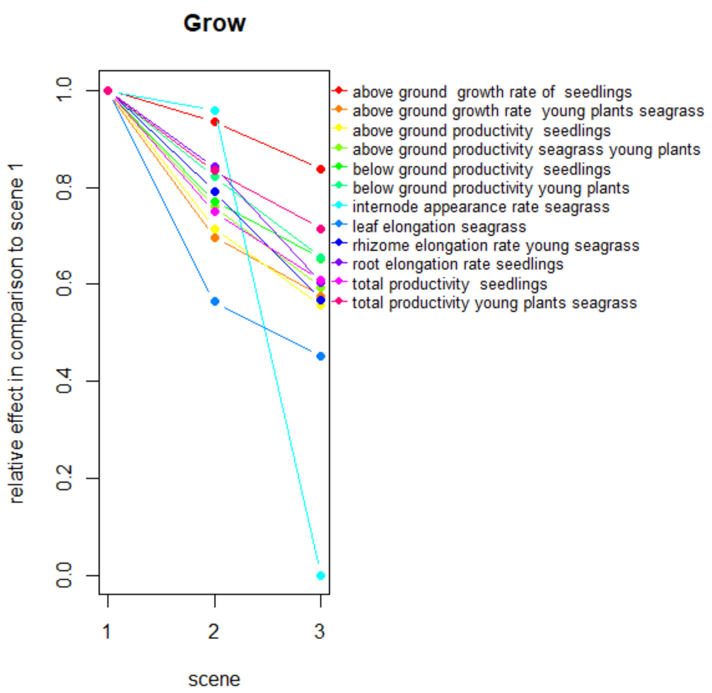

Figure 34 Results for effects on growth variables based on the intermediate scenario (scene 2) and the high-pressure scenario (scene 3) with respect to a reference scenario

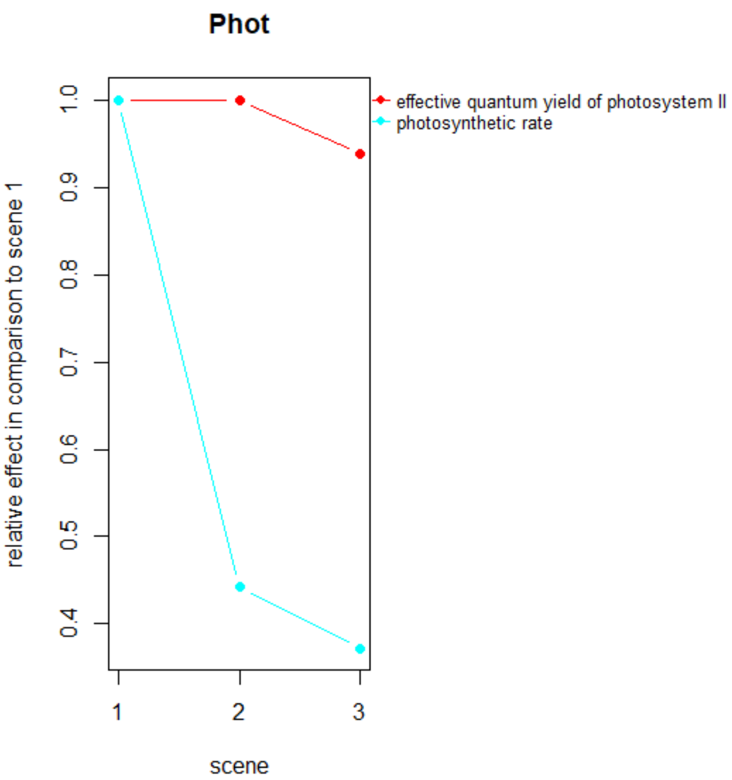

Figure 35 Results for effects on photosynthesis based on the intermediate scenario (scene 2) and the high-pressure scenario (scene 3) with respect to a reference scenario

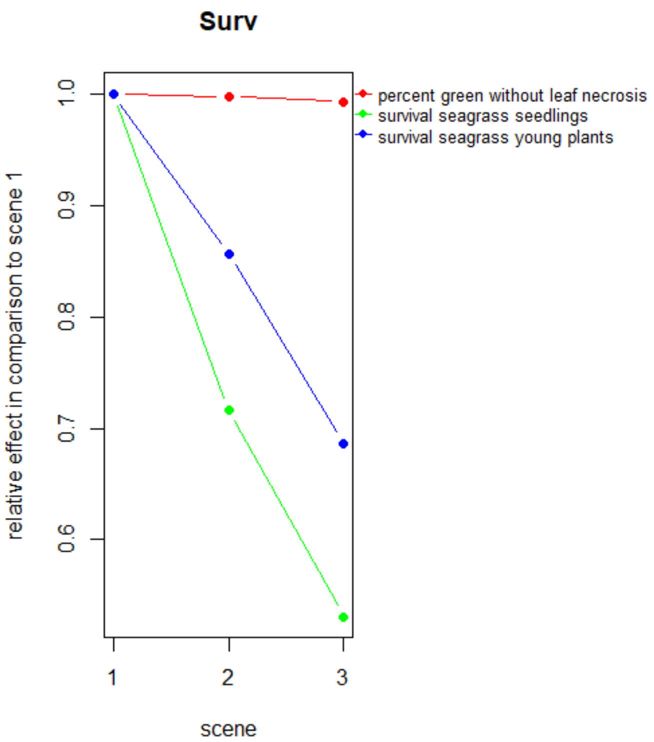

Figure 36 Results for effects on survival and necrosis based on the intermediate scenario (scene 2) and the high-pressure scenario (scene 3) with respect to a reference scenario

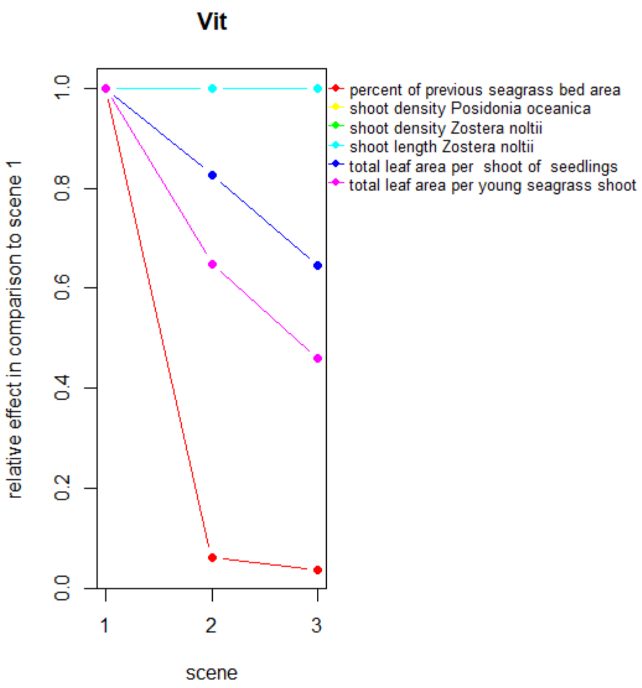

Figure 37 Results for effects on variables concerning vitality based on the intermediate scenario (scene 2) and the high-pressure scenario (scene 3) with respect to a reference scenario

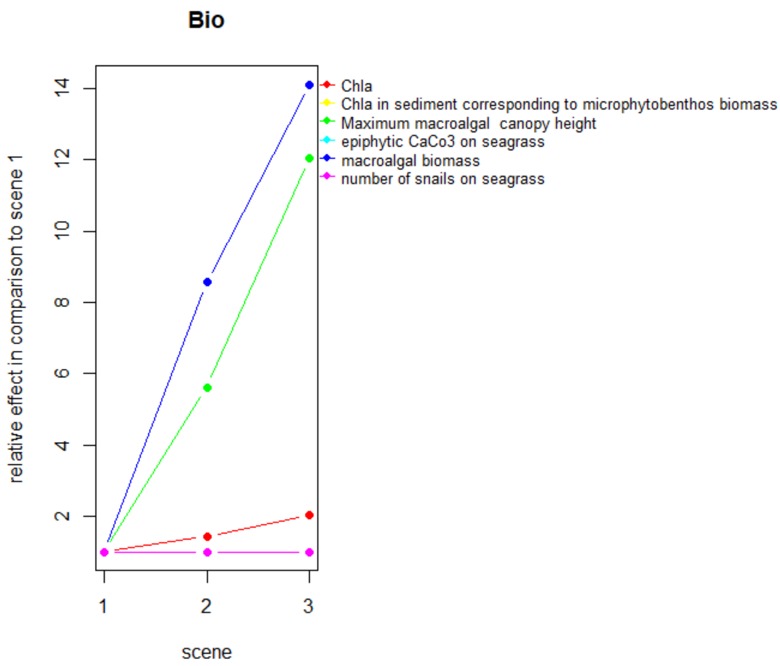

Figure 38 Results for effects on biological variables based on the intermediate scenario (scene 2) and the high-pressure scenario (scene 3) with respect to a reference scenario

5.5 Discussion

The application of the method developed revealed the state of art of cumulative effects of anthropogenic pressures and human activities on seagrasses. The generalized approach for the analysis of cumulative effects in an automated fashion led to new insights of the nature of cumulative effects indicating the relevance of effect addition, multiplicative response and the magnitudes of the contribution of different stressors to the observed effects.

5.5.1 Literature search

The literature search revealed the most frequently studied effects and pressures. The results indicate a strong relevance of the pressures eutrophication, hydrodynamics, climate change and chemical pollution for seagrass meadows. The online tool LiACAT with the inbuilt Sankey diagram[21] served as a suitable tool to save, structure and to present the literature data in a concise way, while providing traceability. We identified knowledge gaps for pressures such as fisheries and the introduction of non-indigenous species. Although these impacts clearly affect seagrass meadows, we only found a few publications about these topics. Moreover, even though many relationships concerning chemical pollution were applicable for the model, those comprised only few substances. Moreover, particularly data for low concentrations and long exposure times were missing. The lack of these ranges affected also the suitability for the test for correlations as the data were non-uniformly distributed. Overall, data about many theoretical possible effects were lacking and only a small percentage of datasets comprised effects of influences of two or more stressors. One reason for that lied in the pre-requirement that the dataset needed to contain different intensities of each of the influencing stressors to be included in the model. Such datasets were rare. However, we needed those for the model to capture the influence of stress intensity and thus to analyze if they had a linear or non-linear influence. Moreover, we cannot not guarantee that all existing literature, which fulfilled the criteria, was included in the model and the database, because we did the literature search at different time points during the last years. Therefore, e.g. some recent publications might be missing. The literature search revealed pressures, which affect the same target but did not belong to the same dataset and thus to the same experimental setup. It would be interesting to figure out if interaction effects occur for these in experimental tests and to describe these mathematically. Thus, the literature search provided inspiration for new research topics.

5.5.2 ACIM

The structure of the modeling tool with its special combination of automatic model selection, model optimization, and generation of a network based on literature datasets together with a link option to an online literature database complemented other existing tools for network generation and model development (e.g. Liu et al. 2008, Courtney and Bianconi 2016). Thereby, the model has a focus on applied nature conservation and environmental assessments.

It allowed comparing different datasets with respect to their potential cumulative effects by calculating parameters indicating the nature and the strength of interactions. The application of the network for the assessment of seagrass meadows revealed new insights concerning system related impacts. Several drivers and a feedback loop for example indirectly influenced the predicted effect on chlorophyll-a concentration in sediment, which reflected the microphytobenthos biomass. However,

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[21] https://kladia.info/klados/

we need more data to populate the network. It is also important to identify between which environs no direct relationship exists. To build the network, we could only use a subset of existing data because the input variables should have different intensities. Additionally, the pre-requirement of an adjusted R^2 value of > 0.6 further excluded some models. This method discarded in some cases even complete data sets, because none of the pre-selected models fulfilled this criterion for them. Therefore, the network created with ACIM, was less complex and had less relationships and inner nodes than the network shown as Sankey diagram, which included all relationships reported regardless of these requirements. This highlighted that many relationships are generally known, but are not quantifiable, at least not under the consideration of varying stress-intensity and exposure time. The program (ACIM) itself could handle a larger amount of datasets (about 100 or more). It is advantageous that the modeling tool updates the network model by creating new models only for the newly added datasets and loads the other models from a special folder. In general, the setup of the script enabled the handling of many data files in a time-saving manner by a decoupling of model- and network generation and scenario testing. Model optimization took the most time and we could not reduce it. Thus, if one enters hundreds of new data sets to the same time, additional computational power could be useful.

We need more data to review and to improve the fitting of the models. Morover, a review of the models for plausibility of an expert would be useful. However, this should rather be the exception and only be done for clearly implausible models to mitigate the integration of subjectivity in this method. The advantage of a strictly automatic procedure was the strong reduction of pre-assumptions and subjectivity. The automated method triggered new insights about relationships and system behavior and revealed data gaps, needed to understand the whole spectrum of the anthropogenic impact on seagrass meadows.

For further developments, it would be helpful to integrate a unit conversion module based on agreed standard units. HELCOM developed such for the Baltic Sea Reports[22] (HELCOM2017). For some conversions, special methods need to be developed for those with a similar meaning and effect but based on different measurements, which were not directly convertible. One example was 'erosion': the magnitude of erosion was reported in the literature as loss of sediment in cm as well as loss of sediment mass. To calculate the mass lost due to erosion, it would be necessary to integrate additional information such as sediment composition.

The complexity of the model was moderate. However, the management of the names of the sources and targets is a potential limitation of the model, when a large amount of datasets would be integrated. Therefore, a prober management of environ names e.g. by grouping by themes and by providing lists of names to choose from, would be a helpful tool as the system grows.

5.5.3 Discussion of decisions made during the development of the modeling tool

To minimize potential problems for the target environs arising due to the mix of data sets dealing with different species and to allow comparability between target topics, we applied a normalization procedure based on optimum values to handle the different inputs. However, in ecotoxicology, lethal concentrations of substances at which half of the individuals die (LC50) are commonly used to unify stressor intensities (Hoekstra 1991). This would have been another option to normalize the data. In contrast, in biological assessments, ecological quality ratios values are often used to normalize the

[22] http://www.helcom.fi/Documents/Action%20areas/Monitoring%20and%20assessment/Manuals%20and%20Guidelines/Manual%20for%20Marine%20Monitoring%20in%20the%20COMBINE%20Programme%20of%20HELCOM.pdf

effects of anthropogenic pressures (WFD 2000/60/EC), which resembles our normalization procedure. We chose to apply a method similar to the latter because it provides a more goal-oriented system perspective. We chose such a perspective to keep in mind that the overall aim to protect seagrass meadows not only means to prevent seagrass species from dying but also to protect the functions they provide. For the protection of seagrasses as habitats and its associated typical biodiversity, some traits of seagrasses are indispensable such as a certain shoot density, which responds to increased anthropogenic pressures.

Based on the concept of concentration addition (Folt et al. 1999, Backhaus and Faust 2012, Loewe et al. 1927), we aggregated the results of different sub-models by summing them up as default method in the network model, when no data about multiple stressor effects were available. We chose to calculate the sum for aggregation also because Vijver et al. 2011 found that this is the most frequently observation for multiple pressures in a meta-study for different taxa. However, this assumption becomes less relevant and the uncertainty of the model will decrease when more data become available. If sufficient data about several influences are available, the optimization procedure of the modeling tool identifies if an interaction occurs and with which weight each of the different sub-models contributes to the result. Compared to other methods (2.3), we consider this as further development of model generation in the context of conservation biology and environmental assessments.

We propose to summarize the results of the network model by calculating the means for target groups of effects or even to calculate an overall value for the impact on a habitat for a given scenario. This way it would also be possible to conduct statistical tests about the significance between different scenarios. However, there were not enough data available to perform such a test and to gain a meaningful result, because many relationships remained unexplored.

Moreover, some of the targets belong to the same topic but may have different implications. This is particularly relevant in the target group comprising biological targets. A large leaf could for example on the one hand indicate a good overall condition of a plant or on the other hand be a result from a high competition pressure affecting the overall condition of the plant and be a response to that. Thus, the grouping of targets always needs careful consideration with respect to the research question addressed with the modeling tool.

In contrast to random forest models (Holon et al. 2018), the present model is a tool to explore particularly the interlinked complexity between multiple anthropogenic pressures and the variety of traits and characteristics of a habitat being affected by them. Moreover, the present model goes beyond categorical results and estimates predicted effects as continuous numbers. However, the present model needs to be validated with data and should therefore rather be seen as a starting point to give a first idea about potential cumulative impacts on seagrass meadows with a focus on ecological relevance. The model should be continuously be fed with new insights and new knowledge to overcome the limitations of its current limited scope due to data gaps and relationships, which are not yet understood.

The present model provides insights to the influence of anthropogenic pressures independent of their geographical distribution. We expected that the entanglement of the pressure intensity from the geographical distribution increases the understanding of the system and therefore focused on this. However, we suggest that next the geographical cumulative aspects, should be integrated. This argument is particularly relevant for anthropogenic pressures with a high intensity but a local distribution at crucial patches such as newly colonized patches with a low biomass and small area covered with seagrasses. Such patches can be of high relevance for the spread and thus the recovery of seagrass meadows. The modeling tool is in its current state already applicable for a geographical approach by using input data for pressure intensities with coordinates. The modeling tool would then calculate results for each geographical spot and the results could serve as input data for impact maps.

We constructed the modeling tool based on a selection of commonly applied models in biology. The modeling tool identified the best model only based on mathematical aspects. With an increasing amount of available data, the modeling tool might select in some cases other types of models as the best model. Adams et al. 2017 recently raised the question how abstract the selection of a best model should be and compared arbitrary chosen parameters with biological meaningful parameters. They concluded that the most useful models were those, which not only had a good fit to the data but also had biological meaningful parameters. A biological meaning did neither play a role for the best model selection nor for the parameter definition. Therefore, it is particularly important to use the model only within the range of the literature data. We suspect that the consideration of biological meaning for the parameters would expand the scope of the model and refine the output. Adams et al. 2017 presented how a best model selection can be done both with consideration of statistical criteria as well as on the 'ease of obtaining biological-meaningful parameters' with an example for a model for the effect of temperature on photosynthesis. For our model, we would need to develop such a method for each relationship. This would be time-consuming and reduce automatism, but be feasible.

5.5.4 Frequency of models

The clear dominance of selected non-linear models suggested that such models are useful for the description of effects caused by anthropogenic stressors and thus need more attention in environmental assessments. We could not explain the high frequency of the hyperbolic models by overfitting because they had the same number of parameters as the linear model. Hence, the solely use of linear models would have oversimplified the response of seagrasses towards anthropogenic pressures by the neglect of other potential models. Moreover, the influence of exposure time was not always linear. This result was not surprising as acclimation processes play an important role in many response patterns and those are typically non-linear (Villazán et al. 2016). The fact that the modeling tool selected several composite models over one-variable models illustrated that the consideration of several influencing factors increased the explanatory power of observations. Hence, the integration of several influencing factors in risk analyses and assessments might increase predictability of effects caused by anthropogenic pressures. However, we need more data for further testing this hypothesis.

If the best model selection of the present paper would be correct, what would this mean from a biological point of view? In a study dealing with foraging, Green and Myerson (1996) interpreted a hyperbolic response as follows: 'A foraging environment is one in which the hazard rate decreases with increases in waiting time' [...] 'the rate of temporal discounting will vary depending on the characteristics of a species environment as well as how that environment impacts individuals at different stages in their life history' and highlighted that the 'discounting rate varies inversely with the amount'. Following this thought, this would mean that seagrasses were able to slow down the rate of detrimental effects (in case of hyperbolic models for exposure time) or that they responded e.g. with defense mechanisms, adjusted dependent on the magnitude of the stress intensity they were exposed to. Opposed to that, to some types of anthropogenic pressures seagrasses could not respond in such a way and could thus not mitigate the effect rate caused by the anthropogenic pressure resulting in the choice of an exponential decay model instead of a hyperbolic one. Enzyme kinetics, which are commonly described by the Michaelis-Menten function, a special form of the hyperbolic model

(Michaelis-Menten 1913), also play a substantial role in response patterns and likely shaped the response curve of some datasets.

We expected the selection of a Gaussian model for copper, as copper is an essential metal and is needed in low concentrations for various biological processes, e.g. for plant growth (Lajayer et al. 2017). However, the modeling tool chose in most cases the hyperbola, in three cases linear models and once the exponential decay model for describing responses to copper. We assume that the modeling tool did not select a Gaussian model because there was a lack of data for very low concentrations of copper as the publications focused on the analysis of copper as a stressor. Likewise, seagrasses have an optimum for velocity. On the one hand, they need low velocity so that water movements cannot flush away their roots; on the other hand, they need some velocity for the distribution of their seeds (Ruiz-Montoya et al. 2012). Here, the modeling tool selected a Gaussian curve only for one dataset, whereas it selected a hyperbola for most relationships involving velocity. We assume that the reason lies in the uneven distribution of velocity values covering more high than low velocities.

Surprisingly, the modeling tool selected a Gaussian model for the response to some herbicides and to burial when we chose the least square error as a method. However, when we applied the AIC, the modeling tool selected more exponential decay models for all herbicides indicating that overfitting could have been an issue in these datasets. However, for all these cases, it needs to be considered if the relevant range of stress intensities and exposure times was covered. A part of a Gaussian curve behaves nearly linear in a particular range and certain parts of the exponential decay model, the hyperbolic model and the Gaussian model share a similar shape for the part where the response variable decreases. Thus, to answer the question which model would be suited best to describe a relationship with certainty, data for all characteristic parts of the model need to be available.

5.5.5 Additive and multiplicative interactions

The analysis of the nature of cumulative effects did not show a general pattern for the combination of influencing variables. We could not confirm the hypothesis that multiple stressors usually lead to additive responses. Neither could we confirm that a multiplicative model would better suit response patterns due to anthropogenic pressures. Instead, many relationships seemed to contribute with different weights to the response. Moreover, the results indicated that a multiplicative part as well as an additive part could reflect response patterns. This could mean that an interaction pertained in some cases only as a certain part of the amount of the stressor or that time-dependent influences on the stress-intensity such as defense mechanisms or self-accelerating processes only addressed a part of the stress the organism experienced.

5.5.6 Comparison between the network model including composite models and the network model only comprising models with one influencing variable

The modeling tool preferred multi-variable models to one-variable models. We assume that the input of more information improved the outcome of the model making it more realistic. However, it is not possible to figure out if this assumption held true with the present study due to the lack of suitable data for model validation. A comparison of the mean of the adjusted R^2 values showed no significant difference between the network model including composite models and those excluding composite models. However, this does not prove a lack of a significant difference between these two model setups. Instead, the clear dominance of single input models in the model setup allowing cumulative models, could just as well be the explanation for a lack of significant difference between the mean of adjusted R^2 values. As the number of publications dealing with cumulative effects increased during the last years, this test should be repeated with more evenly distributed datasets.

The comparison of the network model including multi-variable models with a network model only based on one-variable models revealed that the choice of method mattered. There was a difference in the response of some targets towards increasing pressure intensities with regard to the model behavior as indicated by the test of correlation as well as a difference in the magnitude of the predicted effects on the targets. The difference between these two methods was in most cases dependent on the stress intensity. It remains unclear, which of the models provided results that are more accurate. However, the results highlighted the relevance of cumulative effects and questioned the hypothesis that effect addition is generally the best option to predict cumulative effects.

In conclusion, the assumption of linear effects that add up without consideration of exposure time and cumulative effects would oversimplify the complexity of the response of seagrasses towards multiple anthropogenic threats.

5.5.7 Uncertainty of the model

The comparison between the target values set as reference values and the results of the first scenario differed for some targets by more than 50%. The reason for that might be the different experimental conditions applied for the different datasets used and the fact that in some experiments seagrasses did not reach its full capacities in the datasets derived from publications (e.g. seagrasses did not reach a survival of 100% (Villanzan et al 2016), which was the value we set as the reference target value). Moreover, many linkages in the network model are unknown. We also integrated data of different species of seagrasses in the model and some datasets were very sparse. Thus, there is some uncertainty in the outcome of the models. Lastly, 'scenario one' does not reflect the best conditions for all of the targets. For example, a lower nutrient availability will have rather a positive effect on some targets and a negative on others. Instead of creating an optimum scenario for seagrasses, the motivation for the reference scenario was to create a scenario with oligotrophic conditions and low anthropogenic pressures. Especially for the biological targets, it is difficult to define reference values. We chose those values to reflect good conditions for seagrasses based on corresponding literature. In the future, the overall target for the construction of scenarios, which regard to ecological assessments, should generally rather mirror a healthy ecological balance than to reflect a certain abundance of single species or species groups. Given a higher data availability, the application of biodiversity indicators would be a good option to achieve this.

A validation of the model results is still pending. To achieve this, it would be necessary to set up a large-scale experiment with multifactorial design and to test it with literature datasets comprising all relevant relationships. This was not possible within the scope of my thesis due to time constrains as well as due to the lack of corresponding facilities.In general, the literature search showed that there is a lack of data about the relationships between anthropogenic pressures and effects on seagrasses. In particular, the number of different intensities and exposure times could improve the robustness of the model. This might lead to a selection of different best models as well as to different estimations for parameter values compared to the estimated ones in our model run. Further, if sufficient data become available, we could set up separate network models for different seagrass species. This way we could identify potential species-specific cumulative effects. In the present study, our focus was on setting up the structure of the network and we chose a broader perspective referring to effects on seagrasses in general. To allow the consideration of a larger number of pressures, we sacrificed the species-specific view. Based on some literature (Boscutti et al. 2015, Burkholder 2007) we assume that pressures having an adverse effect on one seagrass species are likely are have adverse effects on other seagrass species as well. We argue that the addition of data from an additional species added information of likely effects on seagrasses in general. Jayathilake and Costelle 2016 showed this kind of effect. Their model predicted best the global distribution when they pooled data from different seagrass species, genera and families compared to a model based on a distinction between species. A similar comparison test should be performed with the present model as soon as enough data will be available to figure out if this holds true also for our model focusing on cumulative effects.

Our model gives a good overview about the current knowledge about quantifiable cumulative effects in seagrass meadows, but for the analysis of single effects, other models would be more useful as they analyze these effects more in detail. For a detailed analysis of single influencing factors, well developed detailed models such as MIKE[23] could be used. In the future, such models and the ACIM model could be combined to generate very powerful, holistic and data-driven simulations of the effects of anthropogenic pressures.

5.6 Conclusions

The overall aim was to develop a procedure to assess cumulative effects of anthropogenic pressures on habitats quantitatively with a flexible method, which allows a continuous update of results as soon as new scientific insights become available. We solved this by establishing a structure facilitating the self-assembling of a network based on available datasets and statistical criteria.

The results of the 'reference scenario' implied that even under lower nutrient conditions, at the absence of herbicides and without any sediment disposal, the conditions can be suboptimal for seagrasses. In particular, the value set for velocity in the reference scenario reflecting common conditions in the North Sea led to several normalized target values below one indicating negative effects on seagrasses. In the scenario three, the increased velocity enhanced the detrimental effects of anthropogenic pressures. This was likely because they partly affected the same targets as anthropogenic pressures and due to interaction effects with them. The relevance of velocity for seagrasses is well known (Schanz and Asmus 2003, Ruiz-Montoya et al. 2012, Villazán et al. 2016). The present model underlines how strongly the effect is interwoven with other pressures. With regard to the management, this means that areas, in which seagrass is exposed to multiple anthropogenic pressures as well as to increased velocity, need special emphasis.

The model setup is very general and is applicable for any habitat and species. There are no limitations for this from a conceptual point of view. Instead, the use of the model is rather limited by data availability restricting the ranges for the modeling simulation. Further, so far, no feedback-loops are included in the model. Feedback loops might though be important. We expect for example density dependent effects in the field. At densities below 30%, the recovery potential of seagrasses is reduced, whereas higher densities promote recovery (Dolch et al. 2017, Kohlus 2008). In order to better understand how different stressors act in concert, how they share the weight of relevance for certain species or species groups, the gap between lab and field experiments should be significantly be reduced. Here, we presented an approach for first quantitative estimations of cumulative effects based on current knowledge. Now, more data are necessary to refine the model and to increase robustness and reliability.

[23] https://www.mikepoweredbydhi.com/

The modeling tool is not only applicable for cumulative effects assessment of anthropogenic pressures, but also usable to analyze any potential network structure consisting of relationships comprising several influencing variables.

5.7 Literature

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6 General discussion

6.1 Discussion of assumptions in cumulative effects modeling

Additive and multiplicative models are often applied to predict cumulative effects of anthropogenic pressures on organisms quantitatively. This is particularly the case in toxicology. However, these models do not always provide accurate predictions of observed effects, as predictions under- or overestimate observed effects (synergistic or antagonistic effects) (e.g. Crain et al. 2008, Crofton et al. 2005, Coors et al. 2012, Faust et al. 2003). Backhaus and Faust (2012) suggested choosing between an additive and a multiplicative model based on the mechanism. However, this method is often not applicable as in many cases no information about the mechanism is available. Based on the results of my thesis, I argue that cumulative effects, which are solely based on the additive or the multiplicative model, are not sufficient for cumulative effects assessment and explain what would be necessary to improve the predictions to mitigate over-and underestimations of predicted effects.

As shown by the literature research about general observations of cumulative effects (see introduction) as well as by the data collected for the species model and the habitat model, interactions between stressors are common and frequently observed in experiments (reviewed in Holmstrup et al. 2010, Ban et al. 2014, Cote et al. 2016, this thesis). The results of the practical tests of the models for blue mussels and for seagrass meadows and the literature research of this thesis further showed that cumulative effects contribute substantially to observed and predicted effects (see literature and results of chapters 4 and 5). Hence, interaction effects should be considered in cumulative effects assessment.

I propose that one should acknowledge that the nature of interactions might change in dependency of the intensity of the contributing stressors. This became evident in the ACIM model as well as in the DEB model (see chapter 4 and 5), which were driven by experimental data derived from scientific literature (e.g. Elliot et al. 1986, Fitzer et al. 2015). Neglecting this dependency can lead to seemingly discrepancies of experimental results, where e.g. two stressors interact in one experimental setup synergistically and in another antagonistically (see e.g. Elliot et al. 1986). Therefore, the dependency of intensity of stressors on cumulative effects should be kept in mind when interpreting categorized interaction types as published by Crain et al. (2008).

The results of my thesis highlight that interaction effects are not static and that they can behave nonlinearly. The comparison of the purely additive DEB model and the DEB model with integrated interaction effects showed for example that the cumulative effect of Pb on the organism was most of the times higher than the predicted effect of Pb only, but that in some occasions it was the opposite (see 4.4). Moreover, the ACIM model revealed that in some cases an additive part as well as a multiplicative part with a dependency of stressor intensities could best explain the literature data. Possibly these interactions are characterized by direct as well as by indirect interaction effects corresponding to the additive model and the multiplicative model. Further research is necessary to understand these patterns and to reveal the biological mechanism explaining these. The results of the ACIM model also indicate that the relative contribution of stressors to an impact might matter. One stressor might have a stronger effect on the organism, contribute more to the overall effect on the organism or has a severe effect on the organism than another one. One stressor might also increase the effect of other stressors as my literature search indicated.

The two developed models (chapter 4 and 5) allow to include these kinds of effects and can serve as tools to analyze in which way multiple stressors act in concert on an ecosystem component. However, much more data of cumulative effects are needed and the models need to be tested more thoroughly with these. For this, interaction effects should be investigated experimentally for different stressor intensities. This will help to understand how stressor intensities and ratios of stressor intensities influence cumulative effects. The modeling tool ACIM offers a method to reveal such dependencies and is usable to analyze corresponding experimental data. Possibly, this way, the modeling tool might in the future help to reveal patterns, which are characteristic for certain ecosystem components or special pressure combinations. As many interaction effects occur on a molecular level, some species independent mechanisms might be identified and possibly aligned with species-specific characteristics such as protein composition to derive species-specific predictions.

The results of the analysis of cumulative effects in seagrass meadows highlight the relevance of time for observed effects, which influenced the observed effect for several single stressors (see chapter 5). In applied assessments, the importance of time is often neglected. Instead, LC50 values, which are in most of the cases derived by short-term experiments with high concentrations play a major role in practical assessments. In contrast, Pörtner (2010) acknowledged the influence of time and proposed to use the product of exposure time and stressor intensity to predict effects of any exposure time and any stressor intensity. This might be an interim solution as long as not enough experimental data are available to identify a model for the influence of exposure time. However, the interaction between time and stress intensity might also be non-linear and these cases require a different kind of model to predict effects. A non-linear relationship could for example be expected due to acclimation processes (compare chapter 4). Moreover, the uptake rate of chemicals differs between high and low concentrations. Thus, time-dependent effects need to be investigated with respect to stressor intensities and experimental tests are needed to figure out what kind of influence exposure time has on single stressor effects as well as on interaction effects. These aspects need to be integrated in experimental setups as well as in cumulative effects assessment to increase the predictability of cumulative effects substantially.

6.2 Comparison between the two model approaches

For the analysis of cumulative effects of anthropogenic pressures on ecosystem components, I developed two methods: one with a focus on species level (cumulative DEB model) and the other one with a focus on habitat level (ACIM). Even though both methods address different levels of biological organization there are some similarities. The conclusions of these comparisons result in ideas for further developments and highlight what aspects are relevant when developing new models for cumulative effects assessment.

The two proposed models both allow the integration of interaction effects. However, the methods applied for this differ from each other as well as the results. The whole organism responses predicted by the cumulative DEB model indicated more severe effects for the test data set when I integrated interactions into the model. In contrast, the results of ACIM showed that the strength of an effect depends on the model applied as well as on the stress intensity of the test scenario. In some cases, the effect of the model output excluding composite models increased with increasing stress intensity and the effect became more severe than for the model run including composite models. In other cases, the model predicted the opposite. Furthermore, for some observations, the integration of composite models led to a more severe effect in all tested scenarios and vice versa. The results of the 'netto effect' of some stressors in the cumulative DEB model further indicated that sometimes the additive model predicted stronger effects than for the case when interactions where included. Thus, the results of both models indicated that the inclusion or exclusion of interaction effects does not determine the strength of the effect alone and that the integration of interaction effects does not always lead to a prediction of a stronger effect compared to a model only including additive effects and vice versa. This confirms that cumulative effects are hard to predict as stated by Dubé (2003). However, both methods developed here for cumulative effects assessment offer options to estimate cumulative effects based on current knowledge providing quantitative predictions for test scenarios.

Apart from interaction effects, the results of the thesis highlights that the exposure time plays a major role for the strength of an effect. However, both models threat this aspect a bit differently: Whereas the influence of exposure time is required in the cumulative DEB model as an input variable for the single stressor model, this is not a precondition in ACIM. However, in the cumulative DEB model the influence of exposure time is generally not included for the calculation of the interaction factors, whereas exposure time is integrated as a possible influence on the interaction effects in ACIM and is included when enough data are available and the defined statistical requirements are fulfilled. I propose to also include the influence of the exposure time for interaction effects in the cumulative DEB model if corresponding data are available as the results of ACIM indicated that the exposure time influences interaction effects. However, the data applied in ACIM are sparse and it would be important to entangle the influence of exposure time on the single stressors and on the interaction itself. To reveal if the exposure time has an influence of an interaction and to understand if this influence can be explained by the influence on the exposure time on single stressors as assumed in the cumulative DEB model, experiments with many different exposure times are needed and both models need to be tested with these data sets. Currently, in both models too few exposure times were tested and this lead to some uncertainty in the model output. Actually, all model results referring to predictions beyond the exposure times of the data included in the model should be interpreted with caution as any modeling in the future is risky and short-term effects might differ from long-time effects.

Similarly, in both models many data from studies using high stress levels were included despite the aim to preferably use data with a long exposure time and rather low stress intensities. The reason is that such data are rarely available as such experiments require more resources. Further, a test with high stress increases the likelihood to get significant results, which are usually easier to publish (Lin and Chu 2018). However, the predictability of effects outside the range of tested stress intensities is limited and if there is a big gap between experimental setups and field conditions, this may lead to severe problems and unrealistic predictions. This is particularly a problem when applying such predictions for assessments. Therefore, more data are needed for lower stress intensities and longer exposure times.

Whereas ACIM is purely data driven and has no biological mechanism included, the cumulative DEB model is characterized by a fixed set of equations describing different biological processes. The method applied in ACIM has the advantage that many datasets are applicable as long as they fulfill the predefined criteria whereas in the cumulative DEB model, datasets of single stressors need to be measured for different stressor intensities and exposure times. Moreover, for the cumulative DEB model, information about DEB parameters need to be available. For species, for which these data are not available, such a cumulative effects assessment cannot be conducted without deriving these data with experiments and modeling procedures requiring a lot of resources. On the other hand, results from DEB models are due to the uniform structure more comparable with each other. Furthermore, the analyses of parameters and the results give indications for possible general response patterns related to a biological meaning and possibly inspire to test corresponding hypotheses experimentally. One example in the test run with the blue mussel is the difference of the parameter for acclimation between essential and non-essential metals. In contrast, the parameters optimized with ACIM are rather abstract and are not directly related linked to a biological meaning. However, also the parameters describing the relative contribution of a stressor to the overall effect may lead to hypotheses of molecular processes, which can then be further investigated. For example, a parameter indicating only multiplicative interactions points to direct interactions whereas a parameter value of zero for the multiplicative part of the model and positive weighing factors indicating an additive interaction points to different molecular pathways of the two stressors (see 2.1.6, 2.1.7 and Backhaus and Faust 2012). The DEB model does not provide this kind of information. The lack of biological meaning aligned to the other parameter values derived by the modeling with ACIM is in a way an advantage as the abstract nature provides some openness for relevant aspects. In contrast, the general shape of the model for the response pattern for the single stressor model is relatively fixed. This freedom bears the risk of wrong models, which are determined with ACIM: Some models might explain the data very well but lack a biological explanation. A reason for the choice of the 'best model' could be for example an outlier, which occurred due to a rare event and special conditions, which were irrelevant for the question investigated. This raises the question if it would be better to apply a model where we know the biological meaning of every parameter or if the application of a method as applied in ACIM would be more appropriate for cumulative effects assessment. From my point of view, we need both approaches. It is important to utilize as much data as possible to get a good overview of the current knowledge and to get a first impression of relationships occurring in an ecological network, which is affected by anthropogenic pressures. On the other hand, it is important to aim at an understanding of the relationships and to try to align general biological meanings for parameters. Further, it is an advantage to have a fixed frame for models describing the effects of anthropogenic stressors on ecosystem components and for the most relevant biological processes to provide comparability between different ecosystem components. However, this is only possible if sufficient data about those processes are available. ACIM provides a good possibility to not only provide a picture of the state of knowledge and first results but is also applicable as a method to set up the basic frame for a model describing the effects of anthropogenic stressors on a habitat because it identifies all known relevant processes.

In both models, only quantifiable effects are considered. This may lead to some bias as the relevance of certain influences might have already been observed but not yet quantified. The matrix, applied in the DEB model providing a color code is very useful for these cases as it summarizes for which interaction effects between the stressors information is available, what kind of interactions were observed so far, and thus should be used complementary to the quantitative models.

Both models suffer from uncertainty when data are extracted from figures, which can be inaccurate especially when many observations are presented in one figure. In addition, the application of a log scale for the presentation leads to inaccurate data extraction. One alternative is to derive data from data portals for data publishing such as 'PANGAEA[24]. It is also possible to contact authors directly and ask them to provide data, but this is potentially time-consuming. For the tests of the models, I extracted data with the freeware tool WebPlotDigitizer[25], as this is always possible. This way I could apply the same method for all datasets.

For none of the models I conducted a quantitative uncertainty assessment. I only calculated the percentage of unknown interaction effects of the matrix for the cumulative DEB model. Instead, I described the uncertainties qualitatively. As both models are relatively complex, a quantitative uncertainty assessment is not trivial, because uncertainty arises at very different levels. Therefore, it

[24] https://www.pangaea.de/

[25] https://automeris.io/WebPlotDigitizer/

would require a development of special method to assess it adequately. The development of such methods should be considered for further developments of the methods.

6.3 Combining the cumulative DEB model and ACIM

Even though both models address different levels of biological organization, a combination of both models is conceivable to gain the advantages of both model types. As described above, the exposure time is not yet considered for interaction effects in the cumulative DEB model. However, if corresponding data become available, those can be analyzed with ACIM and included with the derived parameter values into the cumulative DEB model for the description of an interaction between two stressors. It is possible to compare corresponding results with the current model setup and this way evaluate if the consideration of the exposure time for each of the stressors would correlate with the influence of exposure time derived from a combined dataset with both stressors.

Conversely, it is possible to integrate the outputs of the DEB model into ACIM as input values for species interactions. For example, species using *Mytilus edulis* as food sources can be affected by changes of released gamets or an altered growth of *Mytilus edulis*. The altered food availability may further alter the sensitivity of the predator to some anthropogenic pressures. This way it would be possible to include the results of several cumulative DEB models for different species. However, it is important that the stress scenarios in the DEB model and the ACIM model are in this case the same to provide consistency throughout the larger ecosystem model.

6.4 Applicability of the concept

The overall concept is applicable for environmental assessments, for management, and for the planning of new research projects. It is applicable to fulfil the requirement of the MSFD to analyze cumulative effects with regard to the MSFD descriptor 1 – biodiversity, for which the status of different ecosystem components including species and habitats need to be assessed. However, the cumulative assessment cannot replace other assessments of the status of ecosystem components because the cumulative assessment is associated with a high uncertainty due to lack of knowledge for many theoretical possible interactions. Nevertheless, the assessment of cumulative effects may contribute to the understanding of the reasons for a certain status and give hints for the contribution of single pressures on the overall impact on the ecosystem components. This is a useful information for the management of the species and for the decision of prioritization of measures for improving the status of an ecosystem component. It is further possible to run the models based on different input values for the stressors corresponding to different degrees of possible reductions of anthropogenic pressures. This way one can calculate how the different management options might improve the situation of an ecosystem component. Moreover, it is possible to identify areas of concern for the ecosystem components by comparing different monitoring stations with each other. These areas might need special attention and a focused management to improve the environmental status. On the other hand, the identification of areas, where the cumulative pressures are comparably low and which might serve as refugee habitats, is also possible.

The matrix gives a broad overview of the current state of the art for interaction effects. Because of its flexible structure, it is not only applicable for descriptor 1 but also for other descriptors. For example, the matrix estimates can be used to give an impression how different anthropogenic pressures influence the effects of eutrophication (MSFD descriptor 5). The matrix highlights also knowledge gaps and is therefore useful for the planning of new research projects focusing on the interactions, which cannot yet be quantified or about which utterly nothing is known yet. The LiACAT further provides a framework and a structure to sort and to visualize the current state of the art. The grouped Sankey diagram reveals for example not only the known relationships but also gives an impression of the amount of literature data available about each of the relationships.

All of the methods described above can be lifted to a geographical perspective as described in chapter 2. Such a geographical perspective is useful for marine spatial planning. Marine spatial planning requires the ecosystem based approach including also the assessment of cumulative effects (HELCOM 2018, Altvater et al. 2019). Cumulative assessments based on the spatial analyses of different ecosystem components as well as an analysis of the cumulative impacts due to all occurring anthropogenic pressures reveal for example in which areas additionally human activities are most critical for the environment. The tested methods for cumulative effects assessment are not only applicable for the assessment of the impact based on measured data but also for testing of scenarios. Thus, if the pressures related to a certain project such as the planning of an offshore windfarm are known, the input values for the model should be altered correspondingly and this way it is possible to calculate at which spatial spot the impact is predicted as a minimum with respect to the cumulative impact.

The matrix as well as ACIM are applicable for almost any ecosystem component. In contrast, the DEB model in its current form is specialized for the blue mussel *Mytilus edulis.* Nevertheless, an adjustment of the model for other species is possible. If the biology is relatively similar, this requires only little changes of the DEB parameter values and the application of corresponding other input values based on a literature research about the sensitivity of the species towards environmental parameters and anthropogenic pressures. I tried this for the introduced oyster *Crassostrea gigas* and compared the model results for these two species this way. Also for many other species, only few adjustments of the model would be necessary. DEB parameter values are now available for many species. However, as shown by Lorena et a. 2010, dynamic energy budget modeling of e.g. microalgae requires the consideration of special processes .Therefore such a DEB model differs to a larger degree from the standard DEB model and thus also from the DEB model for *Mytilus edulis*. Hence, the more the biology and the physiological processes differ from *Mytilus edulis*, the more effort is necessary to adjust the model accordingly. On the other hand, DEB models for different kinds of species are available already and these models are applicable for cumulative assessments when completed by the module for single stressor responses and the module for integrating interaction effects. The analysis of cumulative effects of numerous species and habitats to provide input for a comprehensive geographical analysis was not possible within the context of the PhD due to time limitations and are a proposal for the future. It is also unclear how much resources will be available to conduct such analyses for regional assessments. As an alternative, intermediate solution, I propose to calculate a simple index based on a matrix analysis, which is integrated in the framing tool LiACAT. This will provide a first impression about the cumulative effects.

Finally yet importantly, any model is dependent on its input values and the reliability of it fixed characterizing parameters. This applies also for the models proposed in this thesis and limits its interpretability correspondingly.

6.5 Outlook

The models proposed can be further developed by linking them more strongly to environmental processes and biological interactions. The results of the DEB model can for example be used as input values for a population model. This way, for example also density dependent effects can be integrated. Further, the outputs of DEB models are suitable as input values for food web models, because both types of models use carbon as the main element for the calculations. There is already some experience about linking DEB-models to ecological models. Similarities and differences between the ERSEM (European Regional Seas Ecosystem Model) and DEB models have been analyzed (Marques et al. 2014) and a model has been set up between ERSEM and a DEB model (Saraiva 2014). While ERSEM focuses on the microbial food web and its biogeochemistry[26], a link between DEB models with food web models of larger organisms should work equally well. In order to reach that, cumulative DEB models need to be developed at least for the most important food web elements. For such a model, further, active and passive model movements of species would need to be considered. Moreover, anthropogenic pressures such as hazardous substances might be drifted away due to currents and sediment movements. Those aspects need to be taken into account as well.

Another possible further development is the application of traits in ecosystem models. ACIM as well as the DEB models provide information about altered traits as a response to the influence of anthropogenic pressures. In ACIM, these changes of traits could be inputs for further processes. This way, ACIM is applicable as a kind of ecosystem model itself. However, the information about traits can also be extracted and used as input values for other models with a spatial dimension. Spatial data can be easily generated by ACIM by adding coordinate data to scene numbers.

The models also foster experimental research. It is possible to test the results of the models under controlled experimental conditions in mesocosm experiments to figure out if the model predictions reflect the observations. Further, the overall concept together with the single methods reveal possible missing links for a comprehensive understanding of the response of ecosystem components to anthropogenic pressures. The results provided by ACIM further gave insight to the relevance of exposure time and the patterns of interactions. This might motivate to explore the response of some more exposure times and stressor intensities to be able to test relationships beyond linearity. The identified type of model for a relationship might also lead to further questions about the processes determining the observed pattern, and trigger experiments on the molecular level to find possible explanations.

The more data are entered into the database and the more analyses will be run, the higher the likelihood that general patterns of responses to anthropogenic pressures will be revealed. The results of the cumulative DEB model for example indicated that there is possibly a different response to essential metals than to non-essential metals. This needs to be tested experimentally. To the same time, an understanding of general patterns such as this, might improve our understanding and the predictability of likely responses to anthropogenic pressures without the need to test all possible combinations. Models, which link a biological meaning to parameters, are advantageous for that.

Major questions, which arose from the literature research and the analyses of the data dealt with the dynamics of interaction effects. Experimental tests might reveal possibly turning points for a switch between a synergistic and an antagonistic interaction in dependency of stressor intensities and exposure times. The identification of the corresponding thresholds for such turning points would also be relevant for environmental management.

With regard to the application of the general concept and the models, more ecosystem components need to be analyzed and literature need to be continuously updated. This would foster the integration of the results in environmental assessments, the consideration in management plans and for marine spatial planning. In the future, it might further be possible to integrate the results of ACIM, cumulative DEB models and matrixes into existing approaches for cumulative effects assessment such as the Baltic

[26] https://www.pml.ac.uk/Modeling_at_PML/Models/ERSEM

Sea Impact Index (HELCOM 2018) based on Halpern et al. (2008). Moreover, the overall concept provides a technical framework applicable with a few adjustments for the general networks needed for the Bow-Tie analysis[27], a method proposed by an OSPAR group for cumulative effects assessment in the North Sea, which links human activities and anthropogenic pressures to effects on ecosystem components. Thererby, it considers possible mitigation actions to the same time.

6.6 Conclusions

- The application of an online literature database (LiACAT) allows reproducibility, comparability and transparency of cumulative effects assessments, as it combines tools for data extractions, visualization of literature data and integrated models. Thereby, the literature based analysis of cumulative effects on habitats and species can be traced to the original literature sources so that the user can retrace the procedure. The concept for cumulative effects assessment was adapted in a way that allowed the application of existing monitoring data as input data for the models by applying temporal and spatial interpolation methods.
- Two unifying schemes were identified to present cumulative effects: A matrix showing all relevant interactions and providing a general cumulative index value and Sankey diagrams showing the pathways from human activities to effects on ecosystem components. Both methods also provide an overview of the state of the art.
- The results of the cumulative DEB model focusing on effects of anthropogenic pressures on species level showed a difference between a reference scenario and a scenario including anthropogenic effects. Thereby, it mattered if interaction effects between stressors were included into the model or if a simple additive approach was applied.
- The matrix, which was applied as a preparation for the cumulative DEB model, showed which kinds of cumulative effects likely occurred during the study period. The results showed a reduced growth, impacts on reproduction including a delayed maturation, delays of spawning events and reduced reserve biomass due to the anthropogenic pressures throughout the life cycle of *Mytilus edulis*. Cumulative effects connected with acidification, increased temperature, increased copper and increased zinc concentration contributed most to the overall cumulative effect on *Mytilus edulis*.
- The method developed for the assessment of cumulative effects on habitats predicted adverse effects under increasing pressure scenarios for different response variables. Thereby, the model predicted different outcomes depending on the in- or exclusion of composite models comprising two or more influencing variables to explain response variables.
- It was not possible to answer the question whether additive or multiplicative effects occur more frequently in seagrass meadows with certainty because too few data were available representing the influence of at least two different stressors. However, for the datasets available in most cases an additive part as well as a multiplicative part of interaction contributed to the overall observed effect according to the model results. Most composite models consisted of an influence of exposure time and stress intensity.

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[27] https://oap.ospar.org/en/ospar-assessments/intermediate-assessment-2017/chapter-6-ecosystem-assessment-outlook-developing-approach-cumul/

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7 Appendices

7.1 Appendices chapter 4

7.1.1 Criteria for the selection of literature data

Criteria for the selection of data sets from scientific literature for modeling the effect of single stressors (part of the DEB-model)

These minimum requirements needed to be fulfilled for the integration of literature data:

- The data describe effects of the selected stressors on Mytilus edulis
- Different exposure times and stressor intensities were tested

The following criteria were used to choose between different data sets. The data set, which fulfilled the criteria best, was selected:

- The experimental conditions such as salinity and pH resembled the conditions common in the southern North Sea.
- Certain quality standards were fulfilled (e.g. provision of statistical data).
- Data from peer-reviewed journals were used preferentially.
- The model species were collected in the North Sea.
- Accessibility of the paper through online accessibility, services of the University of Oldenburg, the University of Hamburg or by direct contact to the authors.

To select values for parameters and for equations for the for the DEB-model the following criteria were applied:

- Mytilus edulis was the model organism.
- Publications providing many DEB-parameter values and equations were preferred over publications containing only a few parameter values.
- The experimental conditions such as salinity and pH resembled the conditions common in the southern North Sea.
- Peer-reviewed journals were used preferentially.
- The model species were preferentially collected in the North Sea.
- Accessibility of the paper through online accessibility, services of the University of Oldenburg, the University of Hamburg or contact to the authors.

For the selection of literature sources for deriving equations describing interactions between stressors, the following criteria were applied:

- The model species was Mytilus edulis

- The publication dealt either with an interaction between two stressors and Mytilus edulis or with an interaction between two stressors in the water or in the sediment affecting Mytilus edulis.
- Data were available for the effect of a single stressor as well as data about the combination effect of two stressors.
- The experimental conditions such as salinity and pH resembled the conditions common in the southern North Sea.
- Certain quality standards were fulfilled (e.g. provision of statistical data).
- Peer-reviewed journals were used preferentially.
- Accessibility of the paper through online accessibility, services of the University of Oldenburg, the University of Hamburg or contact to the authors.

During the literature research, it turned out that the nature of interactions is sometimes dependent on the intensities of each of the stressors as well as on the ratio of the two stressor intensities to each other and it became hard to choose which data to choose to calculate the interaction factor. To resolve this problem, I defined additional criteria for the selection of literature:

- Preferably data of an experiment are used with a ratio of the stressor intensities that resembled the conditions of the scenario tested.
- If this criterion is fulfilled equally good, in several datasets the concentrations of the single stressors were compared to the concentrations of the test scenario. However, in case of a short exposure time (<14 days) and relatively low concentrations, the next tested concentrations is to be used because an interaction effect may not show under these conditions although it could in nature, where the organisms are exposed for long time periods. I refer here to the thesis by Pörtner (2010), who related exposure time and concentration to each other to explain the magnitude of observed effects and transfer this thought for interaction effects.
- If several datasets exist, that resemble in the stressor intensities and their ratios of stressors intensities to each other, further criteria to select one publication were a preferentially long exposure time and a good fit of experimental conditions with the test scenario.
- If all these criteria equal each other, a mean is calculated to derive the interaction factor.
- Whole animal responses are favored to molecular responses as they are considered to give a better overall impression of the health of the organism.
- Datasets with significant interactions were preferentially used.

7.1.2 Information of interactions used in the DEB model

Table 10 Models used for the interactions

Influence	Influenced stressor	Formula/ method for the calculation of the interaction factor	Statistical values	Source	comment
Increased Cu concentration	Increased Cd concentration	$$Intfact = \frac{combEff - SinglEff}{SinglEff}$$	n.a.	Elliot et al. 1986	
Increased Zn concentration	Increased Cd concentration	$$Intfact = \frac{combEff - SinglEff}{SinglEff}$$	n.a.	Elliot et al. 1986	
Decreased pH	Increased Cd concentration	After transformation of the data, the data were pre-treated and afterwards the interaction factors for the different pH values were calculated with the formula $$Intfact = \frac{combEff - SinglEff}{SinglEff}$$ Then derivation of the relationship between pH value and interaction factor, resulting in the equation $$Intfact = -0.1476 \cdot pHvalue + 1.2212$$	R^2: 0.44	George 1983	The formula was derived based on an analysis of the literature data. More information below
In the experiment, George tested how much percent of the Cd concentration was bound to granules and to metallothionein in a cell-free environment with isolated tertiary lysosomes from *Mytilus edulis* kidneys. In the experiments about interaction effects, the influence of pH was tested for values between 7.23-8.08. Data for the binding of Cd to granules and metallothionein provided in the corresponding figures. Application of the data: The percentage of Cd bound compared to the binding at a pH of 7.5 was plotted for the different pH values and a linear model was fitted to the data points based on the pooled data. The pooling of the data resulted in a worse R^2 value compared to a separate fitting of the datasets but was thought to better reflect an overall relationship independent of the measured effect. The binding of Cd is a positive effect. Thus, the data were transformed according to the following logic: The smaller the pH value the less Cd is bound. Therefore, the adverse effect of any Cd effect would increase by the difference between the Cd bound at pH 7.5 compared to the Cd bound at the reduced pH. The data calculated this way for the different pH values, representing the combined effects, were used to calculate the interaction factor.					

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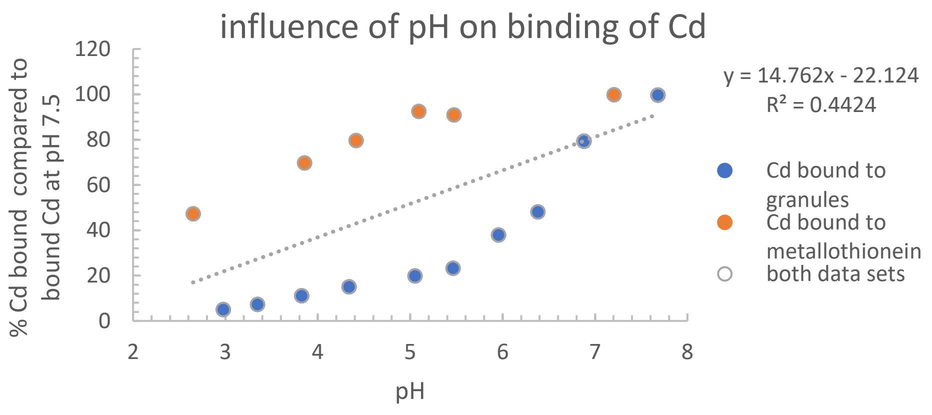
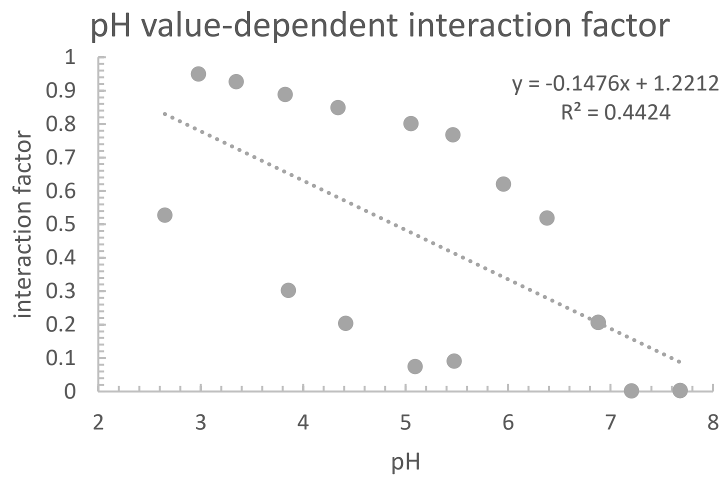
<tr><td colspan="6">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<tr>
<td>Altered temperature</td>
<td>Increased Cd concentration</td>
<td>After transformation of the data, the data were pre-treated and afterwards the interaction factors for the different temperature values were calculated with the formula

$$Intfact = \frac{combEff - SinglEff}{SinglEff}$$

Then the derivation of the relationship between temperature and interaction factor was performed, resulting in the equation

$$Intfact = p1 * x^4 + p2 * x^3 + p3 * x^2 \, p4 * x + p5$$

with p1 = 2.644e-05, p2 = -0.001708, p3 = 0.04012, p4 = -0.3779, p5 = 1.23</td>
<td>SSE: 0.00108,
R-square: 0.9938,
Adjusted R-square: 0.969,
RMSE: 0.03287</td>
<td>Fischer 1986</td>
<td>The formula was derived based on an analysis of the literature data</td>
</tr>
<tr><td colspan="6">In the experiment the condition index of Mytilus edulis was tested for temperatures between 5 and 25°C at a Cd concentration of 1µg/L and lasted for 8 weeks. As the condition index is a positive effect the data were transformed and normalised with the highest CI at the optimum temperature at approximately 7.3 °C. The relative differences between the highest CI and the CI at different temperatures were added to 1 (optimum) and the interaction</td></tr>
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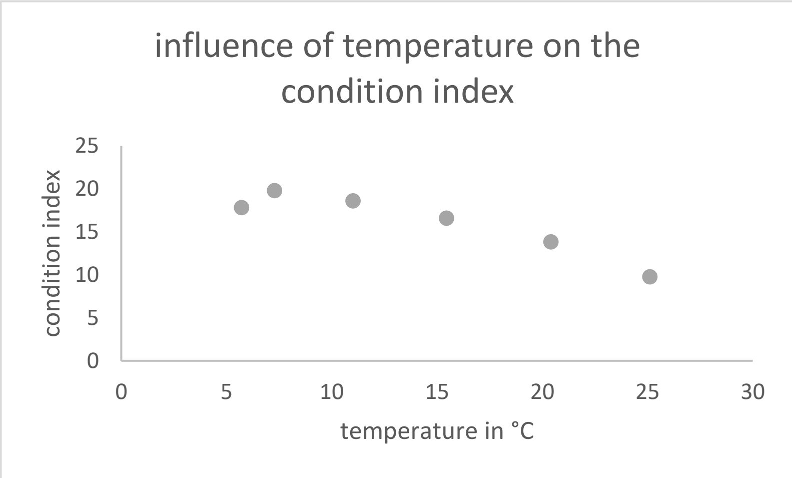
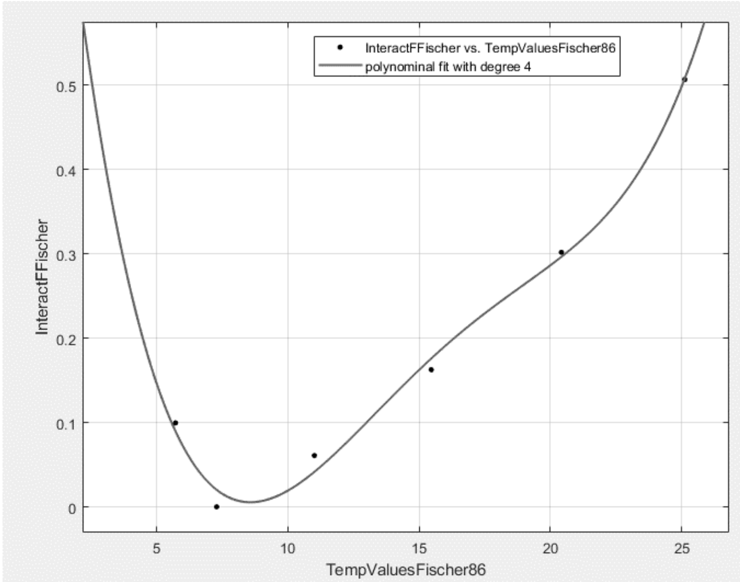
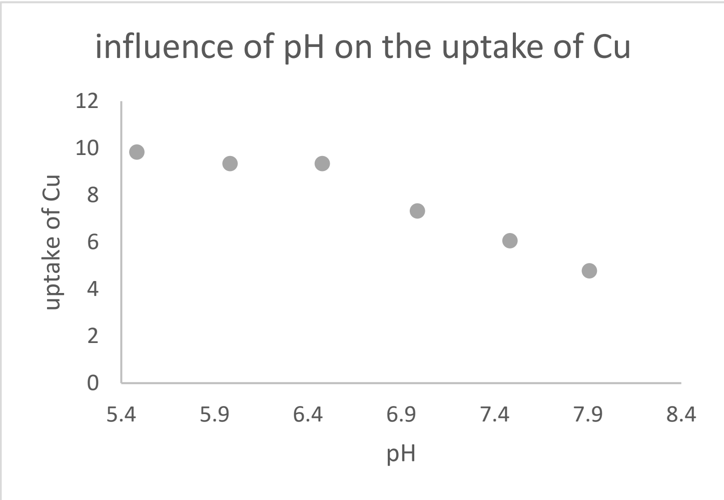
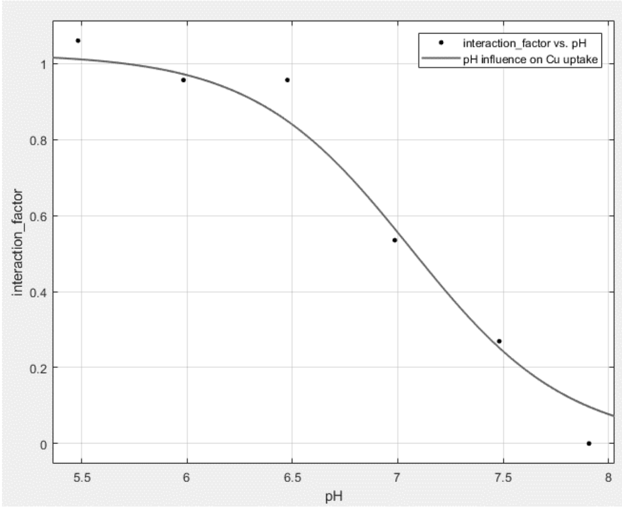
<tr><td colspan="6">factor was calculated with the formula stated above. In a second step, the calculated interaction factors were plotted against the different temperature values and a formula for describing the relationship between the temperature and the CI derived with the cftool of matlab.



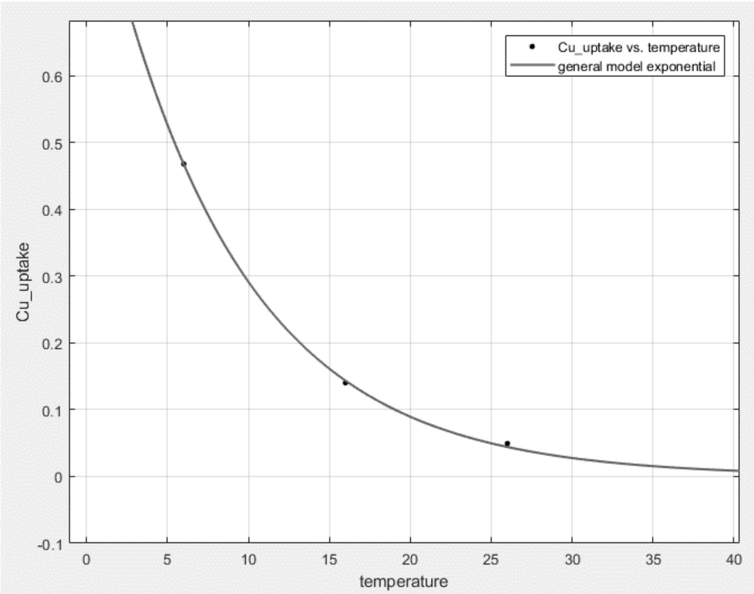


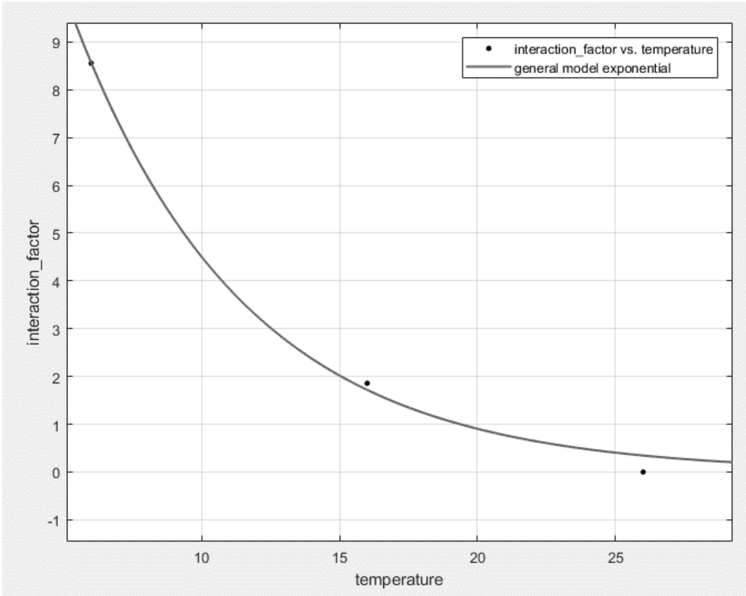
</td></tr>
<tr><td>Increased Cd concentration</td><td>Increased Cu concentration</td><td>$$Intfact = \frac{combEff - SinglEff}{SinglEff}$$</td><td></td><td>Elliot et al. 1986</td><td></td></tr>
<tr><td>Increased Zn concentration</td><td>Increased Cu concentration</td><td>$$Intfact = \frac{combEff - SinglEff}{SinglEff}$$</td><td></td><td>Elliot et al. 1986</td><td></td></tr>
<tr><td>Altered pH</td><td>Increased Cu concentration</td><td>First the interaction factors for the different pH values were calculated with the formula
$$Intfact = \frac{combEff - SinglEff}{SinglEff}$$
Then the derivation of the relationship between temperature and interaction factor was performed, resulting in the equation</td><td>SSE: 0.02485
R-square: 0.9734
Adjusted R-square: 0.9557
RMSE: 0.09101</td><td>Akberali et al. 1985</td><td>The formula was derived based on an analysis of the literature data</td></tr>
</table>

<table>
<tr><td></td><td></td><td>$$Intfact = \frac{1.027}{1 + 6.306 \cdot 10^{-9} \cdot \exp(-2.674 \cdot -pHvalue)}$$</td><td></td><td></td><td></td></tr>
<tr><td colspan="6">Akberali et al. 1985 observed the uptake of Cu at different pH values between 5.4 and 8. The uptake of Cu decreased with increasing pH. Even though Cu is needed in small concentrations, here the uptake of Cu is interpreted as a negative effect because here high concentrations of Cu are considered.


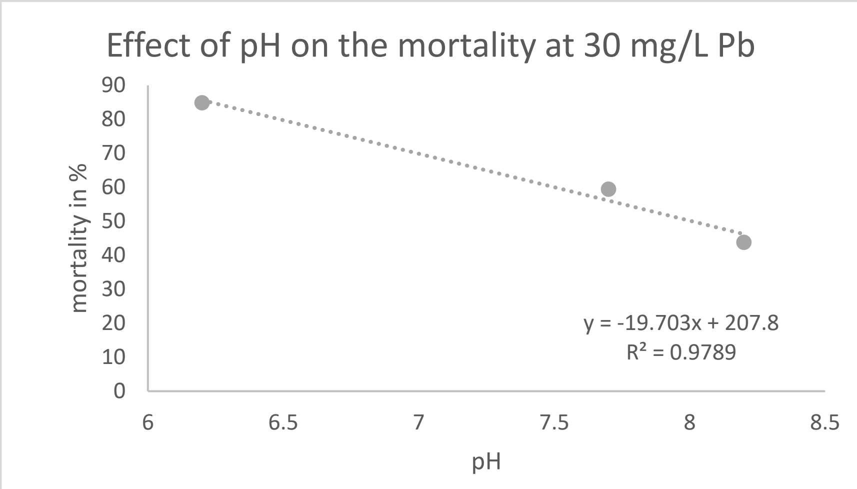

</td></tr>
<tr><td>Increased temperature</td><td>Increased Cu concentration</td><td>First the interaction factors for the different temperature values were calculated with the formula
$$Intfact = \frac{combEff - SinglEff}{SinglEff}$$
Then the derivation of the relationship between temperature and interaction factor was performed, resulting in the equation
$$Intfact = 22.45 \cdot exp^{-0.1606 \cdot temp}$$</td><td>SSE: 0.1383
R-square: 0.9966
Adjusted R-square: 0.9932
RMSE: 0.3719</td><td>Mubiana et al. 2007</td><td>The formula was derived based on an analysis of the literature data</td></tr>
</table>

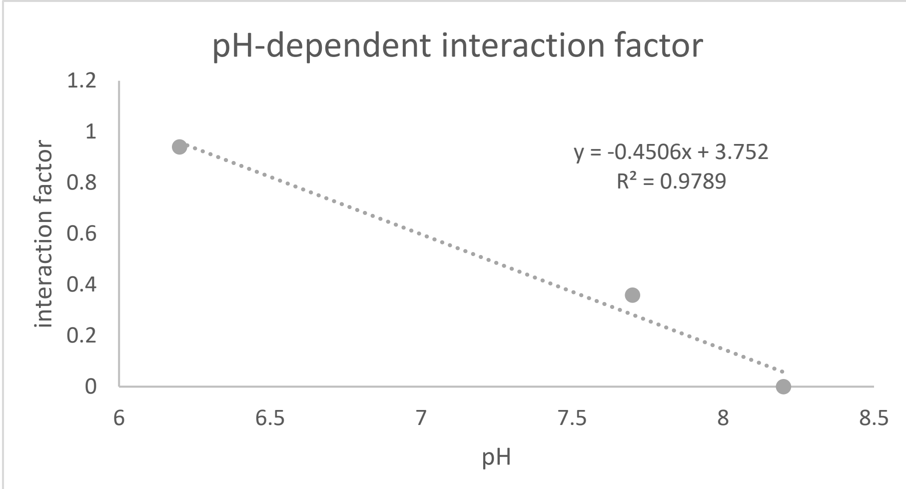
Mubiana et al. 2007 measured the uptake rate of Cu at three different temperatures. The uptake rate decreased with increasing temperature. The uptake rate was differed significantly between the different temperatures. As described above, the uptake of Cu is seen as an adverse effect due to the comparably high concentrations, which are relevant in the model. The best fit for the model was an exponential model approaching an uptake rate of zero. An optimum value for the uptake rate was defined as the uptake rate at 26°C as this represented the lowest uptake rate in the experiment and the water at the monitoring station usually does not reach a higher water temperature. This optimum value for the uptake rate was treated like an effect of a single stressor and used this way to calculate the interaction factor. This procedure is justified by the fact that no uptake would take place without any Cu in the water. Therefore, the uptake itself is an effect due to the presence of Cu.

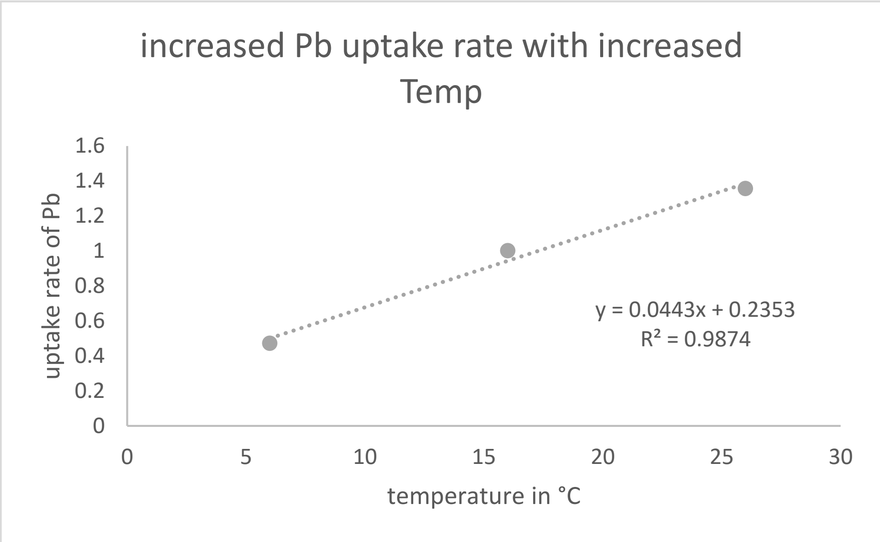
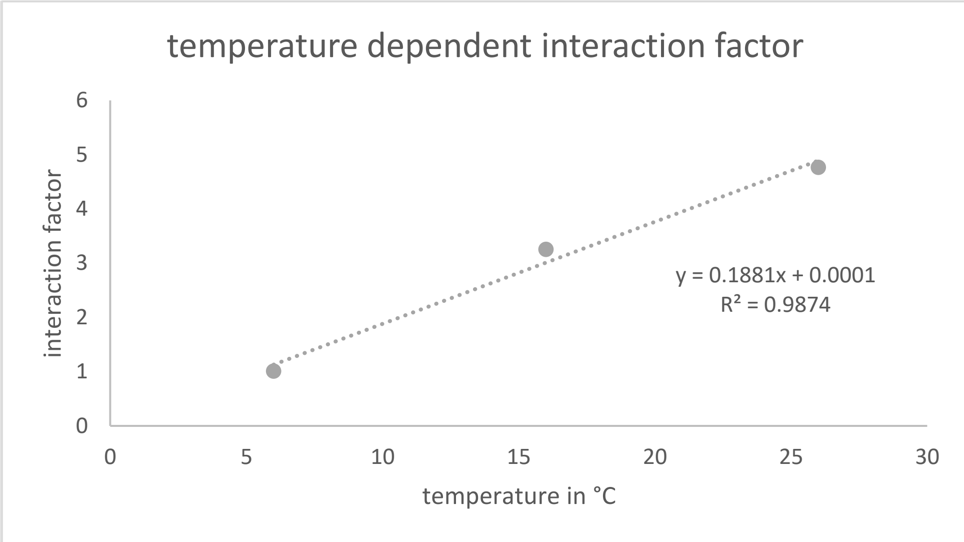


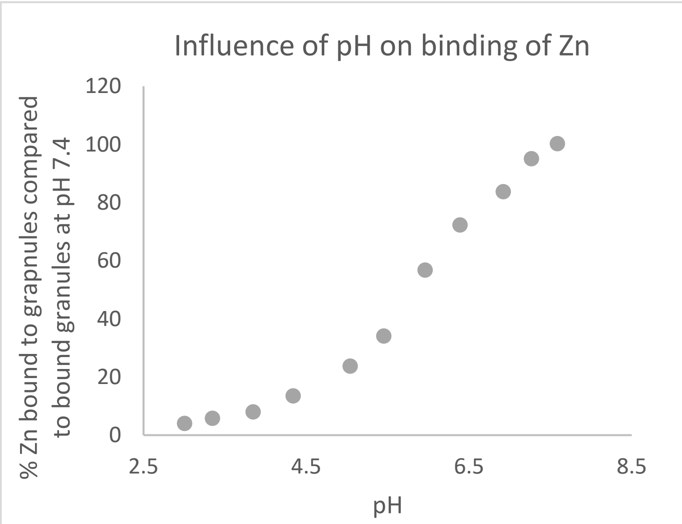


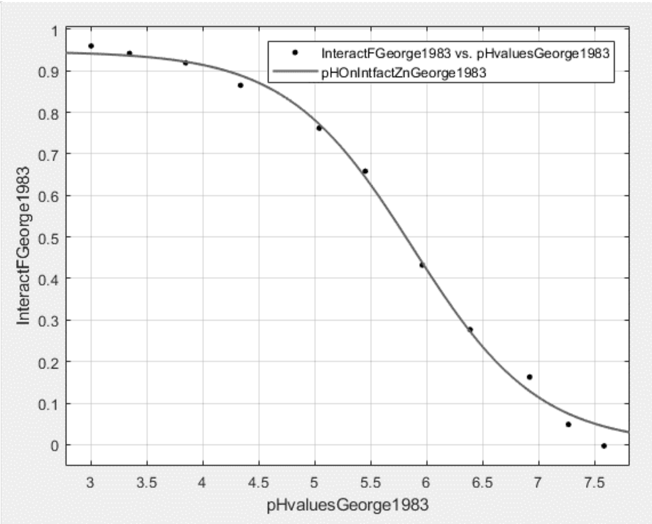
Increased Cd concentration	Increased Pb concentration	$$Intfact = \frac{combEff - SinglEff}{SinglEff}$$			
Alterered pH	Increased Pb concentration	First the interaction factors for the different pH values were calculated with the formula $$Intfact = \frac{combEff - SinglEff}{SinglEff}$$	R^2: 0.9789	Han Zhao-Xiang et al. 2013	The formula was derived based on an analysis of the literature data

<table>
<tr><td></td><td></td><td>Then the derivation of the relationship between pH and interaction factor was performed, resulting in the equation
$$Intfact = -0.4506 \cdot pHvalue + 3.752$$</td><td></td><td></td><td></td></tr>
<tr><td colspan="6">Han Zhao-Xiang investigated how the pH value influenced the mortality due to Pb pollution. As a pH value of 8.1 is considered to be optimum for *Mytilus edulis*, the mortality at a pH value of 8.2 in the experiment, which is closest to this value was seen as the value for mortality without additional influence of pH.


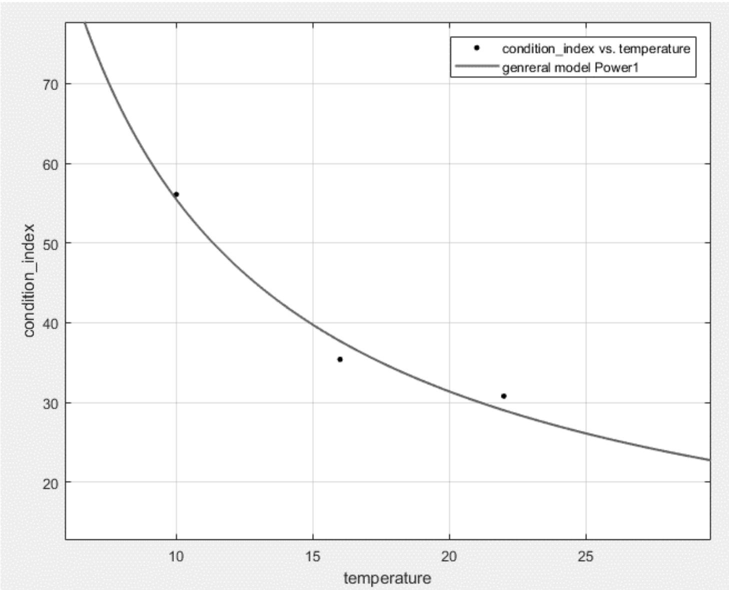

</td></tr>
<tr><td>Increased temperature</td><td>Increased Pb concentration</td><td>First the interaction factors for the different pH values were calculated with the formula
$$Intfact = \frac{combEff - SinglEff}{SinglEff}$$
Then the derivation of the relationship between temperature and interaction factor was performed, resulting in the equation
$$Intfact = 0.1881 \cdot temp + 0.0001$$</td><td>R^2: 0.9874</td><td>Mubiana et al. 2007</td><td>The formula was derived based on an analysis of the literature data</td></tr>
</table>

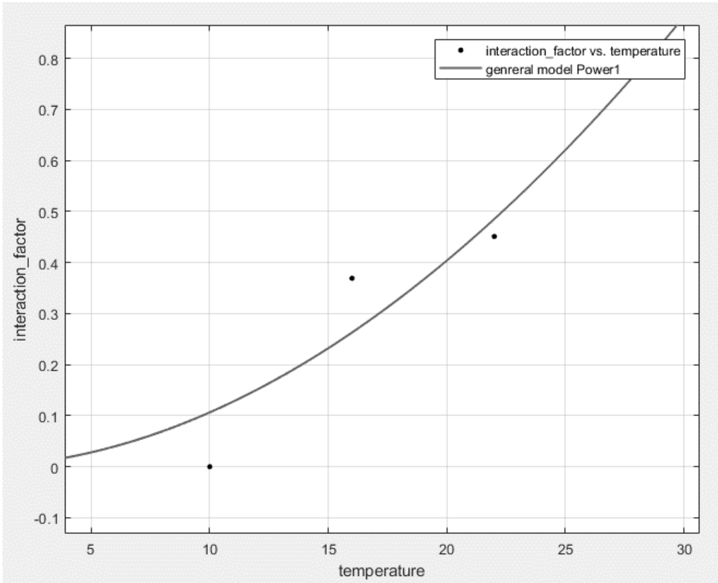
Mubiana et al. observed the uptake of Pb at different temperatures. In contrast to Cu, the uptake rate for Pb increased with increasing temperature. The uptake rate was calculated for 0 °C representing the coldest water temperature possible and used as a proxy for the likely effect of Pb alone without an acceleration due to increased temperature.  					
Increased Cd concentration	Increased Zn concentration	$Intfact = \frac{combEff - SinglEff}{SinglEff}$		Vercauteren and Blust 1999	
Increased Cu concentration	Increased Zn concentration	$Intfact = \frac{combEff - SinglEff}{SinglEff}$		Elliot et al. 1986	
Decreased pH	Increased Zn concentration	First calculation of interaction factors for the different pH values with $Intfact = \frac{combEff - SinglEff}{SinglEff}$	R^2: 0.9962, Adj. R^2: 0.9953, SSE: 0.00532, RMSE: 0.02579	George 1983	

<table>
<tr><td></td><td></td><td>Then derivation of the relationship between pH value and interaction factor, resulting in the equation
$$Intfact = \frac{0.947}{1 + 3.02 \cdot 10^{-5} \cdot \exp(-1.771 \cdot -pHvalue)}$$</td><td></td><td></td><td></td></tr>
<tr><td colspan="6">See method description for the influence of pH on the effect of Cd. The same procedure was followed for the influence on Zn effects. However, only data for the binding of granules were available.


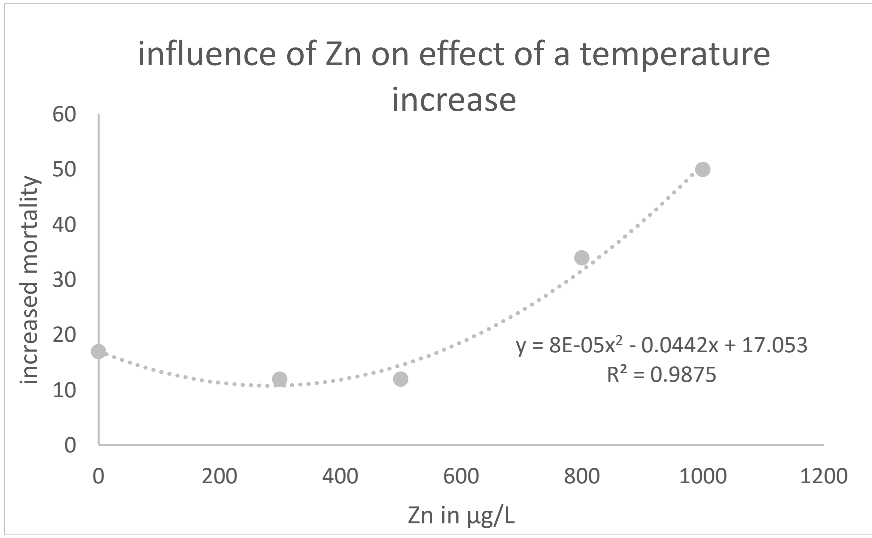

</td></tr>
<tr><td>Increased temperature</td><td>Increased Zn concentration</td><td>After transformation of the data, the data were pre-treated and afterwards the interaction factors for the different temperature values were calculated with the formula
$$Intfact = \frac{combEff - SinglEff}{SinglEff}$$</td><td>SSE: 0.02375
R-square: 0.7942
Adjusted R-square: 0.5885
RMSE: 0.1541</td><td>Cotter et al. 1982</td><td>The formula was derived based on an analysis of the literature data</td></tr>
</table>

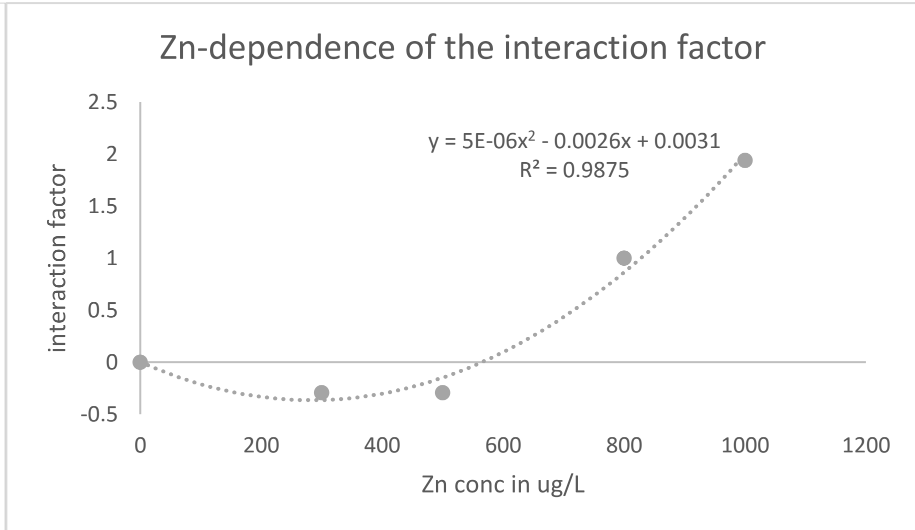
<table>
<tr><td></td><td></td><td>Then the derivation of the relationship between temperature and interaction factor was performed, resulting in the equation
$$Intfact = 0.001262 \cdot temp^{\,1.925}$$</td><td></td><td></td><td></td></tr>
<tr><td colspan="6">Cotter et al. 1982 investigated the influence of temperature on the condition index and on mortality under different increased Zn concentrations (0.3 mg/L and 1 mg/L). An influence of temperature was indicated by the experiment with 1 mg Zn/L with regard to the condition index and by the experiment with mussels from the field with an increased Zn concentration in the body when the water temperature was increased up to 31 °C.

Because the experiment for the observation of the condition index lasted longer (11 days) and the temperature values were more comparable to field conditions than in the experiment testing the mortality at very high temperature values between 29.7 and 31 °C, the data for the condition index were chosen. A high condition index represents a positive observation. Therefore, data were transformed so that they presented an adverse effect. First, the data were normalized with the best condition index in the experiment and then the difference between the normalized highest condition index (1) and the normalized measured condition index at the two increased temperatures was calculated and added to 1. Based on these values, the interaction factors were calculated and the relationship between temperature and interaction factor modeled.




</td></tr>
</table>

<table>
<tr>
<td>Increased Zn concentration</td>
<td>Increased temperature</td>
<td>First calculation of interaction factors for the different Zn concentrations with
$$Intfact\ = \frac{combEff - SinglEff}{SinglEff}$$
Then derivation of the relationship between the Zn concentration and interaction factor, resulting in the equation
$$Intfact = 5 \cdot 10^{-6} \cdot Zn - 0.0026 \cdot Zn + 0.0031$$</td>
<td>R^2: 0.9875</td>
<td>Cotter et al. 1982</td>
<td>The formula was derived based on an analysis of the literature data</td>
</tr>
<tr>
<td colspan="6">Cotter et al. 1982 tested in a short-term experiment how blue mussels responded to increased temperatures and increased Zn concentrations in the water. To extract the influence of Zn on the effect of increased temperature, first the difference between the mortality at 29.7°C and the mortality at 31°C was calculated for each of the tested Zn concentrations. The difference between the observed mortality values at the two temperature were plotted against the increased Zn concentrations and described by a formula.




</td>
</tr>
</table>

Table 11 Effects of single stressors with regard to DEB processes or variables.

Pressure/ stressor	Observed effect	Literature	notes	Affected process/ corresponding variable in DEB model
Cu	Decreased scope for growth	Anderlini 1992	Mixture of Ag, Cu, Pb, and Zn	growth
	Stress response (induction of heat shock protein 70, which is energetically costly)	Radlowska and Pempowiak 2002	Single stressors and mixture of Cd, Pb, and Cu	energy
	Mortality, metallotheonein production (which is energetically costly), decreased phagocytosis	Han et al. 2014	Single stressor and in combination with reduced pH, Shandong province, China	energy
	Inhibitory effect on sperm and egg respiration	Akberali et al. 1985	Effect of single stressors and in combination with reduced pH	reproduction
	Increased cytotoxicity of the algal toxin okadaic acid	Traore et al. 1999	Chemical mixture of the metals Al, Cu, Pb, Hg and Cd	-
	Decreased growth, condition index and increased mortality	Grout and Levings 2000	Field study (transplantation study), elevated concentrations of Cu (main cause for the effect)	growth
	Decreased growth	Strömgren 1982	Effect of metals (Zn, Hg, Cu, Pb, Ni and Cd) tested separately	growth
	Mortality of larvae	Wisely and Blick 1967	Effects of metals (Hg, Cu and Zn) tested separately	reproduction
	Decreased sperm motility,	Earnshaw et al. 1986	Effect of Cu and Zn tested separately	reproduction
	Decreased heart an filtration rate	Grace and Gainly 1987		filtration rate
	Decreased survival rate, behaviour	Sunila 1981	Effect of Cu and Cd tested separately	Data used to model the exposure time- and

	response, byssogenesis			intensity dependency of the mussel as response to Cu
	Inhibition of embryo development	Beiras and Albentosa 2004	The experiment was conducted with *Mytilus galloprovincialis*, effects of Pb, Zn, Cd, Cu and Hg were analysed separately. An additive model could explain the combined effects of Cu and Zn.	reproduction
	Reduced filtration rate	Abel 1976		Filtration rate
Cd	Stress response (induction of heat shock protein 70, which is energetically costly)	Radlowska and Pempowiak 2002	Single test and mixturof Cd, Pb, and Cu	energy
	Mortality, metallotheonein production (which is energetically costly), decreased phagocytosis	Han et al. 2014	Single stressor and in combination with reduced pH, Shandong province, China	energy
	Increased cytotoxicity of the algal toxin okadaic acid	Traore et al. 1999	Chemical mixture of the metals Al, Cu, Pb, Hg and Cd	-
	Decreased growth	Strömgren 1982	Effect of metals (Zn, Hg, Cu, Pb, Ni and Cd) tested separately	growth
	Adverse effects on development, growth and mortality of larvae	Lehnberg and Theede 1979	Mussels from the Baltic Sea, interactive effects with salinity and temperature	Growth, development, reproduction
	Increased respiration rate, increased excretion rate, increased heat shock protein induction, reduced scope for growth	Tedengren et al. 1999	Short-term effect. Respiration could also become depressed at greater intensities or at longer exposure time as shown for other mussels	Respiration, energy, growth

	decreased clearance rate, respiration rate, and scope for growth	Mubiana and Blust 2007	Effect of chemical mixture of Cd, Cu, Co and increased temperature	Clearance rate, respiration rate, growth
	Decreased survival rate, behaviour response, byssogenesis	Sunila 1981	Effect of Cu and Cd tested separately	Data used to model the exposure time- and intensity dependency of the mussel as response to Cd
	Immunological response, growth	Sheir et al. 2013		growth
	Adverse effects on the serotonin system	Fraser et al. 2018	Serotonin regulates sexual differentiation, gamete production and spawning	reproduction
	Inhibition of embryo development	Beiras and Albentosa 2004	The experiment was conducted with *Mytilus galloprovincialis*, effects of Pb, Zn, Cd, Cu and Hg were analysed separately	reproduction
	mortality	Ashanulla 1976	Effects of Zn and Cd were analysed separately	mortality
Pb	Stress response (induction of heat shock protein 70, which is energetically costly)	Radlowska and Pempowiak 2002	Single test and mixturof Cd, Pb, and Cu	energy
	Mortality, metallotheonein production (which is energetically costly), decreased phagocytosis	Han et al. 2014	Single stressor and in combination with reduced pH, Shandong province, China	Energy, mortality can be incorporated in a population model
	Increased cytotoxicity of the algal toxin okadaic acid	Traore et al. 1999	Chemical mixture of the metals Al, Cu, Pb, Hg and Cd	-
	Decreased growth	Strömgren 1982	Effect of metals (Zn, Hg, Cu, Pb, Ni and Cd) tested separately	growth

	Scope for growth	Anderlini 1992	Mixture of Ag, Cu, Pb and Zn, New Zealand	growth
	Adverse effects on the serotonin system	Fraser et al. 2018	Serotonin regulates sexual differentiation, gamete production and spawning	reproduction
	Toxicity to embryos	Nadella et al. 2013	The experiment was conducted with *Mytilus trossulus* and *M. galloprovincialis*, effects of Pb and Zn were analysed seperately	reproduction
	Inhibition of embryo development	Beiras and Albentosa 2004	The experiment was conducted with *Mytilus galloprovincialis*, effects of Pb, Zn, Cd, Cu and Hg were analysed separately	reproduction
Zn	Inhibitory effect on sperm and egg respiration	Akberali et al. 1985	Effect of single stressors alone (O2, Cu, Zn, pH), in combination with reduced pH no difference in effect compared to the effect of the metal alone	reproduction
	Decreased sperm motility,	Earnshaw et al 1986	Effect of Cu and Zn tested separately	reproduction
	Inhibition of embryo development	Beiras and Albentosa 2004	The experiment was conducted with *Mytilus galloprovincialis*, effects of Pb, Zn, Cd, Cu and Hg were analysed separately	reproduction
	Decreased mitochondrial respiration	Akberali nd Earnshaw 1982		energy
	Adverse effects on the embryogenesis success	Beiras and Albentosa 2004	Effects of single stressors as well as combinations. An additive model could explain the combined effects of Cu and Zn. Galician	reproduction

			coast. *Mytilus galloprovincialis* was used as model organism	
	Adverse effects on byssal attachment, acute inflammatory reaction in the gills, dilation of branchial veins, swollen postlateral cells, necrosis of hemocytes, decreased opening response of the shells	Hietanen et al. 1988	Experiments were conducted with *Mytilus edulis* from the Baltic Sea	Respiration, energy
	Abnormal development of embryos	Nadella et al. 2013	Experiments conducted with *Mytilus galloprovincialis* and *Mytilus trossolus*	reproduction
	Decreased growth	Strömgren 1982	Effect of metals (Zn, Hg, Cu, Pb, Ni and Cd) tested separately	growth
	Mortality of larvae	Wisely and Blick 1967	Effects of metals (Hg, Cu and Zn) tested separately	reproduction
	Effects on respiration (increased after exposure), decreased growth, decreased survival	Hanna et al. 2013	Experiments conducted with *Mytilus galloprovincialis*	Growth
	Reduction of valve movement (reduced opening time)	Fdil et al. 2006	Effects of metals (Cu, Hg, Cd, Zn) tested separately, experiments conducted with *Mytilus galloprovincialis*	Filtration rate
	Reduced filtration rate	Abel 1976		Filtration rate
	Increased condition index with increased temperature, mortality	Fischer 1986	Interactive effect with Cd as the process of binding Cd in soft tissues is accelerated with increased temperature	growth
	Effects on development, growth and mortality of larvae	Lehnberg and Theede 1979	Mussels from the Baltic Sea, interactive	Growth, development, reproduction

			effects with salinity and Cd	
Temperature	Increased heart rate and oxygen consumption with increased temperature	Bakhmet 2017		Metabolic rates
	Metabolic depression	Lesser 2016	Effects of the stressors temperature and acidification analysed separately and in combination	Metabolic rates
	Reduced clearance rate, adverse effects of elevated temperature on growth energy balance	Tateda et al. 2015	Experiments conducted with *Mytilus galloprovincialis*	Clearance rate, growth
	Temperature dependent metabolic rate	Thyrring et al 2015		Metabolic rates
	Glycogen storage tissue and gametogenis temperature dependent	Fearman and Moltschaniwskyj 2010	Experiments conducted with *Mytilus galloprovincialis*	Production of gametes
	Percentage of unfertilized eggs	Riba et al. 2016	Effect of reduced pH and metal mixture (Cr, Ni, Cu, Zn, Cd, and Pb) at pH less than 6.5, Bay of Cadiz	reproduction
	Inhibitory effect on sperm and egg respiration	Akberali et al. 1985	Effect of reduced pH alone, more adverse effect in combination with Cu	reproduction
	Inhibition of egg fertilisation	Riba et al. 2016	*Mytilus edulis*, effects of pH alone and in combination with metals tested. Acidity increased the concentrations of Cu, Zn Cd and Pb but were less available for organisms	reproduction
pH	Metabolic depression	Lesser 2016	Effects of the stressors temperature and acidification	Metabolic rates

			analysed separately and in combination	
	Altered metabolism	Zittier et al. 2015		Metabolic rates
	Decreased calcification rate, induced expression of biomineralization-related genes	Li et al. 2015	Interactive effects with temperature, energetically costly process	Energy
O2	Reduced condition index at low oxygen levels	Fischer 1986	Mussels from Kieler Förde	growth

Table 12 Literature data used to characterize the single-stressor model (optimization process)

Experimental data sets for estimating the parameters	Literature and notes	Observed effect	Exposure time	range
Cd	Sunila et al. 1981	survival	21 days	0-25000 µg/L
Cu	Sunila et al. 1981	survival	21 days	0-5000 µg/L
Pb	Schulz-Blades 1972	survival	130 days	0-5000 µg/L
Zn	Hietanen et al. 1988	survival	41 days	0-100000 µg/L
pH	Bamber et al. 1990	survival	31 days	5.8-6.6
O2	Wang and Widdows 1991	growth	10 days	0.6-8.7 mg/L
Temperature	Widdows 1973,	oxygen consumption rate	21 days	10-25 °C

Table 13 Stressor-specific parameters used for the single stressor model

Parameter	stressor	Value/ model	Literature and notes
Growth rate		0.848	Van der Veer et al. 2006
Uptake rate	Cd	0.578	Wang and Fischer 1997, value for mussels of 2.5 cm length used
	Cu	0.2921	Calculated based on Adema 1981 and van Haren et al 1990
	Pb	0.132	Mubiana and Blust 2007, mean of uptake rate at 6°C and 16°C
	Zn	1.464	Wang and Fischer 1997, value for mussels of 2.5 cm length used
Efflux rate	Cd	0.0324	Wang and Fischer 1997, value for mussels of 2.5 cm length used
	Cu	0.0990	Calculated based on Adema 1981 and Van Haren et al 1990
	Pb	0.013	Schulz-Baldes 1974
	Zn	0.0141	Wang and Fischer 1997, value for mussels of 2.5 cm length used

Tolerance value (transition threshold)	Cd	16.91 µg/g	growth inhibition after 9 days of exposure at 5 µg/L (Strömgren 1982), Increased heart rate at 10 µg/L (Bakhmet et al. 2013), corresponding internal concentration calculated with uptake model based on concentration in Strömgren 1982
	Cu	2.37 µg/g	3 µg/L , Growth inhibition after 12 days (Strömgren 1982), Long term growth experiment (21 months) effect at 10 µg/L (Calabrese 1984), corresponding internal concentration calculated with uptake model based on concentration in Strömgren 1982
	Pb	8.16 µg/g	Zhao-Xiang Han et al. 2013
	Zn	227 µg/g	10 µg/L , growth inhibition after 22 days of exposure (Strömgren 1982), corresponding internal concentration calculated with uptake model based on concentration in Strömgren 1982, internal concentrations measured in the south eastern North Sea: between about 75 and 245 µg/g (Borchardt et al. 1988)
	pH	7.7 (lower limit)	lower limit: reduced shell increment, altered respiration rates, energy loss by increased excretion (Thomsen and Melzner 2010)
	O2	6 mg/L (lower limit)	decreased respiration rate (Tang and Riisgard 2018)
	Temperature	25°C (upper limit)	upper limit: MO2, oxygen consumption rate (Zittier et al.2015) , lower limit: several years with winters with long periods of ice cover (Witte et al. 2013), lower limit could not be integrated in the model
Optimum	metals	0	It is assumed that the amount of certain metals needed (e.g. for enzyme synthesis compared to the concentrations tested here is negligibly small. Therefore, the optimum concentration of all metals is assumed to be zero for all chosen metals.
	pH	8.1	Heinemann et al. 2012
	O2	9.29	Fischer 1986
	Temperature	15.8	Lauzon-Guay et al. 2006
Growth rate		0.848	Van der Veer et al. 2006

Uptake rate	Cd	0.578	Wang and Fischer 1997, value for mussels of 2.5 cm length used
	Cu	0.2921	Calculated based on Adema 1981 and van Haren et al 1990
	Pb	0.132	Mubiana et al. 2007, mean of uptake rate at 6°C and 16°C
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Efflux rate	Cd	0.0324	Wang and Fischer 1997, value for mussels of 2.5 cm length used
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	Pb	8.16 µg/g	Zhao-Xiang Han et al. 2013
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	pH	7.7 (lower limit)	lower limit: reduced shell increment, altered respiration rates, energy loss by increased excretion (Thomsen and Melzner 2010)

	O2	6 mg/L (lower limit)	decreased respiration rate (Tang and Riisgard 2018)
	Temperature	25°C (upper limit)	upper limit: MO2, oxygen consumption rate (Zittier et al.2015) , lower limit: several years with winters with long periods of ice cover (Witte et al. 2013), lower limit could not be integrated in the model
Optimum	metals	0	It is assumed that the amount of certain metals needed (e.g., for enzyme synthesis compared to the concentrations tested here is negligibly small. Therefore, the optimum concentration of all metals is assumed to be zero for all chosen metals.
	pH	8.1	Heinemann et al. 2012
	O2	9.29	Fischer 1986
	Temperature	15.8	Lauzon-Guay et al. 2006

Table 14 DEB-parameter values used for the main DEB-model

DEB parameter	Unit	Value	Source	note
Structural mass at birth ([MV0])	mol C	3.3×10^{-9}	Van der Veer et al. 2006	
Initial reserve mass at optimal food conditions ([ME0])	Mol C^E	1.48×10^{-10}	Van der Veer et al. 2006	
Maturity at birth (E_{Hb})	J	2.99×10^{-5}	Saraiva et al. 2012	Cumulative Maturity at birth (M^b_H) = $E_{Hb/}$ μ_E
Maturity at puberty (E_{Hp})	J	1.58×10^{2}	Saraiva et al. 2012	Cumulative Maturity at puberty (M^p_H) = $E_{Hp/}$ μ_E
Bivalve structure and reserve specific density (d_v)	dw/cm^3	0.2	Rosland et al. 2009	
Bivalve reserve chemical potential (μ_E)	Joule mol^{-1}	6.97×10^{5}	Saraiva et al. 2012	
Bivalve reserve/ structure relative molecular biomass (w_v)	$g_{(dw)}$ mol^{-1}	25.22	Saraiva et al. 2012	
Shape coefficient δ_M	no unit	0.297	Saraiva et al 2011a	

Energy conductance ($\dot{v}$)	Cm d^{-1}	0.056	Saraiva et al 2011a	
Allocation fraction to growth and somatic maintenance (kap)	none	0.67	Saraiva et al 2011a	
Volume specific somatic maintenance ($[p_M]$)	J d^{-1} cm^{-3}	11.6	Saraiva et al 2011a	
Specific costs for structure (EG)	J cm^3	5993	Saraiva et al 2011a	
Maximum surface area specific clearance rate (CRm)	m^3 d^{-1} cm^{-2}	0.096	Saraiva et al. 2011b	
Algal maximum surface area specific filtration rate JxiFm	mol C^{-1} cm^{-2}	4.8×10^{-4}	Rosland et al. 2009	
Algal binding probability pxiI	no unit	0.9	Value changed from 0.4 in Saraiva et al. 2011a	
Algal maximum ingestion rate JxiIm	mol C d^{-1}	1.3×10^4	Saraiva et al. 2011a	
Algal nitrogen:carbon ratio (nxiN)	mol N mol^{-1} C	0.1509		Calculated from Redfield Ratio
Algal phosphor: carbon ratio (nxiP)	mol P mol^{-1} C	0.0094		Calculated from Redfield Ratio
Chemical composition of bivalve reserve/ structure for P (n_{EP})	mol P mol^{-1}C	0.006	Saraiva et al. 2012	
Chemical composition of bivalve reserve/ structure for N (n_{EN})	mol N mol^{-1}C	0.18	Saraiva et al. 2012	
Reserve fraction in algal mass (fE)	No unit	0.5	Saraiva et al. 2012	
Yield coefficient of reserves in algal structure ($Y_{EX}{}^{v}$)	mol C^E $mol^{-1}C^v$	0.75	Saraiva et al. 2012	
Reference temperature (T_{ref})	Kelvin	293	Van der Veer et al. 2006	
Arrhenius temperature (T_A)	Kelvin	7022	Van der Veer et al. 2006	
Lower temperature boundary range (T_L)	Kelvin	275	Van der Veer et al. 2006	

Upper temperature boundary range (T_H)	Kelvin	296	Van der Veer et al. 2006	
Arrhenius temperatures for rate of decrease at lower boundaries (T_{AL})	Kelvin	45430	Van der Veer et al. 2006	
Arrhenius temperatures for rate of decrease at upper boundaries (T_{AH})	Kelvin	31376	Van der Veer et al. 2006	
Spawning period (R_{spawn})	days	1	Saraiva et al.2012	
Minumum temperature for spawning (T_{spawn})	Kelvin	282.6	Hummel et al. 1989	
Reproduction efficiency (K_R)	No unit	0.95	Kooijmann 2010	
Gonado-somatic ratio to spawn (GSR_{spawn})	mol C^R mol^{-1} C	0.2	Saraiva et al.2012	
Reproduction efficiency (K_R)	No unit	0.95	Kooijmann 2010	

Table 15 Environmental data used for the test of the model

Forcing variables	**Unit**	**Source**	**Additional modeling procedure**
Temperature	Kelvin	HAMSOM model, ZMAW	None additional
Carbon content of phytoplankton	C m^{-3}	NLWKN data set from monitoring program, AquaEcology	Interpolation in Matlab (interpl, pchip)
Metals Cu, Cd, Pb, Zn	µg L^{-1}	NLWKN	Interpolation in Matlab (interpl, pchip)
pH	No unit	NLWKN	Interpolation in Matlab (interpl, pchip)
O2	mg L^{-1}	NLWKN	Interpolation in Matlab (interpl, pchip)

Table 16 Cumulative percent change between the control- and the stress-scenarios

Cumulative percent change	**Control- vs additive scenario**	**Control- vs cumulative scenario**	**Additive scenario vs cumulative scenario**
Bivalve structural mass (M_V)	-52.54	-63.405	-22.892

Bivalve reserve biomass (M_E)	-52.437	-67.373	-31.402
Bivalve maturity investment (M_H)	-16.334	-36.752	-24.405
Bivalve reproduction buffer (M_R)	-52.351	-51.472	1.8444
Gamets produced	-58.183	-71.914	-32.836
Size	-20.236	-26.979	-8.4529

Start value analysis

Beta

Test of ten start values between 0 and 1 (equally distributed) with all possible combinations with the other parameters

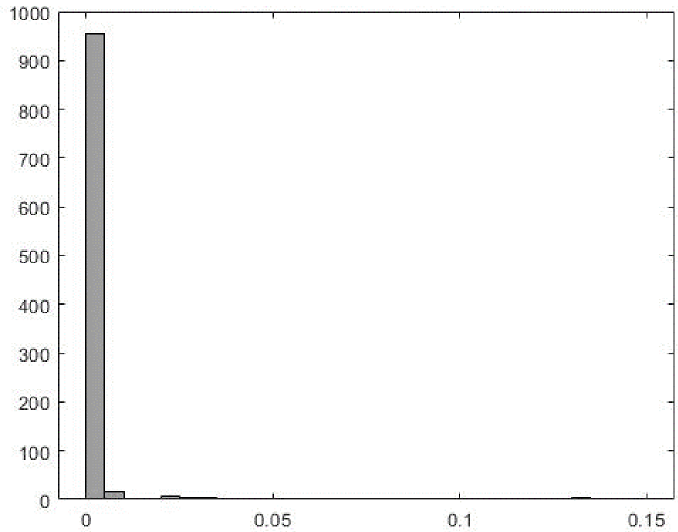

Figure 39 Result of the parameter optimization for beta

➔ Final StartValue: 0.025

Mu

Test of ten start values between 0 and 50 (equally distributed) with all possible combinations with the other parameters

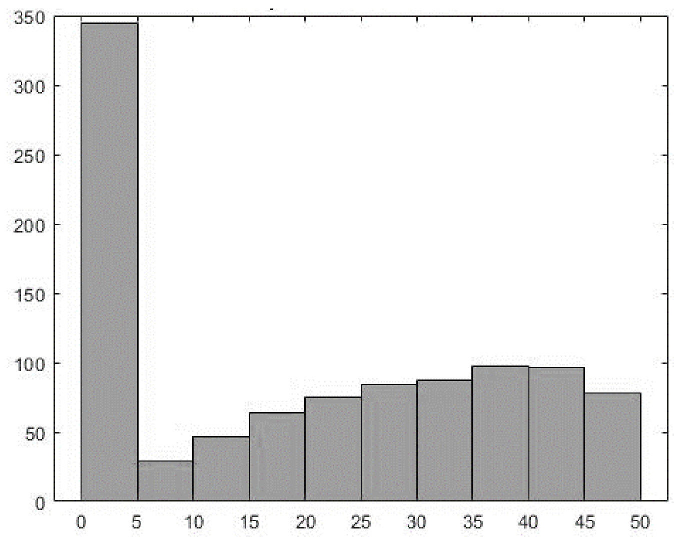

Figure 40 Result of the parameter optimization for mu

- ➔ Final StartValue: 2.5

Alpha

Test of ten start values between 0 and 1 (equally distributed) with all possible combinations with the other parameters

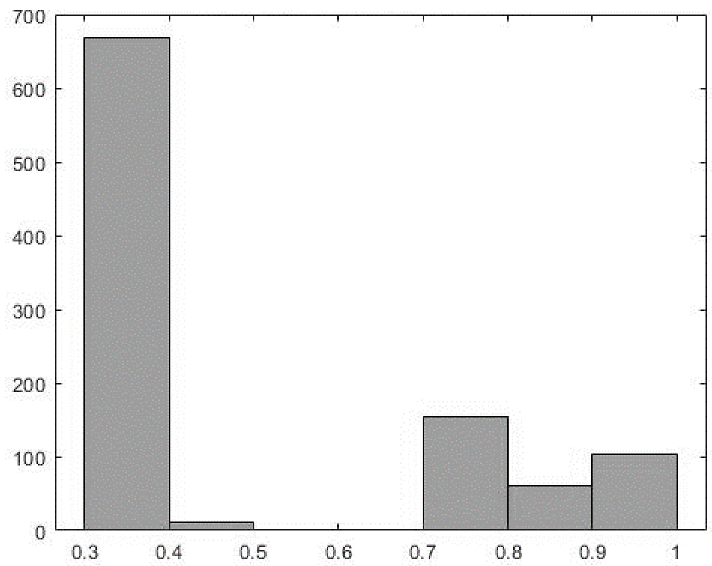

Figure 41 Result for the parameter optimization for alpha

- ➔ Final StartValue: 0.0025

Cd

Beta: start value: 0.002.

final: 0.0016

Mu: start value: 0.002

Final: 0.0051

Alpha: start value: 0.002

Final: 0.9999

Mean error: 13.1004

Mean relative error: 0.1631

Standard deviation: 17.6009

Relative standard deviation: 0.2712

Standard error: 1.2508

Exitflag: 2

Number of iterations: 57

Algorithm: interior-point

```
Nexp
   100   100   100   100   100   100   100   100   100
   100   100   100   100    98    92    97    92    67
   100   100   100   100    93    91    96    91    65
   100   100   100   100    91    90    96    90    64
   100   100    99   100    88    85    96    86    63
   100   100   100   100   100    98    98    98    88
   100   100   100   100   100    94    97    93    71
   100   100    99    99    85    81    95    83    62
   100   100    99    99    82    80    95    81    62
   100   100    99    98    80    78    95    81    61
   100   100    99    97    60    67    95    78    61
   100   100    99    97    47    55    93    67    60
   100   100    99    97    21    31    92    50    60
   100   100    99    97    12    24    92    41    58
   100   100    99    90    10    11    90    27    52
   100   100    98    85     9     5    86    18    47
   100   100    98    85     7     3    85    11    40
   100    99    99    82     5     3    80     8    28
   100    98    99    82     4     2    68     6    13
   100    98    98    81     2     2    65     2    11
   100    98    98    81     2     2    53     2    10
   100    98    97    80     1     1    45     1     9
```

Nmod

100	100	100	100	100	100	100	100	100
100	100	100	100	100	100	100	100	100
100	100	100	100	100	100	100	100	99
100	100	100	100	100	100	100	99	98
100	100	100	100	99	99	99	98	95
100	100	100	99	99	98	98	96	91
100	100	99	99	98	97	97	94	85
100	99	99	98	97	96	95	90	78
100	99	98	97	96	94	93	86	70
100	99	98	96	94	92	90	81	61
100	99	97	94	92	89	86	75	52
100	98	96	92	89	86	83	69	43
100	97	95	90	86	82	78	63	35
100	97	94	88	83	78	74	56	28
100	96	92	85	79	74	69	49	22
100	95	91	83	75	69	63	43	17
100	94	89	79	71	64	58	37	13
100	93	87	76	67	60	53	32	10
100	92	85	73	63	55	48	27	8
100	91	83	69	59	50	43	23	6
100	89	80	66	54	46	39	19	5
100	88	78	62	50	41	34	16	3

Diff

0	0	0	0	0	0	0	0	0
0	0	0	0	-2	-8	-3	-8	-33
0	0	0	0	-7	-9	-4	-9	-34
0	0	0	0	-9	-10	-4	-9	-34
0	0	-1	0	-11	-14	-3	-12	-32
0	0	0	1	1	0	0	2	-3
0	0	1	1	2	-3	0	-1	-14
0	1	0	1	-12	-15	0	-7	-16
0	1	1	2	-14	-14	2	-5	-8
0	1	1	2	-14	-14	5	0	0
0	1	2	3	-32	-22	9	3	9
0	2	3	5	-42	-31	10	-2	17
0	3	4	7	-65	-51	14	-13	25
0	3	5	9	-71	-54	18	-15	30
0	4	7	5	-69	-63	21	-22	30
0	5	7	2	-66	-64	23	-25	30
0	6	9	6	-64	-61	27	-26	27
0	6	12	6	-62	-57	27	-24	18
0	6	14	9	-59	-53	20	-21	5
0	7	15	12	-57	-48	22	-21	5
0	9	18	15	-52	-44	14	-17	5
0	10	19	18	-49	-40	11	-15	6

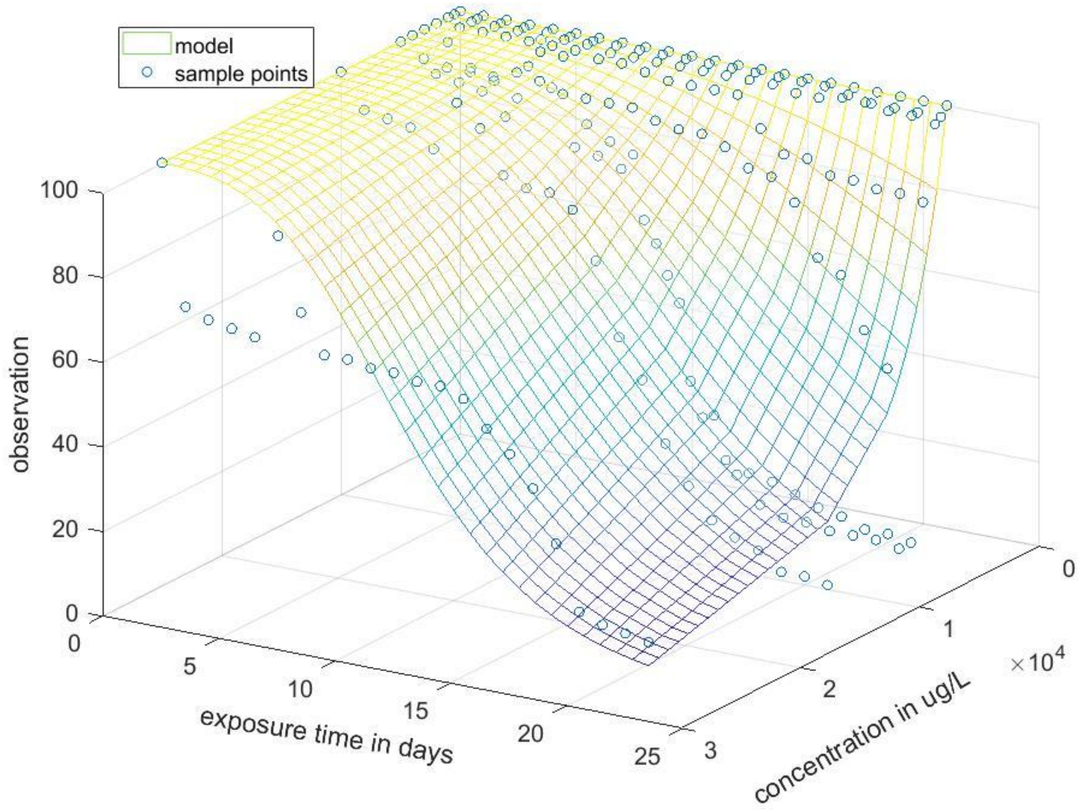

Figure 42 Dose response curves for different exposure times (model and literature data points) for Cd effects on *Mytilus edulis*

Cu

Beta: start value: 0.002

final: 0.0520

Mu: start value: 0.002

Final: 0.0006

Alpha: start value: 0.002

Final: 1.7339e-05

Mean error: 7.8574

Mean relative error: 0.1143

Standard deviation: 9.1868

Relative standard deviation: 0.1876

Standard error: 0.6529

Exitflag: 2

Number of iterations: 56

Algorithm: interior-point

Nexp

100	100	100	100	100	100	100	100	100
100	100	100	100	100	100	100	100	100
100	100	100	100	100	100	99	98	98
100	100	100	100	100	100	98	98	98
100	100	100	100	97	100	98	98	95
100	100	100	100	95	100	98	98	90
100	100	100	100	88	98	95	95	88
100	100	100	100	87	97	91	92	72
100	100	100	100	82	94	82	88	56
100	100	100	100	75	91	77	81	28
100	100	100	100	72	88	61	78	12
100	100	100	97	65	80	49	68	5
100	100	100	97	65	75	31	55	2
100	100	100	96	64	65	28	40	2
100	100	100	96	63	48	27	32	1
100	100	98	96	55	35	25	28	1
100	100	98	96	54	28	20	18	1
100	100	97	95	48	26	19	13	1
100	100	97	95	41	21	15	12	1
100	100	97	95	41	20	12	12	1
100	100	97	95	40	20	10	15	1
100	100	97	95	39	19	11	11	1

Nmod

100	100	100	100	100	100	100	100	100
100	100	100	100	100	100	100	100	100
100	100	100	100	100	100	100	99	98
100	100	100	100	100	99	99	98	94
100	100	100	99	99	99	98	95	88
100	100	99	99	98	98	96	91	80
100	99	99	98	97	96	93	86	69
100	99	98	97	96	94	89	80	57
100	98	97	95	94	92	85	72	44
100	98	96	93	91	89	80	63	32
100	97	94	91	88	86	74	54	22
100	96	92	89	85	82	67	45	14
100	95	90	86	82	78	60	36	8
100	94	88	83	78	73	53	28	4
100	93	86	79	74	68	46	22	2
100	91	83	76	69	63	40	16	1
100	90	80	72	65	58	34	11	0
100	88	77	68	60	53	28	8	0
100	86	74	64	55	48	23	5	0
100	84	71	60	50	43	18	3	0
100	82	68	56	46	38	14	2	0
100	80	64	52	41	33	11	1	0

Diff

0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	-1	-1	0
0	0	0	0	0	1	-1	0	4
0	0	0	1	-2	1	0	3	7
0	0	1	1	-3	2	2	7	10
0	1	1	2	-9	2	2	9	19
0	1	2	3	-9	3	2	12	15
0	2	3	5	-12	2	-3	16	12
0	2	4	7	-16	2	-3	18	-4
0	3	6	9	-16	2	-13	24	-10
0	4	8	8	-20	-2	-18	23	-9
0	5	10	11	-17	-3	-29	19	-6
0	6	12	13	-14	-8	-25	12	-2
0	7	14	17	-11	-20	-19	10	-1
0	9	15	20	-14	-28	-15	12	0
0	10	18	24	-11	-30	-14	7	1
0	12	20	27	-12	-27	-9	5	1
0	14	23	31	-14	-27	-8	7	1
0	16	26	35	-9	-23	-6	9	1
0	18	29	39	-6	-18	-4	13	1
0	20	33	43	-2	-14	0	10	1

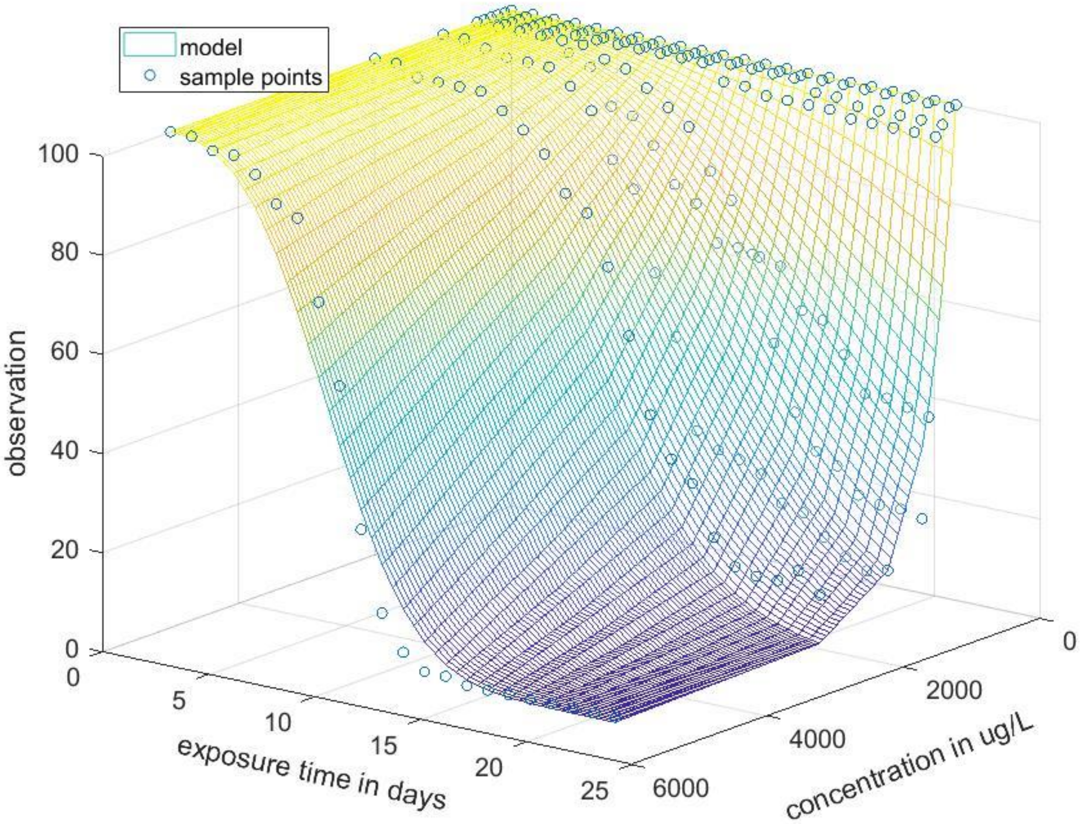

Figure 43 Dose response curves for different exposure times (model and literature data points) for Cu effects on *Mytilus edulis*

Pb

Beta: start value: 0.002

final: 0.0001

Mu: start value: 0.002

Final: 0.0009

Alpha: start value: 0.002

Final: 0.9999

Mean error: 7.1549

Mean relative error: 0.0454

Standard deviation: 8.3190

Relative standard deviation: 0.0549

Standard error: 1.1117

Exitflag: 1

Number of iterations: 16

Algorithm: interior-point

```
Nexp
   100   100   100   100
   100   100   100   100
   100   100   100   100
   100   100   100   100
   100    98   100    99
   100    95    99    95
    99    90    92    85
    97    88    85    74
    95    82    79    69
    92    80    73    65
    90    70    68    57
    85    67    62    49
    81    65    59    38
    78    59    51    30

Nmod
   100   100   100   100
   100   100   100   100
   100   100   100    99
   100   100    99    97
   100    99    99    94
   100    99    98    89
   100    98    96    82
   100    97    94    75
   100    96    92    67
   100    94    89    59
   100    93    86    51
   100    91    83    43
   100    89    79    36
   100    86    75    30

Diff
     0     0     0     0
     0     0     0     0
     0     0     0     1
     0     0     1     3
     0    -1     1     5
     0    -4     1     6
    -1    -8    -4     3
    -3    -9    -9    -1
    -5   -14   -13     2
    -8   -14   -16     6
   -10   -23   -18     6
   -15   -24   -21     6
   -19   -24   -20     2
   -22   -27   -24     0
```

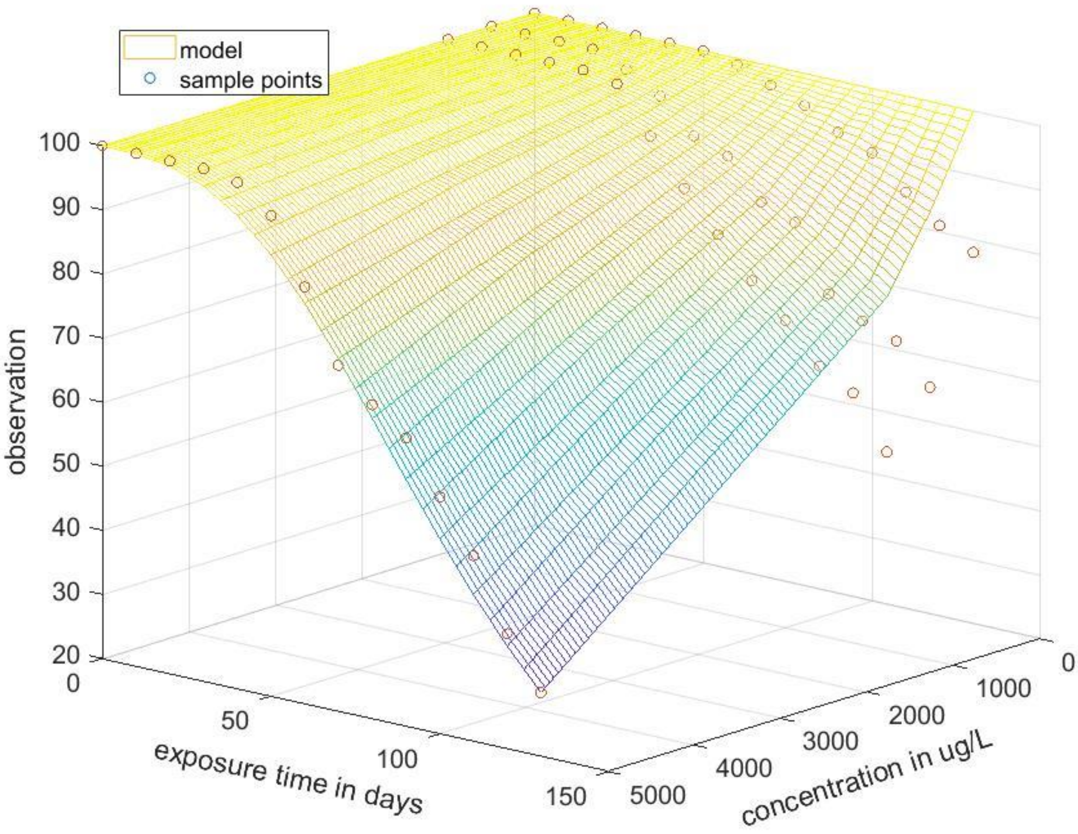

Figure 44 Dose response curves for different exposure times (model and literature data points) for Pb effects on *Mytilus edulis*

Zn

Beta: start value: 0.002

final: 1.309e-05

Mu: start value: 0.002

Final: 49.9763

Alpha: start value: 0.002

Final: 9.9867e-07

Mean error: 4.5701

Mean relative error: 0.0682

Standard deviation: 5.5831

Relative standard deviation: 0.1095

Standard error: 0.4859

Exitflag: 2

Number of iterations: 160

Algorithm: interior-point

Nexp

100	100	100	100	100	100
100	100	100	98	99	80
100	100	99	98	92	74
100	99	98	97	89	71
100	100	99	97	88	68
100	99	98	95	88	59
100	98	98	92	80	49
100	97	97	90	72	40
100	97	95	85	70	38
100	97	92	72	60	27
100	96	91	79	51	20
100	96	90	76	40	15
100	95	89	71	29	12
100	92	90	62	21	8
100	91	90	55	18	5
100	91	86	52	13	5
100	91	86	52	11	5
100	91	83	52	10	4
99	91	82	52	10	4
99	91	82	52	10	4
99	89	82	51	9	4
99	88	81	49	9	4

Nmod

100	100	100	100	100	100
100	100	100	100	99	99
100	100	100	99	98	96
100	100	99	97	95	90
100	99	98	95	91	83
100	99	97	93	86	74
100	98	96	90	80	64
100	98	94	86	74	55
100	97	92	82	67	45
100	96	90	78	61	37
100	95	88	73	54	29
100	94	86	69	48	23
100	93	84	65	42	17
100	92	82	60	36	13
100	91	79	56	31	10
100	90	77	51	26	7
100	89	74	47	22	5
100	87	72	43	19	3
100	86	69	39	16	2
100	85	66	36	13	2
100	84	64	33	11	1
100	83	63	31	10	1

Diff

0	0	0	0	0	0
0	0	0	-2	0	-19
0	0	-1	-1	-6	-22
0	-1	-1	0	-6	-19
0	1	1	2	-3	-15
0	0	1	2	2	-15
0	0	2	2	0	-15
0	-1	3	4	-2	-15
0	0	3	3	3	-7
0	1	2	-6	-1	-10
0	1	3	6	-3	-9
0	2	4	7	-8	-8
0	2	5	6	-13	-5
0	0	8	2	-15	-5
0	0	11	-1	-13	-5
0	1	9	1	-13	-2
0	2	12	5	-11	0
0	4	11	9	-9	1
-1	5	13	13	-6	2
-1	6	16	16	-3	2
-1	5	18	18	-2	3
-1	5	18	18	-1	3

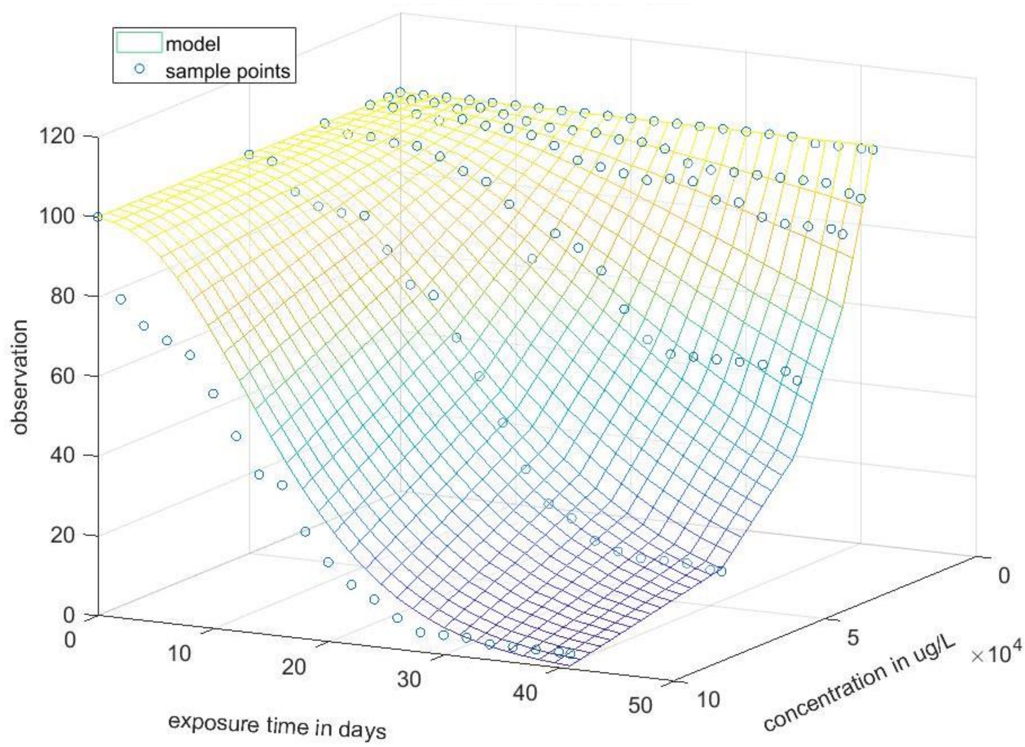

Figure 45 Dose response curves for different exposure times (model and literature data points) for Zn effects on *Mytilus edulis*

Temp

Beta: start value: 0.002

final: 0.0443

Mu: start value: 0.002

Final: 45.6804

Alpha: start value: 0.002

Final: 0.9966

Mean error: 11.5074

Mean relative error: 0.0747

Standard deviation: 14.2911

Relative standard deviation: 0.0937

Standard error: 3.1956

Exitflag: 2

Number of iterations: 52

Algorithm: interior-point

```
Nexp
   99.7110  100.0000   99.7010  100.0000
   57.8070  100.0000   72.8070   48.3520
   68.0550   98.9270   81.6690   69.8730
   79.6210   94.6110   94.7630   52.7610
   87.6500   90.9320   95.2970   62.3630

Nmod
   100   100   100   100
    99    99    98    96
    94    97    87    76
    89    95    78    62
    85    92    70    52

Diff
   -0.2890         0   -0.2990         0
  -41.1930    1.0000  -25.1930  -47.6480
  -25.9450    1.9270   -5.3310   -6.1270
   -9.3790   -0.3890   16.7630   -9.2390
    2.6500   -1.0680   25.2970   10.3630
```

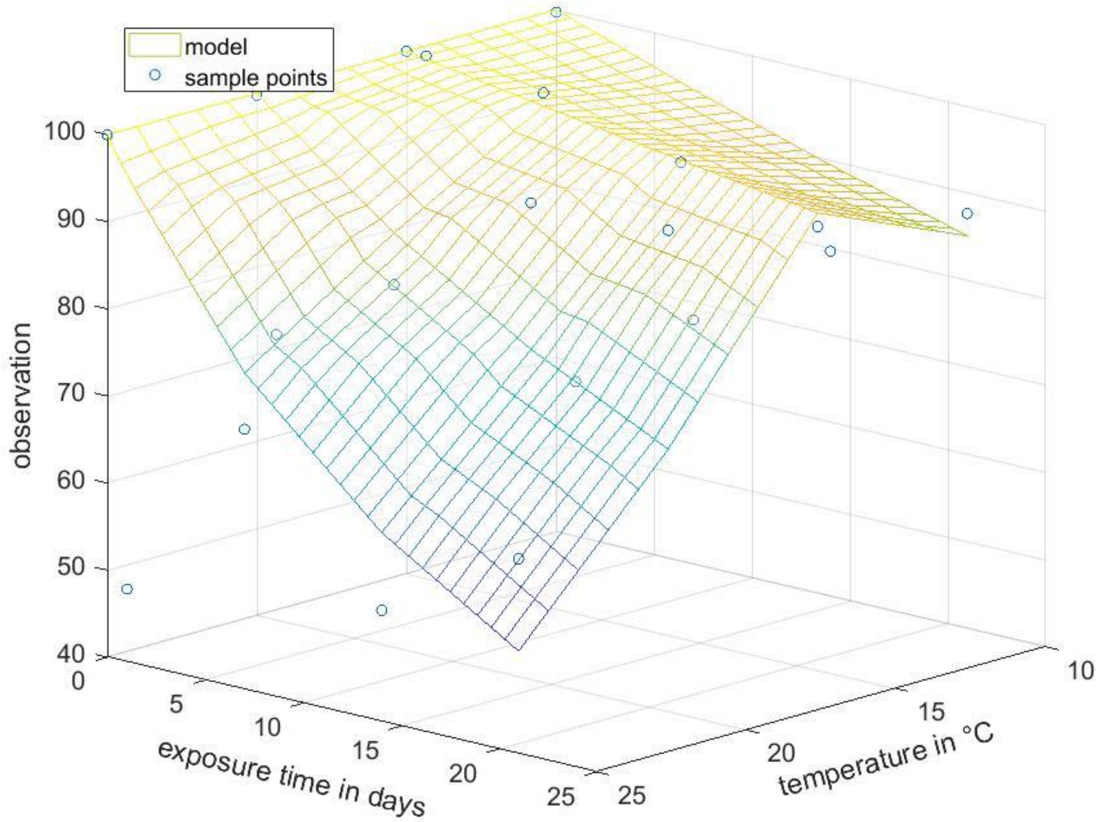

Figure 46 Response curves for altered temperature values and different exposure times (model and literature data points) for effects on *Mytilus edulis*

pH

Beta: start value: 0.002

final: 0.1952

Mu: start value: 0.002

Final: 0.0019

Alpha: start value: 0.002

Final: 8.5448-06

Mean error: 15.1712

Mean relative error: 0.1651

Standard deviation: 14.9605

Relative standard deviation: 0.2627

Standard error: 1.9992

Exitflag: 2

Number of iterations: 123

Algorithm: interior-point

Nexp

100.0000	100.0000	100.0000	100.0000
100.0000	100.0000	100.0000	100.0000
100.0000	100.0000	100.0000	100.0000
100.0000	100.0000	100.0000	100.0000
100.0000	100.0000	100.0000	100.0000
100.0000	97.1130	96.9770	79.5850
100.0000	94.9590	92.2410	43.8710
100.0000	92.7980	89.8090	22.8250
100.0000	86.4460	75.5760	9.6800
100.0000	78.4370	71.2360	7.2440
100.0000	61.0610	53.3130	4.9440
93.5390	55.9030	41.7720	3.1860
91.5070	49.6590	34.4420	0
90.1560	46.5410	30.6440	0

Nmod

100	100	100	100
99	98	98	98
98	97	97	96
96	95	95	93
95	94	93	92
89	87	86	82
85	81	80	76
82	78	76	71
73	67	66	59
72	65	63	56
68	61	59	51
66	59	57	49
64	57	55	46
62	55	52	44

Diff

0	0	0	0
1.0000	2.0000	2.0000	2.0000
2.0000	3.0000	3.0000	4.0000
4.0000	5.0000	5.0000	7.0000
5.0000	6.0000	7.0000	8.0000
11.0000	10.1130	10.9770	-2.4150
15.0000	13.9590	12.2410	-32.1290
18.0000	14.7980	13.8090	-48.1750
27.0000	19.4460	9.5760	-49.3200
28.0000	13.4370	8.2360	-48.7560
32.0000	0.0610	-5.6870	-46.0560
27.5390	-3.0970	-15.2280	-45.8140
27.5070	-7.3410	-20.5580	-46.0000
28.1560	-8.4590	-21.3560	-44.0000

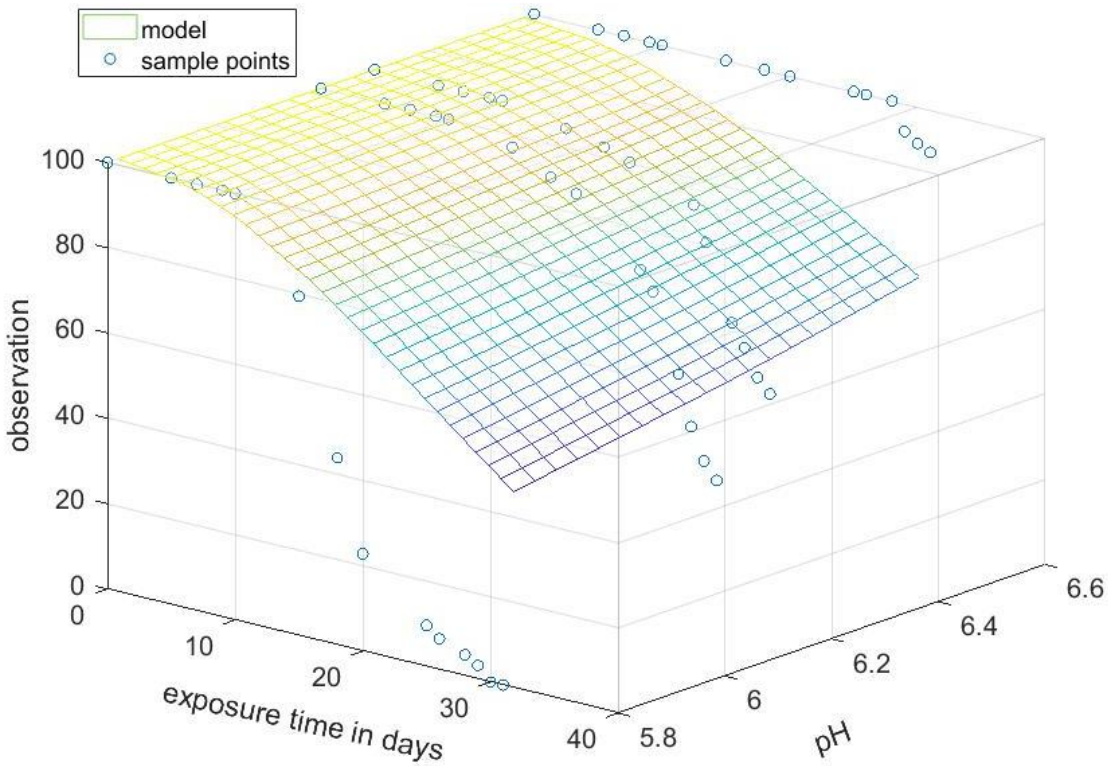

Figure 47 Response curves for altered pH values and different exposure times (model and literature data points) for effects on *Mytilus edulis*

<u>oxygen</u>

Beta: start value: 0.002

final: 0.0210

Mu: start value: 0.002

Final: 0.0196

Alpha: start value: 0.002

Final: 0.5083

Mean error: 2.0293

Mean relative error: 0.0109

Standard deviation: 3.7613

Relative standard deviation: 0.0209

Standard error: 0.7678

Exitflag: 2

Number of iterations: 118

Algorithm: interior-point

```
Nexp
  100.0000  100.0000  100.0000  100.3200
  100.0000  100.1500  101.9800   97.2560
  100.0000  100.0000   99.7050   94.1090
  100.0000   99.4520   99.4520   89.3150
  100.0000   98.2050   97.4360   86.5380
  100.0000   96.0760   95.4820   80.8560

Nmod
   100   100   100   100
   100   100   100   100
   100    99    99    99
   100    99    98    98
   100    97    97    97
   100    96    95    95

Diff
         0         0         0    0.3200
         0    0.1500    1.9800   -2.7440
         0    1.0000    0.7050   -4.8910
         0    0.4520    1.4520   -8.6850
         0    1.2050    0.4360  -10.4620
         0    0.0760    0.4820  -14.1440
```

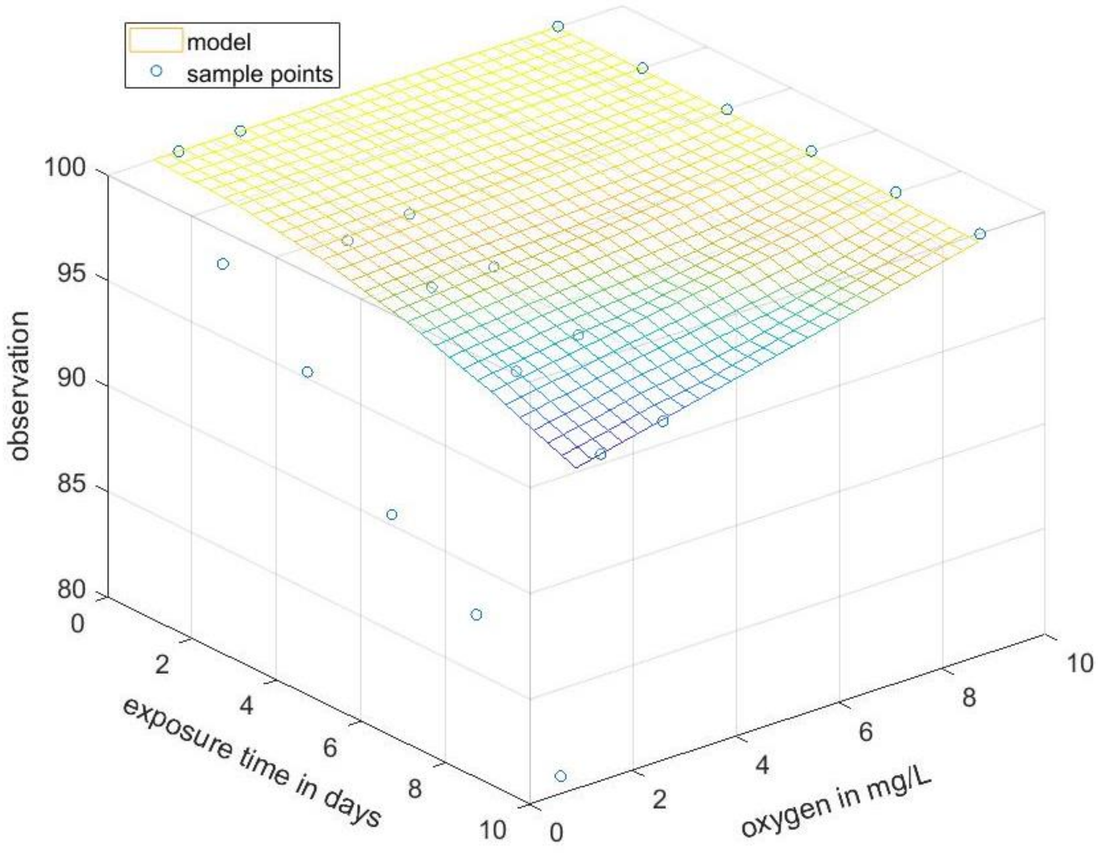

Figure 48 Response curves for altered oxygen concentrations and different exposure times (model and literature data points) for effects on *Mytilus edulis*

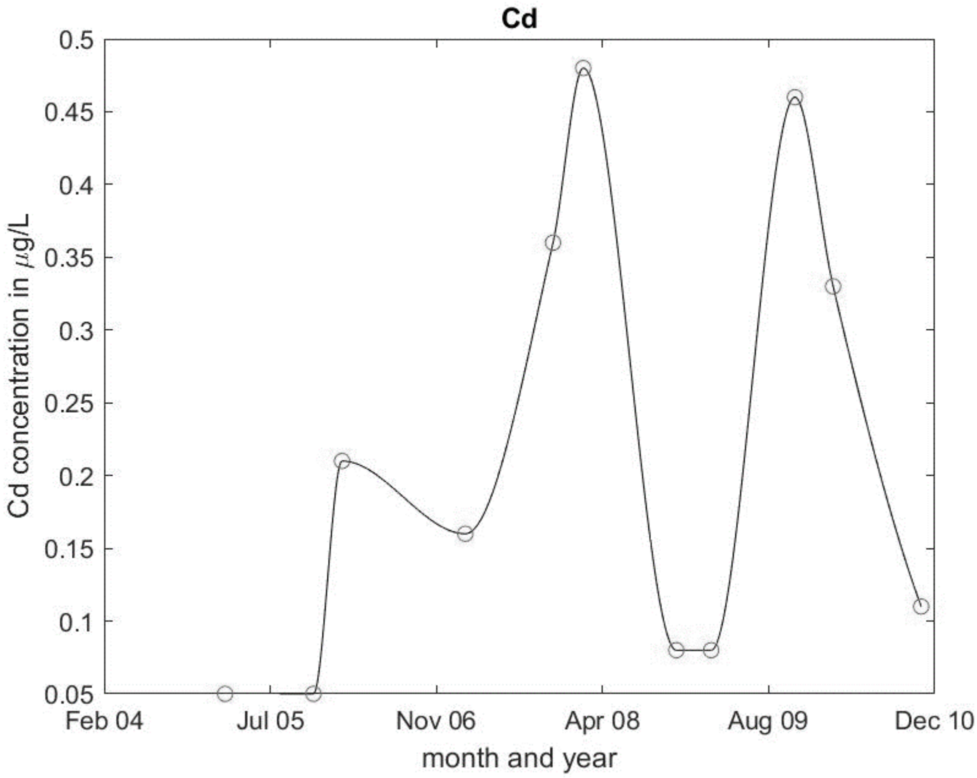

Figure 49 Measured Cd concentration (monitoring program) and interpolated values

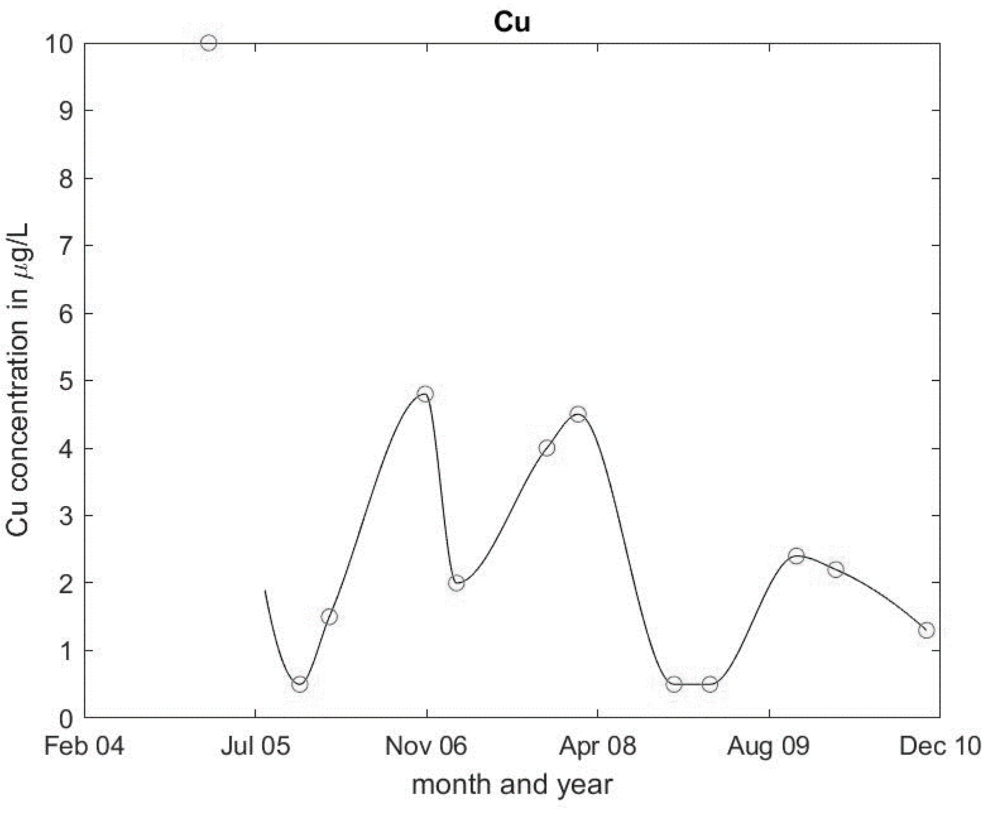

Figure 50 Measured Cu concentration (monitoring program) and interpolated values

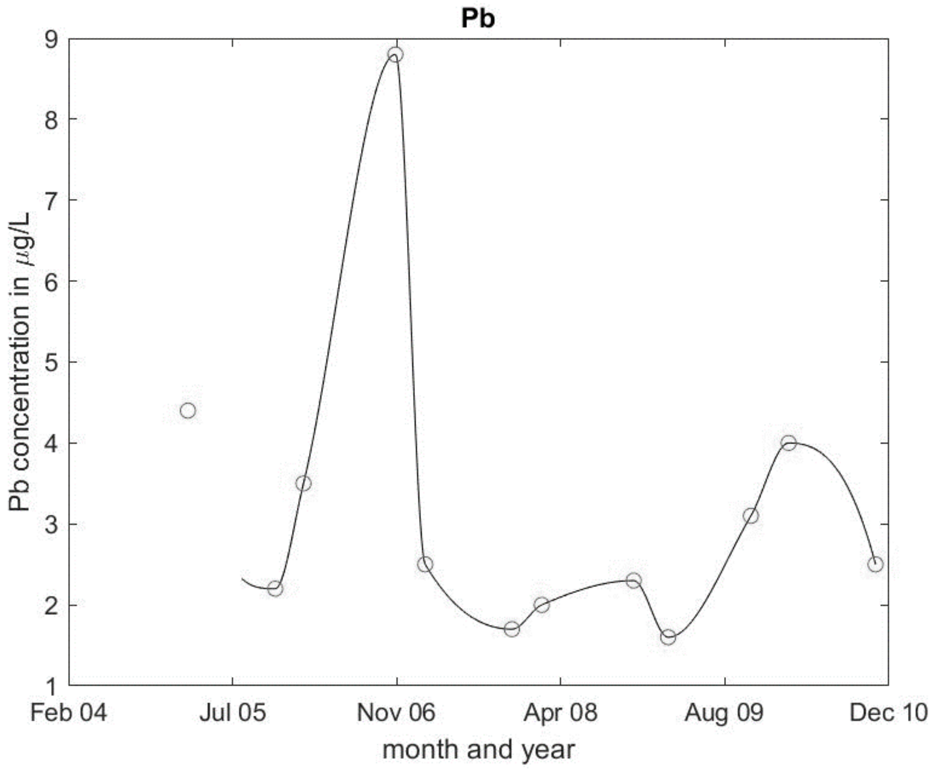

Figure 51 Measured Pb concentration (monitoring program) and interpolated values

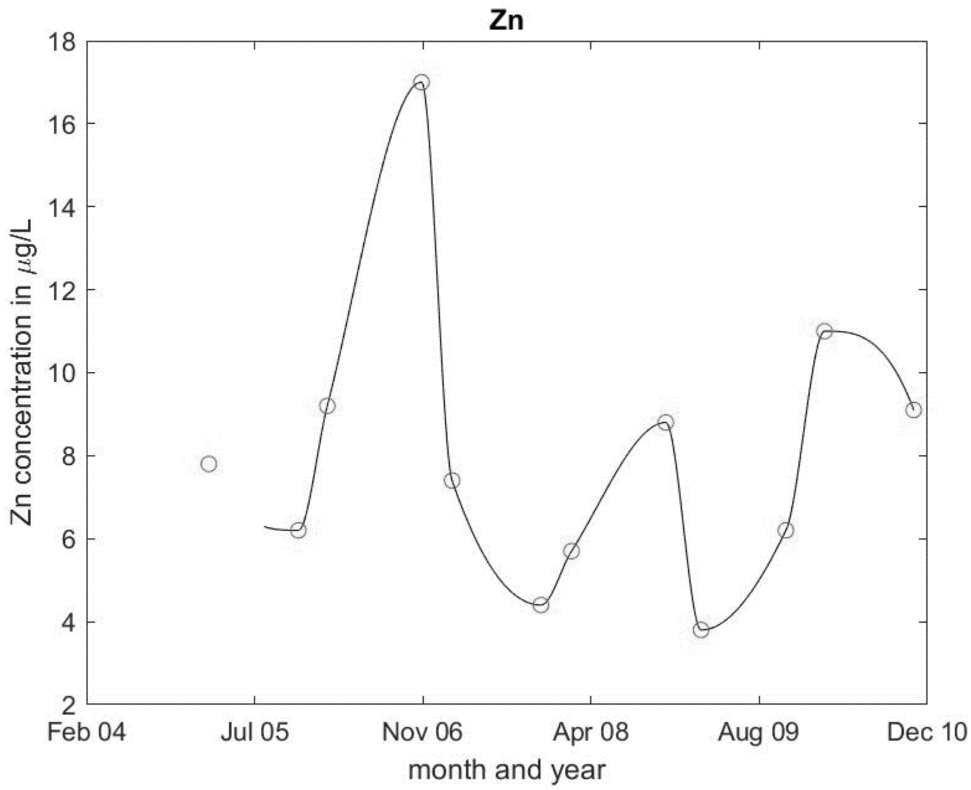

Figure 52 Measured Zn concentration (monitoring program) and interpolated values

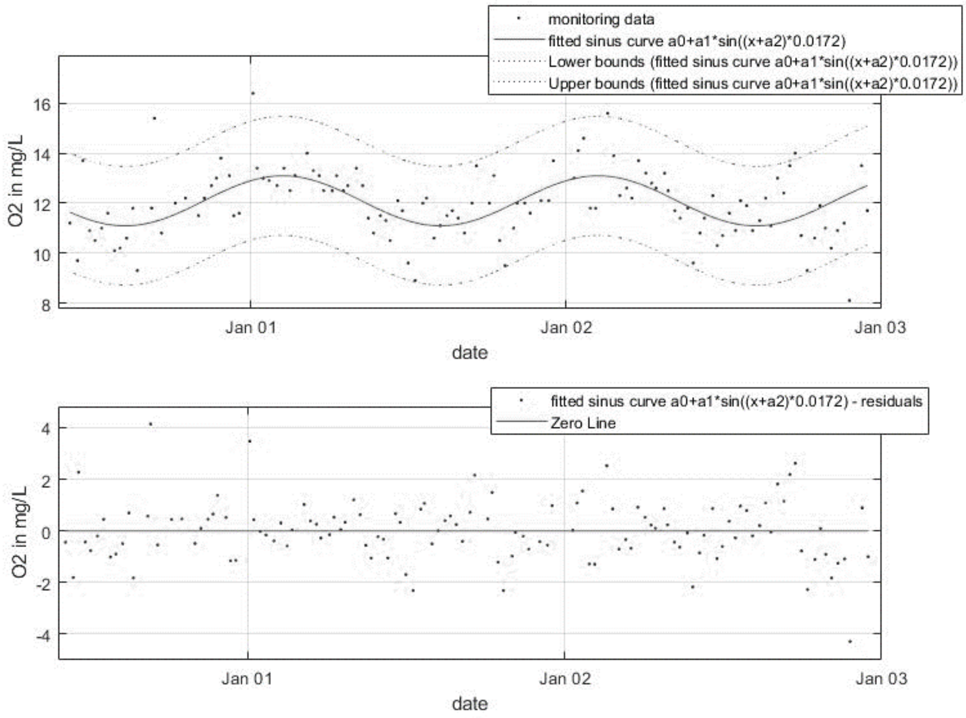

Figure 53 Measured oxygen concentration (monitoring program) and interpolated values between June 2000 and Dec 2002

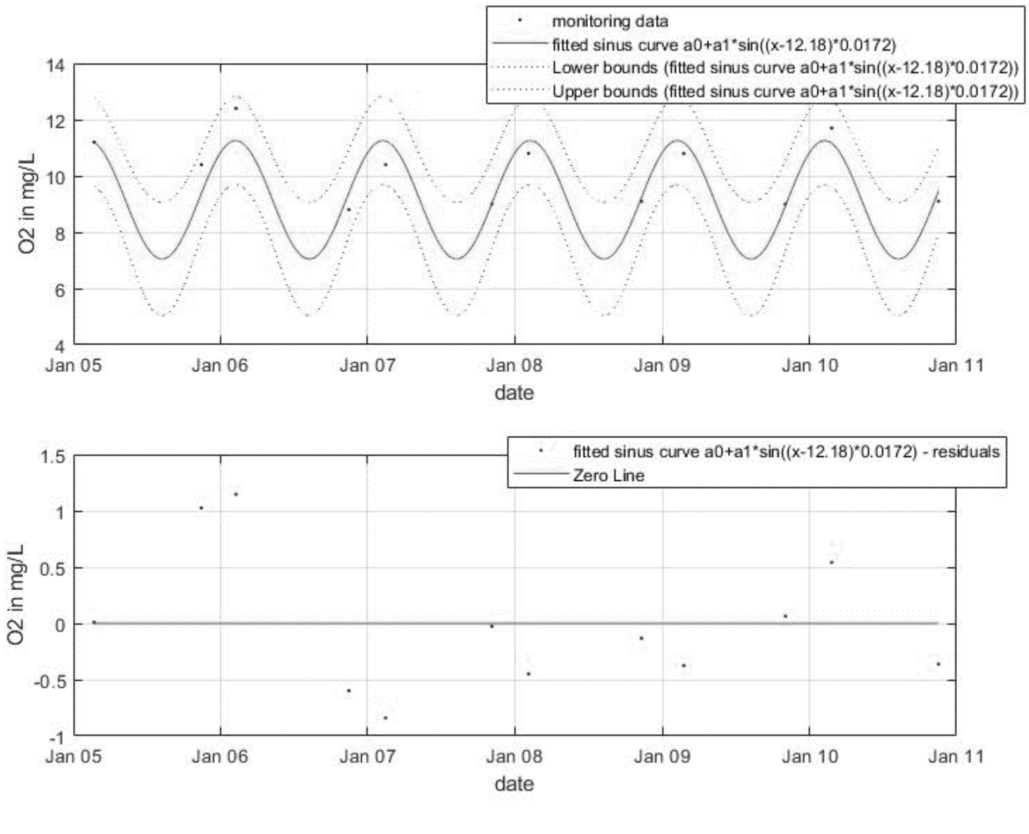

Figure 54 Measured oxygen concentration (monitoring program) and interpolated values between 2005 and 2010

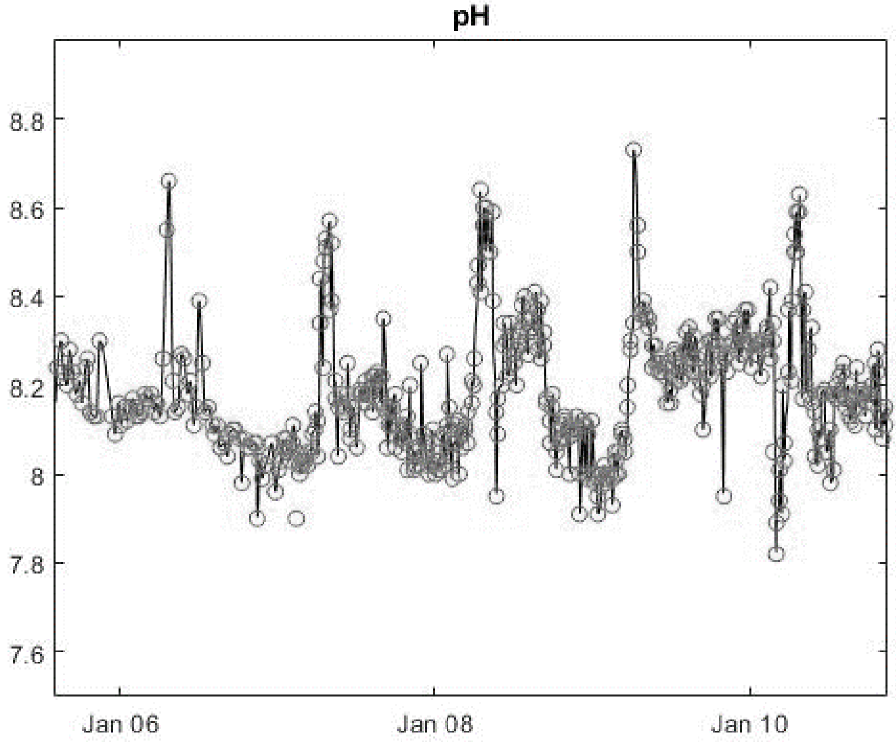

Figure 55 Measured pH values (monitoring program) and interpolated values

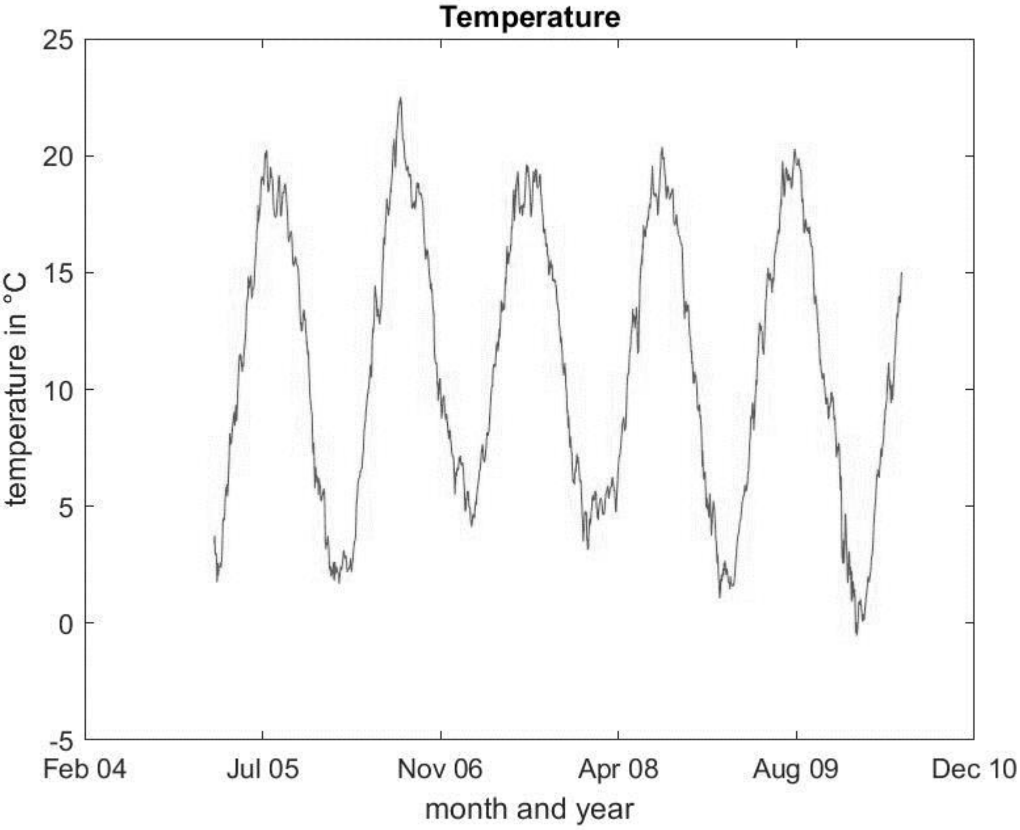

Figure 56 Temperature data from HAMSOM model

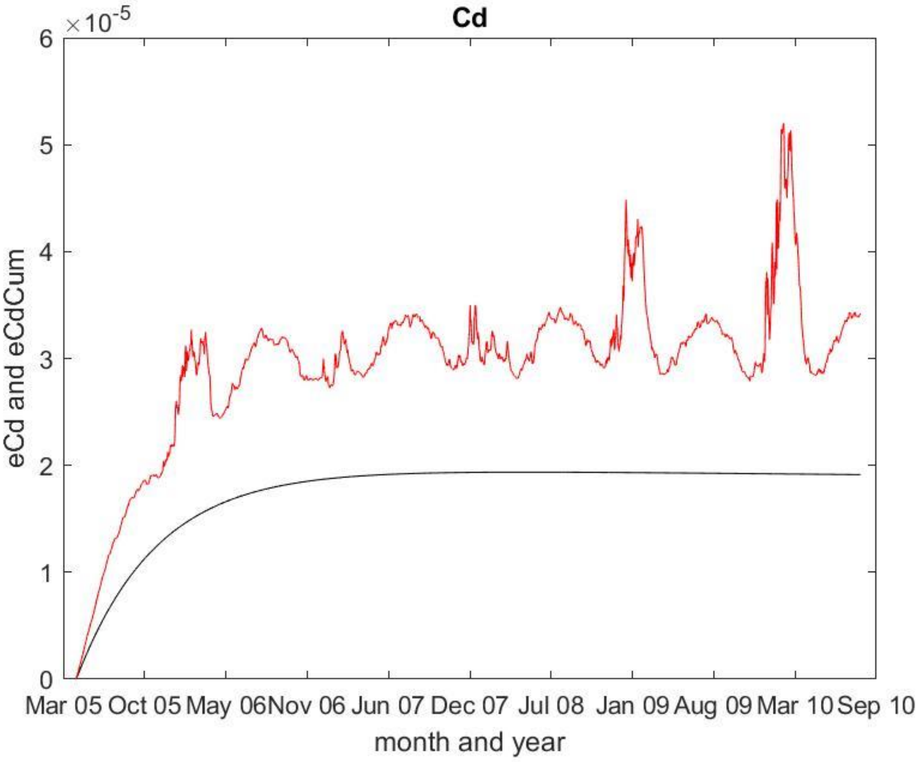

Figure 57 Indicator of stress for *Mytilus edulis* (eCd) due to increased Cd concentrations alone (black line) and with the influences of other stressors on Cd stress (eCdCum)

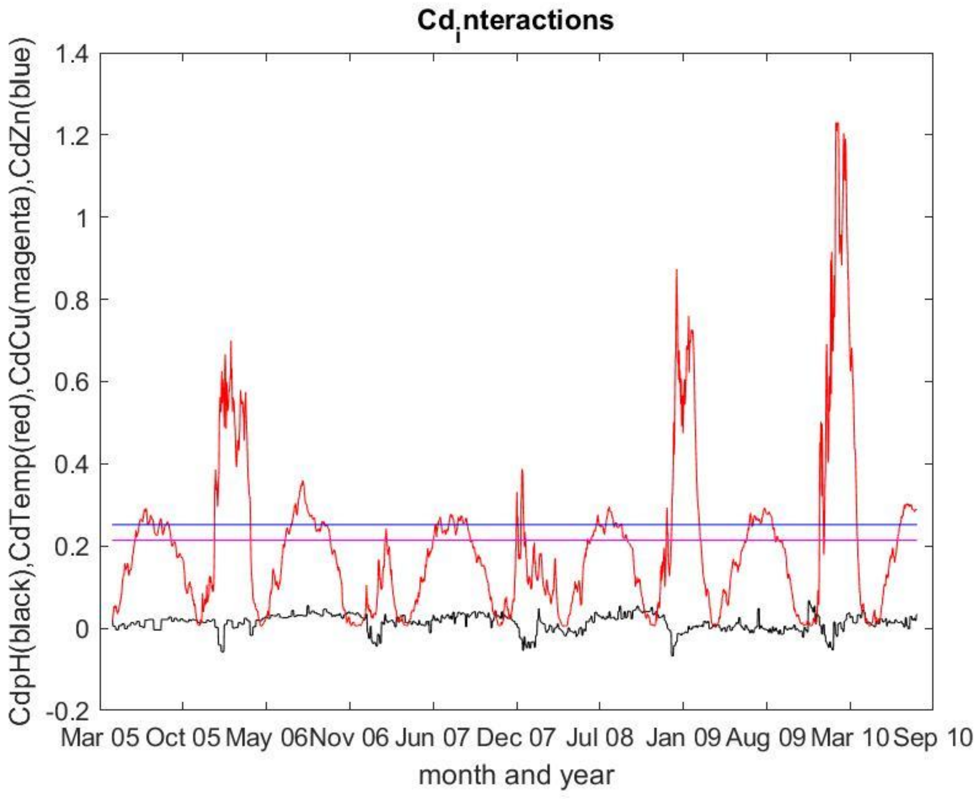

Figure 58 Example for temporal dynamic interactions in contrast to static interaction factors

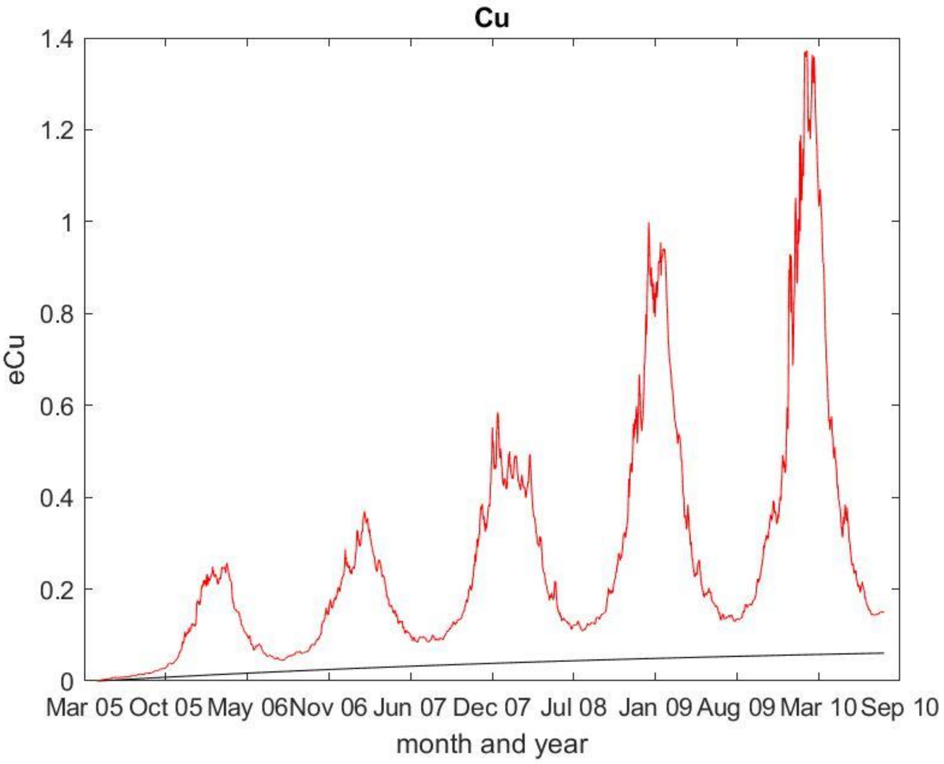

Figure 59 Indicator of stress for *Mytilus edulis* (eCu) due to increased Cu concentrations alone (black line) and with the influences of other stressors on Cu stress (eCuCum)

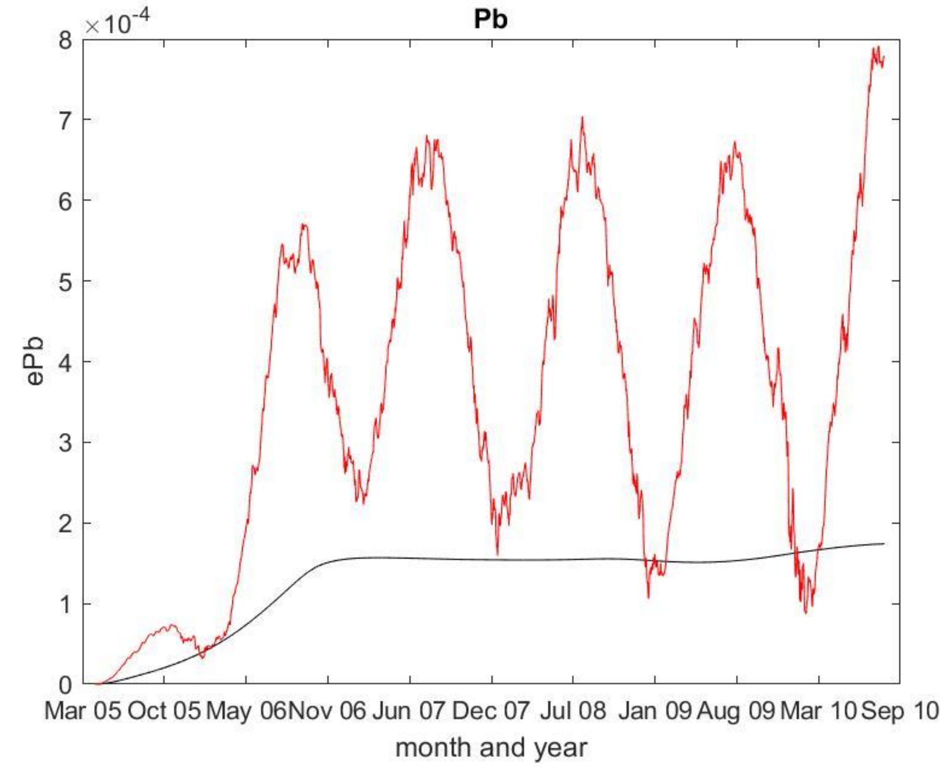

Figure 60 Indicator of stress for *Mytilus edulis* (ePb) due to increased Pb concentrations alone (black line) and with the influences of other stressors on Pb stress (ePbCum)

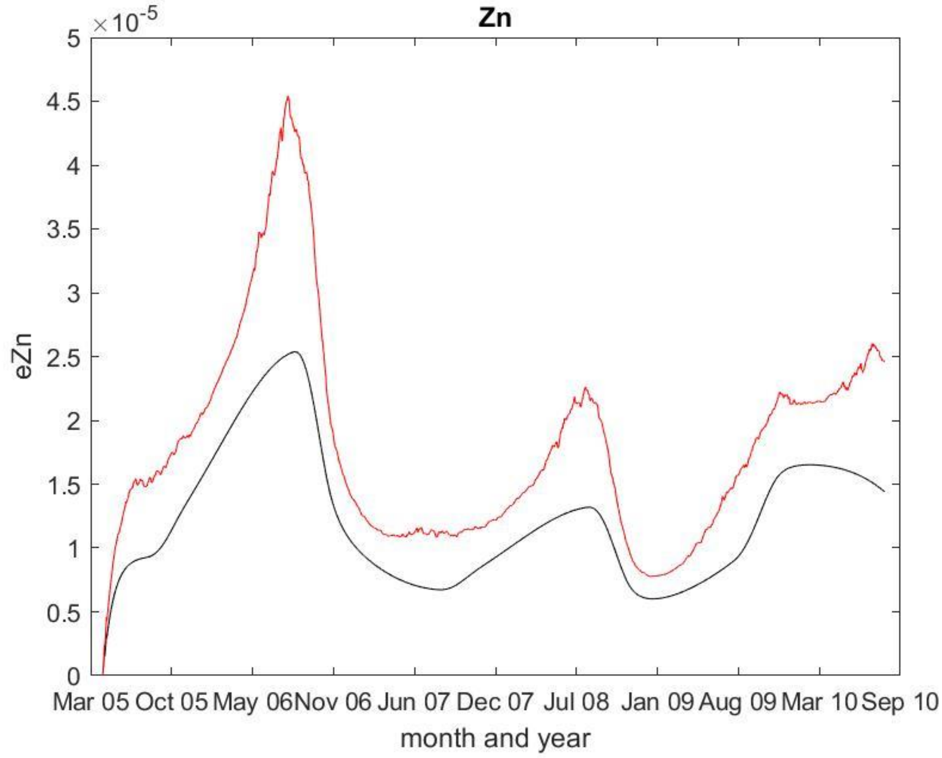

Figure 61 Indicator of stress for *Mytilus edulis* (eZn) due to increased Zn concentrations alone (black line) and with the influences of other stressors on Zn stress (eZnCum)

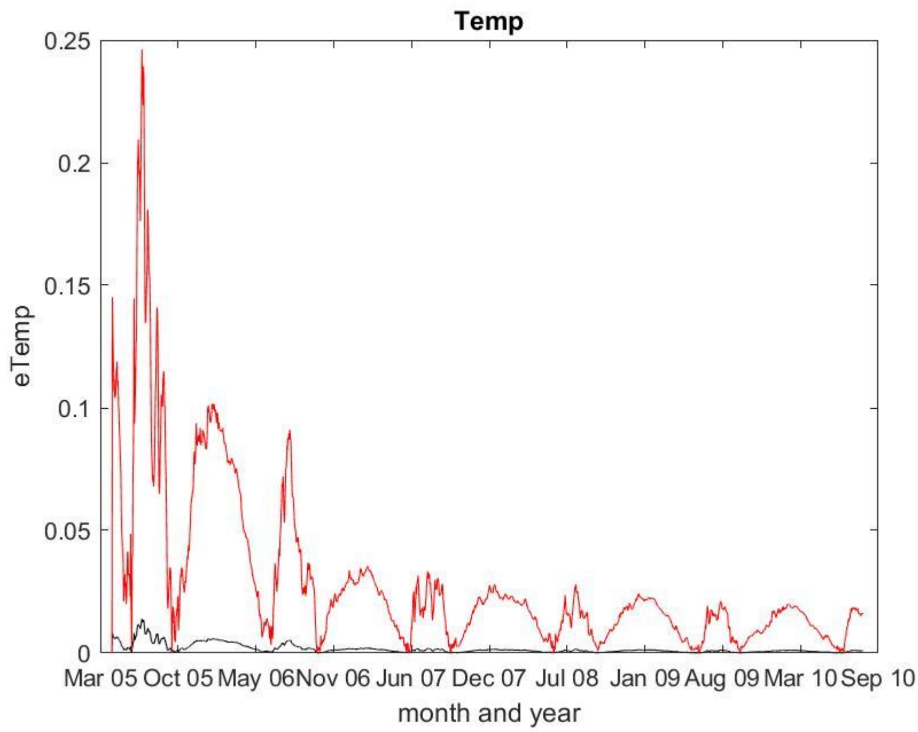

Figure 62 Indicator of stress for *Mytilus edulis* due to increased temperature alone (black line) and with the influences of other stressors on temperature stress (here only Zn)

Interactions between stressors and relevant information from literature and the monitoring station

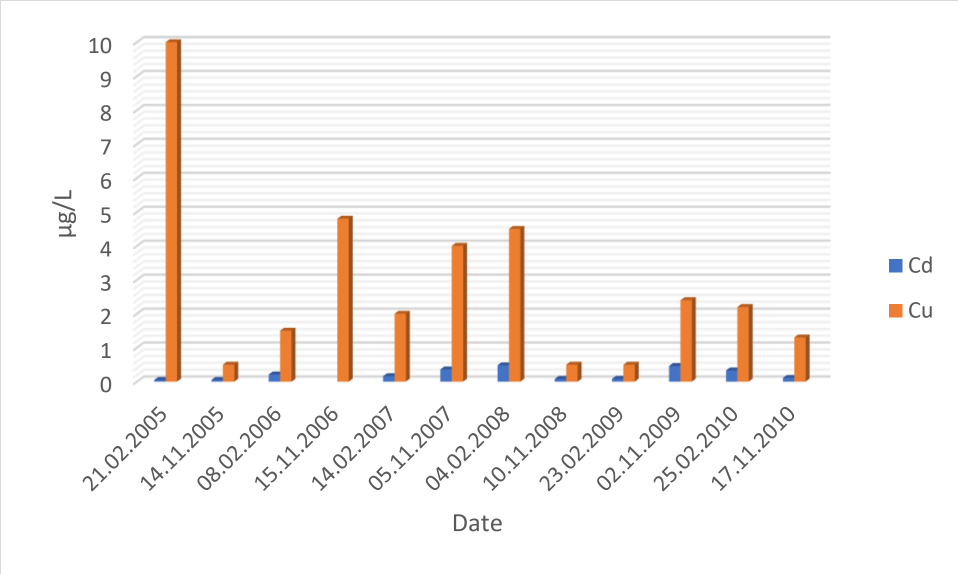

Figure 63 Comparison of the concentrations of Cd and Cu at the monitoring station W1 at Norderney between 2005 and 2010

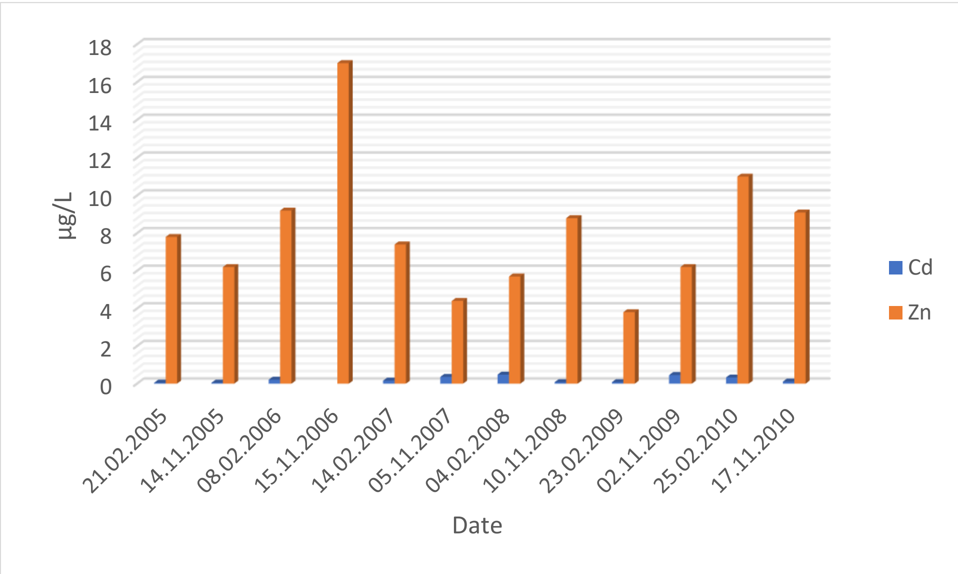

Figure 64 Comparison of the concentrations of Cd and Zn at the monitoring station W1 at Norderney between 2005 and 2010

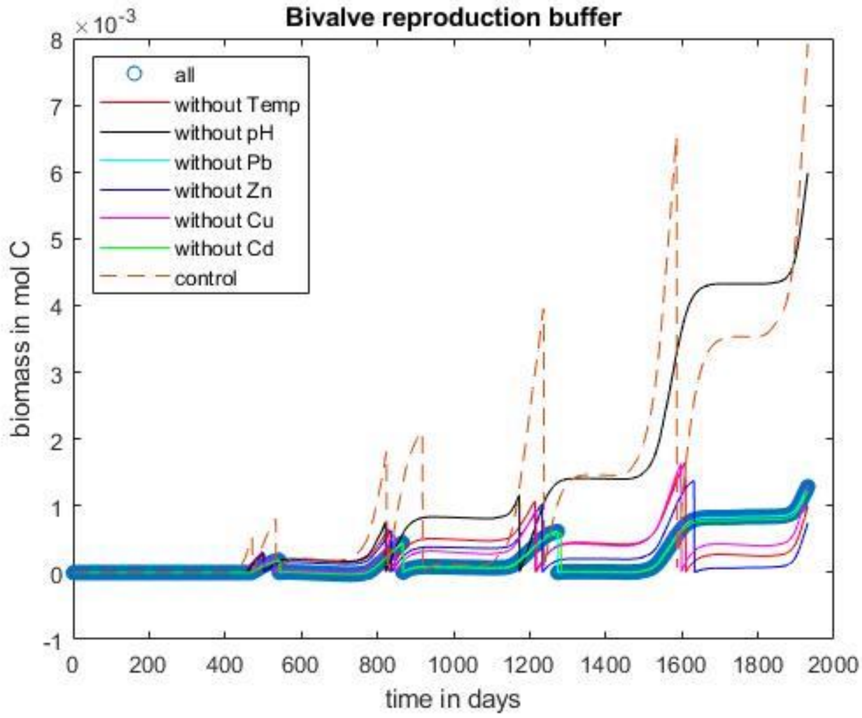

Figure 65 Development of the reproduction buffer based on the model considering cumulative interactions.

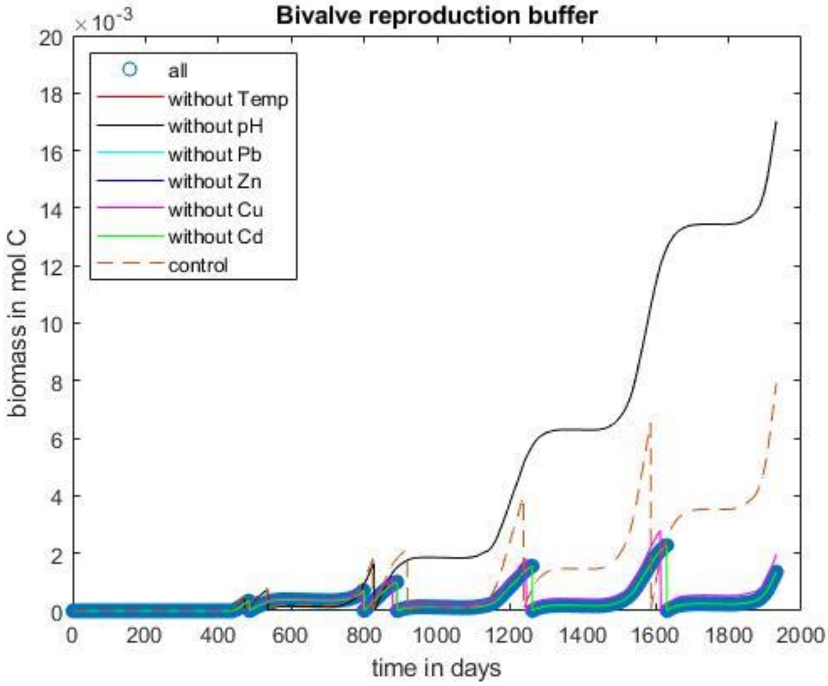

Figure 66 Development of the reproduction buffer based on the model assuming only additive effects and neglecting cumulative interactions.

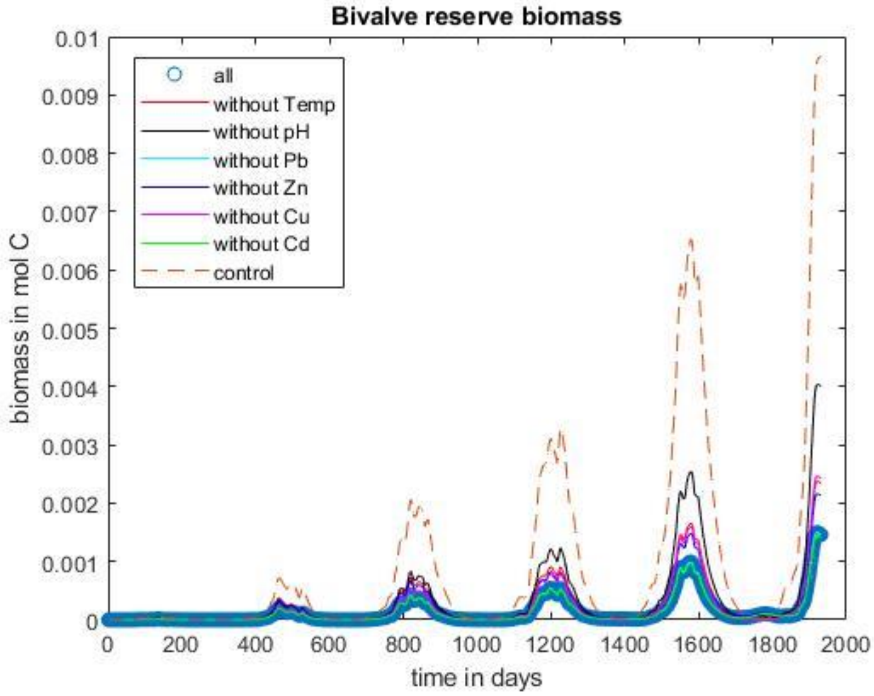

Figure 67 Development of the reserve biomass based on the model considering cumulative interactions.

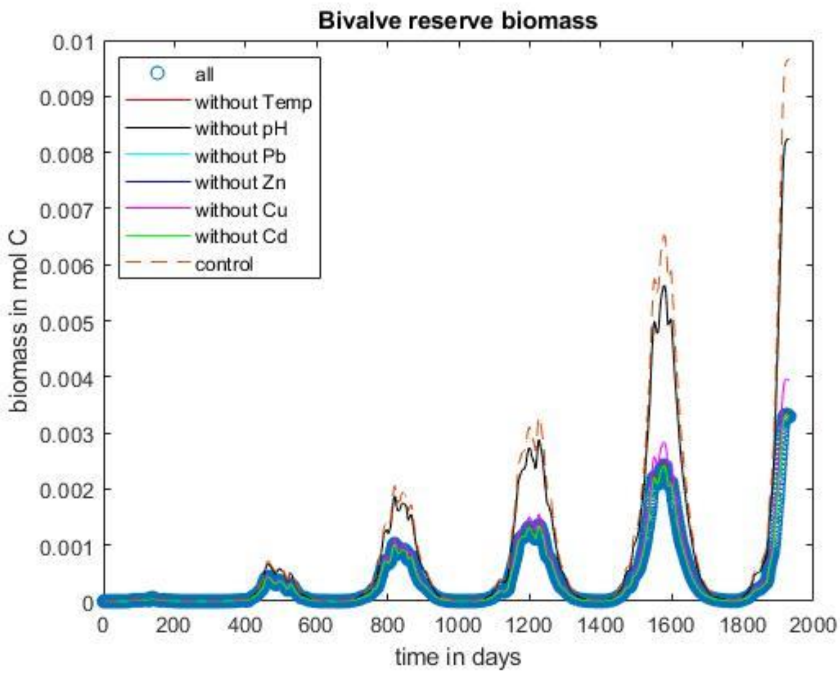

Figure 68 Development of the reserve biomass based on the model assuming only additive effects and neglecting cumulative interactions.

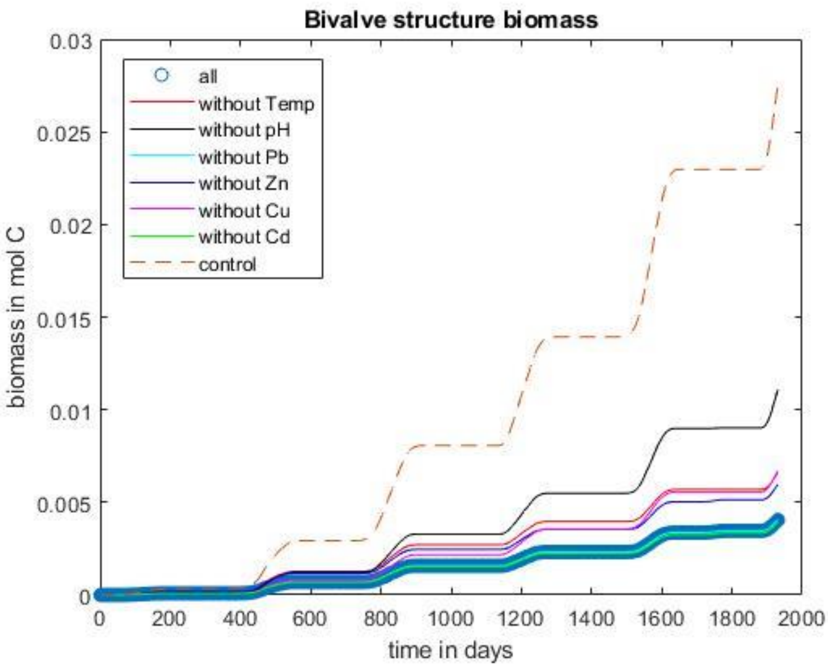

Figure 69 Development of the structural biomass based on the model considering cumulative interactions.

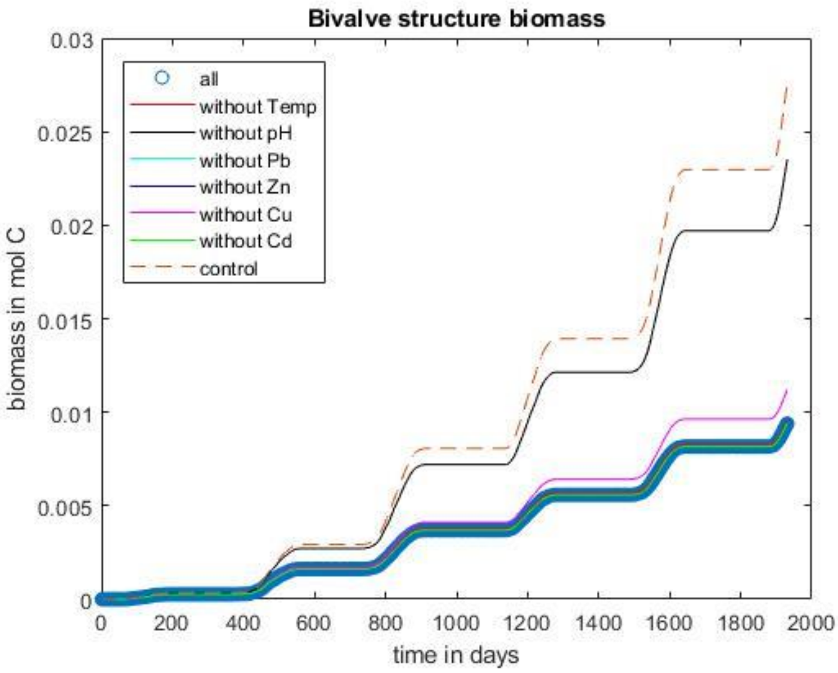

Figure 70 Development of the structure biomass based on the model assuming only additive effects and neglecting cumulative interactions.

Comparison of methods

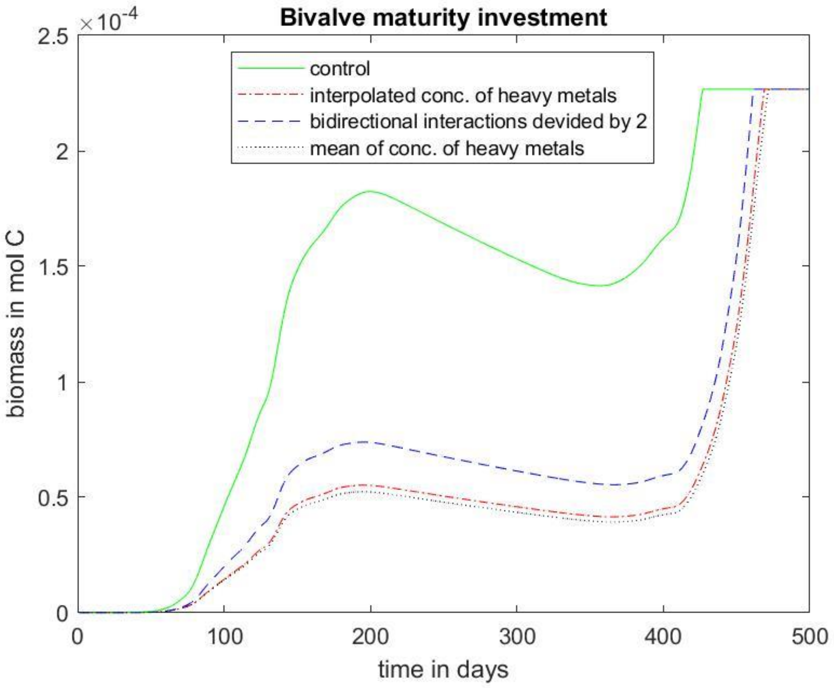

Figure 71 Comparison of methods - maturity investment

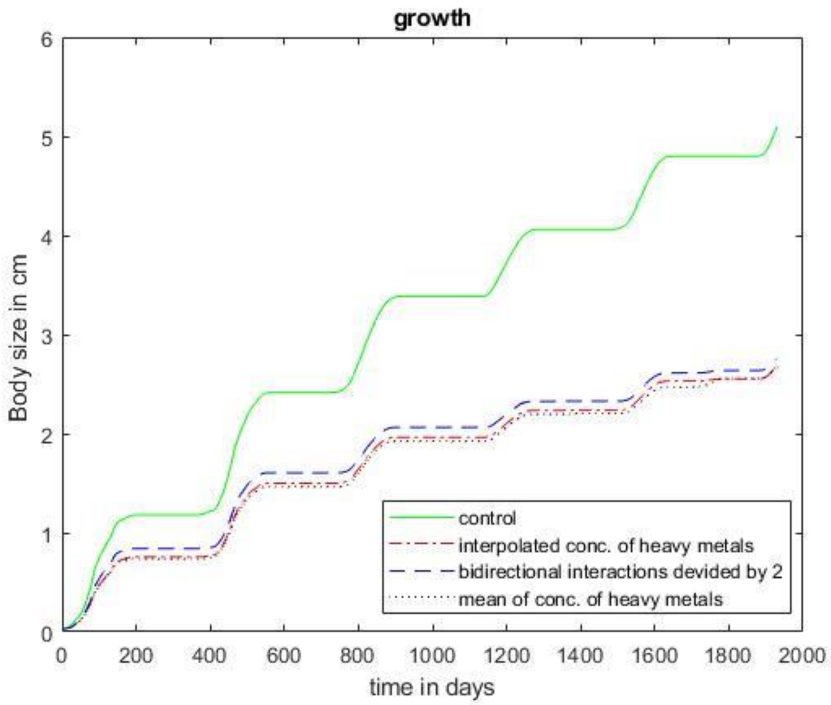

Figure 72 Comparison of methods – growth

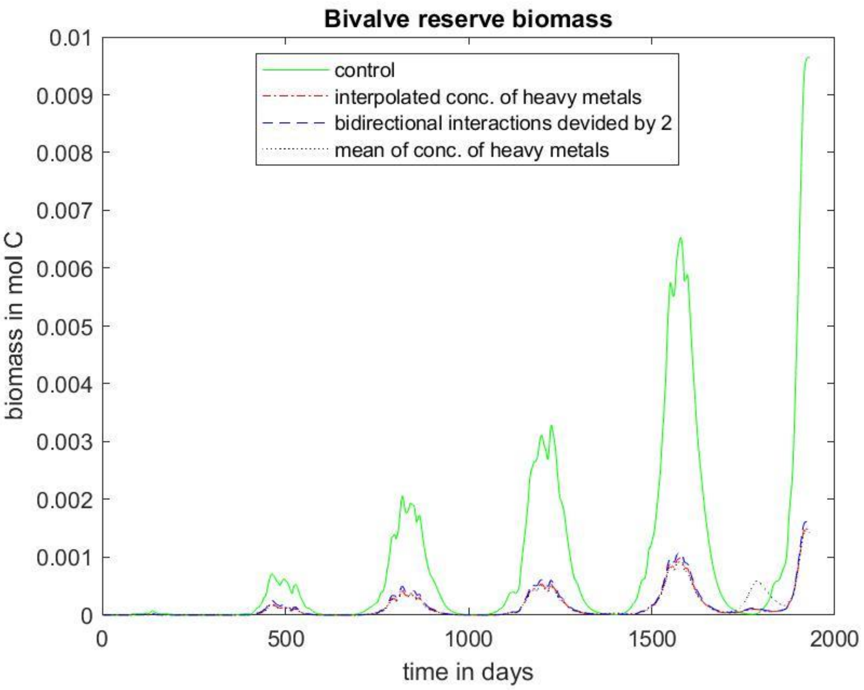

Figure 73 Comparison of methods - reserve biomass

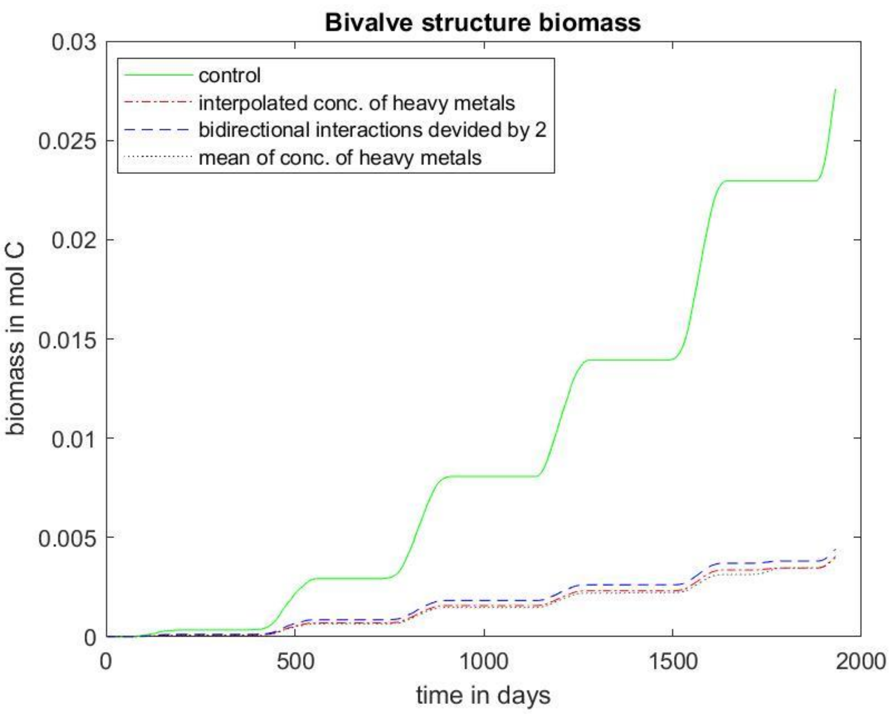

Figure 74 Comparison of methods - structure biomass

Comparison of methods - without control

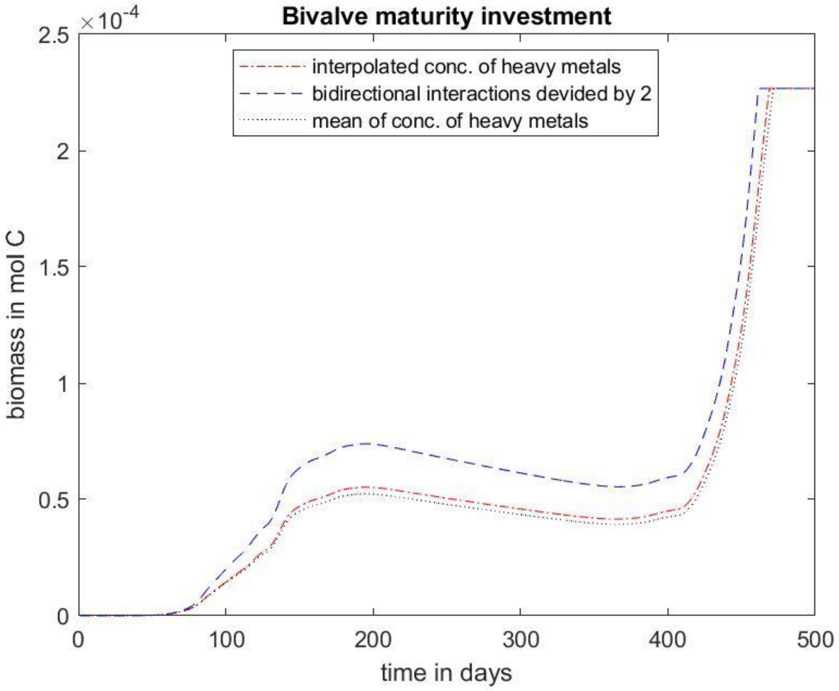

Figure 75 Comparison of methods - growth (without control)

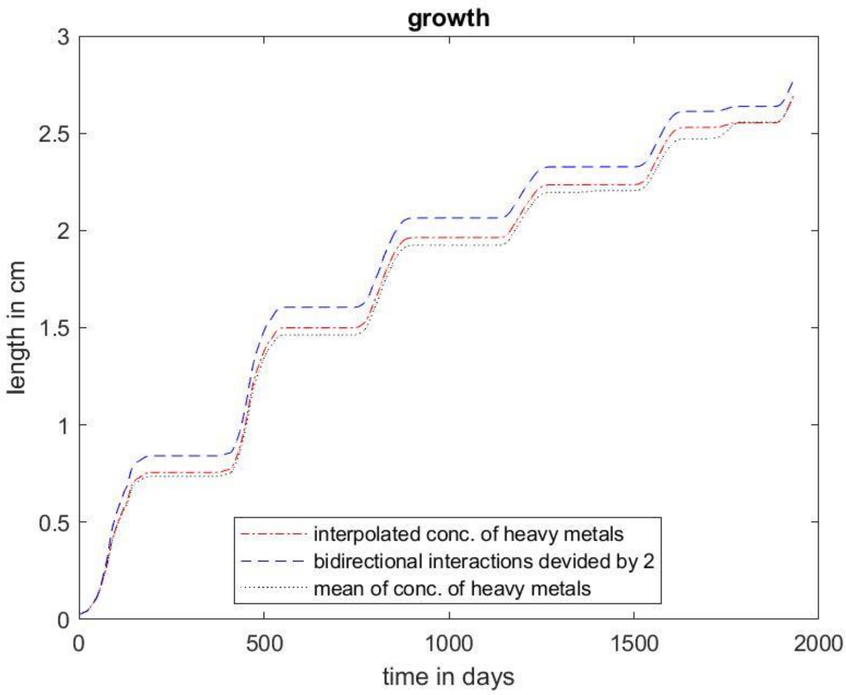

Figure 76 Comparison of methods - growth (without control)

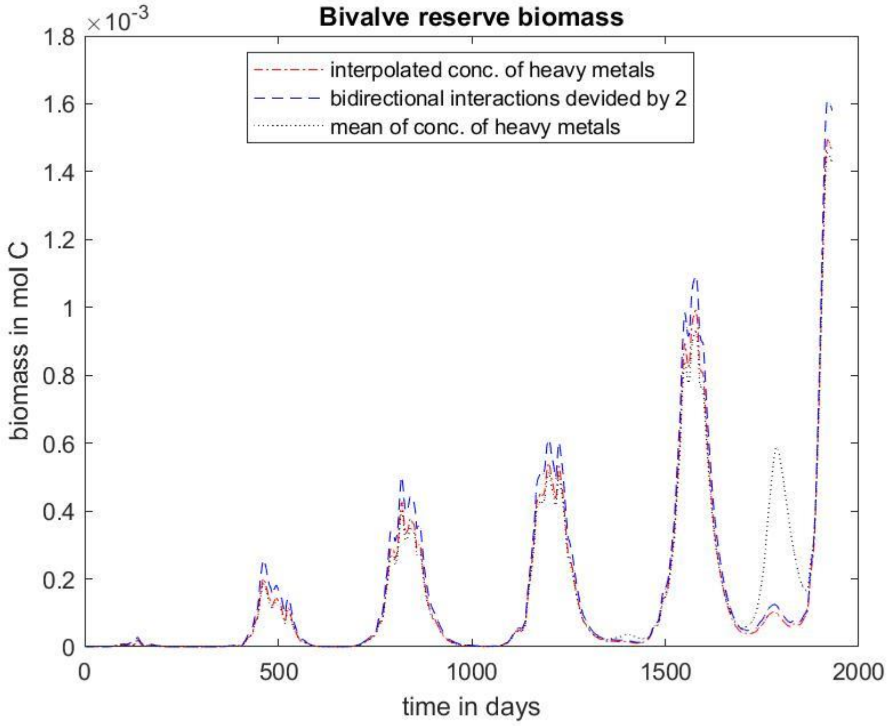

Figure 77 Comparison of methods - reserve biomass (without control)

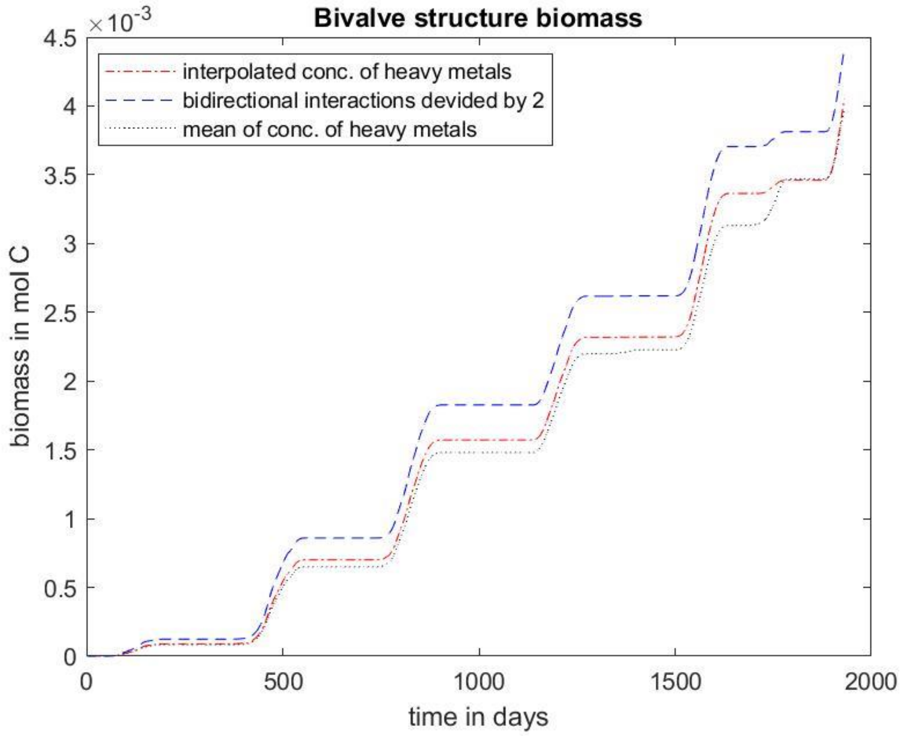

Figure 78 Comparison of methods - structure biomass (without control)

Table 17 Literature about interactions and reasoning for inclusion and exclusion for the model

Source	Comment	influencing stressor	influenced stressor	used for matrix? explanation
Veldhuizen-Tsoerkan et al 1991	In the presence of Cd, survival time for aerial anoxia decreased (Cd conc 50 ug/L, 18°C, aerial exposure: 2 weeks	Cd	anoxia	no, because the exposure time to air was considered too long for being comparable to the conditions of the test scenario.
Weber et al. 1992	Cu depressed median lethal time (LT50) due to anoxia at 200ug/L salinity 32 ppt and temperature 6°C (N2 bubbled seawater)	Cu	anoxia	no, because anoxic conditions were not integrated in the test dataset, the exposure time to air was considered too long for being comparable to the conditions of the test scenario.
Weber et al. 1992	Cu depressed the median lethal time (LT50) due to anoxia at 200ug/L Cu, salinity 32 ppt, temperature 15°C (N2 bubbled seawater)	Cu	anoxia	no, because no anoxic conditions used in the test dataset, the exposure time to air was considered too long for being comparable to the conditions of the test scenario.
Babarro and Zwaan 2002	Survival of anoxia decreased at increased pH (at 8.1 compared to 6.5, no difference of survival between pH 6.5 and 7.3), salinity 31 psu, oxygen below 0.15 mg/L, 10°C, max surivival at pH 8.1: 14 days, at pH 6.5: 15 days under bacterial infection	pH	anoxia	no, due to the additional stress by the bacterial infection
Babarro and Zwaan 2002	survival of anoxia decreased under high salinity conditions, 10 °C, (difference between 31, 27 and 17 psu), anoxia: N2 bubbled seawater, max survival at 17psu: ca. 27 days, under bacterial infection	salinity	anoxia	no, due to the additional stress by the bacterial infection, salinity is not considered in test data

Babarro and Zwaan 2002	survival of anoxia decreased under high salinity conditions, 18 °C, (15 psu compared to 31 psu, no significant difference between 27 and 31 psu), anoxia: N2 bubbled seawater, max survival at 15psu:10 days, under bacterial infection	salinity	anoxia	no, due to the additional stress by the bacterial infection, salinity is not considered in test data
Babarro and Zwaan 2002	survival of anoxia increased under low salinity conditions, 10 °C, (difference between 31, 27 and 17 psu), anoxia: N2 bubbled seawater, max survival at 17psu: ca. 27 days	salinity	anoxia	no, due to the additional stress by the bacterial infection, salinity is not considered in test data
Babarro and Zwaan 2002	survival of anoxia increased under low salinity conditions, 18 °C, (15 psu compared to 31 psu, no significant difference between 27 and 31 psu), anoxia: N2 bubbled seawater, max survival at 15psu:10 days, under bacterial infection	salinity	anoxia	no, due to the additional stress by the bacterial infection, salinity is not considered in test data
Weber et al. 1992	decreased salinity decreased median lethal time due to anoxia at 16ppt and temperature 15°C (N2 bubbled seawater)	salinity	anoxia	no, because no anoxic conditions used in the test dataset, salinity is not considered in test data
Weber et al. 1992	Decreased salinity decreased median lethal time due to anoxia at 16ppt and temperature 6°C (N2 bubbled seawater)	salinity	anoxia	no, because no anoxic conditions used in the test dataset, salinity is not considered in test data
Babarro and Zwaan 2002	Survival of anoxia decreased at increased temperatures (from 10 °C-18°C), salinity 31 psu, oxygen below 0.15 mg/L, pH 8.2, under bacterial infection	Temp	anoxia	no, due to the additional stress by the bacterial infection

Elliot et al. 1986	Increased copper concentrations (10ug) depressed the uptake of Cd at a high concentration of Cd (20 ug/L Cd), 11°C, exposure time:10 days	Cu	Cd	no, because the concentration of Cd in the study system is in comparison with the Cu concentration lower than in the experiment. Data of another experiment of the same publication better resembled the ratio between the stressors. There, the Cu concentration is higher than the Cd concentration.
Elliot et al. 1986	Increased copper concentrations (20ug) depressed the uptake of Cd at a high concentration (20 ug/L Cd), 11°C, exposure time:10 days	Cu	Cd	no, because the concentration of Cd in the study system is in comparison with the Cu concentration lower. Data of another experiment of the same publication better resembled the ratio between the stressors. There, the Cu concentration is higher than the Cd concentration.
Elliot et al. 1986	The presence of copper increased the accumulation of Cd at 10ug/L Cd and at copper concentrations of 10 and 20 ug/L Cu, at 11°C,	Cu	Cd	yes, the experiment was conducted in Tasmania, but the salinity was comparable to the test scenario. At the station at Norderney, Cu concentrations of 10ug/L between the years 2005 and 2010, but Cd concentrations lower than 1ug/L occurred between the years 2005 and 2010. Therefore, only the interaction referring to higher Cu and lower Cd concentrations was applied here. For the lowest concentrations of Cd tested in the experiment, no significant interaction with other metals could be observed (Elliot et al. 1986).

				These data were not used because the exposure time was likely to be too short to show an interaction effect with that concentration. Therefore, the next higher concentration was used.
Elliot et al. 1986	Cu and Zn interactively depressed the uptake of Cd at high concantrations (20 ug/L Cd, 20 ug/L Cu and 200 ug/L Zn, 11°C, exposure time 10 days	Cu and Zn	Cd	no, because this is not a binary relationship (here two stressors influence the effect of one stressor). Only relationships are included were one stressor influences the effect of another stressor
Fischer 1986	At the one hand, at oxygen concentration below 2.5 mg/L, there was a tendency to a decreased condition index (but not significant) during Cd exposure, on the other hand, these low oxygen concentration led to a decreased Cd concentration in soft tissues (significant), and a decreased Cd/ Shell-weight index, At concentrations between 4.2 and 6.5 the Cd/ Shell-weight index and the condition index decreased	oxygen	Cd	no, the range of oxygen concentrations did not fit well to the data of the test scenario (range of the data tested in experiment: 2.5 to 6.5. mg/L, concentraions measured at Norderney between 8.8 and 12.4 mg/L, inconsistency of the results

Sheir et al. 2013	Pre-Pb exposure inhibited phagocytosis activity and increased the neural red uptake (stress indicator), which influenced the effect of Cd; upregulation of methallothiodin expression, mussels from Pb and Fe polluted site were compared to mussels from a reference site with regard to different responses in lab experiments with Cd, 20ug/L Cd, Pb, pH 7.7, salinity 30.8, 1 week acclimation, Pb in water at polluted site: <0.03 but in sediment 54.75 ug/g dry weight, polluted site compared to reference site	Pb	Cd	no, because the effect of Pb could not be entangled from other pollution effects
George 1983	Increased pH resulted in a higher amount of Cd bound to metallothionein	pH	Cd	yes, the conditions of the experiments were comparable with those of the test scenario, data of the binding to metallothioneins and granules were pooled. The relationship between this pooled data could best be described by a linear model. These data were preferentially used over data by Han et al. (2013).
George 1983	Increased pH resulted in a higher amount of metals bound to granules	pH	Cd	yes, the conditions of the experiments were comparable with those of the test scenario, data of the binding to metallothioneins and granules were pooled, compared to Han et al. 2013 more different pH calues were tested. The relationship between this pooled data could best be described by a linear model.

				These data were preferentially used over data by Han et al. (2013).
Han et al. 2013	Increased uptake of the metal at decreased pH	pH	Cd	no, compared to George 1983 fewer pH levels tested
Han et al. 2013	Increased metallothionein concentration at decreased pH at increased metal concentration	pH	Cd	no, compared to George 1983 fewer pH levels tested
Han et al. 2013	Increased percentage of esoinophilic hemocytes at decreased pH at increased metal concentration	pH	Cd	no, compared to George 1983 fewer pH levels tested
Han et al. 2013	29	pH	Cd	no, compared to George 1983 fewer pH levels tested
Han et al. 2013	decrased pH at increased metal concentration led to increased mortality	pH	Cd	no, compared to George 1983 fewer pH levels tested
Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted different to Cd exposure in the lab than mussels from a reference site. Here thinner epithelium of digestive tubules.	pollution	Cd	no, because serveral stressors were involved
Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted different to Cd exposure in the lab than mussels from a reference site. Here increased % of injured tubules/ filaments	pollution	Cd	no, because serveral stressors were involved

Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted different to Cd exposure in the lab than mussels from a reference site. Here decreased fractional area of spermatic follices	pollution	Cd	no, because serveral stressors were involved
Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted different to Cd exposure in the lab than mussels from a reference site. Here decreased fractional area of egg follices	pollution	Cd	no, because serveral stressors were involved
Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted different to Cd exposure in the lab than mussels from a reference site. Here increased necrotic male follicles	pollution	Cd	no, because serveral stressors were involved
Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted differently to Cd exposure in the lab than mussels from a reference site. Here, altered metal concentration of Cu in gills, different stressors(polluted site) mainly Fe and Pb increased in tissues	pollution	Cd	no, because this is not a binary relationship (here several stressors influence the effect of one stressor). Only relationships are included were one stressor influences the effect of another stressor

Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted differently to Cd exposure in the lab than mussels from a reference site. Here altered metal concentration of Cu in haemolymph, different stressors(polluted site) mainly Fe and Pb increased in tissues	pollution	Cd	no, because this is not a binary relationship (here several stressors influence the effect of one stressor). Only relationships are included were one stressor influences the effect of another stressor
Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted differently to Cd exposure in the lab than mussels from a reference site. Here, altered metal concentration of Fe in digestive gland, different stressors(polluted site) mainly Fe and Pb increased in tissues	pollution	Cd	no, because this is not a binary relationship (here several stressors influence the effect of one stressor). Only relationships are included were one stressor influences the effect of another stressor
Struck et al. 1998	internal concentration of Cd was 29% lower in the North Sea than in the Baltic Sea, field data, salinity 7.4-32ppt, note that concentrations of Cd in the water at the different plots sampled might have differed	salinity	Cd	no, salinity is not considered in test data
Mubiana et al. 2007	at increased salinity, internal metal concentration was lower than at low salinities (salinity range: 18-34 ppt), internal concentration of Cd one order of magnitude higher compared to the concentration at18ppt	salinity	Cd	no, salinity is not considered in test data

Fischer 1986	complex interaction effect between Cd and salinity concerning the condition index. The condition index decreased with increased salinities, but variablility of the data was very high and thus the relationship was not significant	salinity	Cd	no, several influencing factors, salinity is not considered in test data, inconsitency in the results (at low salinity of 7,5 psu low tissue concentration and low Cd/ Shell wt index)
Fischer 1986	Low Cd concentration in soft tissue when salinity was lower than 10 psu, but no difference in Cd concentration in soft tissues at salinities between 10 and 35 psu.	salinity	Cd	no, several influencing factors, salinity is not considered in test data, inconsitency in the results (at low salinity of 7,5 psu low tissue concentration and low Cd/ Shell wt index)
Wang et al. 1997	at 0.05 ug/L Cd uptake of Cd was highest at salinity 20 ppt with an influx rate of ca. 0.26 ug/g/d, lowest uptake rate was at 35 ppt, at 15ppt Cd uptake was also reduced, exposure time 10d, 15°C, Cuptake= 25.97*exp(-0.08505*salinity)-27.05*exp(-0.09018*sal)	salinity	Cd	no, several influencing factors, salinity is not considered in test data
Fischer 1986	complex interaction effect between Cd and salinity concerning the Cd/ shell weight index with a maximum of the mean at 10 psu, but variablility of the data was very high and thus the relationship was not significant	salinity	Cd	no, several influencing factors, salinity is not considered in test data, inconsitency in the results (at low salinity of 7,5 psu low tissue concentration and low Cd/ Shell wt index)
Struck et al. 1998	internal concentration of Cd was 40% higher in the Baltic than in the North Sea, field data, salinity 7.4-32ppt, note that concentrations of Cd in the water at the different plots sampled might have differed	salinity	Cd	no, several influencing factors, salinity is not considered in test data

Phillips 1976	at low salinities of 15 ppt the net uptake of cadmium (40ug/L) at 10°C was decreased after 14 days exposure (increase of ug/g wet weight)	salinity	Cd	no, several influencing factors, salinity is not considered in test data
Lehnberg und Theede 1979	complex interaction between salinity, temperature and Cd on development of larvae, ecological niches for larvae	Temp and salinity	Cd	no, several influencing factors, salinity is not considered in test data
Lehnberg und Theede 1979	complex interaction between salinity, temperature and Cd on development of larvae, ecological niches for larvae	Temp and salinity	Cd	no, several influencing factors, salinity is not considered in test data
Mubiana et al. 2007	increased Cd uptake (model: C = a+b*e^-ket, where C is the metal concentration in ug/g in soft tissue, a and b are model estimates, ke is the elimination constant per day and t is time in days), pH 7.8-8.0, O2 7.4-10.4, temperature 6-26, Cd 3.1.3.3, salinity 35 g/L, exposure 28 days	Temp	Cd	no, conditions of the experiment were similar as in test scenario. Netherlands, eastern shelde, but publication by Fischer 1986 provided data for a longer experimental exposure time
Fischer 1986	increased Cd concentration in soft tissue at a Cd concentration of 1.1 ug/L, an at a temperature between 5 an 25°C, equation	Temp	Cd	no, I regarded the observation of the CI a better indicator of overall health of the organism than the uptake rate.
Fischer 1986	decreased condition index at Cd conc 1.1 ug/L, temperature between 5 an 25°C, equation determined with matlab with data derived from diagramm in publication	Temp	Cd	yes, the tested data resembled the stressor intensity of the test scenario, the data by Fischer were used instead of data by Mubiana et al. 2007 because the experiment lasted longer, more temperature values were tested,
Lehnberg und Theede 1979	complex interaction between salinity, temperature and Cd on development of	Temp and salinity	Cd	no, several influencing factors

	larvae, ecological niches for larvae modeled			
Lehnberg und Theede 1979	complex interaction between salinity, temperature and Cd on development of larvae, ecological niches for larvae modeled	Temp and salinity	Cd	no, several influencing factors
Phillips 1976	at low salinities of 15 ppt the net uptake of cadmium (40ug/L) at 18°C and at 10°C was significantly increased (increase of ug/g wet weight)	Temp and salinity	Cd	no, several influencing factors
Vercauteren and Blust 1999	at Zn concentrations above 10 uM Zn, Cd uptake in soft tissues decreased	Zn	Cd	not used because in the experiment by Elliot et al 1986 a longer exposure time was tested
Vercauteren and Blust 1999	at Zn concentrations above 10 uM Zn Cd uptake in gills decreased	Zn	Cd	not used because in the experiment by Elliot et al 1986 a longer exposure time was tested
Vercauteren and Blust 1999	at Zn concentrations above 10 uM Zn Cd uptake in digestive system decreased	Zn	Cd	not used because in the experiment by Elliot et al 1986 a longer exposure time was tested
Vercauteren and Blust 1999	at Zn concentrations above 10 uM Zn, Cd accumulated more in the hemolymph	Zn	Cd	not used because the experiment by Elliot et al 1986 lasted longer
Vercauteren and Blust 1999	at Zn concentrations above 10 uM Zn Cd uptake in soft tissues, gills and digestive system decreased	Zn	Cd	not used because the experiment by Elliot et al 1986 lasted longer
Elliot et al. 1986	increased Zn concentrations decreased uptake of Cd at Zn 200ug/L, Cd 10ug/L, 11°C, exposure time 10 days	Zn	Cd	no, data of another experiment of the same publication better resembled the ratio between the stressors Cd and Zn and the conditions of the test scenario.

				Here, the Zn concentration was considered to be too high
Elliot et al. 1986	increased Zn concentrations decreased uptake of Cd at Zn 200ug/L, Cd 20ug/L, 11°C, exposure time 10 days	Zn	Cd	no, data of another experiment of the same publication better resembled the ratio between the stressors Cd and Zn and the conditions of the test scenario. Here, the Zn concentration was considered to be too high
Elliot et al. 1986	Increased Zn concentration (100ug/L Zn) at a Cd concentration of 10 ug/L increased the uptake of Cd	Zn	Cd	yes, because the ratio between the stressors resembled the ratios between the two stressors in the North Sea and the other experimental conditions resembled those of the test scenario. In the data of the test scenario, the Zn concentration is higher than the Cd concentration, but not 20 times higher as in one of their experiments. Therefore, the interaction with a concentration of 10 µg/L Cd and 100 µg/L Zn was used for the calculation of the relative change. Under these conditions, the increased concentration of Zn exacerbated the effect of Cd on Mytilus edulis.
Elliot et al. 1986	Increased Zn concentration (100ug/L Zn) at a Cd concentration of 20 ug/L increased the uptake of Cd	Zn	Cd	no, because the ratio of the stressors did not resemble the ratio of the test scenario. (at the test

				scenario the concentrations of Zn were much higher than those of Cd)
Griscom et al. 2002	metal assimilation efficiency higher from reduced particles (19% compared to 10%) than from oxidized particles of the sediment	oxygen	Cd, Co	no, because the relationship refers to sediment-bound metals, which were not considered in the present model
Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted different to Cd exposure in the lab than mussels from a reference site. Here altered metal concentration of Fe in gills	pollution	Cd, Fe	no, because serveral stressors were involved
Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted different to Cd exposure in the lab than mussels from a reference site. Here altered metal concentration of Fe in gonads	pollution	Cd, Pb	no, because serveral stressors were involved
Sheir et al. 2013	pre-exposure to pollution influenced the effect of Cd: mussels from a polluted site reacted different to Cd exposure in the lab than mussels from a reference site. Here altered metal concentration of Pb in gonads	pollution	Cd, Pb	no, because serveral stressors were involved
Weber et al. 1992	Anoxia decreased median lethal time (LT50) of Cu at 200 ug/L Cu, 32 salinity and 6°C	anoxia	Cu	no, because no anoxic conditions used in the test dataset
Weber et al. 1992	Anoxia decreased median lethal time (LT50) of Cu at 200 ug/L Cu, 32 salinity and 15°C	anoxia	Cu	no, because no anoxic conditions used in the test dataset

Weber et al. 1992	Anoxia decreased median lethal time (LT50) of Cu at 200 ug/L Cu, 15 salinity and 15°C	anoxia	Cu	no, because no anoxic conditions used in the test dataset
Weber et al. 1992	Anoxia decreased median lethal time (LT50) of Cu at 200 ug/L Cu, 15 psu salinity and 6°C	anoxia	Cu	no, because no anoxic conditions used in the test dataset
Sheir et al. 2013	Increased Cd concentration led to increased uptake of Cu in gills (no significant difference in digestive glands and gonads, difference in heamolymph significant)	Cd	Cu	no, because the experiment was conducted with polluted seawater, data not exact enough (they were provided rounded with too few digits to calculate the difference), better data from the publication by Elliot et al. 1986
Elliot et al. 1986	Cd influenced the accumulation of Cu at 20 ug/L Cu, 10 ug/L Cd, exposure time 10 days	Cd	Cu	yes, because the ratio between the stressors fitted to the data of the test scenario
Elliot et al. 1986	Cd influenced the accumulation of Cu at 20 ug/L Cu, 20 ug/L Cd, exposure time 10 days	Cd	Cu	no, data of another experiment of the same publication better resembled the ratio between the stressors
Elliot et al. 1986	Cd and Zn interactively affected the accumulation of Cu at 10 and 20 ug/L Cu, 10 and 20 ug/L Cd and 100 and 200 ug/L Zn, 11°C, exposure time 10 days	Cd and Zn	Cu	no, because this is not a binary relationship (here two stressors influence the effect of one stressor). Only relationships are included were one stressor influences the effect of another stressor
Koski et al. 2008	pH and Cu concentration in the water were negetively correlated with each other, particularly in the presence of sulfide (e.g. sulfide-rich rocks due to Cu mining)	pH	Cu	no, because the interaction takes place in the water column

Akberali et al. 1985	Under low pH conditions the uptake of Cu was enhanced	pH	Cu	yes, as increased and decreased respiration could both be interpreted as a stress response (increased respiration due to increased oxygen demand and decreased respiration as disturbance of the oxygen supply), I used the increased uptake rate to model the interaction effect between Cu and pH. The relationship between pH and Cu uptake rate was best described with a linear model. I used the corresponding calculated Cu uptake at a pH of 8.2 as a reference for the uptake rate of Cu without the influence of acidification.
Akberali et al. 1985	Only if pH is reduced as well, a reduced egg respiration occurs at Cu 6.355 ug/L, pH 5.5-7.9 (at increased Cu levels alone egg respiration increased)	pH	Cu	no, because both increased and decreased respiration could be interpreted as a stress response (increased respiration due to increased oxygen demand and decreased respiration as disturbance of the oxygen supply)
Riba et al. 2016	Reduced pH altered the Cu concentration in the water (dissolved from sediments), effect on the fertilization of eggs below pH of 6.5	pH	Cu	no, no clear effect, no true control, the effect depended on study site. Due due to the experimental setup, the effects of the single stressors could not be quantified separately and thus this interaction data could not be used for the model.
Han et al. 2013	Increased uptake of the metal at decreased pH	pH	Cu	no, compared to Akberali et al. 1985, fewer pH values tested

Han et al. 2013	Increased metallothionein concentration at decreased pH at increased metal concentration	pH	Cu	no, compared to Akberali et al. 1985, fewer pH values tested
Han et al. 2013	Increased percentage of esoinophilic hemocytes at decreased pH at increased metal concentration	pH	Cu	no, compared to Akberali et al. 1985, fewer pH values tested
Han et al. 2013	Decreased zymosan phagocytosis at decrased pH at increased metal concentration	pH	Cu	no, compared to Akberali et al.1985 , fewer pH values tested
Struck et al. 1998	internal concentration of Cu was 61% lower in the North Sea than in the Baltic Sea, field data (North Sea and Baltic sea, salinity 7.4-32ppt, note that concentrations of Cu in the water at the different plots sampled might have differed	salinity	Cu	no, several influencing factors, salinity is not considered in test data
Struck et al. 1998	internal concentration of Cu was 142% higher in the Baltic than in the North Sea, field data, salinity 7.4-32ppt, note that concentrations of Cu in the water at the different plots sampled might have differed	salinity	Cu	no, several influencing factors, salinity is not considered in test data
Phillips 1976	low salinities at 15 ppt increased the net uptake of copper (20ug/L) at 18°C after 14 days exposure (increase of ug/g wet weight)	salinity	Cu	no, several influencing factors, salinity is not considered in test data
Weber et al. 1992	decreased salinity (16 ppt) increased median lethal time (LT50) due to Cu at 200ug/L Cu temperature 15°C (N2 bubbled seawater)	salinity	Cu	no, because no anoxic conditions used in the test dataset, salinity is not considered in test data

Weber et al. 1992	decreased salinity (16 ppt) increased median lethal time (LT50) due to Cu at 200ug/L Cu temperature 6°C (N2 bubbled seawater)	salinity	Cu	no, because no anoxic conditions used in the test dataset, salinity is not considered in test data
Phillips 1976	the influence of temperature and different salinity regimes on uptake of Cu was erratic, difficult to derive a clear relationship from the data	salinity/ temperature	Cu	no, beauce the relationship was erratic
Mubiana et al. 2007	exponentially increased uptake rates of Cu with increased temperature, salinity 35 g/L, exposure 28 days, at 6° uptake rate (slope) 0.468, at 16°C 0.024, at 26°C 0.015	Temp	Cu	yes,the experimental conditions resembled those of the test scenario, equation derived from data. The interaction could be described by a linear relationship. I used the uptake at 6°C as reference to model the effect of increased temperature on Cu uptake.
Phillips 1976	effect of temperature on uptake rate of Cu, but relationship erratic, difficult to derive a clear relationship from the data	Temp	Cu	no, beauce the relationship was erratic
Elliot et al. 1986	Zn increased the uptake of Cu at Cu conc at Cu 10 ug and 100ug Zn, at 10 ug/ L Cu aand 200 ug/L ZNn, and at 20 ug Cu and 200 ug/L Zn	Zn	Cu	these other influences of Zn not used because the concentrations did not fit to the scanario of Norderney concentrations
Elliot et al. 1986	Zn influenced the uptake of Cu at Cu conc at Cu 20 ug and 100 ug/L Zn	Zn	Cu	yes, because the ratio between the stressors fitted to the data of the test scenario
Sheffrin et al. 1984	at pH 7.8 and 6.8, and a copper concentration of 1-5 ug/L , no interaction effect with regard to behaviour (plantigrade crawling and attachment)	pH	Cu	no, because no interaction was observed

Phillips 1976	different interaction effects between metals	metals	metals	no, datasets not used because the interaction effects could not be entangled from each other (no control for one stressor alone, no full factorial experiment)
Giarratano et al. 2011	Oxygen saturation in the water influenced metal accumulation, field experiment, at higher oxygen concentrations in summer Cu concentrations were lower than in winter, when oxygen concentrations were lower, however, no oxygen depletion occured (oxygen concentration ranged from 9.96 +-0.6 mg/L to 11.48 +-0.73mg/L)	oxygen	Cu	no, because the effect of oxygen could not be clearly entangled from other effects and be quantified.
Giarratano et al. 2011	Oxygen saturation in the water influenced metal accumulation, field experiment, at higher oxygen concentrations in summer Zn concentrations were lower than in winter, when oxygen concentrations were lower, however, no oxygen depletion occured (oxygen concentration ranged from 9.96 +-0.6 mg/L to 11.48 +-0.73mg/L)	oxygen	Zn	no, because the effect of oxygen could not be clearly entangled from other effects and be quantified.

Giarratano et al. 2011	Oxygen saturation in the water influenced metal accumulation, field experiment, oxygen concentrations were higher in summer than in winter, and Cd concentrations were either higher or lower in summer than in winter depending on the site (interactive effect between season and site). No general pattern. No oxygen depletion occured (oxygen concentration ranged from 9.96 +-0.6 mg/L to 11.48 +-0.73mg/L)	oxygen	Cd	no, because the effect of oxygen could not be clearly entangled from other effects and be quantified.
Riba et al. 2016	At decreased pH, the concentration of metal ions (Cr, Ni, Cu, Zn, Cd, and Pb) was increased, but the availability to the organisms was reduced	pH	metals	no, due to the experimental setup: the effects of the single stressors could not be quantified separately and thus this interaction data could not be used for the model
Mubiana et al. 2005	at increased salinity metal concentration was lower than at low salinities (salinity range: 18-34 ppt)	salinity	metals	no, several influencing factors, salinity is not considered in test data
Mubiana et al. 2005	At increased salinity, metal concentration was lower than at low salinities (salinity range: 18-34 ppt), internal concentration of Zn one order of magnitude higher compared to the concentration at 34ppt)	salinity	metals	no, several influencing factors, salinity is not considered in test data

Traore et al. 1999	The presence of a mixture of metals (Al, Cu, Pb, Hg, Cd) increases the cytotoxicity of low concentrations of the algal toxin Okadaic acid: the mixture induced like Okadaic acid (a toxin produced by sponges and dinoflagellates associated with Dinophysis) lipid peroxidation. The presence of a mixture of metals (Al, Cu, Pb, Hg, Cd) increased the lipid peroxidation effect of Okadaic acid, increased the percentage of protein synthesis inhibition and increased the lactate dehydrogenase (LHD) release.	metals	metals, toxin	no, beauce the relationship refers to a mixture (no binary relationship)
Sheir et al. 2013	Increased Cd concentration led to decreased Pb concentrations in digestive glands	Cd	Pb	yes, additional info: the mean was calculated of interaction effect on digestive glands and gills, for the model an average of the observed relative increase due to this interaction as an input for the matrix was used.
Sheir et al. 2013	Increased Cd concentration led to decreased Pb concentrationsin gills	Cd	Pb	yes, additional info: the mean was calculated of interaction effect on digestive glands and gills, for the model an average of the observed relative increase due to this interaction as an input for the matrix was used.

Sheir et al. 2013	Addition of Cd led to a decrease of the effects of Pb and Fe in polluted mussels (necrosis, inflammation and neoplasia), mussels from a polluted site with high Pb and Fe concentrations were compared to mussels from a reference site with regard to different responses in lab experiments with Cd, 20ug/L Cd, Pb, pH 7.7, salinity 30.8, 1 week acclimation, Pb in water at polluted site: <0.03 but in sediment 54.75 ug/g dry weight, polluted site compared to reference site	Cd	Pb	no, because the effect was not be quantified and evaluated statistically
Koski et al. 2008	pH and Pb concentrations in the water are negetively correlated with each other, particularly if sulfide is present (e.g. sulfide-rich rocks due to Cu mining)	pH	Pb	no, because the interaction takes place in the water column
Riba et al. 2016	Altered Pb concentration in the water (dissolved from sediments), effect of reduced pH on the fertilization of eggs below pH of 6.5	pH	Pb	no, no clear effect, no true control, the effect was dependent on study site
Han et al. 2013	Increased uptake of the metal at decreased pH	pH	Pb	no, because mortality was considered to be a better indicator for whole organism reponse (data set from the same publication used)
Han et al. 2013	Increased percentage of esoinophilic hemocytes at decreased pH at increased metal concentration	pH	Pb	no, because mortality was considered to be a better indicator for whole organism reponse (data set from the same publication used)

Han et al. 2013	Decreased zymosan phagocytosis at decrased pH at increased metal concentration	pH	Pb	no, because mortality was considered to be a better indicator for whole organism reponse (data set from the same publication used)
Han et al. 2013	Increased metallothionein concentration at decreased pH (6.6) at increased metal concentration (30 mg/L)	pH	Pb	no, because mortality was considered to be a better indicator for whole organism reponse (data set from the same publication used)
Han et al. 2013	Increased metallothionein concentration at decreased pH (7.7) at increased metal concentration (30 mg/L)	pH	Pb	no, equation derived from other data set of the same publication
Han et al. 2013	increased mortality at decreased pH and increased metal concentration	pH	Pb	yes, several pH values were tested, the experiment was conducted at the Jiaozou bay of the Shandong province in China, but the salinity conditions there resemble those measuered at the monitoring station at Norderney, the data set for mortality as a response variable was preferred over other data set of the same publication, Han et al. 2013 measures the longest exposure time for this interaction compared to other publications listed above
Phillips 1976	High salinities at 35 ppt decreased the net uptake of lead (20ug/L) at 10°C after 14 days exposure	salinity	Pb	no, several influencing factors, salinity is not considered in test data
Struck et al. 1998	No effect of salinity on Pb concentrations in mussels, field data, salinity 7.4-32ppt, note that concentrations of Pb in the	salinity	Pb	no, several influencing factors, salinity is not considered in test data

	water at the different plots sampled might have differed			
Phillips 1976	high salinities at 35 ppt decreased the net uptake of lead (20ug/L) at 18°C after 14 days exposure	salinity/ Temp	Pb	no, because salinity was not considered as a stressor in the test scenario
Mubiana et al. 2007	increased temperature led to increased Pb uptake, pH 7.8-8.0, O2 7.4-10.4, temperature 6-26, Pb 2.2-2.3 ug/L, salinity 35 g/L, exposure 28 days	Temp	Pb	yes,the experimental conditions resembled those of the test scenario, equation derived from data. I used their data to calculate the increase of the uptake rate depending on the temperature and defined the uptake rate at 6°C as reference to calculate the change of the uptake rate with increasing temperature as it was the lowest uptake rate they measured.
Akberali et al. 1985	Addition of Cu reduced the pH in seawater Cu 6.355 ug/L, pH 5.5-7.9	Cu	pH	no, because the interaction refers to an interaction in the water column and for the test scenario we used pH values measured in the water column
Melzner et al 2013	Increased hypoxia lead to a decreased seawater pH, (pH 7.5 pCO2 <0.5-3000uatm, 10°C, 0-350 uM O2); pH = 7.281+0.0024*seawater O2 in uM	oxygen	pH	no, because the relationship refers to an interaction in the water column
Melzner et al 2013	increased salinity led to an increased seawater pH, (pH 7.5 pCO2 <0.5-3000uatm, 10°C, salinity 20-35 psu)	salinity	pH	no, several influencing factors, salinity is not considered in test data

Akberali et al. 1985	Addition of Zn reduced the pH in seawater Zn 6.54 ug/L, pH 7.1-7.9	Zn	pH	no, because the interaction refers to an interaction in the water column and for the test scenario we used pH values measured in the water column
Cotter et al. 1982	increased temperature increased the negative effect of low salinity (mortality) at 10 ppt, temperatures 10, 16, 22°C tested, 80 individuals, exposure time 42 days	Temp	salinity	no, because salinity was not considered as a stressor in the test scenario
Hiebenthal et al. 2013	Shell growth was inhibited at 25°C and increased pCO_2 (1357 uatm CO_2) (however not significant), temperatures 7.5, 10, 16, 20 and 25°C), salinity of 16.4	pH	Temp	no, because the salinity in the experiment did not resemble the conditions of the test scenario
Hiebenthal et al. 2012	growth decreased with increasing temperature at high salinities (35ppt), temperature: 4-25°C, (the sign denotes the increasing severeness of the negative effect)	salinity	Temp	no, several influencing factors, salinity is not considered in test data
Hiebenthal et al. 2012	growth decreased with increasing temperature at low salinities (15ppt), temperature: 4-25°C	salinity	Temp	no, several influencing factors, salinity is not considered in test data
Phillips 1976	low salinities at 15 ppt increased the temperature effect on the net uptake of cadmium (40ug/L) at 6 and 14 days exposure	salinity	Temp	no, several influencing factors, salinity is not considered in test data
Cotter et al. 1982	thermal tolerance was reduced by ca. 1°C at 10000 ug/L Zn (mortality tested), salinity 35 ppt for lower Zn and Salinity 8-16 psu at high Zn	Zn	Temp	no, due to the additional influence of salinity

Cotter et al. 1982	thermal tolerance was reduced (mortality tested), Zn concentrations tested:0, 300, 500, 800 an 1000 ug/L, temp 29.7- 31°C, salinity 35 ppt	Zn	Temp	yes,salinity conditions resembled those found in the North Sea, but temperature values were very high, I used these data to describe the change of the effect of Zn on the thermal tolerance by pooling the data for different temperature values and by determining an equation to calculate the influence of different Zn concentrations on mortality under elevated temperature values.
Bremco and Calabrese 1969	increased temperatures decreased growth of larvae (e.g. at 25°C) at low and high salinities (e.g. at 15, 20ppt and 40ppt)	salinity/ temp	Temp/ salinity	no, several influencing factors, salinity is not considered in test data
Bremco and Calabrese 1969	increased temperatures (e.g. at 25°C) decreased survival of larvae at low and high salinities (e.g. at 15, 20ppt and 40ppt)	salinity/ temp	Temp/ salinity	no, several influencing factors, salinity is not considered in test data
Bremco and Calabrese 1969	decreased growth of larvae decreased temperatures at low and high salinities (e.g. at 15, 20ppt and 40ppt)	salinity/ temp	Temp/ salinity	no, several influencing factors, salinity is not considered in test data
Bremco and Calabrese 1969	decreased temperatures decreased survival of larvae at low and high salinities (e.g. at 15, 20ppt and 40ppt)	salinity/ temp	Temp/ salinity	no, several influencing factors, salinity is not considered in test data
Sheir et al. 2013	Increased Cd concentration led to increased uptake of Zn in digestive glands	Cd	Zn	no, because a comparison with Vercauteren and Blust 1999 showed that the experimental conditions were similar but the experiment by Vercauteren and Blust had no influence of other stressors and comprised more targets.

Sheir et al. 2013	Increased Cd concentration led to increased uptake of Zn in gonads	Cd	Zn	no, because a comparison with Vercauteren and Blust 1999 showed that the experimental conditions were similar but the experiment by Vercauteren and Blust had no influence of other stressors and comprised more targets. We did not calculate a mean to avoid the creation of a bias towards a certain target.
Vercauteren and Blust 1999	At Cd concentrations above 1 M Cd, Zn uptake in soft tissues started to decrease (value for a concentration of 1uM Cd)	Cd	Zn	yes, environmetal conditions comparable with test scenario
Vercauteren and Blust 1999	At Cd concentrations of 10 M Cd, Zn uptake in soft tissues decreased	Cd	Zn	yes,the experimental conditions resembled those of the test scenario, equation derived from data, mean of the different experiments calculated
Vercauteren and Blust 1999	At Cd concentrations of 10 M Cd, Zn uptake in gills decreases	Cd	Zn	yes,the experimental conditions resembled those of the test scenario, equation derived from data, mean of the different experiments calculated
Vercauteren and Blust 1999	At Cd concentrations of 10 M Cd, Zn uptake in the digestive system decreases	Cd	Zn	yes,the experimental conditions resembled those of the test scenario, equation derived from data, mean of the different experiments calculated
Vercauteren and Blust 1999	At Cd concentrations of 10 M Cd Zn uptake in the hemolymph increases 3fold	Cd	Zn	yes,the experimental conditions resembled those of the test scenario, equation derived from data, mean of the different experiments calculated

Elliot et al. 1986	Increased Cd levels increased the accumulation of Zn (at 10 and 20 ug/L Cd, and at 200 ug/L Zn), 11°C, exposure time 10 days	Cd	Zn	no, because data of another experiment fitted better to the data of the test scenario.
Elliot et al. 1986	An increased Cu concentration of 20ug/L increased the uptake of Zn (Zn conc: 100 ug/L)	Cu	Zn	yes, because the ratio between the stressors fitted to the data of the test scenario
Elliot et al. 1986	The presence of 20 ug/L Cu increases the uptake of Zn even at very low concentrations of Zn (11.5 ug/L Zn), 11°C, exposuretime 10 days	Cu	Zn	no, because the ratio of the stressors did not resemble the ratio of the test scenario. (at the test scenario the concentrations of Zn were much higher than those of Cd)
Kaitala 1988	The presence of Cu increased the uptake of Zn at 200 ug/L Cu and 400 ug/L Zn	Cu	Zn	not included, because I didnt have access to the whole articel and don´t know about the experimental conditions such as salinity etc., experiment maybe conducted at Helsinki as the author is from a biological statioj situated there. "A factorial experiment and measurements of the atom absorption spectrometry showed an increase of 25% of the Zn concentration when Cu was present (Kaitala 1988). "
George 1983	Increased pH resulted in a smaller amount of metals bound to granules	pH	Zn	yes, the conditions of the experiments were comparable with those of the test scenario, data of the binding to metallothioneins and granules were pooled, compared to Han et al. 2013 more different pH calues were tested

Riba et al. 2016	Altered Zn concentration in the water (dissolved from sediments), effect of reduced pH on the fertilization of eggs below pH of 6.5	pH	Zn	no, no clear effect, no true control, the effect dependent on study site
Struck et al. 1998	Zn concentrations in mussels collected in the Baltic were higher than Zn concentrations in North Sea, note that concentrations of Zn in the water at the different plots sampled might have differed	salinity	Zn	no, several influencing factors, salinity is not considered in test data
Struck et al. 1998	Internal concentration of Zn was 37% lower in the North Sea than in the Baltic Sea , field data, salinity 7.4-32ppt, note that concentrations of Zn in the water at the different plots sampled might have differed	salinity	Zn	no, several influencing factors, salinity is not considered in test data
Phillips 1976	no effect of salinity on Zn uptake detected (salinities tested: 15ppt, 35ppt, 400 ug/L Zn, exposure: 6-14 d, temp. 10 and 18°C	salinity	Zn	no, several influencing factors, salinity is not considered in test data
Wang et al. 1997	at 2 ug/L Zn uptake of Zn was highest at salinity 20 ppt with an influx rate of ca. 0.26 ug/g/d, lowest uptake rate was at 35 ppt, at 15ppt Cd uptake was also reduced, 15°C, Znuptake = 2.975*exp(-((sal-18.3)/21.93)^2)	salinity	Zn	no, several influencing factors, salinity is not considered in test data
Cotter et al. 1982	mussels were 16% lighter at 22ppt than at 35ppt; Zn conc. 300, 1000, Temp. 10, 16, 22°C	salinity	Zn	no, salinity not considered in test data, salinity is not considered in test data

Struck et al. 1998	internal concentration of Zn was 58% higher in the Baltic than in the North Sea , field data (North Sea and Baltic sea, salinity 7.4-32ppt, note that concentrations of Zn in the water at the different plots sampled might differ	salinity	Zn	no, several influencing factors, salinity is not considered in test data
Cotter et al. 1982	at decreased salinty (22ppt) Zn concentration was 29% higher in soft tissue than at 35ppt, Zn 300, 800, 1000 and 3000, 10000, 30000 ug/L	salinity	Zn	no, several influencing factors, salinity is not considered in test data
Cotter et al. 1982	at decreased salinty (22ppt) condition index decreased compared to than at 35ppt, Zn 300, 800, 1000 and 3000, 10000, 30000 ug/L	salinity	Zn	no, several influencing factors, salinity is not considered in test data
Cotter et al. 1982	increased temperature increased the toxic effect of Zn (but not the uptake), 10, 16, 22°C, salinity 10, 22 and 35ppt, and 100, 1000, 3000 ug/L Zn (decreased lethal time by a factor of 0.96 per °C)	Temp	Zn	yes, study took place at Australia, but salinity conditions were comparable to the test scenario
Lehnberg und Theede 1979	complex interaction between salinity, temperature on development of larvae, ecological niches for larvae modeled	Temp and salinity	Temp and salinity	no, interaction could not be quantified accoding to the sheme applied in the manuscript

7.1.3 Literature:

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7.2 Appendices chapter 5

7.2.1 Applied literature information

Table 18 For those targets, representing possible changes in species composition always the highest number is used. For the aggregation of those the absolute values are used to indicate the average magnitude of predicted change. EQR: Ecological Quality Ratio (add source WFD)* not included in the test models due to a too small R^2 value

Target group	**Targets**	**Target Reference values**	**comment**	**Literature for reference value**
Chemical composition	Chl-a in young plants	1.49 mg/g/fresh wt	Highest measured conc. in *Zostera marina* in experiment (at 4 µg Cu/L)	Zhao et al. 2016
	Chl-a+b in young plants	2.52 mg/g/fresh wt	Highest measured conc. in *Zostera*	Zhao et al. 2016

			marina in experiment (at 5 µg Cu/L)	
	Chl-b in young plants	0.91 mg/g/fresh wt	Highest measured conc. in *Zostera marina* in experiment (at 10 µg Cu/L)	Zhao et al. 2016
	Carotenoid in young plants	0.24 mg/g/fresh wt	Highest measured conc. in *Zostera marina* in experiment (at 4 µg Cu/L)	Zhao et al. 2016
	Sucrose content	399 mg/g dry wt	Highest measured content in *Zostera marina* under control conditions	Munkes et al. 2015
Nutrient uptake	Ammonium uptake	28 µmol/g dw/h	Max value at low velocity was used as this is the preferred condition for *Zostera noltii* (value after 28 days. Experiment lasted 36 days), concentration in water: 0.27 µmol NH_4^+ L^{-1}, NH_4^+ L^{-1}, 1.59 µmol NO_3^- L^{-1}, 0.22 µmol PO_4^{3-} L^{-1}in water + enrichment of 50 µmol NH_4^+ L^{-1} and 5 µmol phosphate every 2 days	Villazán et al. 2016
	Phosphate uptake	2.69 µmol/g dw/h	Conditions see line above, max value for phosphate uptake after 36 days	Villazán et al. 2016

photosynthesis	Photosynthetic rate	196 µmol O_2 /g dry wt/ h	Photosynthetic rate under control conditions with 9.14 mg/ L O_2 (*Zostera marina*)	Holmer and Bondgard 2001
	Effective quantum yield of photosystem II	100 %	100% functioning (no inhibition of photosynthesis) (*Halophila ovalis*)	Wilkinson et al. 2015
growth	Above ground growth rate of seedlings	0.124 cm/ day	At control conditions of 4 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Above ground growth rate young plants seagrass	0.174 cm/ day	At control conditions of 4 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Above ground productivity seedlings	0.058 mg dw/ shoot /day	At control conditions of 4 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Above ground productivity seagrass young plants	0.386 mg dw/ shoot /day	At control conditions of 4 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Below ground productivity seedlings	0.033 mg dw/ shoot /day	At concentrations of 3 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Below ground productivity young plants	0.089 mg dw/ shoot /day	At concentrations of 3 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Leaf elongation seagrass	23.52 mm/m/day	Max value at optimum O_2 concentrations	Holmer and

			of 9.14 mg/L (*Zostera marina*)	Bondgard 2001
	Netto growth rate seagrass	1.64 mg fw/d/experimental unit	Max value at velocity of 0.35 m/sec. , (*Zostera noltii*), (higher velocity improved light capture (de los Santos et al. 2010 in Villazán et al. 2016)	Villazán et al. 2016
	Rhizome elongation rate young seagrass	0.12 mm/shoot/day	At control conditions of 4 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Root elongation rate seedlings	0.39 mm/shoot/day	At concentrations of 5 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Total productivity seedlings	0.145 mg dw/ shoot /day	At control conditions of 4 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Total productivity young plants seagrass	0.52 mg dw/ shoot /day	At control conditions of 4 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Internode appearance rate seagrass	0.05 internode/d/experimental unit	Max value at velocity of 0.35 m/sec. , (*Zostera noltii*), (higher velocity improved light capture (de los Santos et al. 2010 in Villazán et al. 2016)	Villazán et al. 2016
vitality	Leaf abundance seagrass	4.70 no/experimental plant unit	Max value, at velocity of 0.01 m/sec. (*Zostera noltii*)	Villazán et al. 2016

	Leaf length *Zostera noltii*	255 mm	Max length in experiment, (*Zostera noltii*)	Cabaco and Santos 2007
	Leaf length *Zostera marina*	932 mm	Max length in experiment, (*Zostera marina*)	Munkes et al. 2015
	Shoot length seagrass	20.3 cm	Max shoot length, at 0.04 m/sec. velocity (*Zostera noltii*)	Schanz and Asmus 2003
	Total leaf area per shoot of seedlings	0.46 cm^2/shoot	At control conditions of 4 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Total leaf area per young seagrass shoot	0.95 cm^2/shoot	At control conditions of 4 µg Cu/L (*Zostera marina*), max value	Zhao et al. 2016
	Sheath length	51 mm	Max value in experiment, (*Zostera noltii*)	Cabaco and Santos 2007
	Shoot density	7000 no/m^2	Control value, *Zostera noltii*)	Cabaco and Santos 2007
recruitment	Fertile shoot density seagrass	15 no/m^2	max fertile shoot density in experiment	Munkes et al. 2015
	Shoot appearance rate	0.03 shoots/day/experimental plant unit	Max value in experiment, at 0.35 m/ sec. velocity	Villazán et al. 2016
Survival and absence of necrosis	Percent green without leaf necrosis	100%	This would reflect ideal conditions	
	Survival seagrass	100%	This would reflect ideal conditions	
	Survival seagrass seedlings	100%	This would reflect ideal conditions	

	Survival seagrass young plants	100%	This would reflect ideal conditions	
Community composition	Biomass of epiphytes on seagrass	0.15 mg AFDW/cm^2 leaf	Highest value, at low velocity of 0.09 m/sec. (*Zostera noltii*)	Schanz et al. 2002
	Epiphytic CaCo3 on seagrass	45.4 mg/ leaf	Highest value, at pH of 8.15 (*Posidonia oceanica*)	Hall-Spencer et al. 2008
	Microphytobenthos biomass (Chl-a in sediment)	25.8 µg/g dry wt	Highest value, at highest seagrass density (*Zostera noltii*)	Widdows et al. 2008
	Number of snails on seagrass	31745 no/ m^2	Highest value, at low velocity of 0.045 m/sec.	Schanz et al. 2002
	Maximum macroalgal canopy height	5 cm	Maximum macroalgal canopy height in *Zostera marina* meadow, nitrogen loading rate of 20 kg N/ ha/yr	Hauxwell et al. 2003
	Macroalgal biomass	144 g dry wt/m^2	Maximum macroalgal biomass in *Zostera marina* meadow, nitrogen loading rate of 1573 kg N /yr	Deegan et al. 2002
	Chl-a in water	0.21 mg/m^3	At this concentration highest overall biomass and 2nd highest shoot density, (*Thalassia testudinum*)	Green and Webber 2003
Sediment characteristics	Eroded sediment mass	10 g/m^2	Critical threshold for sediment erosion	Defew et al. 2002
	Bed shear stress	0.09 Pa	Critical threshold for the onset of erosion at the	Widdows et al. 2008

			absence of *Zostera noltii*, the value is higher with the presence of *Zostera noltii* and with increased density of it	
Aboveground to below ground ratio	Aboveground to belowground ratio seagrass	0.5	Approximately the mean of different light and velocity conditions	Villazán et al. 2016

Table 19 Sources Add a scenario where the pressure situation increases 3 times. For the test-scenario, the highest values found for the region were used. For the scenario 2, for pH the lowest value was used, for light availability, the max value was used for the reference scenario, oxygen: lowest measured value

Source group	Sources	Input values reference scenario (scenario 1)	Comment and species the literature refers to	Source Reference values	Input values scenario 2	Source test-scenario	Time frame	Geographical range	Input values scenario 3	Comment
pollution	Cu	4 µg/L	Cu is an essential metal, (*Zostera marina*)	Zhao et al. 2016	10 µg/L	Monitoring data, NLWKN	2005-2010	Norderney, German Bight, North Sea	20 µg/L	Value of 2nd scenario doubled
	Simazine	0 µg/L	For all herbicides the absence of them is considered as the optimum		0.003097 µg/L	Max value, Mai et al. 2013	Mai to June 2009 and Mai 2010	German Bight, North Sea	0.3097µg/L	Value of 2nd scenario times 100
	Diuron	0 µg/L			0.0005 µg/L	Max value, Brumovský et al. 2016	October 2014	German Bight, North Sea	0.05 µg/L	Value of 2nd scenario times 100
	Atrazine	0 µg/L			0.003985 µg/L	Max value, Mai et al. 2013	Mai to June 2009 and Mai 2010	German Bight, North Sea	0.3985 µg/L	Value of 2nd scenario times 100
	Ametryn	0 µg/L			0.000532 µg/L	Max value, Mai et al. 2013	Mai to June 2009 and Mai 2010	German Bight, North Sea	0.0532 µg/L	Value of 2nd scenario times 100
	Bromacil	0 µg/L			0.0006 µg/L	Schulten et al. 2006	November 2013	Nordertil, German Bight, North Sea	0.06 µg/L	Value of 2nd scenario times 100

	Hexaxione	0 µg/L			0.002714 µg/L	Max value, Mai et al. 2013	Mai to June 2009 and Mai 2010	German Bight, North Sea	0.2714 µg/L	Value of 2nd scenario times 100
	Prometryn	0 µg/L			0.001688 µg/L	Max value, Mai et al. 2013	Mai to June 2009 and Mai 2010	German Bight, North Sea	0.1688 µg/L	Value of 2nd scenario times 100
Hydro-graphic conditions	pH	7.87	Mean value measured at Kiel Bight	Thomsen et al. 2013	7.87	Thomsen et al. 2013	2008-2010 (measured throughout the year)	Kiel Fjord, German Bight, North Sea	7.87	
	velocity	0.3 m/sec.	Mean value of monitoring station (data from west – east direction and south – north pooled)	Monitoring data, BSH	0.3 m/sec.	Monitoring data, BSH	2005-2007	Norderney, German Bight, North Sea	1.23 m/sec.	Max value or monitoring data, BSH, 2005-2007
	velocity	0.13		Max value from Schanz and Asmus 2003, in North Sea velocity in average higher					0.13	

Eutrophication	Light availability	1482 µmol photons/s/m²	Max value of data set as high light availability is considered as favourable	Scholz und Liebezeit 2012	455.4 µmol photons/s/m²	Scholz und Liebezeit 2012	August 2008	Solthörn tidal flat, German Bight, North Sea	227.7 µmol photons/s/m²	50% reduction of the value of 2nd scenario
	Oxygen concentration	10mg/L	Maximum mean seasonal concentration	Monitoring data, COMP3	2 mg/L	Monitoring data, COMP3, (low value in the range of measured data)	2006-2014 measured mainly during summer (July to September)	Norderney, German Bight, North Sea	1 mg/L	50% reduction of the value of 2nd scenario
	N-Loading rate	20 kg N/ha/yr	Low value	Katwijk et al. 1999, Ems estuary	625 kg N/ha/yr	Katwijk et al. 1999, Hoepner 1991 (data within this range)	1999	Ems estuary, North Sea	1250 kg N/ha/yr	Value of 2nd scenario doubled
others	Burial	0	No disturbance by burial is considered as the optimum for seagrass	-	0	-	-	-	50 cm	Stronkhorst et al. 2003, data from 1996-1997, measured 50-1000m away from a dumping site (8.2 mio m³) near The

											Hague, Netherlands, North Sea
	Seasonal influence	200th day of the year	Arbitrary chosen value	-	200th day of the year	-	-	-			
	time	60 days	Arbitrary chosen value	-	60 days	-	-	-		60 days	

Table 20 Model tests with min and max values from literature sources used in the model

Source group	Sources	Input value min	Input value max	Source Reference values
pollution	Cu	0 µg/L	50 µg/L	Zhao et al. 2016
	Simazine	0.3007 µg/L	310.393 µg/L	Wilkinson et al. 2015
	Diuron	0.100 µg/L	104.126 µg/L	
	Atrazine	5.457 µg/L	87.670 µg/L	
	Ametryn	0.0987 µg/L	31.190 µg/L	
	Bromacil	0.301 µg/L	315.951 µg/L	
	Hexaxione	0.290 µg/L	30.754 µg/L	
	Prometryn	0.290 µg/L	312.649 µg/L	
Hydrographic conditions	pH	6.98	8.17	Hall-Spencer et al. 2008
	velocity	0.01 m/sec.	0.35 m/sec.	Villazan et al. 2016
Eutrophication	Light availability	47.3 µmol photons/s/m²	550 µmol photons/s/m²	Villazan et al. 2016, Hasler-Sheet et al. 2017
	Oxygen concentration	2.015 mg/L	9.142 mg/L	Holmer and Bondgard 2001
	N-Loading rate	4.6 kg N/ha/yr	417 kg N/ha/yr	Burkholder et al 2007
others	Burial	0	20 cm	Cabaco and Santo 2007, Munkes et al. 2015
	Seasonal influence	124^{th} day of the year	236^{th} day of the year	Munkes et al. 2015

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7.3 Contributions

7.3.1 Chapter 3 – Overall concept

Silke Eilers mainly developed the general concept for assessment of cumulative effects. Adorian Ardelean and Thomas Raabe further contributed with some ideas for refinements for the concept. Silke Eilers developed ideas for the main structure of the tool from a user perspective. Adorian designed the architecture for the implementation of the concept for the online tool LiACAT, adjusted the ideas for the tool from a computer scientist perspective, and wrote the corresponding scripts for the implementation of the tool. Thereby some modules of the tool are based on freeware tools. The script for the analysis of cumulative effects of habitats is based on the concept and the script described in chapter 5. Adorian Ardelean adjusted it for the integration in the online tool LiACAT.

7.3.2 Chapter 4 – Matrix model and DEB model, Test with data of the species *Mytilus edulis*

Silke Eilers developed the concept for analyzing cumulative effects with a combination of a matrix and a DEB-model focusing on temporal dynamics. Thereby she developed a scheme to analyze and include different aspects of temporal dynamics of single stressors into a DEB model for blue mussels. Wolfgang Ebenhoe wrote a basic script for the realization of these ideas (one part of the complete script for modeling anthropogenic effects on blue mussels), which Silke Eilers later refined. Silke Eilers wrote the script for the core DEB model of blue models based on published equations of a DEB model (Saraiva et al. 2012) and combined it with the matrix method for the integration of interaction effects. Silke Eilers conducted the literature analysis and applied the test data.

7.3.3 Chapter 5 – ACIM modeling tool – Test with data of the habitat seagrass meadows

Silke Eilers developed the concept for the analysis of cumulative effects on habitats. Silke Eilers and Kiril Schröder wrote the script for the implementation of the concept, whereby Kiril Schröder solved the tricky parts of the implementation. Silke Eilers conducted the literature analysis and applied the test data.

7.4 **Erklärung**

Hiermit erkläre ich, dass die von mir vorgelegte Dissertation selbstständig verfasst und die benutzten Hilfsmittel vollständig angegeben sind.

Die Dissertation wurde noch nicht veröffentlicht. Einige Inhalte der Dissertation habe ich jedoch durch eine Präsentation auf dem Meeresumweltsymposium in Hamburg 2014[28] sowie durch für HELCOM verfasste Texte der Öffentlichkeit zugänglich gemacht. Darüber hinaus habe ich kurze Texte für die BLANO (Bund Länder-Arbeitsgemeinschaft Nord- und Ostsee), für HELCOM und für das Umweltbundesamt verfasst. Desweiteren habe ich zusammen mit verschiedenen Coautoren Projektberichte für das Umweltbundesamt geschrieben (siehe Auflistung unten). Das Rahmenkonzept wurde in der Fachzeitschrift „Wasser und Abfall“ veröffentlicht. Die entsprechenden Publikationen und Berichte sind in der Publikationsliste (7.5) mit einem Sternchen versehen.

Außerdem bestätige ich, dass die Dissertation weder in ihrer Gesamtheit noch in Teilen einer anderen wissenschaftlichen Hochschule zur Begutachtung in einem Promotionsverfahren vorgelegt wurde, ich die Leitlinien guter wissenschaftlicher Praxis an der Carl von Ossietzky Universität befolgt habe und dass ich im Zusammenhang mit dem Promotionsvorhaben keine kommerziellen Vermittlungs-oder Beratungsdienste (Promotionsberatung) in Anspruch genommen habe.

Datum Unterschrift (Silke Eilers)

[28] http://docplayer.org/10040939-Meeresumwelt-symposium-2014.html

7.5 Publications

Publications in journals

Eilers, S. Ardelean, A. Raabe, T. (2017): Kumulative Bewertung des Umweltzustandes nach der Meeresstrategie-Rahmenrichtlinie. ***Wasser und Abfall*** *

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Lannig G., Eilers S., Bock C., Pörtner H.O. & Sokolova I.M. (2010) Impact of ocean acidification on temperature-dependent energy metabolism of oyster, *Crassostrea gigas,* **Marine Drugs**

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Reports

Eilers, S., Raabe, T. (2021): Entwicklung eines Nordsee-Belastungsindex zur Analyse der räumlichen Verteilung und Kumulation ausgewählter menschlicher Belastungen im deutschen Meeresgebiet (in prep.)*

Altvater, S., Lukic, I., Eilers, E. (2019): EBA in MSP – a SEA inclusive handbook. Federal Maritime and Hydrographic Agency (BSH), Rostock. DOI: 10.13140/RG.2.2.27416.55042, ISBN: 978-3-86987-990-1

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Eilers, S., Raabe, T., Ardelean, A., Dürselen, C-D., Burgmer, T., Dierschke, V., Hill, K., Hill, R., Burkhard, B., Hertz-Kleptow, C. (2014): Entwicklung eines Konzeptes zur kumulativen Bewertung anthropogener Belastungen im Rahmen der Umsetzung der Meeresstrategie-Rahmenrichtlinie (MSRL). Endbericht des F&E Projektes FKZ 371125216*

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Lannig, G., Eilers, S., Bock, C., Pörtner, H. O. & Sokolova, I. M. (2010) Impact of elevated CO2 levels on temperature-dependent energy metabolism of oyster, Crassostrea gigas., ***Ocean Sciences Meeting, Understanding Ecophysiological Adaptation Potential to Climate Change: Mechanistic Approaches***, 22.-26. Feb., Portland, USA.

Eilers, S., Lannig, G., Pörtner, H. O., Bock, C., Ivanina, A. & Sokolova, I. M. (2009) Effects of multiple stressors on energy metabolism in oysters ***Society for experimental Biology (SEB) Annual meeting 2009, Glasgow, Scottland***

Kurochkin I.O., Ivanina A.V., Eilers S., Downs C. A., May L. A. & Sokolova I. M. (2009) Effect of cadmium and environmental anoxia and re-oxygenation on metabolism of eastern oysters (Crassostrea virginica). ***Society for Integrative and Comparative Biology (SICB) Annual meeting 2009, Boston, USA***

Kurochkin, I. O., Ivanina, A., S. Eilers, C. A. Downs, L. A. May, and I. M. Sokolova (2009) Cadmium affects metabolic responses to prolonged anoxia and reoxygenation in eastern oysters (*Crassostrea virginica*) ***Society for experimental Biology (SEB) Annual meeting 2009****, Marseille, France*

Data publishing

Lannig, G.; Eilers, S.; Pörtner, H. O.; Sokolova, I. M.; Bock, C. (2011) Seawater carbonate chemistry and biological processes of mussel Crassostrea gigas during experiments, 2011. doi:10.1594/**PANGAEA**.761915

Publishing of scripts

https://github.com/SilkeEilers/MultipleStressorsAnalyses *

7.6 Curriculum Vitae

Doctoral studies and employment at AquaEcology **since Aug 2012**	**Topic for PhD thesis: Development of a concept for the assessment of cumulative effects with regard to the Marine Strategy Framework Directive** Supervisor: Prof. Dr. Helmut Hillebrand (ICMB, University of Oldenburg), Employment: Different projects with regard to the Marine Strategy Framework Directive at AquaEcology GmbH, Oldenburg
Research assistant at the University of Oldenburg **Apr 2011 – Apr 2012**	**Research Project COMTESS** **(Sustainable coastal land management: trade-offs in Ecosystem Services)** Measurement of plant traits and environmental parameters, and species determination, working group: landscape ecology, supervisor: Prof. Dr. Michael Kleyer, Oldenburg
Master program at the University of Lund **Sep 2009 – Feb 2011**	**Major focus: conservation biology/ aquatic biology** Master´s degree project: „Oviposition strategies of the butterfly Oberthür´s Grizzled Skipper (*Pyrgus armoricanus*) in a fragmented landscape" supervisors: Prof. Dr. Erik Öckinger, Sveriges Landbruksuniversitet Uppsala (SLU) and Dr. Lars Pettersson, University of Lund
Bachelor program at Hochschule Bremen **International Degree Course in Industrial and Environmental Biology** **Oct 2005 – Jul 2009**	**Major focus: environmental biology** Bachelor´s degree project: „Effects of hypercapnia and elevated temperature on metabolism of the oyster Crassostrea gigas", Supervisor: Dr. Gisela Lannig Alfred Wegener Institut Bremerhaven in the working group Integrative Ecophysiology, Head: Prof. Dr. H-O Pörtner, supervisors: Dr. Gisela Lannig (AWI), Prof. Dr. Heiko Brunken (HS Bremen) Exchange student at the University of Kalmar in Sweden Internship at the University of North Carolina in Charlotte, USA, supervisor and head: Prof. Dr. Inna Sokolova