Surface waters under multiple stress: monitoring methods, stressor interactions and combined effects

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Summary

Water is essential for life on earth. Fresh and marine surface waters not only provide a great variety of habitats for many aquatic plant and animal species, they also fulfil various essential ecosystem functions, such as the provision of clean drinking water or the regulation of the climate. However, the condition of surface waters is increasingly declining worldwide, which severely threatens their biodiversity and ecosystem functions. We are in the midst of a biodiversity crisis, with over 40,000 plant and animal species being at risk of extinction. In addition, the capacity of surface waters to provide essential functions is decreasing.

This deterioration is driven by increasing human impacts, which have substantially modified the earth's surface and atmosphere to exploit its functions. These impacts drive several stressors affecting biodiversity and ecosystem functions and nowadays, most areas are affected by multiple, co-occurring stressors. Such multiple stressors can act independently, but they can also interact with each other, enhancing or dampening their combined effect. The severe effects of multiple stressors on the global ecosystem require the conservation of intact and restoration of damaged ecosystems.

For successful environmental management, multiple stressor interactions have to be taken into account, as they require specific management measures. However, understanding the stressor-effect relationships and predicting interactions and combined effects of multiple stressors remains a major challenge, making effective management of surface waters difficult.

Assessing the current condition of surface waters and identifying the stressor effects and interactions that cause this condition are crucial for a successful environmental management. The aim of this work is supporting the future conservation and restoration of surface waters by addressing contemporary challenges in environmental assessment and stressor research:

Chapter 1: The assessment of the condition of a specific water body, including potential stressors affecting the area, is the basis for environmental management. Hence, comprehensive monitoring programmes that acquire information on the environmental status of the system are essential. In this chapter, novel methods with the potential to enhance marine monitoring are identified and rated. The main benefits of these methods are the autonomous collection of real-time data with enhanced spatial and temporal resolution as well as data acquisition on ecosystem elements that have not yet been monitored.

Chapter 2: The experimental study of multiple stressors can help to disentangle interactions and effects of specific stressor combinations. Thus, it helps to derive guidelines for effective management. In this chapter, a stream-mesocosm experiment to study the combined effects of fine sediment and a novel insecticide on the decomposition of organic matter as an important ecosystem function is evaluated. Results indicate that both stressors can inhibit organic matter decomposition. An interaction between the two stressors was not detected under the given conditions.

Chapter 3: In order to detect and quantify possible stressor interactions, an understanding of the factors that influence stressor interactions and effects is essential. Several factors relating to ecological processes are already known, but the role of the sampling strategy has not been examined so far. In this chapter, the influence of sample size and stressor gradient length on observed multiple stressor effects is studied. The results indicate that both factors play a significant role in shaping observed multiple stressor effects. This highlights the need for cautious interpretation of observed effects and adaptive environmental management.

For the future conservation and restoration of ecosystems, further research is needed. Shifting from descriptive frameworks towards a mechanistic understanding of multiple stressor effects might improve the prediction and promote the management of multiple stressors. In addition, further studies on stressor mitigation are needed to better assess restoration effects, as current studies mostly focus on the effects of increasing stressor levels.

Global biodiversity as well as ecosystem functions are already severely threatened and trends are alarming, making immediate conservation and restoration measures urgently needed. The effects of measures cannot be predicted with certainty and therefore, they need to be constantly monitored in order to detect possible unintended consequences like adverse effects on the environment and to be able to revise the measures accordingly.

Zusammenfassung

Wasser ist die Grundlage für das Leben auf der Erde. Flüsse, Seen und Meere bieten nicht nur eine große Vielfalt an Lebensräumen für aquatische Pflanzen- und Tierarten. Sie erfüllen auch wichtige ökologische Funktionen wie die Selbstreinigung von Wasser oder die Regulierung des Klimas, die die Grundlage für das Überleben aller Lebewesen sind. Der Zustand der Gewässer verschlechtert sich jedoch weltweit, was ihre biologische Vielfalt stark gefährdet. Wir befinden uns heute inmitten einer Biodiversitätskrise: Über 40.000 Pflanzen- und Tierarten sind vom Aussterben bedroht und die Gewässer verlieren zunehmend ihre Fähigkeit, essenzielle ökologische Funktionen auszuführen.

Angetrieben wird diese negative Entwicklung durch den zunehmend invasiven menschlichen Einfluss auf den Planeten. Seit Jahrhunderten verändern die Menschen die Erde und ihre Atmosphäre massiv, was zu einer Vielzahl an Stressoren geführt hat, die die biologische Vielfalt sowie ökologische Funktionen gefährden. Heutzutage treten meist mehrere Stressoren gleichzeitig auf. Multiple Stressoren können unabhängig voneinander wirken, aber auch miteinander interagieren, was ihre Effekte auf das Ökosystem wiederum verstärken oder abschwächen kann. Die schwerwiegenden Auswirkungen multipler Stressoren auf das globale Ökosystem erfordern ein weitreichendes Umweltmanagement, um intakte Gewässer zu schützen und geschädigte Gewässer zu renaturieren.

Für ein erfolgreiches Umweltmanagement müssen potentielle Interaktionen multipler Stressoren berücksichtigt werden, da diese spezifische Maßnahmen erfordern. Doch die Entschlüsselung der Mechanismen hinter den Interaktionen und die Voraussage ihrer Effekte stellen nach wie vor eine große Herausforderung dar.

Das Ziel dieser Arbeit ist es, ein erfolgreiches Umweltmanagement zu unterstützen, indem moderne Methoden des Gewässermonitorings bewertet werden und neue Erkenntnisse über Interaktionen und Effekte multipler Stressoren gewonnen werden. Damit leistet diese Arbeit einen Beitrag zum dringend notwendigen Schutz und zur Renaturierung von Oberflächengewässern.

Kapitel 1: Die Grundlage für ein erfolgreiches Umweltmanagement besteht darin, den Zustand des untersuchten Gewässers genau zu kennen. Hierfür ist ein umfassendes Monitoring, das Daten über den biologischen, chemischen und physikalischen Zustand des Gewässers liefert, unerlässlich. In *Kapitel 1* werden Methoden dargestellt und bewertet, die das Potential haben,

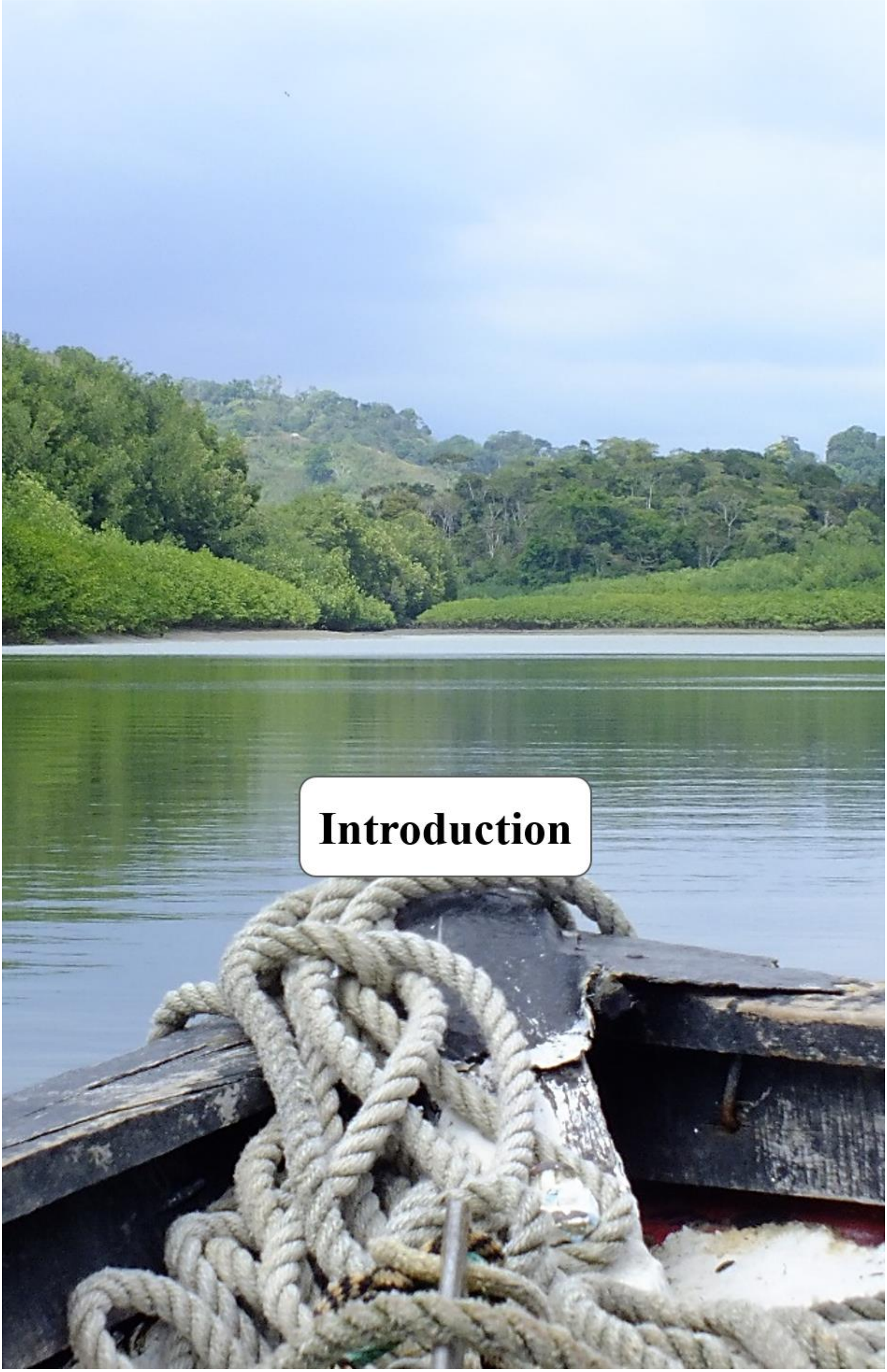
das marine Monitoring zu verbessern. Zu den Vorteilen dieser Methoden gehören vor allem das autonome Sammeln von Echtzeitdaten mit verbesserter räumlicher und zeitlicher Auflösung, sowie eine breitere Datengrundlage.

Kapitel 2: Die experimentelle Untersuchung multipler Stressoren trägt dazu bei, Interaktionen und Effekte spezieller Stresskombinationen zu entschlüsseln. Dies ermöglicht das Ableiten von Leitlinien für ein erfolgreiches Management. In *Kapitel 2* werden die Auswirkungen von Feinsediment und des neuartigen Insektizides Chlorantraniliprol auf den Abbau organischer Stoffe in Fließgewässern untersucht. Die Ergebnisse zeigen, dass beide Stressoren den Abbau organischer Stoffe hemmen können. Eine Interaktion zwischen den beiden Stressoren wurde unter den gegebenen Bedingungen nicht festgestellt.

Kapitel 3: Um die Effekte multipler Stressoren ermitteln zu können, ist es wichtig zu verstehen, welche Faktoren diese beeinflussen. Hierzu zählen beispielsweise die Identität der Stressoren, Wechselwirkungen zwischen Pflanzen- und Tierarten oder evolutionäre Anpassungen an Stressoren. In *Kapitel 3* wird untersucht, welchen Einfluss die Stichprobengröße und die Länge des Stressgradienten bei der Berechnung der Stresseffekte haben. Die Ergebnisse deuten darauf hin, dass beide Faktoren eine wichtige Rolle spielen und beweisen damit die Notwendigkeit, berechnete Stresseffekte vorsichtig zu interpretieren. Außerdem wird durch den Einfluss der Länge des Stressgradienten klar, wie wichtig ein adaptives Umweltmanagement ist, um Maßnahmen gegebenenfalls an sich verändernde Bedingungen anpassen zu können.

Um Gewässer zukünftig zielgerichteter schützen und renaturieren zu können, ist weiterführende Forschung notwendig. Einerseits wird ein tieferes Verständnis der Mechanismen benötigt, wie multiple Stressoren miteinander interagieren und auf das Ökosystem wirken. Andererseits sind verstärkt Studien erforderlich, die sich auf die Auswirkungen von reduziertem Stress konzentrieren, um die Effekte von Renaturierungsmaßnahmen differenzierter beurteilen zu können.

Die globale biologische Vielfalt sowie die ökologischen Funktionen von Gewässern sind bereits stark gefährdet und Prognosen zur zukünftigen Entwicklung sind alarmierend. Deshalb sind sofortige Schutz- und Renaturierungsmaßnahmen dringend erforderlich. Obwohl die Effekte potentieller Maßnahmen bisher nicht sicher vorausgesagt werden können, sollte dies deren Umsetzung nicht bremsen. Stattdessen sollten die Auswirkungen von Schutz- und Renaturierungsmaßnahmen kontinuierlich überwacht werden, um mögliche nachteilige Effekte auf die Umwelt frühzeitig zu erkennen und Maßnahmen entsprechend überarbeiten zu können.


Introduction

General introduction

Surface waters under multiple stress

Water is an indispensable component of the planet on which all living beings depend. Fresh waters, including streams and lakes, hold only 0.01 % of the global water resources and cover 0.8 % of the earth's surface (Gleick et al., 1996). Despite this small share, a high diversity of species lives in these freshwaters (about one-third of vertebrate species and 6 % of all described species; Dudgeon et al., 2006). This disproportionate share of freshwaters as habitats for plants and animals highlights their great importance for biodiversity. In contrast, marine waters constitute the largest ecosystems, holding 96.5 % of the global water and covering 71 % of the earth's surface (Shiklomanov, 1993). The great variety of marine habitats such as seagrass meadows, mangroves, coral reefs or rocky intertidal zones provide habitats for a high diversity of plant and animal species. Apart from their importance as habitats, fresh and marine surface waters (hereafter summarized as 'surface waters') fulfil various ecosystem functions that form the basis for life on earth. Ecosystem functions include all biotic and abiotic processes that transform and shift energy or materials in an ecosystem and represent a significant role for ecosystem health (Paterson et al., 2012). The main ecosystem functions provided by surface waters include habitat creation and maintenance, water purification, nutrient decomposition and cycling, as well as local and global climate regulation (Halpern et al., 2008; Hanna et al., 2018; Kaval et al., 2019).

Biodiversity and ecosystem functions show worldwide declines, which are particularly drastic in surface waters. Global biodiversity has shown a clear negative trend for the past 12,000 years (Boivin et al., 2016; McGill et al., 2015) and nowadays, more than 40,000 species are threatened with extinction (IUCN, 2022). The unprecedented pace of biodiversity loss resulted in a decline by more than half of species populations within the last 50 years (WWF, 2016). The resulting human-induced mass extinction is equal to the five global mass extinctions caused by natural catastrophes in the last 500 million years (Tilman et al., 2017). Many of the threatened species depend on surface water habitats, including amphibians (41 % of species threatened), sharks and rays (37 % of species threatened), or reef corals (33 % of species threatened, Figure 1; IUCN, 2022). In particular freshwater biodiversity shows dramatic trends: Over the last 50 years, more than 81 % of species populations have declined (Vári et al., 2022; WWF, 2016). Associated with the general decline in biodiversity, the capacity of ecosystems to provide functions is decreasing (Rocha et al., 2015). According to the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES, 2019), 14 of the 18 assessed functions have declined over the last 50 years. The functions related to surface waters include, for instance, habitat creation and maintenance or freshwater and coastal water quality regulation.

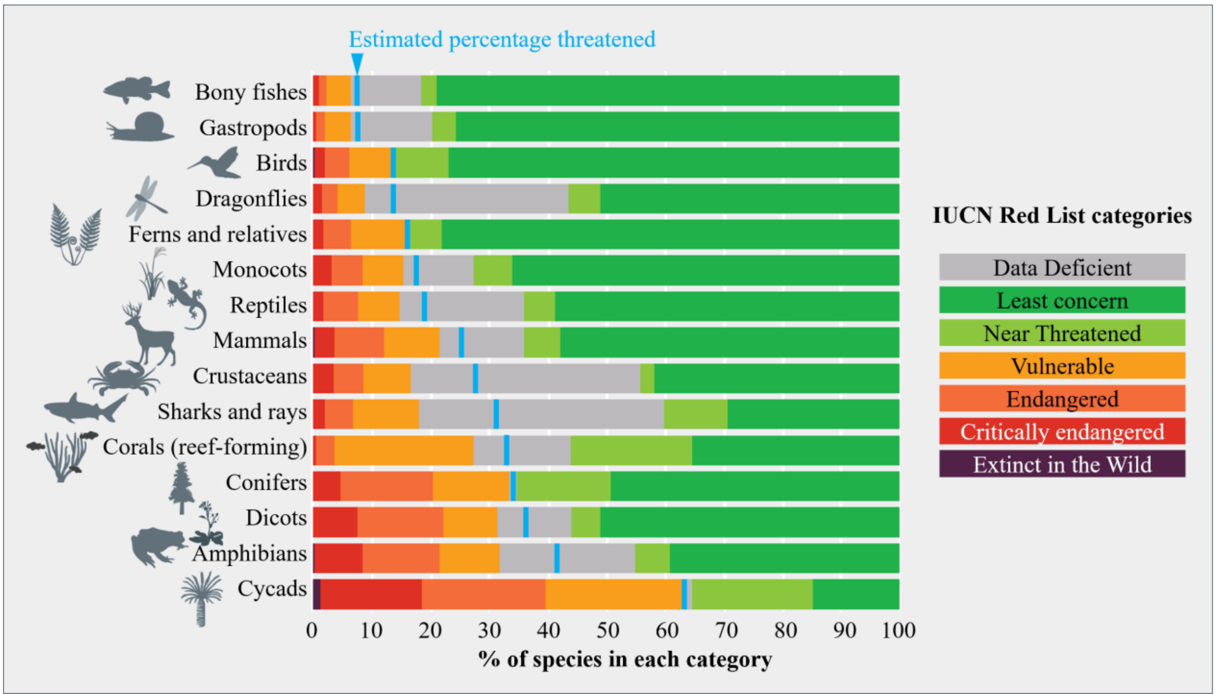


Figure 1: Current global extinction risk for different species groups (modified after IPBES, 2019). Each bar shows the percentage of species threatened with extinction for a specific taxonomic group. From top to bottom, taxonomic groups show an increasing estimated percentage of threatened species (shown by the vertical blue line), assuming that data deficient species show the same extinction risk as species without data deficiency. Data have been collected by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species.

This deterioration is driven by human exploitation of specific functions resulting in multiple stressors impairing ecological processes. Human development entails excessive population growth, technological innovations and rapid growth of economies as well as trade on a global scale (Corbridge, 1986). These dynamics are accompanied by an increasing demand for resources such as food, living space, energy or raw materials. To meet this demand, resources are unsustainably exploited and nowadays, humanity uses the resources of 1.75 earths annually (Global Footprint Network, 2022). In consequence, humans considerably modified the majority of the planet's surface. Nowadays, over 75 % of terrestrial (including freshwaters) and 40 % of marine areas are strongly affected by human activities (IPBES, 2019; UN Waters, 2020). The small fraction of the planet still considered wilderness (23 % of land and 13 % of the ocean), however, are remote and unproductive areas hard to manage efficiently (Halpern et al., 2008; Riggio et al., 2020). Moreover, human activities not only change the planet's surface, they also modify the composition of the atmosphere. Especially the emission of greenhouse gases results in global climate change, which causes pronounced shifts in climatic conditions (IPCC, 2022). These changes in the planet's surface and atmosphere drive several stressors, i.e., anthropogenic perturbations to a system which are either unfamiliar to that system or natural to that system but applied at levels exceeding the natural variability (Barrett et al., 1976). In the past, certain areas were primarily affected by single stressors related to the dominating human use of the surrounding areas, such as water bodies near agricultural land being affected by excessive nutrient inputs or urban streams containing high amounts of pollutants due to urban waste water inflows. With increasing human impact, also the number of multiple co-occurring stressors has been increasing. Nowadays, 84 % of terrestrial and 98 % of marine areas are affected by multiple rather than single stressors (Figure 2; Halpern et al., 2015; Kennedy et al., 2018). In particular stressors related to climate change are gaining relevance, as local stressors due to land use changes are increasingly accompanied by the global effects of climate change. For instance, water bodies in agricultural areas affected by excessive nutrient inputs are nowadays additionally affected by increasing temperatures or changed precipitation patterns.

The main stressors impairing surface waters are land- and sea-use change, overexploitation of food organisms, pollution, the introduction of invasive species and climate change (IPBES, 2019). Due to the position of surface waters within the landscape, all sorts of particles from surrounding areas are washed into their systems (Dudgeon et al., 2006). These land use-related stressors include excessive inputs of nutrients, sediments or contaminants, which induce various effects such as increased algal growth, anoxic water conditions, destruction of habitats, or (sub)lethal concentrations of hazardous substances (Burkholder and Glibert, 2013; Dudgeon et al., 2006; Hauer et al., 2018). In order to integrate water bodies into industrial and agricultural landscapes and utilise their functions, they are straightened, deepened or equipped with dams and hydropower plants. These modifications, combined with unsustainable water abstractions, disrupt natural hydromorphological conditions and damage aquatic habitats (Bolpagni and Piotti, 2015; Elosegi et al., 2018). Moreover, the exploitation of native species for food and the introduction of invasive species due to globalised trade and transport cause shifts in community compositions and trophic interactions (Bailey, 2015; Beauchesne et al., 2021). In addition to the direct impacts, such as declines in fish populations, fisheries also affect populations of sea birds, marine mammals and reptiles due to bycatch (WWF, 2016) and destroy benthic habitats due to trawling (Thrush and Dayton, 2002). Climate change drives stressors such as increased water temperatures or changed precipitation patterns, which not only affect habitat conditions, but also lead to modified ocean dynamics, acidification or sea level rise (EEA, 2018; Hewitt et al., 2016). In summary, these multiple stressors caused by an unsustainable use of prioritised ecosystem functions threaten aquatic ecosystem health and thereby, the basis for life on earth (IPBES, 2019).

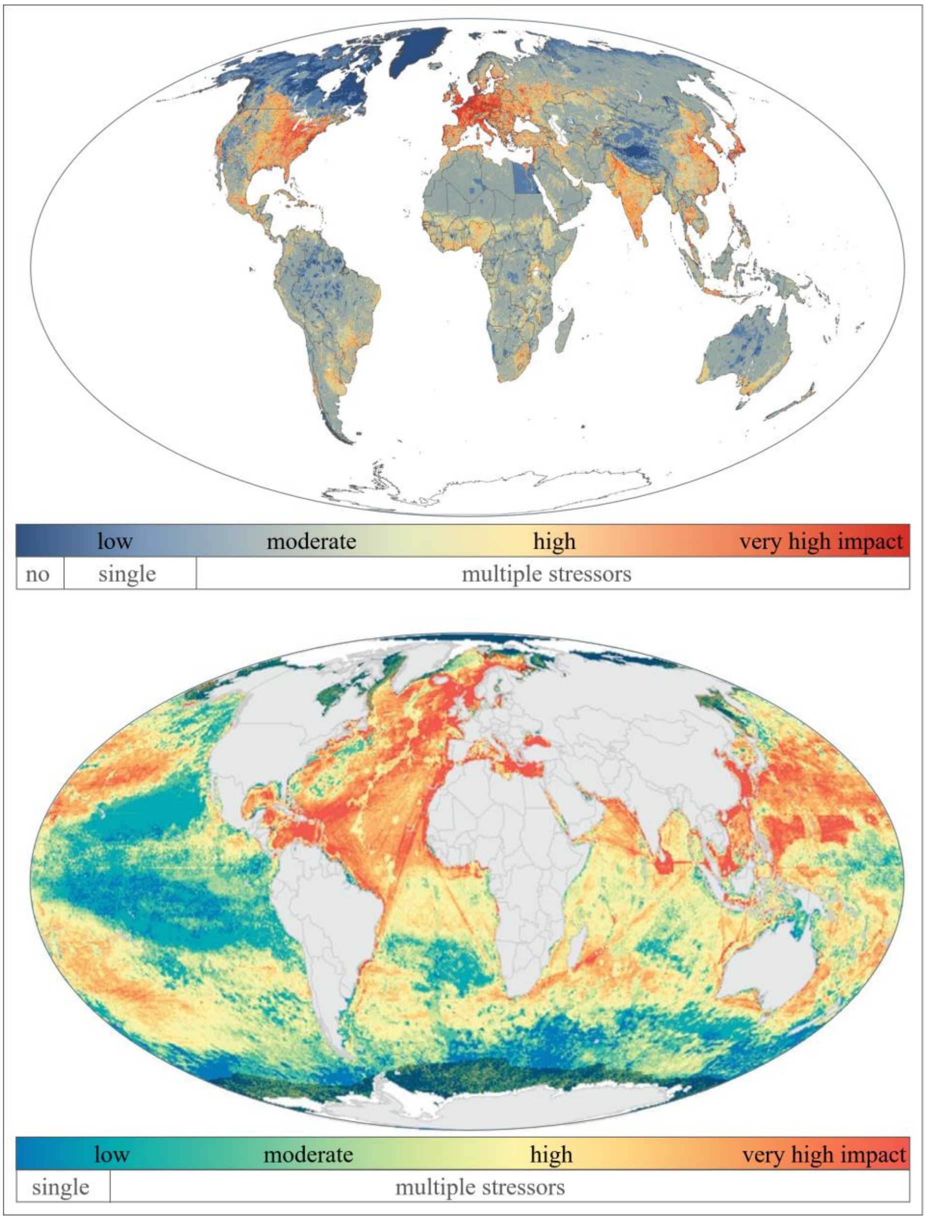


Figure 2: Human impacts across the globe (modified after Halpern et al., 2015 and Kennedy et al., 2018). Global maps show the cumulative human modification affecting terrestrial (top) and marine (bottom) ecosystems. The colours depicting no, single and multiple stressor occurrence are indicated below the coloured bars. The maps show that 84% of land and 98 % of marine ecosystems are affected by multiple stressors.

Stressor interactions: A challenge for environmental management and research

Multiple stressors can interact with each other, causing effects different from their respective individual effects on the environment. When multiple stressors co-occur, they can either act independently or interact with each other. In case of independently acting stressors, their combined effect is identical to the sum of their single effects, called additive stressor effect.

Interacting stressors can mediate each other's effects and lead to complex impacts. The simplest concept to categorise stressor interactions is to groups them into synergistic and antagonistic interactions (Figure 3; Folt et al., 1999). Synergistic stressors reinforce each other's effects, resulting in a combined effect that is higher than the additive effect. For instance, specific pesticides and fine sediment can show synergistic effects when affecting benthic organisms, as pesticides adsorb to the sediment particles, which increases contact exposure of organisms to the pesticide (Sardo and Soares, 2010). Synergistic interactions among stressors are of particular concern, as they have a greater potential to degrade ecosystems compared to antagonistic interactions (Arrigo et al., 2020). Antagonistic stressors dampen each other's effects, resulting in a combined effect that is lower than the additive effect. For instance, increasing water temperatures and sediment loading can both harm corals, but the effect of warming can be weakened by sediment due to inhibited light penetration (Anthony et al., 2007).

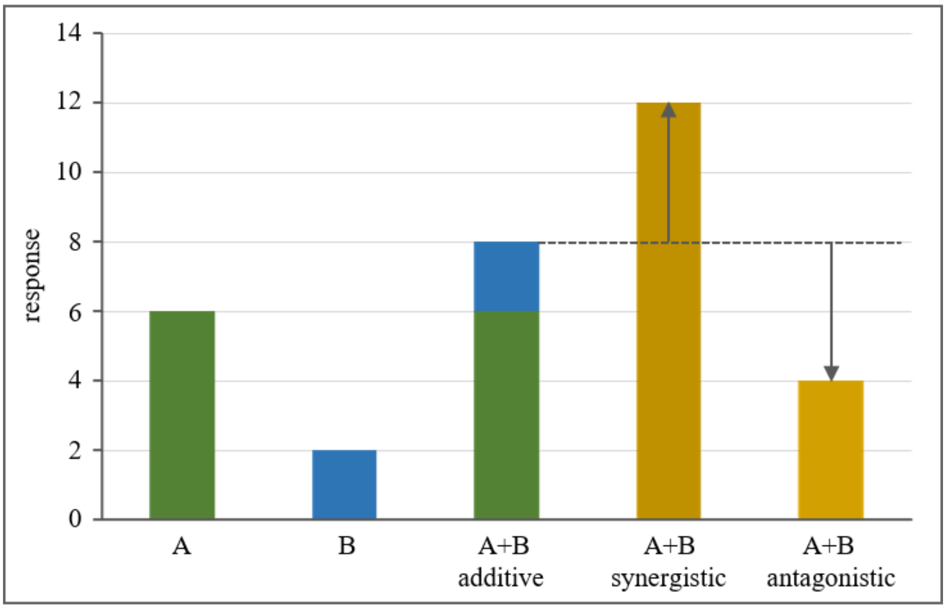


Figure 3: Conceptual approach to interpret multiple stressor interaction types (modified after Piggott et al., 2015b). The individual effects of stressors A and B are 6 and 2, respectively. Their combined effect (A+B) is additive, when it is equal to the sum of individual effects (6+2 = 8). In case of a synergistic interaction, the combined effect is higher than the sum of individual effects (6+2 < 12). In case of an antagonistic interaction, the combined effect is lower than the sum of individual effects (6+2 > 4).

The occurrence of multiple stressor interactions is conceptually well understood and demonstrated in experiments (Figure 3; Schäfer and Piggott, 2018), but remaining challenges are the detection, quantification and management of interacting stressors (Feld et al., 2016). Main challenges for the detection of interactions include that studies differ in their model designs, the used stressor levels and gradients, and sample sizes. All these factors influence study outcomes and thereby, the comparability of results and capability to draw generally valid conclusions from them are limited (Feld et al., 2016; Schäfer and Piggott, 2018; Spears et al., 2021). Moreover, most studies are restricted to pairs of stressors and few response variables, which cannot capture the full complexity of stressor effects on the environment (Gessner and Tlili, 2016). The main challenge for the prediction of interactions is the vast number of factors shaping them. Obvious factors are the identity of stressors and the response variable they affect. In addition, the level of biological organisation plays a role as biotic interactions become more relevant as the level increases (i.e., from organism, population, community to ecosystem level; Galic et al., 2018; Thompson et al., 2018a). Other biotic factors include the trophic level of response organisms (Beauchesne et al., 2021) or the evolutionary adaptation of species subjected to stress (Orr et al., 2021). Also framing conditions such as stressor timing (i.e., stressor duration, simultaneous/sequential occurrence, gradual/abrupt occurrence; Brooks and Crowe, 2019; Pinek et al., 2020; Taherzadeh et al., 2019), the ecosystem type, or the spatial scale (i.e., mesocosms, single basins, multiple basins; Birk et al., 2020) have been shown to influence multiple stressor effects.

In order to successfully counteract the global ecosystem degradation, multiple stressor interactions need to be considered in environmental management. The drastic loss of biodiversity and ecosystem functions indicates that many surface waters have already lost their capacity for self-recovery (Ceballos et al., 2015; IPCC, 2019). To prevent intact surface waters from transitioning into undesirable states and to restore degraded systems, human management is urgently needed (Borja et al., 2020). As a first step in management, it is crucial to identify area-specific stressors and the qualitative condition of the system (Söderström and Kern, 2017). To obtain this information, comprehensive monitoring of preferably all ecosystem elements is essential (Mack et al., 2020). Subsequently, present stressors and potential interactions should be identified. Based on the presence or absence of such interactions, the most important stressor(s) to be mitigated should be identified (Ormerod et al., 2010; Spears et al., 2021). In the case of additive effects, mitigation of one or both stressors should lead to an improved condition of the managed area. In the case of a synergistic stressor interaction, it is most efficient to mitigate only one stressor (the one with the stronger effect), as the reduction of one stressor also lowers the effect of the other stressor. In the case of an antagonistic stressor interaction, it is crucial to mitigate both stressors simultaneously, as the reduction of just one stressor can remove its dampening effect on the other stressor, further deteriorating the condition of the area (Spears et al., 2021).

Despite decades of research, the complex interplay between multiple stressors and the environment is still not fully understood. While many factors influencing multiple stressor effects have been identified and the relationships between some common stressor combinations are well studied (Andersen et al., 2015; Ellis et al., 2017), large gaps in knowledge remain. Many important stressor combinations, such as climate change in combination with local stressors, have not yet been fully assessed (O´Brien et al., 2019). The links between multiple stressors and ecosystem functioning also need to be better understood, as most studies focus on stressor effects on biotic ecosystem elements (e.g. Mayer-Pinto et al., 2018; Schinegger et al., 2016). In general, there are many unresolved questions in stressor research, and therefore the prediction of multiple stressor effects is still a challenging issue (Schäfer and Piggott, 2018).

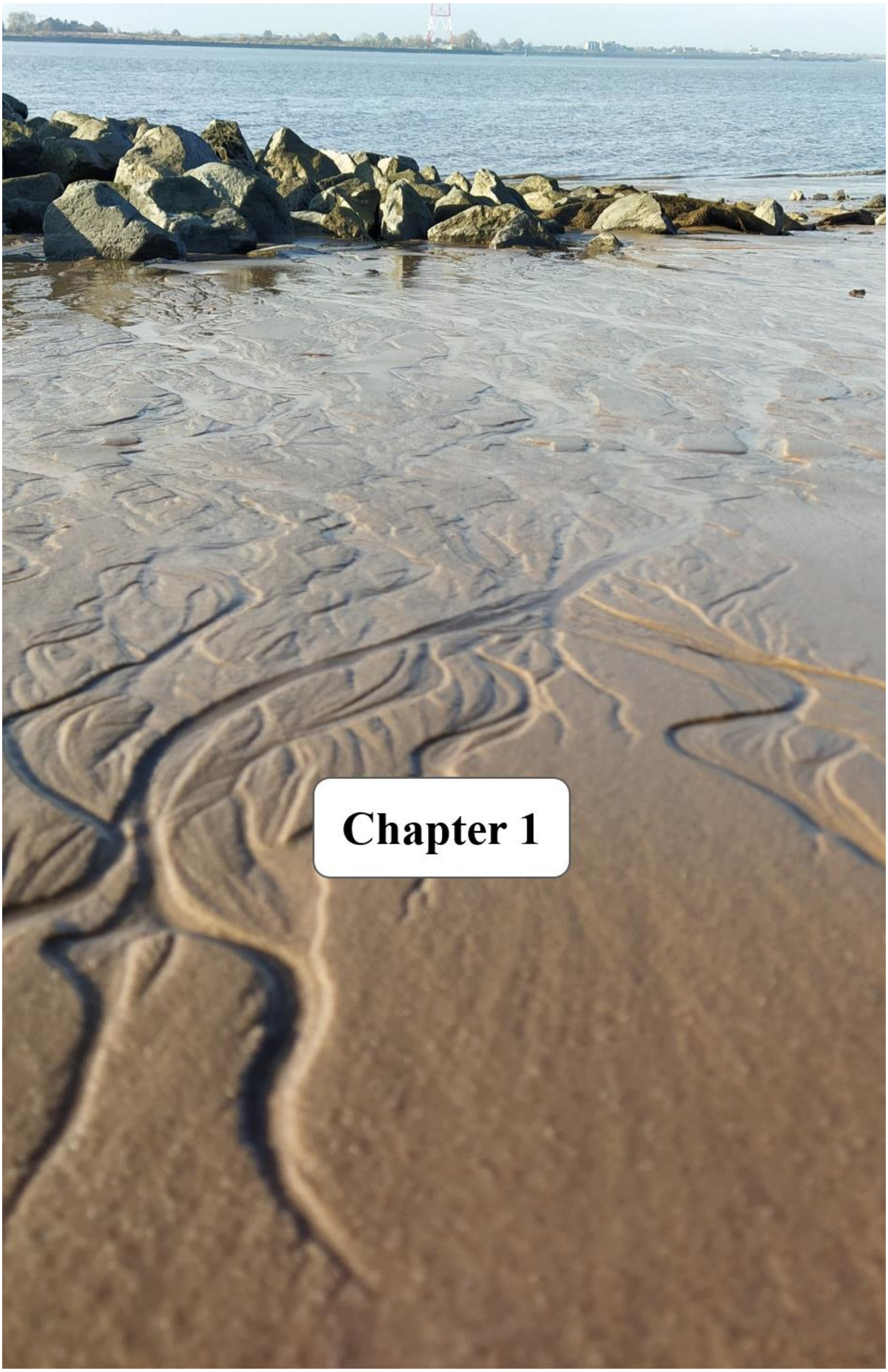
Aim of this thesis

Further research is needed to guide the urgently needed conservation and restoration of global ecosystems. Knowledge on the current condition of surface waters and the stressors that cause this condition are crucial for a successful environmental management. In this work, contemporary challenges in environmental assessment and stressor research are addressed with the aim of supporting the future conservation and restoration of surface waters:

Chapter 1: A comprehensive assessment of the condition of an ecosystem is essential to pave the road for successful environmental conservation and restoration. However, monitoring programmes, such as the one of the Baltic Sea, exhibit gaps that hinder a comprehensive assessment. This chapter explores novel methods with the potential to fill some of these gaps and thereby improve marine monitoring. The methods are described and rated in terms of their costs and applicability to provide environmental managers with an overview of potential methods for advanced future monitoring programmes.

Chapter 2: Understanding the effects of human activities, such as agricultural practices, is important for an effective environmental management. Fine sediment and insecticides are agricultural stressors affecting surface waters around the globe. Their combined effects on biota are well studied, but studies on their effects on ecosystem functions are lacking. In this chapter, the individual and combined effects of fine sediment and a new insecticide, chlorantraniliprole, on organic matter decomposition in streams are studied to gain insights into their specific cause-and-effect relationships.

Chapter 3: Determining the key stressors to be addressed by management measures is crucial for an effective environmental management. These key stressors can be determined by identifying prevalent multiple stressor interactions and the combined effects on the environment. Currently, it is unclear to what extent the sampled data influence the observed stressor effects in addition to ecological processes. To fill this knowledge gap, the role of sample size and stressor gradient length on observed multiple stressor effects is examined by analysing field data from fresh and marine surface waters.



Chapter 1

Chapter 1: A synthesis of marine monitoring methods with the potential to enhance the status assessment of the Baltic Sea

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In the context of this doctoral work, the following manuscript was published in Frontiers in Marine Science as:

Mack, L., Attila, J., Aylagas, E., Beermann, A., Borja, A., Hering, D., Kahlert, M., Leese, F., Lenz, R., Lehtiniemi, M., Liess, A., Lips, U., Mattila, O.-P., Meissner, K., Pyhälahti, T., Setälä, O., Strehse, J. S., Uusitalo, L., Willstrand Wranne, A., Birk, S. (2020). A Synthesis of Marine Monitoring Methods With the Potential to Enhance the Status Assessment of the Baltic Sea. Frontiers in Marine Science 7, 552047. https://doi.org/10.3389/fmars.2020.552047

A multitude of anthropogenic pressures deteriorate the Baltic Sea, resulting in the need to protect and restore its marine ecosystem. For an efficient conservation, comprehensive monitoring and assessment of all ecosystem elements is of fundamental importance. The Baltic Marine Environment Protection Commission HELCOM coordinates conservation measures regulated by several European directives. However, this holistic assessment is hindered by gaps within the current monitoring schemes.

Here, twenty-two novel methods with the potential to fill some of these gaps and improve the monitoring of the Baltic marine environment are examined. We asked key stakeholders to point out methods likely to improve current Baltic Sea monitoring. We then described these methods in a comparable way and evaluated them based on their costs and applicability potential (i.e., possibility to make them operational).

Twelve methods require low to very low costs, while five require moderate and two high costs. Seventeen methods were rated with a high to very high applicability, whereas four methods had moderate and one low applicability for Baltic Sea monitoring. Methods with both low costs and a high applicability include the Manta Trawl, Rocket, Sediment Corer, Argo Float, Artificial Substrates, Citizen Observation, Earth Observation, the HydroFIA®pH system, DNA Metabarcoding and Stable Isotope Analysis.

Introduction

The unique Baltic Sea ecosystem is in critical condition due to strong anthropogenic pressures, therefore, it urgently requires protection and restoration (Andersen et al., 2015; HELCOM, 2017). As one of the largest brackish water bodies worldwide, the Baltic Sea's most distinguishing feature is a pronounced salinity gradient. Marine and freshwater species coexist and interact, creating a unique but sensitive biological community (HELCOM, 2016). The Baltic Sea is shallow with a low water exchange rate with other marine water bodies, which makes it especially vulnerable to human impacts (Szymczychta et al., 2019). Due to effluents draining from nine countries into its basin, main environmental pressures include eutrophication and contamination. In consequence, areas with low oxygen or even anoxic conditions are expanding (Gustafsson et al., 2012; Carstensen et al., 2014). In addition, the ecosystem is highly impaired by marine litter, non-indigenous species, underwater noise, fishing, as well as habitat disturbance and loss (Andersen et al., 2015). Climate change already reduces the extent and duration of ice cover in the Northern parts, as well as increases riverine freshwater inflow (HELCOM, 2018a). The critical condition of the Baltic Sea calls for profound mitigation actions as stipulated by the present environmental legislation.

Several European directives and international conventions address the protection of the Baltic Sea. The most important directives are the Marine Strategy Framework Directive (MSFD; European Commission, 2008) and the Water Framework Directive (WFD; European Commission, 2000). The common aim of these directives is to achieve a good status of the Baltic Sea. "Status" refers to the qualitative condition of the ecosystem, which is classified as good if it deviates only slightly from near-natural conditions (European Commission, 2000, 2008). Further relevant legislations are the Habitats Directive (92/43/EEC), the Birds Directive (2009/147/EC), the Common Fisheries Policy (Regulation EU No 1380/2013) and the regulation on invasive alien species (Regulation EU No 1143/2014). The implementation of the directives is regionally coordinated by the Baltic Marine Environment Protection Commission HELCOM (i.e., "Helsinki Commission"). This intergovernmental organisation has the aim to protect the Baltic Sea, conserve its habitats and biodiversity and ensure sustainable use of its resources (HELCOM, 2018a). Working with an ecosystem-based approach, the understanding of anthropogenic pressures and their impacts on the marine environment and human wellbeing are fundamental (Söderström and Kern, 2017). HELCOM established the Baltic Sea Action Plan (BSAP; Backer et al., 2010) as a joint programme to protect the Baltic Sea and restore the good status of its marine environment by 2021 (HELCOM, 2007).

For an efficient protection and restoration of the Baltic Sea, comprehensive monitoring of all its ecosystem elements is of fundamental importance. Monitoring comprises the acquisition of biological, chemical, physical, hydrological and morphological data of the ecosystem to assess its status (Mack et al., 2019). The assessment of the Baltic Sea's status is following an indicator-based approach (HELCOM, 2018a). Indicators address specific measurable attributes of selected ecosystem elements, allowing to monitor spatial or temporal changes of these elements. Several indicators are defined and grouped into eleven thematic categories addressing characteristic ecosystem features and functions, so-called "descriptors" within the MSFD (Zampoukas et al., 2012).

The current monitoring of the Baltic Sea, however, reveals significant gaps, which conceivably prevent a holistic assessment and impede adequate conservation of the Baltic Sea. Five main gaps were identified by Emmerson et al. (2019) and Kahlert et al. (2020), three of which relate to insufficiently monitored and lacking indicators (i–iii), while two relate to regulation and coordination (iv–v):

i. Insufficient monitoring of existing indicators in space and time, which especially applies to oxygen conditions, phytoplankton, zooplankton, benthic habitats and species, and the monitoring of mobile species.

ii. The lack of indicators that adequately reflect the descriptors of the MSFD, including food webs, sea-floor integrity, contaminants, marine litter, and underwater noise/energy.

iii. Ecosystem elements and drivers of change, which are not monitored so far, including climate change and ecosystem services.

iv. Insufficient regulations on data handling or storage, in particular regarding some descriptors such as biodiversity (i.e., benthic habitats and species), non-indigenous species, bycatch, hazardous substances, and marine litter.

v. The lack in coordination of the monitoring between countries, which especially applies for the descriptors mentioned in the previous gap.

These gaps arise from various circumstances: Traditional methods require relatively high efforts regarding costs and time, and therefore cannot be conducted as often as necessary to meet the data requirements regarding spatio-temporal resolution and coverage. These traditional methods include manual water/sediment sampling from research vessels, sampling and observation by trained divers, manned aircraft surveys or the morphological identification of sampled organisms. An insufficient development of measurable attributes of existing and emerging ecosystem threats leads to a lack in indicators, ecosystem elements or descriptors monitoring. For instance, food webs are currently assessed by proxies like nutritional state, growth rate and size structure of specific organisms, but a straightforward indicator describing the food web length and stability was not developed so far. Technological advances and associated high data volumes evolve faster than data management strategies, resulting in the lack of central data management systems. Furthermore, a lack of coordination results from differing national and international legislation (Birk et al., 2012).

To fill these gaps, novel monitoring methods can be implemented. In this investigation, we define as “novel” those methods which are not yet in general use or applied to some Baltic Sea regions, as well as methods which have been developed recently. Several novel methods have been developed to facilitate autonomous acquisition of real-time data, increased spatial and temporal data resolution and the assessment of novel indicators, ecosystem elements or descriptors (Danovaro et al., 2016). A good example for the integration of novel methods is the use of the HELCOM chlorophyll-a indicator for the assessment of eutrophication status (HELCOM, 2018b). The indicator combines data from traditional research vessel-based sampling with Earth Observation and FerryBox systems. It was used in the latest status report to assess the eutrophication effects in the off-shore areas of the Baltic Sea (HELCOM, 2018c). Another example is Earth Observation that provides near real-time information on water quality parameters and surface water temperature. Some Baltic countries, e.g. Finland, already utilise these observations on daily level in monitoring programmes and also as complementary material for WFD reporting (Attila et al., 2018). The efforts by several EU countries for advancing the use of Earth Observation for WFD were summarised in a recent White Paper (Papathanaopoulou et al., 2019). However, to make a monitoring method operational, it should be operationally feasible and at a reasonable cost. Yet, sometimes a new method is not always transferable to routine monitoring contexts due to several constraints such as the inaccessibility to the equipment required for sample collection or preservation. Also, the study of the cost-efficiency of monitoring methods is not very usual (Abramic et al., 2014; Bellanger and Levrel, 2017; Aylagas et al., 2018).

In this context, as part of the BONUS FUMARI project (”Future marine assessment and monitoring of the Baltic”; 2018-2020; https://www.syke.fi/BONUS_FUMARI/), which aims to propose a renewed monitoring system of the Baltic Sea marine environment, we reviewed novel methods with the potential to enhance the HELCOM monitoring of the Baltic. We identified methods suited to fill the monitoring gaps (i) – (iii) listed above (as these gap types are directly related to monitoring practices) and rated their costs and applicability potential (i.e., possibility to make them operational) for the Baltic Sea monitoring. The methods offer an improvement in comparison to the traditional methods and might replace or supplement them in a future monitoring system. In the methodology section, we describe the procedure of method identification and rating. In the results and discussion section, each method is classified, shortly described and rated based on its costs and applicability for the marine monitoring. In conclusion, the ability of methods to fill the main gaps is assessed. The results from this research can be applied to other regional seas worldwide.

Materials and methods

Identification of novel methods

To increase the overall impact, our BONUS FUMARI project follows an end-user-centric approach, integrating suggestions from key stakeholders in environmental management (i.e., academic researchers, monitoring coordinators or field biologists). Novel monitoring methods were identified by scanning scientific projects, publications and conducting stakeholder surveys. This comprehensive collation was then examined to retain only methods, which fulfilled the following five criteria: 1) technology readiness level of seven or higher (European Commission, 2014); 2) comprehensive and standalone techniques (excluding sensors or analysers depending on a deployment system); 3) filling a gap in the current monitoring; 4) “novel” and not in general use; and 5) evaluated as cost-efficient in terms of their cost-benefit-ratio. For this compilation we considered scientific research projects [all BONUS projects since 2010 and the finished and ongoing projects listed in the Technical guidance on monitoring for the MSFD (JRC, 2014)], scientific publications (JRC, 2013; Danovaro et al., 2016; Filipe et al., 2019; Lehikoinen et al., 2019), and stakeholder suggestions. Stakeholders working in the field of environmental management and research in the Baltic countries were asked for shortcomings in the Baltic monitoring to assess the good status of the region regarding the directives MSFD, WFD and BSAP, and for novel methods with the potential to fill these. This was done during two enquiries from October 2018 to May 2019 and we received 42 responses (Appendix 1A).

Description and rating of novel methods

All methods were described and evaluated in a standardised way. We specified their potential to add to a reference framework/programme (MSFD, BSAP and WFD), the monitored quality elements and the traditional methods potentially replaced or supplemented by the novel method.

Furthermore, we described the general operating principle of the method and its application for the routine monitoring.

For evaluating the costs of a method, we differentiated between investment costs and monitoring costs. For evaluating a methods' applicability, we assessed reliability, environmental impact, added value, limitations and required expertise. These criteria were developed including approaches of JRC (2013), Nygård et al. (2016), and Hering et al. (2018). The novel methods were rated by assigning scores ranging from "--", i.e., "very low" to "++", i.e., "very high" to each of the evaluation criteria. It should be notes that different evaluation criteria were rated using a different range of these scores, depending on their feasibility (Table 1). For instance, the criterion "added value" could not score negative and "limitations" could not score positive. Overall cost and applicability rating was done by averaging the grades for the single ratings. Monitoring costs were given a higher weight (2:1) than investment costs to emphasise annual running costs over one-time investments. The final rating of the methods can be found in Table 2.

Investment costs were defined as one-time investments for equipment and personnel training. This comprised the costs for the monitoring device or deployment system, including necessary equipment like standard sensors or sampling devices, and the expenses for personnel training (personnel costs were set to 70 € per hour). For citizen observations this included costs to start web services and management activities. For remote sensing methods, we also included the costs for the development of a data management system. Since the investment costs of most methods depended on several parameters, like number and type of included sensors or the place of application, the costs were mainly estimated based on expert knowledge. Cost assignments followed criteria put forth by the Joint Research Centre (JRC, 2013; Table 1).

Monitoring costs were defined as the running costs of monitoring, i.e., costs incurring after the initial set up. They include consumables, personnel working time, maintenance and, in case of citizen observations and remote sensing methods, data handling. Due to data availability, we first collected monitoring costs in various dimensions, ranging from the costs for a single monitoring campaign to the costs for an annual monitoring of a specific transect/area. Based on these, we estimated the annual monitoring costs for the whole Baltic Sea. These depend on the monitoring objective and therefore, the costs are given under specific assumptions (Appendix 1B). Since most of the methods collect data on multiple quality elements at the same time, we calculated the monitoring costs for a single quality element to allow for cost comparisons between methods. For research vessel-dependent methods, we did not include ship costs like fuel or rent.

In our cost analysis, we did not take the possibility of sharing facilities/instruments or cooperating the execution among institutes into account. This can potentially reduce investment and monitoring costs and efforts by maximising the use of resources. Furthermore, analysis protocols can be homogenised, which reduces the need for interlaboratory calibrations (JRC, 2013).

Reliability was assessed based on the failure safety of the method itself and the precision of acquired data in comparison to the traditional methods (Appendix 1B). Reliability of the methods was “high,” when resulting data had an improved reliability. This also applied, when the precision of acquired data is comparable to data acquired with the traditional method, but due to the greater amount of data, the reliability can be regarded higher. A high default rate and therefore low reliability of the method was assessed as “low.”

Environmental impact of the methods was rated ranging from “beneficial” to “moderate.” Hereby, “beneficial” means a positive impact on the environment, e.g., by removing litter from the beaches. A “low” impact is caused by small organismic sample sizes and damages to the physical habitats, e.g., by anchoring devices to the sea floor. Methods causing damages of the physical habitats or/and lethal sample treatment of a relatively big sample size were rated with a “moderate” impact.

Added value describes the type of novelty, which a method adds to the routine monitoring. It was rated as “high” for methods with higher spatial and/or temporal data resolution, e.g., due to their autonomous measurement, and therefore, filling gap (i). A “very high” added value was assigned to methods including the monitoring of novel quality elements, ecosystem elements or descriptors and therefore, filling gaps (ii) and (iii). Furthermore, methods with an added social or environmental value, e.g., by rising the environmental awareness of the population, were rated as “very high."

Limitations describe disadvantages and shortcomings of a method. We rated limitations as “none,” i.e., having no or an easily manageable effect on method applicability, or “moderate,” i.e., causing slight restrictions to method applicability. Methods that scored “high,” i.e., limited method applicability, require further research to improve method applicability.

Required expertise describes the level of expertise needed to conduct field sampling/surveying or sample analysis. It was rated following categories proposed by JRC (2013): “Low” indicates the need for trained personnel without specific professional education, “moderate” requires trained personnel with specific professional education and “high” requires special skills.

Table 1: Evaluation criteria and rating scores of the novel methods. In the first column, the quantitative rating is shown, assigned to scores from ‘+ +’ as best to ‘- -’ as worst rating.

Rating	**Investment Costs (in k€)**	**Monitoring Costs (in k€)**	**Reliability**	**Environmental impact**	**Added value**	**Limitations**	**Required expertise**
+ +	≤ 1	≤ 1	–	–	Very high	–	–
+	1– 10	1 – 50	High	Beneficial	High	–	Low
0	10 – 50	50 – 100	–	–	–	None	Moderate
-	50 – 100	100 – 150	Low	Low	–	Moderate	High
- -	> 100	> 150	–	Moderate	–	High	–

Results and discussion

Method classification

Monitoring typically comprises three steps: 1) field sampling/surveying in which *in situ* or remote samples/data are gathered; 2) sample analysis, which refers to the treatment of gathered samples/data to extract quantitative or qualitative information; and 3) data analysis, the treatment of quantitative and qualitative information to interpret the resulting data, e.g., by calculating metrics or applying class boundaries. We classify methods for field sampling/surveying as either 1a) *in situ* research vessel-dependent (sampling within the water, the whole deployment time is depending on the operation of a (research) vessel); 1b) *in situ* research vessel-independent (operation is independent of a research vessel, though devices may be deployed and recovered using one); 1c) citizen observation; or 1d) remote sensing. Methods for sample analysis comprise 2a) field analysis; and 2b) laboratory analysis. Methods for data analysis were not included in this work.

Method descriptions

We identified twenty-two methods to be reviewed, half of the methods were mentioned in both the stakeholder survey and literature, half by stakeholders only. In the following, we give a short description of each novel monitoring method. Furthermore, we list the MSFD descriptor(s), as they can provide information on and highlight the gaps the respective methods can fill (Table 3). Comprehensive method descriptions can be found in the Appendix 1B or on the BONUS FUMARI methods database (http://freshwaterplatform.eu/fumari/).

1a) Methods for field sampling/surveying - In situ, research vessel-dependent:

The **Moving Vessel Profiler (MVP)** is a free-falling "fish," which generates near-vertical high-resolution profiles of the water column. The fish is attached to a winch on board of a research vessel and operated while the vessel is moving (Figure 4a; Furlong et al., 2006). In marine monitoring the MVP can be operated between stations, and therefore increase the number of profiles generated during a monitoring cruise. These additional data can be used for a more comprehensive assessment of eutrophication and hydrographical conditions due to the enhanced spatio-temporal resolution and coverage of monitoring data.

The **Remotely Operated Towed Vehicle (ROTV)** is a towed profiler, which is deployed from a research vessel and can be operated in three dimensions in the water column (Figure 4b; Floeter et al., 2017). In marine monitoring ROTVs enhance the spatio-temporal resolution and coverage of monitoring data used to assess eutrophication and hydrographical conditions. Furthermore, it can be used to obtain high-resolution data or additional information in a specific area of interest, when steered manually. For instance, ROTVs can be operated for the detection and identification of warfare relicts dumped in the sea, and sampling of contaminated water and sediment (Beldowski et al., 2018).

A **Manta Trawl** is a net-based sampling device to collect marine surface microlitter bigger than 300 mm (Figure 4c). While being dragged on the water surface, it collects water with its opening. The water is filtered through a fine net and the litter is stored in the cod end, a removable collecting bag (Setälä et al., 2016; Tamminga et al., 2018). In marine monitoring the Manta Trawl can be deployed between monitoring stations to routinely collect data on microlitter in surface waters. In particular, the possibility for a standardised monitoring of microplastics, which is not included in the current monitoring directives (HELCOM, 2018a; Kahlert et al., 2020), gives the Manta a high relevance for the Baltic Sea monitoring.

The **Rocket** is an example for an encapsulated through-flow filtration device used to sample waterborne microplastics in the upper water layers (Figure 4d; Lenz and Labrenz, 2018). The concept is based on suction of water through fine stainless-steel cartridge filters to retain any suspended particulate matter larger than the applied pore size (i.e., 10 mm). The mobile design allows for application at field sites, as well as application aboard a vessel to take open water samples. The Rocket is a valuable tool for addressing monitoring needs for pollution of smaller microplastic particles (i.e., < 300 mm) and to cover locations where the application of trawling systems is impractical or impossible.

A **Sediment Corer** like the GEMAX is a gravity corer to sample soft sediments (Figure 4e). It is deployed from a research vessel and when released, the corer falls down the water column and vertically cuts into the sediment (Charrieau et al., 2018). A closing mechanism automatically locks the sediment in the system when recovering the corer. The GEMAX is an efficient sampler for monitoring purposes as it takes two sediment cores at the same time doubling the sampled volume of the sediment compared to the more common single-core corers. In marine monitoring it can be deployed at sampling stations with soft sediment to sample microlitter deposited in sediments. The Corer is especially valuable since it offers a standardised method for the monitoring of microplastics.

1b) Methods for field sampling/surveying - In situ, research vessel-independent:
The **Argo Float** is a free-floating platform, which generates vertical profiles of the water column (Figure 4f). Since it floats freely, its horizontal range and path is defined by the currents. It frequently surfaces by changing buoyancy due to an oil filled bladder. In general, an Argo Float profiles at 10-day intervals, but intervals can also be programmed to generate, for instance, multiple profiles a day (Roiha et al., 2018; Siiriä et al., 2019). In marine monitoring Argo Floats can be deployed to autonomously obtain high-resolution vertical profiles of the water column to assess eutrophication and hydrographical conditions.

The **Glider** is an autonomous underwater vehicle used to generate horizontal profiles of the physico-chemical and biological state variables along its route being defined by an operator (Figure 4g). It can move down to 1,500 m by changing buoyancy due to an oil-filled bladder (Liblik et al., 2016). Since Gliders can autonomously move underwater, they can also be operated in ice-covered areas and under harsh conditions (Brito et al., 2014; Meyer et al., 2018). Obtained water quality data can be used for a more comprehensive assessment of eutrophication and hydrographical conditions and Gliders can also be used for the detection of warfare relicts dumped in the sea.

A **FerryBox** is an automatic flow-through system for the continuous measurement of water parameters (Figure 4h). This system is specifically developed for the permanent operation on non-research vessels like ferries, which regularly ship their transit routes (Petersen, 2014; EuroGOOS, 2017). Regarding the marine monitoring, the FerryBox enables the acquisition of long-term time series on a constant route and, therefore, the monitoring of temporal changes in food webs, eutrophication and hydrographical conditions like ocean acidification (Lips and Lips, 2017; Schneider and Müller, 2018).

Profiling Buoys for the automatic measurement of water quality profiles are moored platforms, floating on the water surface (Figure 4i). For profiling, a multi-parameter probe is lowered in the water column to conduct measurements either continuously or stopping at specific heights. Profiling frequency, intermediate profiling steps and the maximal depth can be programmed (Liu et al., 2019; Venkatesan et al., 2019). Due to the generation of frequent profiles at a given station, the changes in water conditions within the day can be recorded over long periods and, therefore, Profiling Buoys can be used to assess biodiversity, eutrophication and hydrographical conditions (Lips et al., 2011).

Bottom-mounted Profilers for the automatic measurement of water quality profiles are platforms moored to the sea bed (Figure 4k). For profiling, either the whole platform or the multi-parameter probe is rising. With profiling frequencies of 3–8 h, the changes in biological and physico-chemical water conditions within the day can be recorded over long periods. Thus, bottom-mounted profilers can be used for an enhanced assessment of biodiversity, eutrophication and hydrographical conditions (Prien and Schulz-Bull, 2016; Stoicescu et al., 2019).

For **Active Biomonitoring with Blue Mussels**, the bivalves are used as sentinel species in the monitoring of bioavailable pollutants (Figure 4l). Mussels enable monitoring the pollution of a specific location, as they accumulate environmental chemicals in their tissues. Therefore, mussels without former pollution are translocated to a specific area of interest (Schöne and Krause, 2016; Strehse et al., 2017). For marine monitoring purposes, this method enables a more comprehensive monitoring of eutrophication and contamination due to the collection of time-weighted average concentrations of bioavailable pollutants, including nutrient and carbon isotopes (Briant et al., 2018) and dumped munitions (Strehse et al., 2017; Appel et al., 2018).

Passive Samplers like the **Chemcatcher®** and **Polar Organic Chemical Integrative Sampler (POCIS)** are collecting contaminants based on molecular diffusion and sorption to a binding agent (Figure 4m). They are deployed at a specific location and accumulate the contaminants in the surrounding environment over time (Vrana et al., 2005). The Chemcatcher® collects in-/organic substances of polar or non-polar nature (Charriau et al., 2016), while the POCIS is selective for polar organic chemicals (Harman et al., 2012). Passive Samplers can be used to enhance the monitoring of contaminants, including dumped munitions, due to the collection of time-weighted water concentrations of pollutants (Belden et al., 2015; Lotufo et al., 2019).

Artificial Substrates are sampling devices mimicking complex habitats to collect biological communities over years (Figure 4n). The **Autonomous Reef Monitoring Structure (ARMS)** mimics the complex structure of hard benthic habitats like rocks or coral reefs, while the **Artificial Substrate Unit (ASU)** resembles soft corals or sponges (DEVOTES, 2013; Cahill et al., 2018). The analysis for community characterisation can be coupled to molecular techniques such as DNA metabarcoding. The applicability of both ARMS and ASUS in marine monitoring is highly valuable, since they enable a standardised sampling of the hard-bottom benthic communities across countries and therefore comparable monitoring data (DEVOTES, 2013).

1c) Methods for field sampling/surveying - Citizen Observations:

In **Citizen Observations**, voluntary observations are made by non-professional observers (Figure 4o). Coordinated by researchers, engaged citizens are integrated into environmental science, including the observation of various environmental phenomena, which are transmitted to specific platforms using the smartphone or computer. Several programmes have been established at local scale (Palacin-Silva et al., 2016), including the monitoring of Secchi depth (https://www.havaintolahetti/), non-indigenous species (https://www.invasive-alien-species-finland), phytoplankton (https://Leväbarometri), and several local, national and European wide campaigns for the prevention of marine litter near the shore and in the coastal waters (https://www.siistibiitsi.fi/ and https://www.eea.europa.eu/marine-litterwatch). Besides added value for the acquisition of monitoring data, the integration of citizens into the environmental monitoring can strongly increase the societal environmental awareness.

1d) Methods for field sampling/surveying - Remote Sensing:

Unmanned Aerial Vehicles (UAVs), commonly known as “drones,” are measurement platforms collecting data while flying over the area of interest (Figure 4p). For operations in marine and coastal environments, different types of UAVs are used with varying flight duration and modes of operation (autonomous or manual; Colefax et al., 2017; Setlak and Kowalik, 2019). In marine monitoring UAVs can be used to increase the spatio-temporal resolution and coverage of monitoring data for parameters used to assess biodiversity, eutrophication, commercial fish and shellfish, hydrological conditions, contaminants and marine litter.

In a monitoring context, **Earth Observation** means the use of satellites for the remote sensing of biological and physicochemical properties of the upper water layer (Figure 4q). Europe-wide satellite missions are performed by the European and North American Space Agencies, which are offering free access to their satellite images (Harvey et al., 2015; Attila et al., 2018). In marine monitoring Earth Observation profoundly increases the temporal resolution and spatial coverage of data on water quality parameters, giving a more comprehensive picture on environmental conditions related to biodiversity, eutrophication and hydrographical conditions (Anttila et al., 2018; Attila et al., 2018).

In **Remote Electronic Monitoring (REM)**, video and sensor technology are combined to provide a comprehensive overview on the fishing activity and catch handling on fishing trawlers (Figure 4r; WWF, 2015, 2017). The analysis of the REM data can be coupled to computer-based identification and quantification of organisms. In marine monitoring REM can be used to enhance data acquisition on all activities of fishing trawlers, enabling a more comprehensive monitoring of biodiversity, commercial fish and shellfish, and food webs (Kindt-Larsen et al., 2012). Furthermore, fishing practices might become more sustainable due to the continuous surveillance on board of the trawlers (WWF, 2017).

2a) Methods for Sample Analysis - Field Analysis:

The CONTROS **HydroFIA®pH** system is autonomously conducting flow injection analysis to determine the pH of water (Müller et al., 2018; Figure 4s). The system was developed for the continuous long-term measurement of pH of the surface water and is therefore suitable for both the operation on research vessels and non-research vessels like ferries (Aßmann et al., 2011; Müller et al., 2018). In marine monitoring the system can be deployed independently or in combination with a FerryBox to obtain pH long time series for locations along a set route. This enables spatial and temporal monitoring of ocean acidification (Müller et al., 2018).

Imaging Flow Cytometry (IFC) platforms are used to analyse phytoplankton communities (Figure 4t) by combining traditional flow cytometry and automated imaging to analyse large sample sizes with high speed (Karlson et al., 2016). The different instruments include laboratory applications, instruments included into a FerryBox, or autonomous *in situ* platforms at fixed stations. Machine Learning algorithms can be used for analysing the acquired images (González et al., 2019). IFC platforms enhance the assessment of biodiversity, nonindigenous species, food webs and eutrophication, and can also be important components early-warning-systems for harmful algal blooms (Anderson et al., 2019).

2b) Methods for Sample Analysis - Laboratory Analysis:

(e)DNA Metabarcoding is a molecular-based methodology that allows the simultaneous identification of several species within a sample using high-throughput sequencing technologies (Figure 4u). To attain species lists of complete biological communities simultaneously, DNA metabarcoding can be applied. In addition, metabarcoding can be used to detect species inhabiting a certain habitat using environmental DNA (eDNA) extracted from water or sediments (Pawlowski et al., 2018; Zhang et al., 2020). In marine monitoring DNA metabarcoding has the potential to improve the monitoring of biodiversity, non-indigenous species, commercial fish and shellfish and eutrophication indicators (Jeunen et al., 2019).

Stable Isotope Analysis (SIA) can be performed to derive food web structures and energy pathways within communities (Michener and Kaufman, 2007; Figure 4v). The stepwise enrichment of 15N compared to 14N with increasing trophic level enables the estimation of the food chain lengths (of number of trophic transfer steps within the food web). The stability of the food web can be monitored by comparing the 13C/12C isotopic ratios of predatory fish among years (Michener and Kaufman, 2007; Jardine et al., 2017). So far, parameters to assess food webs are still under development (Rombouts et al., 2013) and the monitoring of food webs can be improved using the proposed novel indicator “food web length and stability” and assessed using SIA. Furthermore, the assessment of eutrophication can be supported by identifying anthropogenic nitrogen and carbon inputs (Briant et al., 2018; Ziółkowska et al., 2018).

For the **computer-based identification and quantification of organisms**, computer systems are trained to autonomously identify and count sampled organisms using algorithms (“Machine Learning”; Figure 4w; Kelleher et al., 2015). For the identification of larger organisms (e.g., fish), an algorithm can be trained based on an image recognition system. After successful training, the algorithm can be used to identify and quantify caught species/bycatch (Williams et al., 2012) or evaluate indicator-related metrics (Uusitalo et al., 2016). For marine monitoring, computer-based identification of organisms offers an automated method to increase the speed and accuracy of data acquisition (Osterloff et al., 2019).

Figure 4: Novel monitoring methods with the potential to enhance the marine monitoring. a) Moving Vessel Profiler © AML Oceanographic; b) Remotely Operated Towed Vehicle © MacArtney; c) Manta Trawl © Maiju Lehtiniemi, SYKE; d) Rocket © Robin Lenz, IOW; e) GEMAX Corer © Maiju Lehtiniemi, SYKE; f) Argo Float © www.argo.uscd.edu; g) Glider © Kimmo Tikka, FMI; h) FerryBox © modified after 4HJena and SLU; i) Profiling Buoy © Tiina Sojakka, UTU; k) Bottom-mounted Profiler © Siim Juuse; l) Active Biomonitoring using Blue Mussels © Jana Ulrich, CAU; m) POCIS © Heidi Ahkola, SYKE; n) ARMS (left) and ASU (right) © AZTI Tecnalia; o) Citizen Observations © Vanessa Riki, SYKE; p) Unmanned Aerial Vehicle © Jan Eric Bruun, SYKE; q) Earth Observation © ESA Copernicus Sentinel Data

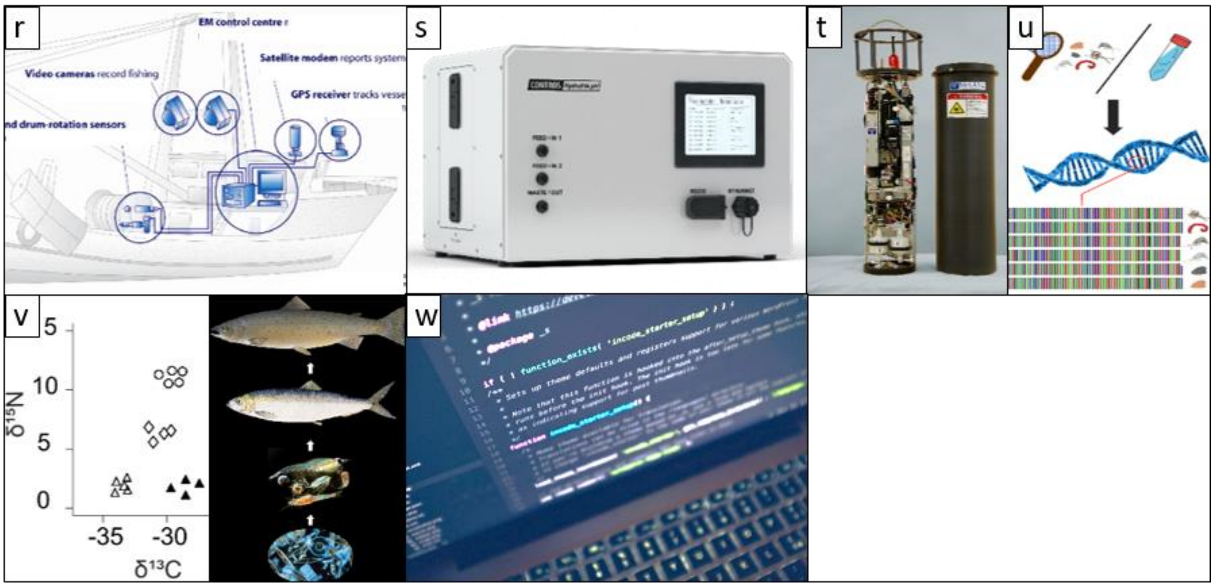

Figure 4 (cont.): Novel monitoring methods with the potential to enhance the marine monitoring. r) Remote Electronic Monitoring © Archipelago Marine Research; s) HydroFIA®pH © Kongsberg Maritime Contros; t) Imaging Flow CytoBot © McLane Research Laboratories; u) DNA metabarcoding © Leoni Mack, UDE; v) Stable Isotope Analysis © Leoni Mack, UDE; w) Computer-based identification of organisms © Luca Bravo

Rating of methods

Costs of novel monitoring methods

Two thirds of the analysed novel methods require very low to low overall costs, while four methods require moderate and two high overall costs (Table 2). There is no clear pattern between the different categories of monitoring methods. Citizen Observations is the only method with very low overall costs. This is due to the voluntary field sampling of citizens (data acquisition at no cost) with only the web services and management activities requiring personnel time. Gliders and REM have high overall costs. For Gliders, this is due to very high investment costs (100,000 € for purchasing a Glider). Regarding REM, the installation and maintenance of the system on a single trawler is of low costs with 17,000 €. However, to create equal economic conditions between the trawlers, according to WWF (2017) the system needs to be installed on at least all big sized (> 12 m) trawlers of the Baltic Sea. In the whole area, there are 558 registered big sized trawlers (ICES, 2018), resulting in the high overall costs of REM. It should be noted that the sharing of facilities and instruments offers the possibility to reduce investment and monitoring costs. For instance, Gliders are such instruments, and their investment costs could be reduced by splitting them between institutions.

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There is a broad range of costs across the different methods, which also reflects the diversity of methods addressed. Investment costs of 80,000 € on average are required for the initial set up of a novel method. With about 500 €, the Artificial Substrates require the lowest investments, while the ROTV and MVP require about 350,000 and 400,000 €, respectively. This wide range in costs is also reflected in the monitoring costs: Based on the assumptions made (Appendix 1B), the use of a method for the annual monitoring of the whole Baltic Sea requires on average 93,000 €. Using computer-based identification and Citizen Observations have the lowest costs with 300 and 600 € annually, while the use of Gliders and REM require 85,000 and 1,116,000 €, respectively.

Monitoring costs could not be assessed for three methods. For the MVP, the extrapolation of monitoring costs on Baltic-wide coverage could not be estimated. These costs depend on the total number of installed devices on research vessels, as well as the frequency, at which these are deployed. Other methods can be used for various different applications, including the ROTV and UAV. The ROTV can be deployed automatically to receive profiles, or in manual mode, steering it in three dimensions to monitor a small area at a very high resolution. Especially the manual deployment is very specific in its time, area and frequency. In case of UAVs, the monitoring costs heavily depend on the kind of vehicle (fixed-wing or multi-rotor and size of vehicle), the place of deployment (open sea or coastal), the mode of operation (manual or automatic operation) and monitoring objective (long-term deployment of a specific area or occasional snapshots). All these variables cannot be estimated for all countries of the Baltic Sea.

We could compare the monitoring costs using novel methods to the costs using traditional methods only for DNA metabarcoding and REM. Regarding metabarcoding, the costs for the molecular identification per sample are decreasing with an increasing number of samples, while the morphological approach has a fixed price (and waiting time) per sample (Aylagas et al., 2018). Metabarcoding is thus most cost-efficient when a sufficiently high number of samples is analysed, which is met under the assumptions made (Appendix 1B). This also applies when identifying hard-bottom benthic macroinvertebrate communities sampled by ARMS and ASU in the Baltic Sea. Assuming that three replicates of ARMSs and ASUs are deployed per southern sub-basin (11 stations, since in the southern Baltic mainly soft bottom is prevalent) and three replicates per northern WFD water body (32 stations, since in the northern Baltic hard substrate is more common), this adds up to 43 sites and 260 samples. Based on these assumptions, the annual costs for DNA metabarcoding sum up to about 45,000 €. The traditional morphological identification of ARMS and ASU samples costs 455 € per sample, summing up to about 118,000 €. See Aylagas et al. (2018) for a detailed calculation of the costs for metabarcoding and traditional identifications. If eDNA metabarcoding is applied rather than bulk sample analysis, the whole costs decrease further for eDNA because no sorting is needed but DNA is directly extracted from water or sediment. Furthermore, WWF (2017) made comprehensive analyses on different methods for the monitoring of fisheries and concluded that REM is the most cost-efficient method for this purpose.

For the remaining methods, the costs could not be compared to the traditional methods due to various reasons. For traditional methods that are research-vessel based, monitoring costs could not be determined due to the case-specific costs (e.g., number of sampled stations per cruise; length of cruise; personnel on board). Methods like ROTV, MVP, FerryBox, or Earth Observation collect data on multiple parameters and, therefore, could replace or supplement more than one traditional method. Methods like the Manta Trawl, Rocket, Sediment Corer or SIA collect data on novel quality parameters and, therefore, there is no traditional method to be compared to. This is a common problem when assessing cost efficiency of novel methods (Hyvärinen et al., 2021).

Applicability of novel monitoring methods

Twelve methods are rated with very high and five with high applicability, while four methods are rated as moderate and one as low. The applicability rating showed no patterns among monitoring method categories. The methods with very high and high ratings can be recommended for Baltic routine monitoring. Here, single disadvantages in monitoring methods (e.g., a moderate environmental impact or a high expertise required) are overruled by their specific advantages. The Glider, Active Biomonitoring using Blue Mussels, IFC platforms and computer-based identification of organisms gained an overall “moderate” rating, while UAVs gained an overall “low” rating. These methods can be recommended for specific monitoring tasks and/or need further technical development to achieve a higher applicability.

Most of the methods are rated with respect to a specific monitoring objective or application and therefore, the rating is dependent on specific assumptions. Regarding DNA metabarcoding, the rating refers to the analysis of the species composition in bulk samples. The approach is limited as it does not allow storage of samples and does not reveal absolute abundances of the organisms (Leese et al., 2018). However, some studies have demonstrated relationships between the number of reads and species abundance when calculating DNA-based indices (Aylagas et al., 2018; Ushio et al., 2018; Schenk et al., 2019). In general, molecular methods have the potential to enhance the monitoring of several MSFD descriptors and thus are a promising approach for future marine monitoring (Danovaro et al., 2016; Valentini et al., 2016; Weigand et al., 2019; Filipe et al., 2019).

In case of Gliders, the technology seems not advanced enough for a cost-efficient monitoring so far, but due to the high potential of this method, research is conducted to further enhance its applicability (e.g., Meyer, 2016; Alenius et al., 2017). The method needs further improvement, since about 41% of the missions in shallow water currently fail due to platform loss or technical defects (like leakages or failures in the power supply or buoyancy; Brito et al., 2014). But due to their high spatiotemporal resolution and coverage in data acquisition, the Gliders offer a high potential to improve the marine monitoring, which cannot be achieved by conventional underwater vehicles or research vessel-based methods (Brito et al., 2014). Furthermore, Gliders can be used to detect warfare relicts dumped in the sea, increasing the importance of further technical development.

In case of the UAVs, limitations such as relatively short operating times and civil aviation restrictions cause the low applicability rating. But UAVs have a high potential to sample small areas with high spatio-temporal resolution, also in remote areas. The sampling is less time consuming and less infrastructure is needed compared to research vessel-based sampling. Currently, there is a lot of research to improve the technology of the platforms and available sensors and in turn improve the cost-efficiency and applicability (e.g., Colefax et al., 2017). In conclusion, all the methods included in this analysis were identified as promising novel monitoring methods by stakeholders and therefore each method may have specific advantages for use in a novel monitoring system.

Table 2: Overall rating of the costs and applicability of novel methods. Overall costs are divided into investment and monitoring costs, whereas applicability comprises of reliability, environmental impact, added value, limitations and required expertise. '+ +' indicates a very high, '+' a high, '0' a moderate, '-' a low and '- -' a very low rating.

Step in monitoring	Classification	Method	Investment costs	Monitoring costs	Overall costs	Reliability	Environmental impact	Added value	Limitations	Required expertise	Applicability
Field sampling/ surveying	*In-situ*, research vessel-dependent	Moving Vessel Profiler	- -			+	0	+	-	0	+
		Remotely Operated Towed Vehicle	- -			+	0	+ +	-	0	+ +
		Manta Trawl	+	+	+	+	-	+ +	- -	+	+
		Rocket	+	+	+	+	-	+ +	-	+	+ +
		GEMAX corer	0	+	+	+	-	+ +	0	+	+ +
	In-situ, research vessel-independent	Argo Float	0	+	+	+	0	+	-	0	+
		Glider	- -	0	-	-	0	+ +	-	0	0
		FerryBox	-	+	0	+	0	+	0	+	+ +
		Profiling Buoy	- -	+	0	+	0	+	-	+	+ +
		Bottom-mounted Profiler	- -	+	0	+	0	+	-	+	+ +
		Active Biomonitoring using Blue Mussels	+ +	+	+	+	- -	+ +	-	0	0
		Passive Samplers: Chemcatcher and POCIS	+	0	0	+	-	+ +	0	0	+ +
		Artificial Substrates: ARMS and ASU	+ +	+	+	+	-	+ +	-	0	+
	Citizen Observations	Citizen Observations	+	+ +	+ +	+	+	+ +	-	0	+ +
	Remote sensing	Unmanned Aerial Vehicles	0			+	-	+	- -	0	-
		Earth Observation	+	+	+	+	0	+ +	0	-	+ +
		Remote Electronic Monitoring	0	- -	-	+	0	+	- -	+	+
Sample analysis	Field analysis	HydroFIA®pH	0	+	+	+	0	+	-	+	+ +
		Imaging Flow Cytometry platform	- -	+	0	+	0	+	-	-	0
	Laboratory analysis	DNA Metabarcoding	0	+	+	+	-	+ +	-	0	+
		Stable Isotope Analysis	+ +	0	+	+	-	+ +	0	0	+ +
		Computer-based identification	0	+ +	+	+	0	+	-	-	0

Coverage of gaps by the novel methods

The novel methods can be used to fill gaps of type (i)–(iii) regarding all MSFD descriptors except seafood contamination (Table 3). The main gaps of type (i) can be compensated using several of the reviewed methods, which partly allow for a higher spatial and/or temporal resolution of monitoring data due to their autonomous measurement (Table 3). We want to emphasize the use of Earth Observation to facilitate the observation of the entire assessment area in the open sea, which is a highly relevant topic in the MSFD.

The lack of indicators, and therefore gaps of type (ii), can be addressed for all descriptors but underwater noise/energy. Using (e)DNA metabarcoding, the monitoring of several descriptors can be enhanced, since new indicator species are made accessible. A promising method for an improved monitoring of food webs is SIA, using the novel indicator “food web length and stability.” Regarding sea-floor integrity, the Artificial Substrates ARMS and ASU can be used to generate a more comprehensive data basis by monitoring the presence of particularly sensitive and/or tolerant species, but still, indicators for a better reflection of the descriptor remain to be developed. The monitoring of dumped munitions was addressed by several recently finished and ongoing research projects (e.g., MODUM - Towards the Monitoring of Dumped Munitions Threat, 2013–2016; UDEMM, 2016 - Environmental monitoring for the delaboration of munitions on the seabed, 2016–2019; DAIMON - Decision Aid for Marine Munition, 2019–2021). For the detection and identification of dumped warfare relicts, ROTV and Gliders can be deployed, while the concentrations of leaking contaminants in the water can be monitored employing Active Biomonitoring using Blue Mussels or Passive Samplers. Regarding microplastics, the constant fragmentation and the large diversity within types of particles cannot be adequately addressed by a single method (Potthoff et al., 2017). Therefore, novel methods can be combined, e.g., by sampling larger particles using the Manta Trawl and complement these data by Rocket filtration samples and Sediment Corers. The applicability of the Earth Observation techniques to measure marine litter have been under study in recent years and methodologies are evolving (Martinez-Vicente et al., 2019).

Ecosystem services and climate change were also identified as missing descriptors and are addressed in recent research (HELCOM, 2013). The monitoring of these descriptors is a wide and complex field and therefore, was not addressed in this work. However, in the course of the BONUS FUMARI project, indicators for the monitoring of ecosystem services and novel methods to measure these were collected on the novel methods website http://freshwaterplatform.eu/fumari/.

Table 3: Novel methods and the Marine Strategy Framework Directive descriptor(s), which these methods can be used to monitor. "○" indicates methods that can fill a gap type i), "●" indicates methods that can fill a gap type ii), methods with the potential to fill a gap type iii) (in this case: Climate Change and Ecosystem Services) are highlighted using *italic* font. The descriptors with main gaps identified by Emmerson et al. (2019) and Kahlert et al. (2020) are also highlighted using *italic* font.

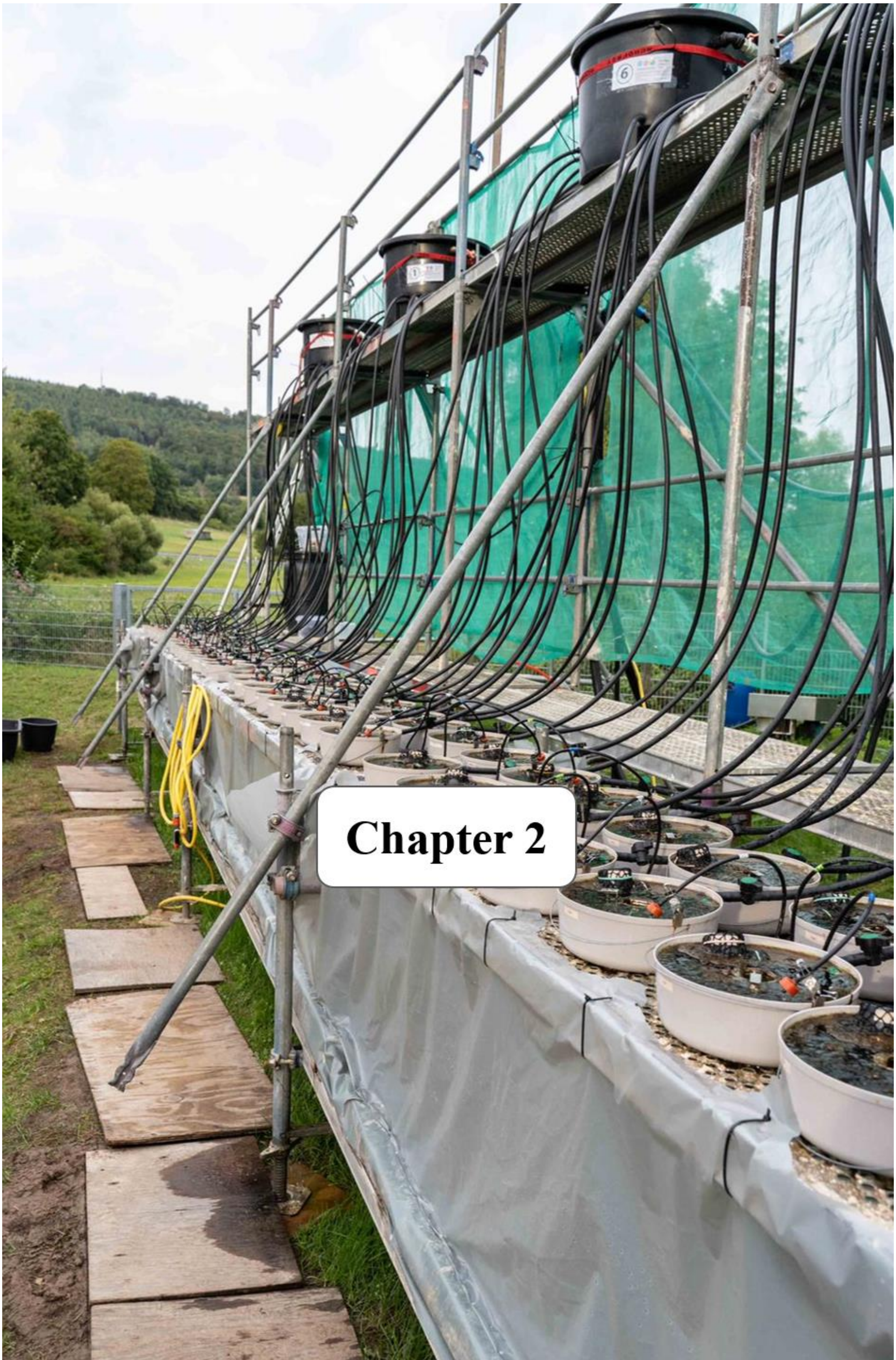
	Biodiversity	**Non-indigenous species**	**Commercial fish/shellfish**	***Food webs***	***Eutrophication***	***Sea-floor integrity***	***Hydrographical conditions***	***Contaminants***	**Seafood contamination**	***Marine litter***	***Underwater noise/energy***
Moving Vessel Profiler					○		○				
Remotely Operated Towed Vehicle					○		○	○/●			●
Manta Trawl										○/●	
Encapsulated Filtration Device										○/●	
Sediment Corer								○		○/●	
Argo Float					○		○				●
Glider					○		○	○/●			●
FerryBox				○	○		○				
Profiling Buoy	○				○		○				
Bottom-moored Profiler	○				○		○				
Active Biomonitoring					○			○/●	○		
Chemcatcher and POCIS								○/●			
Artificial Substrates: ARMS and ASU	○/●	○			○	○					
Citizen Observations		○			○		○			○	
Unmanned Aerial Vehicles	○		○		○		○	○		○	
Earth Observation	○				○		○			○/●	
Remote Electronic Monitoring	○		○	○							
HydroFIA®pH	○				○		○				
Imaging Flow Cytometry platform	○	○		○	○						
(e)DNA Metabarcoding	○/●	○/●	○/●		○/●	○/●					
Stable Isotope Analysis				●	○						
Computer-based identification	○	○	○								

The need for adaptable monitoring practices

With this overview and evaluation of monitoring methods, we strive to support the decision making and implementation of environmental monitoring. Even though the focus of this study is on filling the gaps in the Baltic monitoring, the addressed methods can also be applied for specific research questions and in other marine regions worldwide. An in-depth comparative analysis of the methods for the environmental status assessment was not envisaged by this study and is not necessarily feasible, due to their heterogeneous nature and the wide range of applications possible. Furthermore, the cost-efficiency of the addressed novel methods was not compared to the traditional methods they might replace or supplement. This is due to the wide applicability of the novel methods that can address several ecosystem elements and indicators, the high number of factors influencing the costs, and the lack of cost analyses regarding the specific methods. An alternative option for the cost evaluation, which is not addressed here, would be using depreciation costs rather than investment costs. We defined investment costs as the costs for the monitoring device or deployment system, including the necessary equipment, and personnel training; this limits the application of depreciation costs.

New game-changing or more effective novel methods may produce high value data that are not fully comparable to these produced by traditional methods and, thus, may fail to meet current legislative demands. Very often the suggested implementation of such novel methods may be dismissed due to a lack of full comparability, but it seems unreasonable to assume a full comparability of novel methods with old ones. The current legislation driving routine monitoring of the Baltic Sea was drafted against the backdrop of the scientific knowledge and the methods available at the time of drafting. In fact, such a tacit prerequisite may hinder both scientific progress as well as effective future management of the Baltic Sea.

While we do not advocate abrupt changes in monitoring or the replacement of old by novel methods in Baltic monitoring per se, we stress the need to review whether there is a need to re-examine and adapt our current monitoring. With this contribution, we want to highlight the need for adaptable monitoring practices. For this, parallel and standardised comparisons are of central importance (Blackman et al., 2019). However, such novel methods need to be mature enough to have gained considerable consensus within the scientific community as well as HELCOM and also to have resulted in standardised and replicable devices and process chains. In order to implement novel methods as parts of monitoring programmes, their applicability and cost-efficiency needs to be demonstrated in the considered ecosystem, the operational practices and data flows need to be managed, and guidance for the interpretation of the acquired data needs to be given (preferably including indicators that can use the data to assess the ecosystem status). The use of the novel method should be calibrated and standardised internationally to allow for comparison between different areas or deployments.



Chapter 2

Chapter 2: Fine sediment and the insecticide chlorantraniliprole inhibit organic matter decomposition in streams through different pathways

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In the context of this doctoral work, the following manuscript will be submitted for publication in Aquatic Sciences as:

Mack, L., Buchner, D., Brasseur, M. V., Leese, F., Piggott, J. J., Tiegs, S. T., Hering, D. (unpublished). Fine sediment and the insecticide chlorantraniliprole inhibit organic matter decomposition in streams through different pathways.

Intensive agriculture drives an ongoing deterioration of stream biodiversity and ecosystem functioning. Key agricultural stressors include the flushing of fine sediment and insecticides from adjacent land into streams, whose individual and combined effects on the aquatic flora and fauna are well studied. The functional consequences of the biodiversity loss associated to agricultural stressors, however, has been less frequently addressed. We address this knowledge gap, examining the effects of fine sediment and different concentrations of the insecticide chlorantraniliprole on organic matter decomposition in an outdoor mesocosm experiment. The stream mesocosms contained standardised cotton strips, which were used to assess organic matter decomposition in terms of decay rates of strip tissue and microbial respiration of the strips' biofilm.

The decomposition of strips buried under fine sediment was inhibited, which we relate to the limited accessibility for invertebrate and microbial feeding, as well as the limited nutrient and oxygen exchange. Also chlorantraniliprole inhibited decay rates, which we relate to missing invertebrate grazing and excessive algal growth. In contrast to decomposition rates, we did not observe any stressor effect on microbial respiration. But effects on respiration due to changed microbial organic matter decomposition might be overshadowed by other shifts in metabolic activities. An interaction of fine sediment and the insecticide chlorantraniliprole was not identified, however, this might result from the study design.

Introduction

The ongoing deterioration of stream habitats due to intensive agriculture has detrimental consequences for aquatic biodiversity and ecosystem functioning. Freshwaters are among the most endangered ecosystems and the loss in aquatic biodiversity is proceeding at a peerless rate (Butchart et al., 2010), resulting in damaging consequences for ecosystem functioning. Agriculture drives diverse impacts on physical, chemical and habitat-related conditions of streams, which affects biota and associated functions through various pathways: Hydromorphological modifications associated with straightening, removal of riparian vegetation and water abstraction can result in changed flow velocities, increased erosion and rising temperatures within the streams. Furthermore, the disconnection of streams from their floodplains and tributaries disrupts the characteristic lateral and longitudinal continuity of streams (Bolpagni and Piotti, 2015). Nutrients and insecticides, as well as fine sediments are washed from agricultural lands into streams and initiate a cascade of effects, ranging from blanketing of macrophytes and habitats by sand and silt, enhanced algae growth and associated oxygen depletion at night times (Burkholder and Glibert, 2013; Hauer et al., 2018).

Fine sediment and insecticides are key stressors resulting from agricultural activities and their effects on aquatic biota are well studied. Fine sediment (inorganic particles <2 mm in diameter) is a natural component of streams, but when it excessively enters streams due to surface run-off or bank erosion, it can have strong negative effects: An increased turbidity of water and a blanketing of macrophytes can inhibit plant growth, subsequently reducing oxygen levels and food availability for herbivorous organisms. The small particles can clog fish gills and the interstitial spaces in stream beds, leading to respiration problems for fish and serious degradation in habitat quality for all biota. Especially the loss of spawning habitats can have detrimental long-term consequences for aquatic communities (Dunlop et al., 2005; Hauer et al., 2018; Li, 2013; Pulg et al., 2013). Insecticides to fight crop damage from terrestrial insects can be flushed into streams and harm aquatic insects. Consequences can range from sub-lethal effects, like a reduction in feeding and metabolic activity, to lethal effects. Furthermore, insecticides can be highly persistent and therefore accumulate within organisms, leading to biomagnification along the food chain (Lavtižar et al., 2015; Rodrigues et al., 2015; 2017). When co-occurring, fine sediment and insecticides can interact in their effect on aquatic communities: Insecticides can adsorb to fine sediment and thus be associated with its input into surface waters (Hauer et al., 2018). Within the water, insecticides can accumulate in stream bank sediment, reducing pelagic and enhancing benthic insecticide exposure (Chará-Serna et al., 2019; Sardo and Soares, 2010).

The functional consequences of fine sediment and insecticides have been less frequently addressed, leaving knowledge gaps on the single and combined effects on organic matter decomposition. Studies on the effects of fine sediment on organic matter decomposition revealed contradicting effects: Fine sediment can limit the exchange of oxygen and nutrients between the biofilm and the water column, suppressing microbial breakdown (Besemer et al., 2007; Cornut et al., 2014). In contrast, physical abrasion of organic matter by fine sediment can stimulate decomposition (Ferreira et al., 2020). Insecticides were shown to have a negative effect on organic matter decomposition through inhibition of invertebrate shredding (Chara-Serna et al., 2017; Rodrigues et al., 2018). We are aware of a single study on the combined effects of fine sediment and an insecticide on organic matter decomposition. Chará-Serna et al. (2019) conducted an outdoor pond-mesocosm experiment, investigating the individual and combined effects of fine sediment and imidacloprid (a neonicotinoid insecticide that also binds to sediment) on freshwater community structure, net ecosystem production and organic matter decomposition. They found an antagonistic interaction on zooplankton but no effect on net primary production or organic matter decomposition (Chará-Serna et al., 2019). New agents are constantly developed to effectively manage crop damage. A relatively novel insecticide is chlorantraniliprole, which is expected to gain increasing importance due to the ban on several neonicotinoid insecticides and its high effectiveness and selectivity to insects. Its negative effects on various aquatic species have been studied (Lavtizar et al., 2015; Rodrigues et al., 2015, 2017), but there is a lack of studies on its individual and combined effects with other agricultural stressors on stream functioning.

In this study, we address the current knowledge gaps on the individual and combined effects of fine sediment and the insecticide chlorantraniliprole on organic matter decomposition. Organic matter decomposition is a central component of stream ecosystem functioning (Ferreira et al., 2020). It can be divided into three interdependent phases (Cummins, 1974): i) leaching of soluble compounds, ii) microbial conditioning, due to the colonisation and activity by bacteria and fungi, and iii) fragmentation by invertebrates and physical abrasion. To analyse the organic matter decomposition, the cotton strip assay has shown to be a valuable technique. Cotton strips are mainly made of cellulose, which is a highly relevant carbon source for stream ecosystems, since it is the most abundant polymer and the main constituent of plant litter (Tiegs et al., 2013). Furthermore, the biofilm on strips can be used to examine the microbial activity and community taking part in organic matter breakdown.

We conducted a stream-mesocosm experiment in a 2x4 factorial design including a temporal gradient of two and three weeks. As measures for organic matter decomposition we investigated the loss of tensile strength of cotton strips, as well as the microbial respiration of the biofilm on the cotton strips. We expected:

1. Fine sediment and insecticide addition will suppress tensile strength loss due to an inhibited microbial activity (Cornut et al., 2014) and missing invertebrate shredders (Rodrigues et al., 2018).
2. For both stressors in combination, we expect the tensile strength loss to be higher than additive individual effects: When the insecticide binds to the fine sediment, the exposure of benthic invertebrates to the toxin is enhanced, reducing invertebrate shredding and therefore, tensile strength loss (Chara-Serna et al., 2017).
3. The addition of fine sediment will decrease microbial respiration and diversity due to an inhibited oxygen and nutrient availability for the biofilm (Cornut et al., 2014) and conversely, increasing insecticide concentrations will enhance microbial respiration and diversity due to the inhibition of invertebrate grazing.
4. For both stressors in combination, we expect the microbial respiration and diversity to be less than additive individual effects due to the binding of the insecticide to fine sediment (according to the mechanism explained in hypothesis 2) and the limited accessibility of cotton strips covered by sediment to insecticides.
5. From day 28 to day 35, we expect stressor effects to increase due to the longer incubation time.

Materials and methods

Study site and experimental design

Our study was conducted using a streamside mesocosm setup (ExStream System) fed by the stream Bieber (Germany, Hessen, 50°09'38.9''N, 9°17'58.6''E). The Bieber, a fine material-rich, siliceous central upland stream (water body type 5.1 according to the German river typology; Pottgießer and Sommerhäuser 2004), is in good ecological state and part of a long-term monitoring project providing extensive data on physico-chemical characteristics and benthic invertebrate communities.

The ExStream system consisted of 64 stream channels with a similar setup to the ExStream systems used in New Zealand (Piggott et al., 2015a), Germany (Beermann et al., 2018), and China (Juvigny-Khenafou et al., 2020) and ran from 09 August to 19 September 2020 for 41 days. A schematic illustration is given in Figure 5. The water from the Bieber, including drifting invertebrates, algae and microbes (< 4 mm), was continuously pumped into four header tanks. Each tank fed 16 stream channels (circular mesocosms with outer diameter 24.5 cm, inner diameter 5.1 cm, volume 3 L, and area of 450 cm^2) with a flow of 2 L/min, resulting in a flow velocity of about 0.1 m/s. Channels were filled with a) sediment from the Bieber, reflecting the sediment composition of stream areas with the flow conditions of the stream channels to provide habitat (600 g gravel < 1 cm, 600 g gravel 1-3 cm, 300 g stones > 3 cm and 3 larger flat stones); b) leaf tubes containing 2.67 ± 0.12 g alder leafs (*Alnus glutinosa*); c) two cotton strips (2.5 cm x 8 cm) for assessing organic matter decomposition; d) a 3 g pack of alder sticks; and e) a ceramic tile (35 x 35 mm) for biofilm aggregation in order to provide habitat and food for invertebrate grazers and shredders (Figure 6). The water outflow of the stream channels was the inner circular opening, which was equipped with a sieve (≤ 1 mm) to catch drifting animals. To supplement natural drift colonisation of the channels, we added stream macroinvertebrates by kick-net sampling (Elbrecht et al., 2016). Samples contained high amounts of *Gammarus pulex* and *Lepidostoma basale*, and we additionally collected five individuals of *Ephemera danica* per mesocosm. Other species present at the site and potentially being collected using the kick-sampling are listed in Appendix 2, Table A2-1.

The experiment comprised of a colonisation phase (day -21 until day -1) and a stressor phase (day 0 until day 21). Before the start of the colonisation phase, the channels were filled with sun-dried stream substrate. On day -21, water flow started and the channels were equipped with leaf tubes, wood sticks and ceramic tiles. Cotton strips were added on day -17. On day -7, sampled macroinvertebrates were added, which then had a 6-day period for acclimatisation until day 0. The stressor phase comprised of an acute stressor phase from day 0 to day 4, followed by a reduced stressor phase from day 5 to day 21. This was done to follow natural dynamics of sediment and insecticide introduction into streams, where rainfall events lead to short-term but high exposure, followed by long-term low exposure of aquatic organisms to introduced stressors.

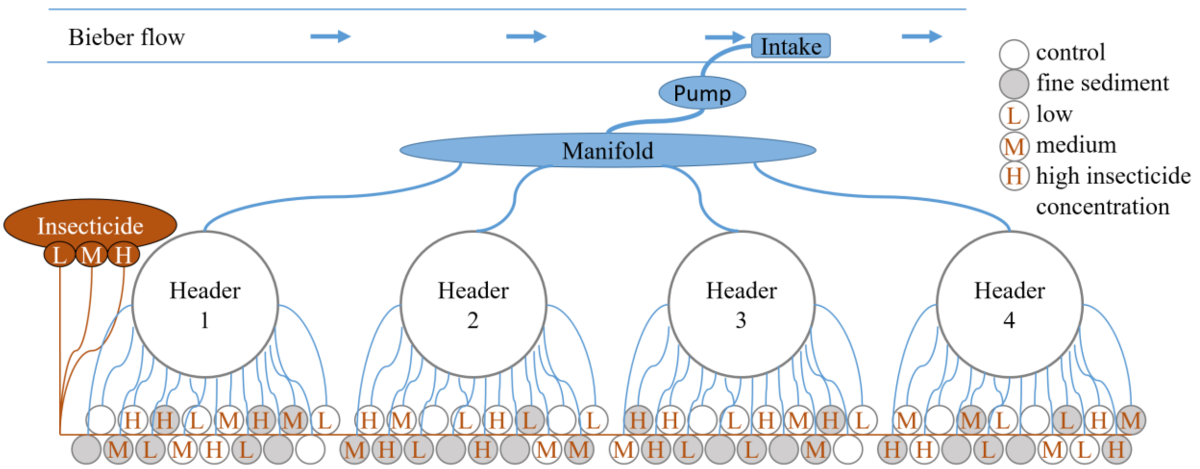


Figure 5: Scheme of the ExStream setup. The stream water of the Bieber is filtered with a mesh size of 4 mm and pumped into four header tanks, which feed the single stream channels with a constant flow of 2 L/min. The 64 stream channels show a 2x4 factorial design with fine sediment (presence/absence) and insecticide (low/medium/high) addition as stressors.

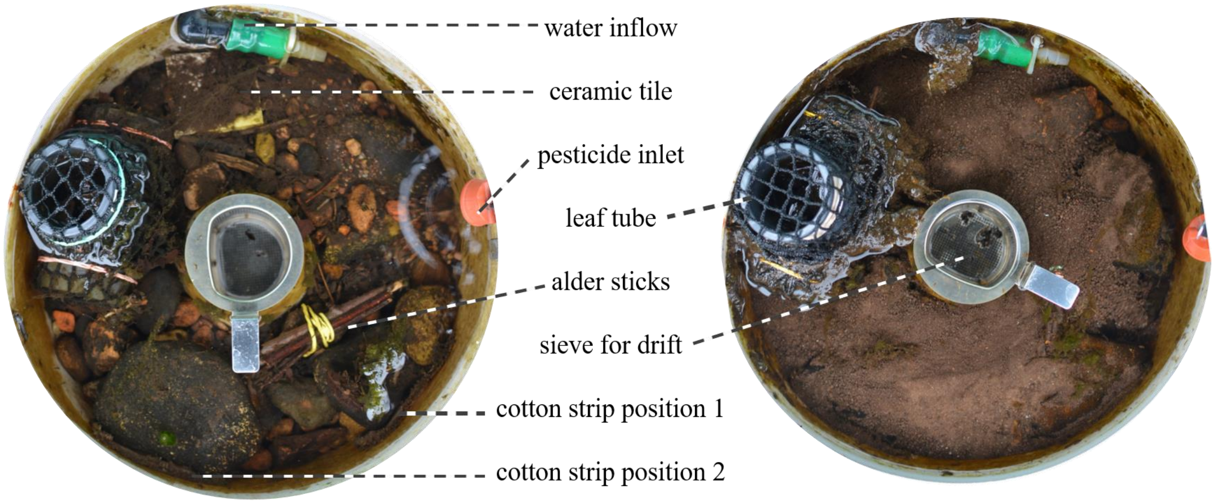


Figure 6: Examples of stream channels without (left) and with (right) fine sediment treatment. The positions of the components of the stream channels are indicated. For channels with fine sediment treatment, most of the channel substratum was buried under fine sediment.

Stressor manipulation

In a 2x4 factorial design, we applied fine sediment (presence/absence) and three concentrations of an insecticide (low, medium, high) as stressors. Two of the eight replicates for each stressor combination were randomly assigned to each header tank. The fine sediment (< 2 mm) was collected from the area around the stream, sieved and sun dried for 24 h. On day 0, 450 mL of fine sediment were added into the channels while turning off the water inflow for about 5 min. On day 0, 100 % of the stream channels' substrate was buried under fine sediment, but due to the high flow velocity, fine sediment shifted during the stressor phase (Appendix 2, Figure A2-1). In the acute stressor phase, the insecticide chlorantraniliprole (Coragen, DuPont) was applied in the concentrations 0.2 µg/L, 2 µg/L and 20 µg/L, followed by the reduced stressor phase, where concentrations were lowered by a factor of 10. The insecticide was pumped into the mesocosms from one stock solution, achieving the different concentrations by adjusting different pumping rates.

Cotton strip assay

To assess the ecosystem function of organic matter decomposition, cotton strips were deployed in the stream channels. The standardised cotton strips are made of heavy-weight cotton fabric (Style 548; Fredrix, Lawrenceville, GA, USA) and 2.5 cm x 8 cm in size (Tiegs et al., 2013). The strips were deployed in the stream channels to allow for organic decomposition, which was then assessed by determining the respiration rate and microbial community of the biofilm on the strips, as well as tensile strength measurements.

Deployment in the mesocosms

Strips were handled gently at all times using forceps by the fray, rather than the woven part of the strip. On day -16 of the stressor phase, two strips were deployed in each mesocosm. They were placed behind each other on the outer wall of the mesocosms opposite of the jet inflow. By clamping them behind two of the big stones of the substratum they were held in place.

Removal of cotton strips from the mesocosms

The strips were retrieved after two different incubation times, i.e., at day 12 and day 19 of the stressor phase (total incubation time of 28 and 35 days; Figure 7). To minimise the effect of the strip position within the stream channel, we randomised the sampling of the first or second position. At each retrieval, respiration of the biofilm on the cotton strips was measured, DNA/RNA samples were taken, the strips were dried, weighed and tensile strength was measured.

Respiration determination

To study the microbial activity of the biofilm colonising and decomposing the cotton strips, the respiration rate on each strip was measured. A tank with a continuous flow of fresh stream water (respiration tank) was prepared for the incubation of the respiration chambers (chambers with one strip to measure the respiration rate of the biofilm). For each mesocosm, two respiration chambers were filled with water from the respiration tank. A single strip was removed from a mesocosm using sterile forceps and gently shook to dislodge loose sediment and dead algae without damaging the biofilm. The cotton strip was put into one of the respiration chambers and the lid was closed tightly while submerging the chamber in the respiration tank to eliminate any visible air bubbles. The second chamber without a cotton strip (control chamber) was filled and closed in an identical fashion. Both respiration chambers were then wrapped in tin foil to shield off any light and stop photosynthesis. For incubation within the respiration tank, they were tied together with a rubber band. The time of deployment and temperature within the respiration tank was recorded.

After an incubation time of 2-4 h, oxygen concentration was measured in both respiration chambers. For the final oxygen measurement of 60 seconds, the oxygen probe was put directly into the respiration chamber, connecting the probe to the opening of the respiration chamber in an airtight fashion to yield off dissolved oxygen drift. To minimise photosynthesis, the chambers were still wrapped in tin foil while oxygen determination. To remain a constant flow, a stirring fish was added into the chamber, which was placed on a magnetic stirrer working on a constant velocity. The time of incubation, temperature in the respiration tank and final dissolved oxygen concentrations of both respiration chambers were recorded.

The dissolved oxygen consumption of the biofilm on the cotton strips, R_{strip}, is expressed as the difference in dissolved oxygen concentration between the control chamber (respiration of the stream water) and the strip biofilm, correcting for differences in cotton-strip dry mass and the duration of respiration in the chambers (Eq. 1).

$$R_{strip} = (DO_{control} - DO_{strip}) * V_{H2O} / m_{strip} / t \qquad (1)$$

$DO_{control}$ is the concentration of dissolved oxygen in the control chamber after the incubation period, while DO_{strip} is the dissolved oxygen concentration in the cotton-strip chamber, which are both expressed in mg/L. V_{H2O} is the volume of water in the respiration chamber, expressed in L, m_{strip} is the cotton-strip dry mass, expressed in g (for determination of dry mass see below), and t is respiration incubation time in hours.

Drying and weighing of the strips

To remove adhering sediment and biofilm from the remaining strips and inhibit further biological decomposition, the strips were gently brushed with a paintbrush in an ethanol bath (each side for 20 seconds). Strips and respective labels were placed in individual tin foil trays and dried at 40 °C for ≥ 24 h. The dry strips were weighed to the nearest 0.001 g using a laboratory balance and placed in tin foil envelopes until shipping. These envelopes were stored in air-tight plastic bags along with silica gel as desiccant until tensile strength measurement.

Tensile strength determination

Tensile strength of all cotton strips was determined using a Mark-10 MG100 tensiometer (Tiegs et al., 2013). The ends of each strip were placed in the grips, ensuring the strips did not slip during the measurement. With a rate of 2 cm/min the strips were pulled and the maximum tensile strength, at which the strips tore, was recorded for subsequent analysis.

We calculated the decay rate (k in the unit 1/day) for each cotton strip using the following exponential decay model (Eq. 2):

$$k = -(\ln(x_{strip}/x_{control}))/t \qquad (2)$$

x_{strip} is the tensile strength of the cotton strip, $x_{control}$ is the mean tensile strength of 10 strips that were not incubated, but cleaned with ethanol and stored in a desiccator, t is the deployment time in days.

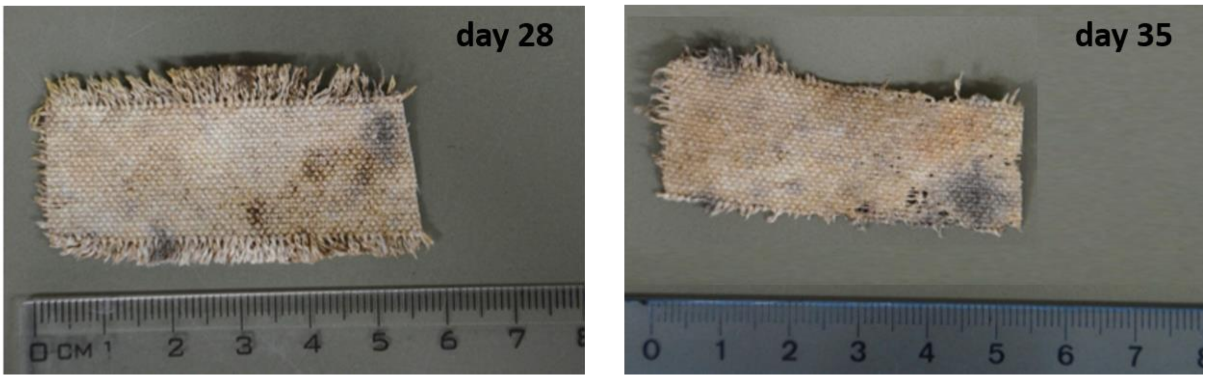


Figure 7: Examples of cotton strips after the deployment periods of 28 (left) and 35 days (right) in the stream channels. The strips were deployed in channel 6 with no fine sediment and medium insecticide treatment.

Statistical analysis

All analyses to study the single and combined effects of the stressors sediment and insecticide were conducted in R (version 4.1.0, R Core Team, 2021).

We tested for differences in tensile strength loss and respiration rate between cotton strips with different incubation times, positions in the stream channel, and coverage of sediment, as well as for the stressor applications of fine sediment addition and different insecticide concentrations using Mann-Whitney U-tests and Kruskal-Wallis tests (R Core Team, 2021).

Single and combined stressor effects on the tensile strength loss of the cotton strips and the respiration rate of the biofilm were analysed using linear mixed effect models (Kuznetsova et al., 2017). To identify relevant stressor and interactions effects, the significances of regression coefficients were used (t-test; $p < .05$). We applied the models to the samples of day 28, day 35 and a combination of both sampling events. As random effects, the position within the stream channel, the header tank number, the days of incubation and the sediment coverage of the strip were used, if applicable. Gaussian errors and an identity link were used as link function and error distribution of the model. Model evaluation included the examination of residuals

(Shapiro-Wilk Test for the correlation of fitted and normal distributed values; R Core Team, 2020) and the model fit (marginal and conditional R^2 as the proportion of explained variance by the fixed effects; Barton, 2020).

Results

Decay rate is inhibited by sediment coverage and chlorantraniliprole

The median tensile strength loss after the incubation was at 86.6 % with a decay rate of 0.07. The decay rate significantly increased with deployment time (Figure 8; Mann-Whitney U-Test, p = .014). Also the position within the stream channel showed an influence, with the first position showing a higher decay rate than the second position (Figure 8; Mann-Whitney U-Test, $p < .05$).

The addition of fine sediment to the stream channels showed no general effect on the decay rate (Figure 9). But strips that were fully buried under fine sediment showed a significantly lower decay rate compared to strips not buried (Figure 8; Mann-Whitney U-Test, $p < .05$).

While we did not observe an effect of chlorantraniliprole after 28 days, results show an inhibition of organic matter decomposition after 35 days of incubation (Figure 9; Kruskal-Wallis test, $p < .05$): tensile strength loss decreased from 96.3 to 95.0, 90.6 and 85.6 % for none, low, medium and high insecticide concentrations, respectively.

Linear regression analysis did not reveal stressor effects after 28 days of incubation. After 35 days, insecticide addition and the coverage by fine sediment showed significant negative effects on tensile strength loss ($R^2 = 0.28$). Models did not indicate any stressor interaction.

Respiration rate does not respond to the addition of fine sediment or insecticide

In general, the median respiration rate after the incubation was at 0.35 $mg_{O2}/g_{strip}/h$. Coverage by fine sediment, the position of the strip within the stream channel and the deployment time did not influence the respiration rate.

Also the addition of fine sediment and the insecticide showed no significant effect on the respiration rate (Figure 10; Mann-Whitney U-Test, $p > .05$).

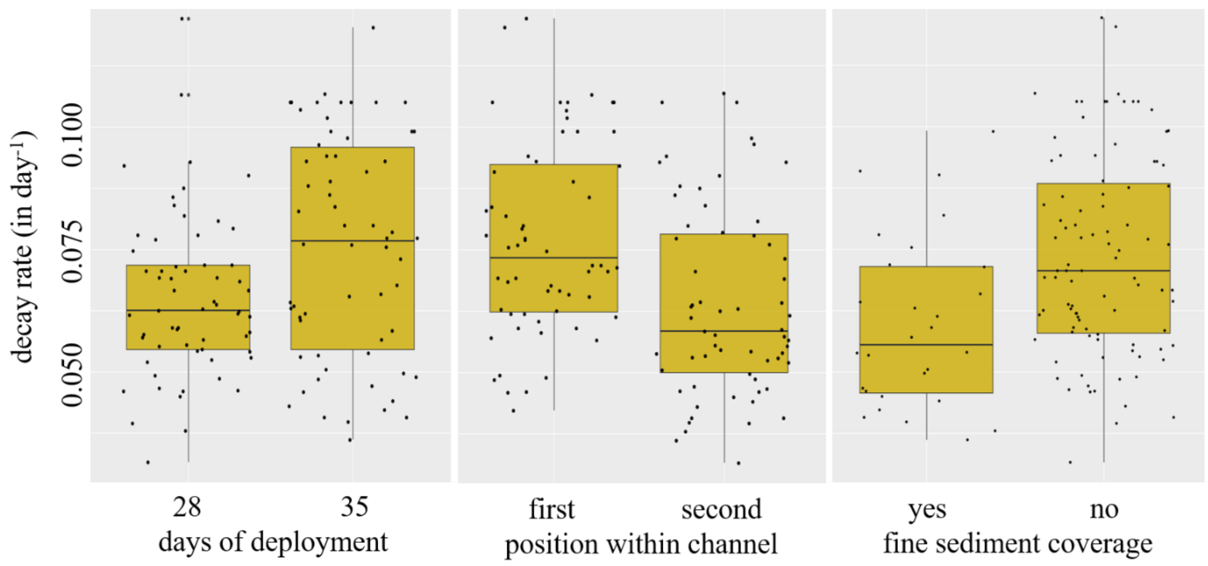


Figure 8: Decay rate of strips with different deployment time (left), positions within the channel (middle) and sediment coverage (right). The decay rate significantly depended on all these variables: It was increasing with deployment duration, higher for the first position in the channel compared to the second position, as well as higher for strips not covered compared to strips covered by fine sediment (Mann-Whitney U-Test, $p < .05$).

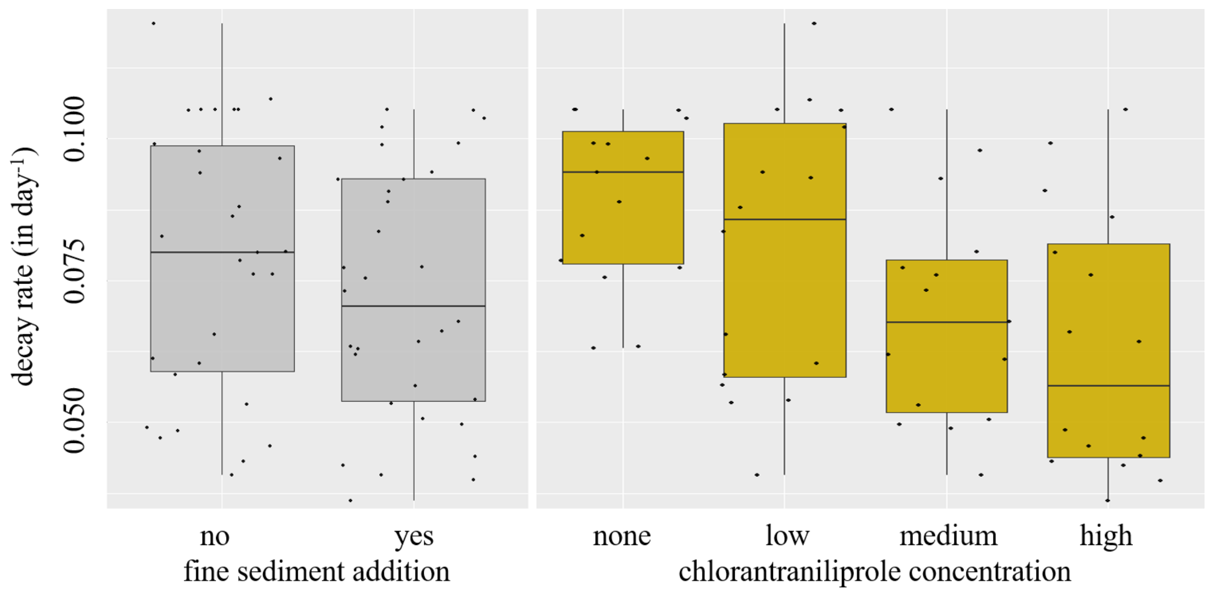


Figure 9: Decay rate for different stressor applications after 35 days of deployment. While results indicate no effect of fine sediment addition (left; Mann-Whitney U-Test, $p > .05$), chlorantraniliprole significantly affects tensile strength loss (right): With increasing insecticide concentrations, the cotton strip decomposition is increasingly inhibited (Kruskal-Wallis test, $p < .05$).

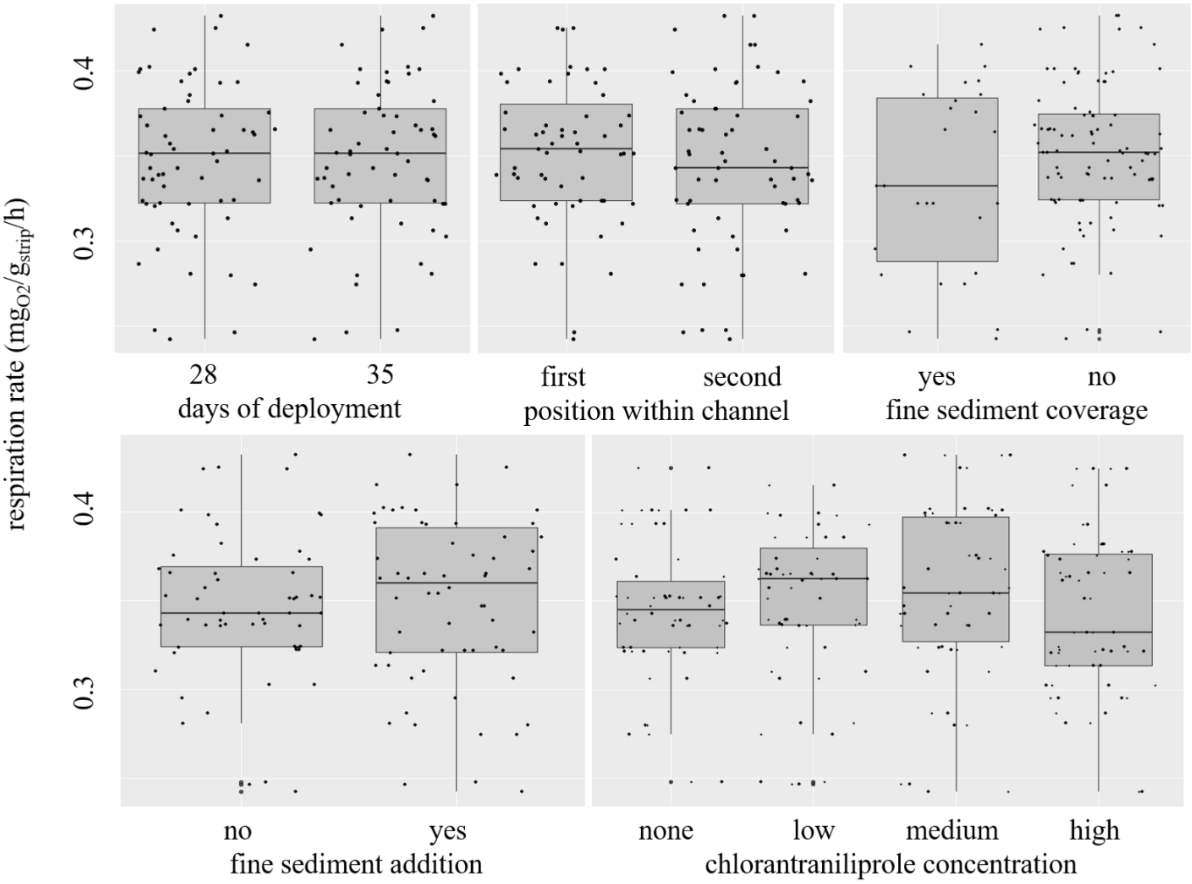


Figure 10: Effects on the respiration rate. Effects of the deployment duration, position within channel and sediment coverage (top), as well as stressor effects (bottom) on the respiration rate of the biofilm on cotton strips (for days 28 and 35 combined)**.** None of the effects was significant.

Discussion

We expected that both fine sediment and insecticide addition suppress tensile strength loss (hypothesis 1). This expectation was supported for insecticides but rejected for fine sediment addition. Accordingly, hypothesis 2 of synergistic effects of both stressors on cotton strip decomposition was rejected, too. Although we found no significant effects of fine sediment addition in general, we observed that fine sediment flushed into streams negatively affects the decomposition of organic material when covering the tissue: The coverage creates a barrier between the water column and the strip tissue, limiting accessibility of the tissue and nutrient and oxygen exchange, which inhibits invertebrate and microbial feeding. Furthermore, anoxic conditions within the fine sediment may result, which additionally hinders invertebrate and microbial activity (Ferreira et al., 2020). However, as only few of the cotton strips were covered by fine sediment, there was no significant overall effect of fine sediment addition on decomposition.

Another possible mechanism of fine sediment affecting decay rate is increased physical abrasion by moving fine sediment particles (Ferreira et al., 2020). The flow velocity of 0.1 m/s was high enough to cause continuous fine sediment transport within the channels (Appendix 2, Figure A2-1) and therefore, physical abrasion of the cotton strip tissue by fine sediment may have occurred. Further, we observed higher decay rates for strips in the first compared to the second position in the mesocosm, indicating an abrasion effect due to higher velocities in the front area. But as our data show no general effect of fine sediment on the decay rate (Figure 9), we cannot confidently attribute this higher loss in tensile strength to physical abrasion by sediment. In line with our findings, Bastias et al. (2020) found a stimulation of leaf litter decomposition at flow velocities of 0.0092 m/s, but they could not identify if this was due to the physical abrasion by sediments, currents, or due to stimulated microbial activity (by enhanced oxygen and nutrient exchange).

Our expectation that decay rate is inhibited by insecticide addition was supported. We assume this is a consequence of changes in invertebrate and algae abundance. The insecticide causes a decrease in aquatic invertebrate activity and abundance, which in turn leads to decreased shredding of the cotton strip tissue. This is in line with findings of Rodrigues et al. (2018), who observed a decreased organic matter decomposition due to decreased invertebrate abundance. In general, the involvement of invertebrates in the decomposition of cotton strips is not yet fully clear. Tiegs et al. (2013) assume no influence of invertebrate activity, whereas Clapcott and Barmuta (2010) found evidence of invertebrate feeding on cotton strip tissue. High numbers or *G. pulex* and *L. basale* were present in the mesocosms, which are both known as shredders of fallen leaves and plant tissue (Appendix 2, Tables A2-1, A2-2). In addition, we observed several *L. basale* specimens on the strips, and therefore, we assume that invertebrate shredding had an effect on the decomposition of the cotton strip tissue.

Organic matter decomposition can also be inhibited by high algal growth within the mesocosms: After eight days, we observed excessive algal growth in mesocosms with medium and high insecticide concentrations (with a median coverage of 2, 1, 16 and 74 % for none, low, medium and high concentrations; Appendix 2, Table A2-3, Figure A2-2). We relate this increase in algal growth to missing invertebrate grazing. In general, the direct contribution of algae to organic matter decomposition is uncertain, with contradicting observations in different studies. Howard-Parker et al. (2012) observed stimulation, Halvorson et al. (2019) observed inhibition, while Elosegi et al. (2018) observed no effects at all. Halvorson et al. (2019) argue that algae can reduce the decomposition of organic matter by providing labile carbon, which fungi invest into growth and reproduction rather than decomposition. In addition, Pascoal and Cássio (2004) explain this inhibiting effect by algal-induced low dissolved oxygen concentrations at night time, reducing the capacity and efficiency of microbial enzymatic activity. We assume the dense algae cover in our experiment limited oxygen availability for microbes and thus contributed to the reduced decomposition.

The limited organic matter decomposition, as reflected by limited tensile strength loss, is not reflected in changed respiration rates. We expected decreasing microbial respiration with fine sediment input and an increase in respiration with increasing insecticide concentrations (hypothesis 3). Both parts of the hypothesis were rejected, as respiration rate responded neither to fine sediment nor to insecticide addition. Chlorantraniliprole is highly selective to the insect receptor ryanodine, which is not present in microbes. But apparently there was also no indirect effect of reduced macroinvertebrate grazing on microbial activity. Also fine sediment addition did not influence respiration rates, although several other studies detected an inhibition of microbial activity due to reduced oxygen and nutrient transport between the water column and the biofilm (Niyogi et al., 2003; dos Reis Oliveira, 2019; Ferreira et al., 2020).

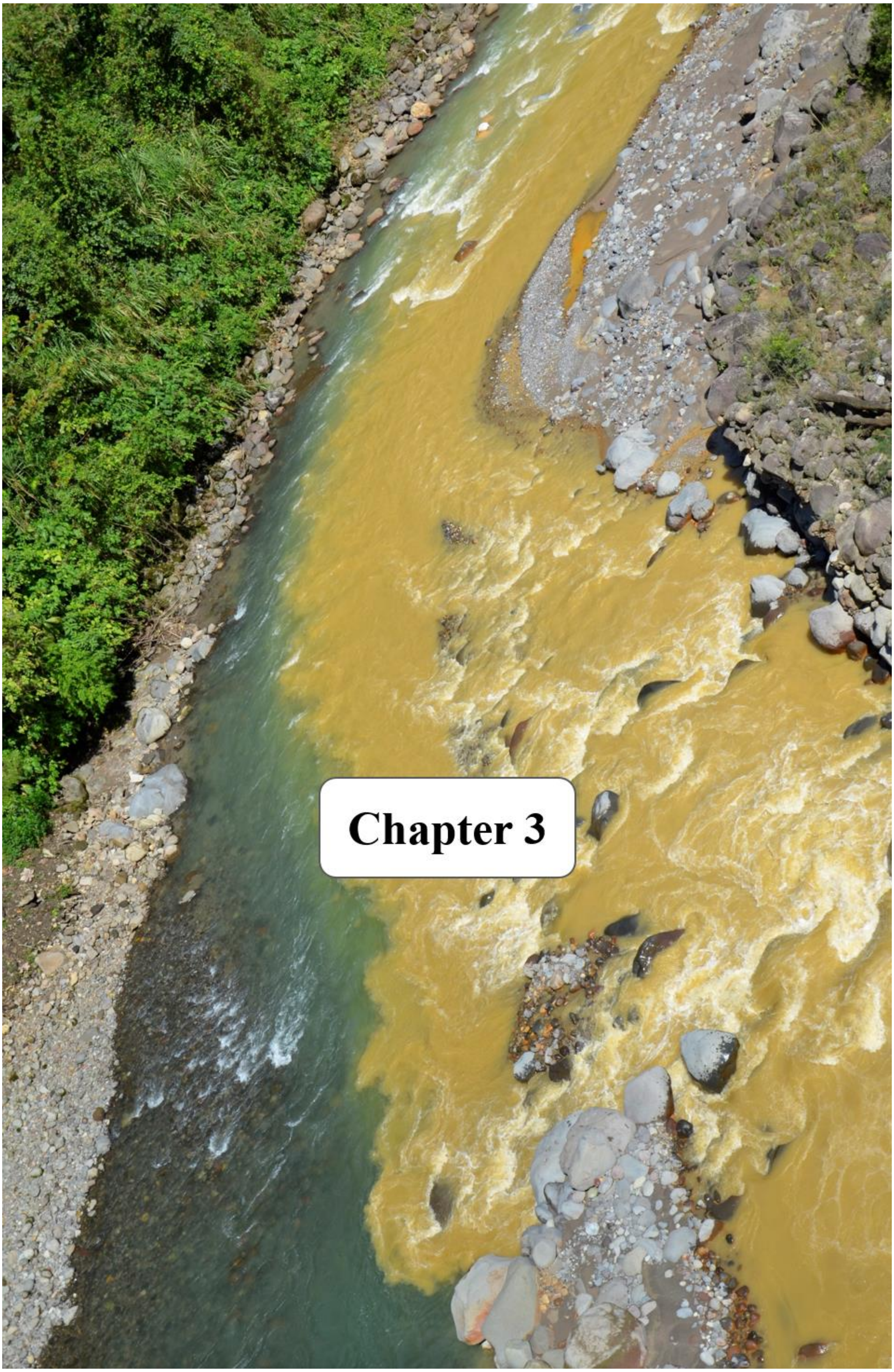
The loss in tensile strength and the microbial respiration both indicate organic matter decomposition, but there were strong differences in stress effects on these two indicators. In our experiment, the reduced loss of tensile strength under insecticide exposure is most likely related to invertebrate grazing, which is not necessarily influencing microbial respiration. In addition, tensile strength loss is related to heterotrophic microbial activity for cellulose decomposition, integrating the stream conditions during the incubation time. Respiration, in contrast, reflects a snapshot of the auto- and heterotrophic activity at the time of measurement (Tiegs et al., 2013) and includes cellulose decomposition as well as other metabolic processes such as growth and reproduction. Therefore, the stressors could very well influence microbial organic matter decomposition, but the respiration activity does not reflect this influence, as shifts in other metabolic activities mask these changes. For instance, an increased growth and reproduction of fungi (due to labile carbon excretions by algae) may compensate for reduced cellulose decomposition. Microbial communities are highly dynamic in their structure, diversity and functionality and therefore can change their response patterns to stressors rapidly (Juvigny-Khenafou et al., 2020).

Finally, we expected that stressor effects increase with incubation time (hypothesis 5). This hypothesis was supported for tensile strength loss, but not for microbial respiration. Similar reasons as discussed above can be hold responsible for this difference, as the observed effects are obviously related to invertebrate grazing. We conclude that tensile strength loss is a more sensitive and integrative measure for organic matter decomposition compared to respiration.

The design of the ExStream system has some disadvantages that limit the transfer of the results to field conditions. We applied the stressors directly to the stream mesocosms, missing the natural flushing of the insecticide and fine sediment into the stream. Thus, we cannot address a possible interaction of the stressors related to the flushing into streams, but only the interaction when both stressors are already present in the water. Under natural conditions, the fine sediment can cause an increased flushing of insecticide into streams, but due to the individual application of the two stressors into the stream mesocosms, this effect could not be tested for. Furthermore, the long-term effects of the insecticide addition could not be addressed in this study. The insecticide was shown to migrate deep into the soil profile and leach into the water column for up to two years after the input (Kolupaeva et al., 2019), but the stressor phase of the ExStream was run for two weeks. The expected interaction between the two stressors was therefore not observable in our short-term experiment, but might nevertheless occur under field conditions.

Conclusions

In summary, our results support the conjecture that both, insecticides and fine sediments, can limit organic matter decomposition, most likely through different pathways. Insecticides reduce invertebrate density and activity, thus limiting the impact of invertebrate feeding on microbial population growth and on direct destruction of the tissue. Reduced invertebrate feeding can also increase algae cover that can limit microbial activity. Fine sediments are likely to impact bacteria and fungi more directly, in particular if they cover the organic matter. On the other hand, moving fine sediment can cause abrasion that mechanically contributes to decomposition. Interactions between both stressors could not be observed, but possibly occur under field conditions.



Chapter 3

Chapter 3: Observed multiple stressor effects depend on sample size and stressor gradient length

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In the context of this doctoral work, the following manuscript was submitted for publication in Water Research as:

Mack, L., Fernández de la Hoz, C., Penk, M., Piggott, J. J., Crowe, T., Hering, D., Kaijser, W., Aroviita, J., Baer, J., Borja, A., Clark, D. E., Fernández-Torquemada, Y., Kotta, J., Matthaei, C. D., O'Beirn, F., Paerl, H. W., Sokolowski, A., Vilmi, A., Birk, S. (submitted). Observed multiple stressor effects depend on sample size and stressor gradient length.

Multiple stressors are continuously deteriorating surface waters worldwide, posing many challenges for their conservation and restoration. Effect types of multiple stressors range from single-stressor dominance to complex interactions. Identifying prevalent effect types is critical for environmental management, as it helps prioritise key stressors for mitigation. However, it remains unclear whether observed multiple stressor effects reflect true ecological processes or simply the sampling strategy. We examined the role of sample size and stressor gradient lengths in 158 paired-stressor response cases with over 120,000 samples from rivers, lakes, marine and transitional ecosystems around the world. For each case, we split the stressor gradient into two partial gradients and investigated associated changes in stressor effects.

Sample size influenced the identified effect types, and stressor interactions were less likely for cases with fewer samples. After splitting gradients, 40 % of cases showed a change in effect type, 30 % no change, and 31 % showed a loss in stressor effects. These findings suggest that identified effect types may often be statistical artefacts rather than representing ecological processes. In general, non-linear responses were more pronounced for organisms at higher trophic levels.

We conclude that observed multiple stressor effects are not solely determined by ecological processes, but also depend on sampling design. Observed effects are likely to change when sample size and/or gradient length are modified. Our study highlights the need for improved monitoring programmes with sufficient sample size and stressor gradient coverage. Our findings emphasize the importance of adaptive management, as stress reduction measures or further ecosystem degradation may change multiple stressor-effect relationships, which will then require associated changes in management strategies.

Introduction

Multiple stressors are damaging ecosystems worldwide and, for successful conservation and restoration of surface waters, they need to be addressed in concert (Nõges et al., 2016). Human-induced pressures operate locally (e.g. modified land use) and globally (climate change), all leading to critical declines in biodiversity and function of terrestrial and aquatic ecosystems (Dirzo et al., 2014). Surface waters are particularly vulnerable ecosystems and suffer from various stressors, such as nutrient and contaminant loadings, hydro-morphological alterations, rising temperatures and acidification (EEA, 2018; IPCC, 2019). Most aquatic ecosystems are affected by multiple, co-occurring stressors, which can interact and thereby, change their combined effects on biological communities (Breitburg and Riedel, 2005; Grizzetti et al., 2017; Reid et al., 2019; Schinegger et al., 2016). Conceptually, ecologists distinguish between dominant, additive and interactive (synergistic or antagonistic) multiple stressor effect types (Folt et al., 1999). Interactions can occur when one stressor modifies the effect of the other stressor or modifies the sensitivity of the affected organism to the other stressor. Identifying stressor interactions is particularly important for the design of effective mitigation measures in environmental management, as different interaction types require different management approaches (Côté et al., 2016; Ormerod et al., 2010; Spears et al., 2021). Mitigating a stressor that interacts synergistically with other stressors can have a pronounced positive effect on ecosystem health. For antagonistic stressor interactions, by contrast, the management of a single stressor may lead to further ecological degradation (Spears et al., 2021). Studies investigating the occurrence of stressor interactions have not revealed consistent patterns (Birk et al., 2020; Côté et al., 2016; Jackson et al., 2015; Kroeker et al., 2017), limiting the prediction of multiple stressor impacts.

Several factors that influence the effects of multiple stressors on species communities and ecosystems have already been identified. In particular, the specific stressors and the affected organisms determine multiple stressor effects (Ban et al., 2014; Côté et al., 2016). In addition to characteristic stress sensitivity/tolerance of response organisms, factors such as the level of biological organisation (Beauchesne et al., 2021; Galic et al., 2018; Thompson et al., 2018b), biotic interactions (Kroeker et al., 2017; Thompson et al., 2018a) and adaptive evolution of organisms (Cambronero et al., 2018; Orr et al., 2021; Zhang et al., 2018) can play a vital role. Independent of the stressor pairs and organism groups, framing conditions such as the timing, sequence and duration of stressors (Brooks and Crowe, 2019; Debecker et al., 2017; Jackson et al., 2021; Lange et al., 2018), ecosystem type and spatial scale (Birk et al., 2020) can also be important.

The dependence of effect types on scales suggests that observed effect types are not solely dependent on the environmental setting, but also on the sampling strategy. An increase in scale can be associated with an increase in the size of datasets or the stressor gradient length (e.g. an increase in the temperature gradient length from 15 – 22 °C to 15 – 31 °C). Feld et al. (2016) showed that sample size and stressor gradient in survey-based multiple stressor studies needed to be sufficient to accurately detect the stressor effect type (sample size ≥ 150 and gradient length ≥ 75 % of the full gradient). However, systematic analyses of the role of the stressor gradient length on multiple stressor effects are lacking. Such knowledge is needed to support the conceptual and operational understanding of multiple stressor-effect relationships and the design of novel frameworks in multiple stressor research. Ultimately, this knowledge can improve the prediction of stressor mitigation effects in environmental monitoring, as stressor mitigation often leads to a shortening of the stressor gradient length.

Our overall aim was to elucidate how sample size and stressor gradient length influence observed multiple stressor effects, in order to advance multiple stressor understanding and support environmental management. We collected existing datasets representing 158 cases of stressor pairs affecting plants and animals from rivers, lakes, marine and transitional ecosystems. For each full case (covering the entire stressor gradient), we divided the gradient of the first stressor (the one with the greater effect) into two equal parts, creating a lower and an upper gradient (including lower and higher first stressor levels; Figure 11). To identify patterns of whether and how multiple stressor effects change with sample size and along the first stressor gradient, we examined the changes in multiple stressor effects from the full gradient compared to each of the partial gradients. In addition, we investigated if these changes in stressor effects depended on specific grouping categories, including ecosystem domain, water category, response organism group and kingdom, response category, stressor categories, and effect types of the full cases. We did not formulate specific hypothesis, as we expected effects but the nature of these effects was obscure prior to our analysis and could not be retrieved from the relevant literature.

Materials and Methods

Data collection and characterisation

We searched for primary data on multiple stressors and their biological effects in surface waters to collect paired-stressor response combinations (hereafter referred to as ‘cases’) fulfilling the following criteria: a) data originating from field measurements, b) at least two stressors related to land use and/or climate change, c) more than four stressor levels for each stressor, d) aquatic animals or plants as response variables, and e) lakes, rivers, marine waters or transitional waters (surface water bodies at the transition zone from rivers to coastal areas, which are partly saline and substantially influenced by freshwater flows; European Communities, 2000) as water categories.

We define a stressor as an anthropogenic perturbation to a system which is either unfamiliar to that system or natural to that system but applied at levels exceeding the natural variability (Barrett et al., 1976). Stressors included in this study belonged to seven categories (Table 4): i) nutrient stressors, including concentrations of nitrogen and phosphorus components, ii) thermal stressors, including water and air temperatures, iii) morphological stressors, including morphological modifications of water bodies and their surroundings, iv) hydrological stressors, including modifications of the hydrological regime, v) physico-chemical stressors, including dissolved oxygen, pH, salinity and chloride, vi) toxic stressors, including xenobiotic compounds such as heavy metals or pesticides, and vii) light stressors, including alterations in irradiance.

Response organisms included metrics on five organism groups: i) benthic flora (20 cases), ii) phytoplankton (53 cases; including some specimens of the kingdom *Chromista*), iii) zooplankton (5 cases), iv) benthic invertebrates (61 cases), and v) fish (19 cases). These groups belonged to the categories a) biodiversity metrics, including indices that reflect proportions of taxonomic groups in the community, b) biomass/abundance, including biomass or total abundance measures such as counts, concentrations, density or coverage, and c) functional traits, including absolute or relative abundances of functional groups of phytobenthos, benthic invertebrates and fish.

Data of individual cases are openly available in GitHub at https://github.com/leonimack/Multiple_stressor_gradient_analysis. An overview of the analysed cases and their references is given in the Appendix 3 (Table A3-1).

Modelling multiple stressor effects

The effects of paired stressors on biological responses were determined by linear regression modelling, which has been widely used in studies analysing multiple stressor impacts of aquatic biomonitoring data (e.g. Birk et al., 2020; Ellis et al., 2017; Jackson et al., 2016; Piggott et al., 2015b; Spears et al. 2021; Verbeek et al., 2018). All analyses were conducted in R (version 4.0.3, R Core Team) based on the approach suggested by Feld et al. (2016) to assess the impacts of multiple stressors and the analytical procedure detailed in Birk et al. (2020). The following provides a short overview of the data processing, modelling, model evaluation and statistical synthesis. The codes to run the linear regression model and the gradient split are openly available in GitHub at https://github.com/leonimack/Multiple_stressor_gradient_analysis.

Table 4: Overview of the number of cases with specific stressor combinations. Freshwater cases included lakes and rivers, salt water cases included transitional and marine waters.

	Number of cases	
Paired stressors	**Freshwater**	**Saltwater**
Nutrient | Morphological	41	3
Nutrient | Thermal	23	7
Nutrient | Physico-Chemical	9	20
Nutrient | Hydrological	9	0
Nutrient | Toxic	6	2
Thermal | Physico-Chemical	8	17
Thermal | Hydrological	4	0
Physico-Chemical | Physico-Chemical	1	2
Physico-Chemical | Hydrological	1	0
Physico-Chemical | Light	0	3
Morphological | Hydrological	1	0
Morphological | Toxic	1	0

Data processing included transformation and standardisation of continuous stressor and response variables to a near-normal distribution (centered and scaled to have a mean of zero and variance of one) using Box-Cox transformation (Fox and Weisberg, 2019). We identified the two key stressor variables for each analytical case: In datasets with three to six stressors, we applied the dredge function for automated model selection, identifying the two stressors which provide the best account of the data (Barton, 2020). In datasets with more than six stressors, Random Forest analysis (Liaw and Wiener, 2002) was performed to identify the six most relevant stressors, followed by application of the dredge function. Further, stressor correlation was investigated using a correlation matrix chart (Peterson and Carl, 2020). Cases with a Spearman correlation of ≥ 0.7 were excluded to avoid collinearity problems (Feld et al., 2016).

Linear regression modelling was conducted to identify the effect of each stressor and the stressor interaction term on the biological response. Depending on the nature of stressor and response data, we used generalised linear models (GLM) or generalised linear mixed models (GLMM), following the statistical procedure in Birk et al. (2020). Model evaluation was conducted using the coefficient of determination explained by the stressor effects (marginal R^2). Models with an $R^2 < 0.2$ (weak relationships) were excluded from the analysis.

Classification of multiple stressor effects

Multiple stressor effect types were evaluated using standardised effect sizes (= regression coefficients) and their significance (t-test, $p < .05$; Table 5). Dominance was assigned to cases with only one stressor showing a significant effect. An additive effect was assigned to cases with both stressors showing significant effects. Interaction was assigned to cases with the stressor interaction showing significant effects, regardless of whether the first and second stressor main effects were significant or not.

The type of interaction for interactive cases was classified based on whether the overall stressor effect (sum of effect sizes of both stressors and their interaction) was greater or smaller than the additive stressor effect (sum of first and second stressor effect sizes). Synergistic effects were assigned to cases where the overall effect was greater than the additive effect, and antagonistic effects were assigned to cases where the overall effect was smaller than the additive effect (Table 5).

Table 5: Classification of multiple stressor effect types and interaction types. Classification depends on the standardised effect sizes of the first stressor (b_1), the second stressor (b_2) and the stressor interaction (b_3). For effect types, '**y**' denotes a significant effect (t-test, p < .05), whereas '**-**' denotes a non-significant effect.

	b_1	b_2	b_3	Classification of multiple stressor effect type
Multiple stressor effect type	**y**	–	–	dominance of the first stressor
	–	**y**	–	dominance of the second stressor
	y	**y**	–	additive stressor effects
			y	interaction between stressors
Type of interaction	$\|b_1 + b_2\| < \|b_1 + b_2 + b_3\|$			synergistic interaction
	$\|b_1 + b_2\| > \|b_1 + b_2 + b_3\|$			antagonistic interaction

Gradient split

Each original gradient was split into two 'partial gradients' (Figure 11). Based on the stressor effects modelled using the original stressor gradients, the stressor with the greater standardised effect size was identified as the 'first stressor'. We conducted the gradient split by cutting the transformed data set of the original gradient at the median of the first stressor levels. Thereby, we created a lower and an upper gradient case with similar sample sizes, with the median values included in the lower gradient case. To ensure that the split primarily affected the first stressor gradient, we excluded 36 partial cases where the length of the second stressor gradient was reduced by more than one third. For the remaining cases, the median gradient length of the second stressor was reduced by only 6 %. Therefore, we can expect the changes in multiple stressor effects to be primarily related to the splitting of the first stressor gradient.

Initial analyses indicated that effect types were related to sample size. To rule out the possibility that observed changes in multiple stressor effects were due to the reduced sample size from original compared to partial gradients, we also created full gradients with halved sample sizes (referred to as the 'full gradient' henceforth). This was done by deleting every second measurement along the first stressor gradient of the original cases.

All partial gradients were analysed with the same modelling approach (GLM/GLMM) as for the respective full gradient, to estimate the changes in effect types and effect sizes (see sections 2.2 and 2.3 above). After the gradient split and regression analysis, 158 full cases and 275 partial (137 lower and 138 upper) cases remained for synthesis analysis.

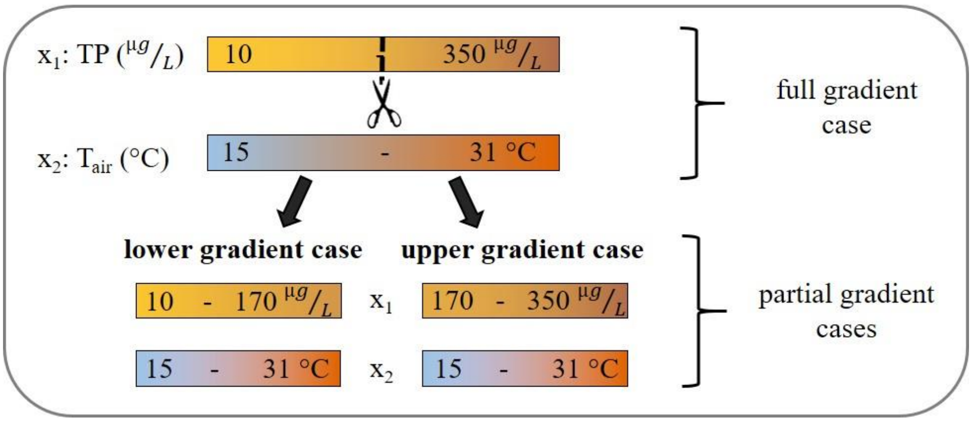

Figure 11: Exemplified gradient split. The full gradient cases covered the entire gradient lengths for the first stressor (x_1) total phosphorus (TP) and the second stressor (x_2) air temperature (T_{air}). Partial gradient cases included the lower or higher levels of the first stressor and (at least two thirds of) the entire gradient length of the second stressor.

Synthesis analysis

To study the dependence on stressor gradient length, we determined the changes in multiple stressor effects from full to partial gradients with similar sample sizes. The following analyses were performed:

1. Correlation between the sample size and effect type, by plotting a correlation chart (Peterson and Carl, 2020) and conducting pairwise Mann-Whitney U-tests.

2. Changes (e.g. from dominance to additive) in effect types or a loss in stressor effect after gradient splitting. A loss in stressor effect was defined as models with an explanatory power below 5 % or without any significant effects.

3. Switches in stressor effect directions, from stimulation to inhibition of the response organism or vice versa.

4. Changes in stressor effect sizes. We conducted a meta-analysis of changes in the standardised effect sizes of both stressors and their interaction in response to the gradient splitting using

OpenMEE software (Wallace et al., 2017). Variance of each standardised effect size was calculated as the product of associated standard errors from GLM(M) and the square root of the sample size, raised to the power of two. Effect sizes of these comparisons and their variances were then computed for each of the stressor/interaction variables from each individual study as the differences between the full and the lower, as well as between the full and the upper gradient. The significance of these comparisons (*Z*-test, $p < .05$) was then tested across all studies and for different grouping categories (see below). Using the same approach, we also compared full gradients to the original gradients (with twice the number of measurements) to investigate if sample size alone affected the effect size. For the meta-analysis on effect size changes, we excluded cases with an explanatory power below 5 %.

5. To support the above analyses with information on increases or decreases in model performance, median changes in the explanatory power (marginal R^2) of models were compared using pairwise Mann-Whitney U-tests.

Finally, we investigated if the above changes in the stressor effects depended on the following grouping categories: a) the first stressor gradient part (lower versus upper partial gradient), b) ecosystem domain (fresh- or saltwater), c) water category (river, lake, marine, transitional), d) response organism kingdom (plants or animals) and group (benthic flora, phytoplankton, benthic invertebrates and fish; excluding zooplankton cases due to their low number), e) response category (biodiversity, biomass/abundance or functional traits), f) first stressor categories (nutrient stressors, thermal stressors, morphological stressors, hydrological stressors, physico-chemical stressors, toxic stressors; excluding light stressors due to their low number), and g) effect types (dominant, additive, synergistic, antagonistic) of the full cases. We tested for significant differences between the grouping categories using chi^2-tests.

Results

After gradient splitting, we found pronounced changes in effect types and sizes. In a consistent pattern throughout all analyses, changes were significantly weaker for plants compared to animals, following the pattern phytoplankton/benthic flora < benthic invertebrates < fish. We therefore focused on differences between these response organism categories. Results regarding other grouping categories (i.e., first stressor gradient part, ecosystem domain, water category, response category, and first stressor category) are only reported in the following if considered noteworthy.

The data presented in the results section can be found in GitHub at https://github.com/leonimack/Multiple_stressor_gradient_analysis.

Influence of sample size on effect types

We found a significant influence of the sample size of the original or the partial gradients on the effect type (Figure 12). Cases with smaller sample sizes generally resulted in dominant effect types, while cases with larger sample sizes resulted in additive and interactive effect types (Kruskal-Wallis test, $p < .05$).

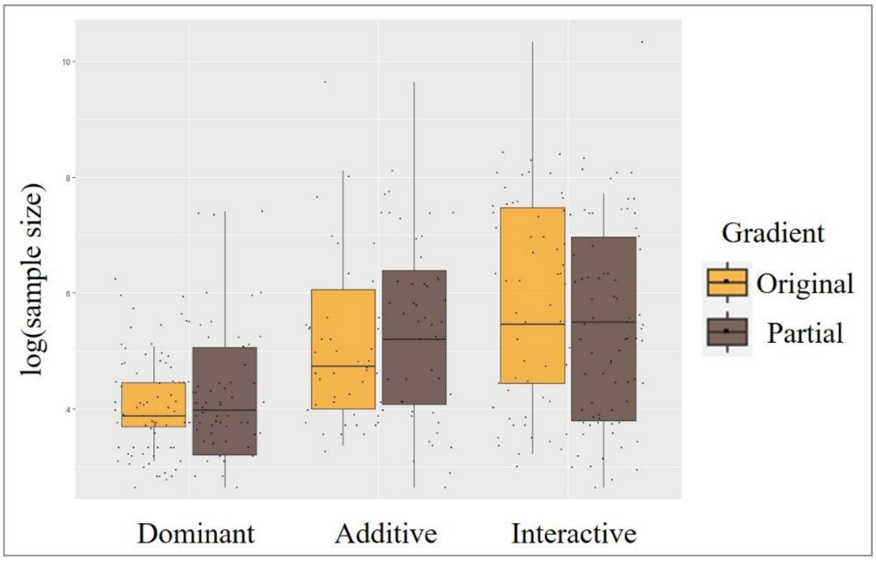

Figure 12: Influence of sample size on effect types. Sample sizes of cases with dominant, additive and interactive stressor effect types, for the original cases before the gradient split and partial gradients (upper and lower partial gradients combined). The sample size significantly influenced the identified effect types (Kruskal-Wallis test, $p < .05$).

Gradient-dependent changes in effect types

From full compared to partial gradients, 40 % of cases showed a change in effect type, 30 % no change and 31 % showed a loss in stressor effects. We did not observe different patterns in effect type changes for the lower versus upper partial gradients (Appendix 3, Table A3-2). The frequency of changes depended on the effect type before the split (chi^2-test, $p < .05$, Figure 13): dominant effect types mainly remained dominant or lost the stressor effect, with 38 % of cases still being dominant after the split, 23 % changing in effect type and 38 % showing a loss in effect. Additive cases changed in effect type most frequently and lost their stressor effects least frequently, with 31 % of cases not changing, 53 % changing and 16 % showing a loss in stressor effects. Synergistic and antagonistic effects mostly changed in effect type: 24 and 19 % remained the same, 46 and 43 % showed a change, and 30 and 38 % lost their stressor effects, respectively.

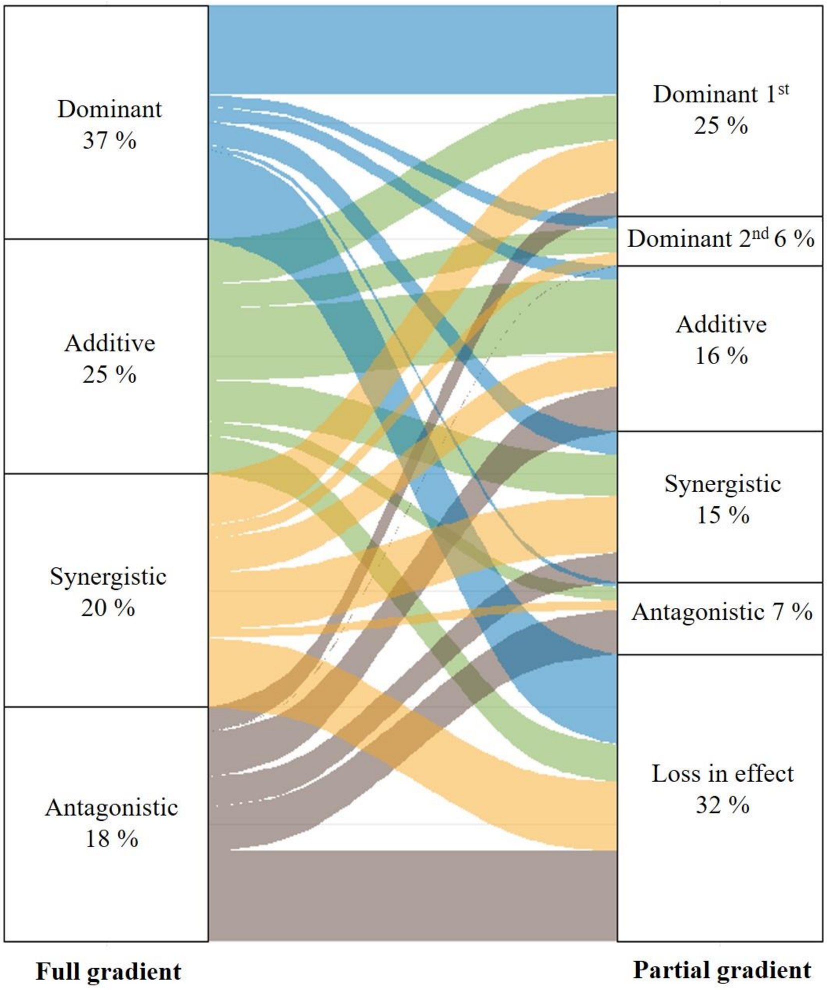

Figure 13: Changes in effect types from full to partial gradients (with both gradients having similar sample sizes). Dominant cases mainly remained dominant or lost the stressor effect in reduced gradients. Additive cases mainly changed in effect type and lost stressor effects with the lowest frequency. Synergistic and antagonistic cases changed in effect type most often, followed by a loss in effect and non-changing cases.

Gradient-dependent switches in effect directions

After gradient splitting, 58 % of cases showed a switch in the direction of at least one stressor/interaction effect from the full compared to the partial gradient. There were significant differences between organism kingdoms, with a switch in stressor direction in 73 % of animal cases and in 41 % of plant cases (Figure 14; chi^2-test, $p < .05$).

The first stressor effects only switched direction when reflecting nutrient or thermal stressors. Cases with physico-chemical, morphological, hydrological and toxic first stressors showed no switches. Moreover, the frequency of switches increased with phytoplankton/benthic flora < benthic invertebrates < fish (chi^2-test, $p < .05$).

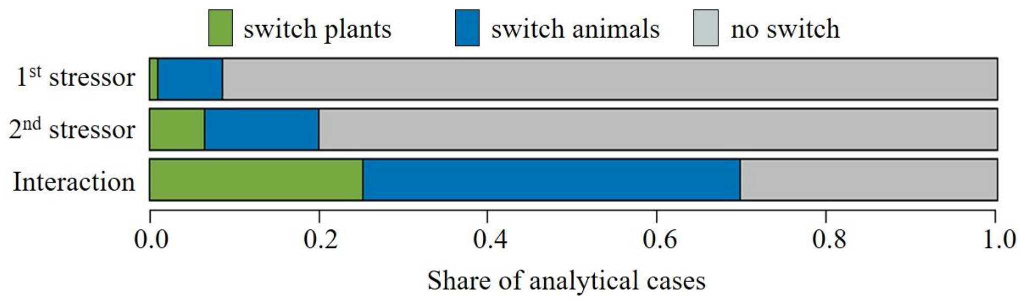

Figure 14: Switches in stressor effect directions upon gradient split. The bars show the proportion of cases with a switch/no switch in the effect direction of the stressors/interaction from full compared to partial gradients. Cases affecting animals account for a higher proportion of switches than those on plants (chi^2-test, $p < .05$).

Quantitative changes in effect sizes

When comparing the effect sizes of the original (all samples included) to those of the full gradients (halved sample size), we found no significant differences. Thus, sample size alone did not influence the effect sizes of the individual studies.

Across all cases combined, the effect size of the first stressor did not significantly change with reduced gradient lengths, though there was a tendency of an increase in effects from the full towards the upper gradient (Figure 15). Effect sizes of the second stressor significantly increased with reduced first stressor gradient, whereas the stressor interaction term only increased from full to the upper gradients (Z-test, $p < .05$).

In general, the changes in all stressor/interaction effect sizes (except for second stressor changes towards the lower gradient) showed the pattern phytoplankton/benthic flora < benthic invertebrates < fish cases for both partial gradients. Changes in the first stressor effect sizes were significant for certain organism groups. From full to lower gradients, the first stressor effect size did not change for plant groups, while it showed a pronounced increase in fish cases. From full to upper gradients, benthic flora cases showed a pronounced decrease, while benthic invertebrate and fish cases showed a pronounced increase.

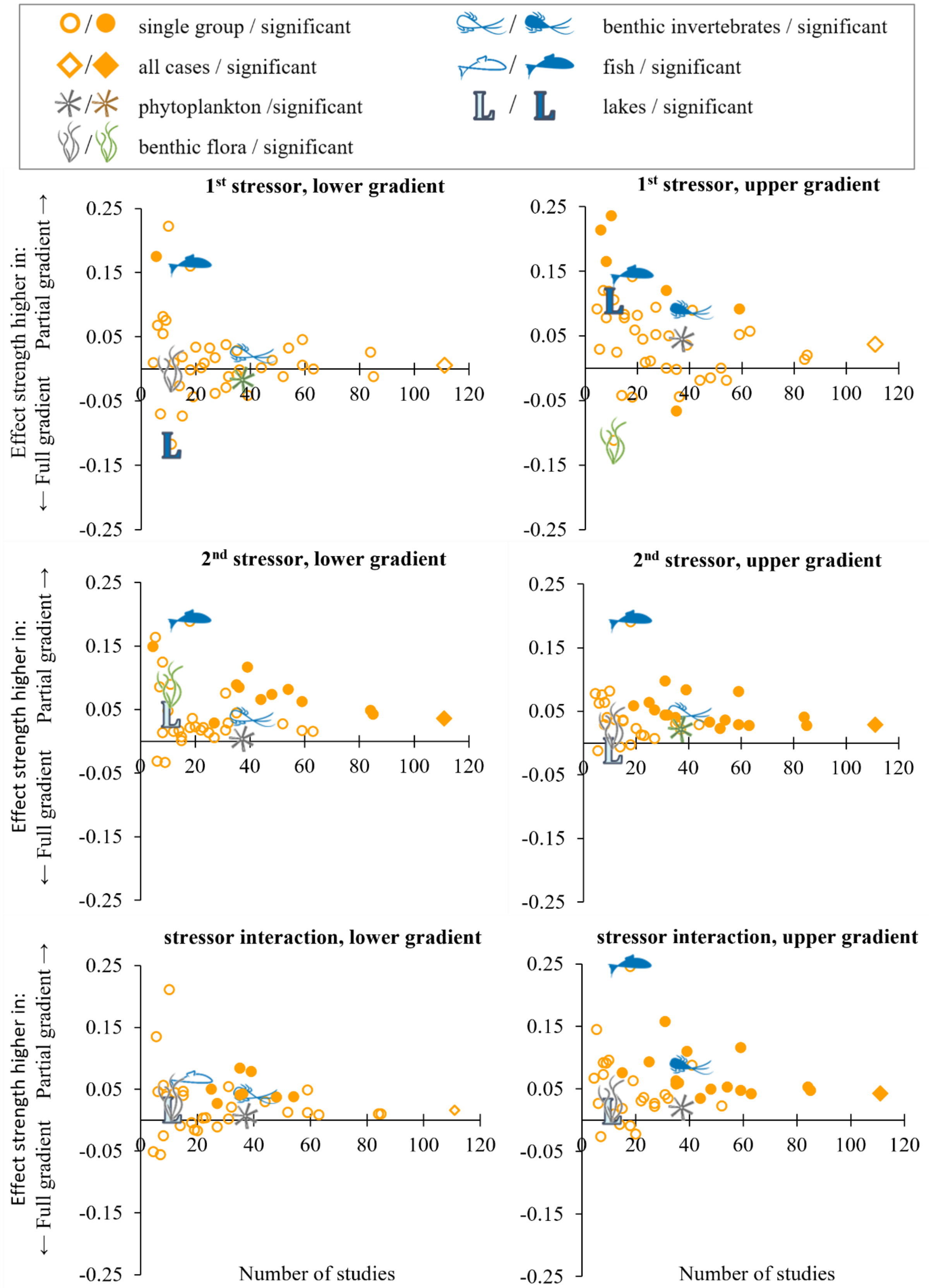

Figure 15: Changes in effect sizes upon gradient split. Changes are shown for the first stressor (top), second stressor (middle) and stressor interaction (bottom). Symbols show the effect sizes of partial gradients minus the full gradients of specific groups (as in the different grouping categories), and thereby indicate if (and by how much) the effect size was stronger in the full (negative values) or the partial gradient (positive values). For example, for the 11 lake cases, the first stressor effect size decreased in lower gradients and increased in upper gradients.

Changes in the explanatory power of models

After the gradient split, the median explanatory power of models decreased from 0.35 to 0.23 of explained variance. The magnitude of this decrease in explanatory power showed no significant differences between any grouping categories.

There were some cases (17 %) where explanatory power increased, but decreases (83 %) were much more frequent (Table 6). Organism groups and kingdoms revealed different patterns: in the lower gradients, the frequency of cases with increasing explanatory power was significantly higher for animal than for plant groups (chi^2-test, $p < .05$).

Table 6: Changes in the explanatory power of models. Shares of cases with an increase or decrease in the explanatory power (R^2) for all cases as well as organism kingdoms and organism groups separately. Significant differences between kingdoms and organism groups are highlighted in **bold** (chi^2-test, $p < .05$).

Cases	Lower gradient		Upper gradient	
	increase	decrease	increase	decrease
All	0.19	0.81	0.15	0.85
Plants	**0.08**	**0.92**	0.14	0.86
Animals	**0.28**	**0.72**	0.17	0.83

Discussion

In general, our findings demonstrate that observed multiple stressor effects in survey-based studies are not only determined by ecological processes, but also by sample size and stressor gradient length. Important implications for resource managers are addressed in the concluding section of our paper.

Effect types often result from insufficient data or the statistical approach

The obvious relationship between sample size and effect type detected in our study highlights the need for careful interpretation of modelled effect types. Definition of effect types based on thresholds of *p*-values can be misleading because *p*-values are correlated with sample size (Greenland et al., 2016; Wasserstein and Lazar, 2016). This relationship was clearly observed in the cases included in our study; consequently, stressor effect types detected in many survey-based multiple stressor studies could be a result of the size of datasets rather than of ecological processes. We therefore agree with the widely cited recommendation that scientists should not rely solely on significance levels and categorical interpretations of effect types, but should put more emphasis on stressor effect sizes (Nakagawa and Cuthill, 2007; Spears et al., 2021).

We controlled for the influence of sample size in all gradient split comparisons by adjusting sample sizes of full and partial gradients. Examining the changes in effect types after gradient split indicated that, for dominant cases, the second stressor did not affect the response variable at all. The majority of cases remained dominant or lost stressor effects when the stressor gradient was split, indicating that strong second stressor gradients were underrepresented in the data. Additive cases showed a large contrast to the dominant ones: A high share of cases changed in effect type, which can be explained by the small difference between first and second stressor effect sizes (Appendix 3, Figure A3-1), as even small changes in effect sizes are likely to lead to switches in the stressor importance, potentially resulting in a changed effect type. Furthermore, there was a low share of cases without stressor effects, which can be explained by the definition of additive cases, where both stressors have to show a significant effect on the response. Therefore, in case the effect of one stressor is lost, the other stressor still shows a significant effect. Interactive cases mainly changed towards dominant or additive effect types, likely due to the loss of specific stressor levels required for detecting stressor interactions (Birk et al., 2020). We conclude that in our analysis, besides true ecological processes, also insufficient data or the statistics underlying the analysis significantly influence changes in effect types.

Switching effect directions point to unimodal stressor effects

More than half the cases showed switching stressor effect directions, indicating non-linear multiple stressor-effect relationships. All these cases concerned either nutrients or temperature as the first stressor. This observation indicates that stressor impact is not always monotonously increasing with stressor intensity, as organisms show bell-shaped tolerance curves for certain environmental variables (e.g. Erofeeva, 2021; Harley et al., 2017). Favourable nutrient concentrations or temperatures stimulate productivity of animals and plants. However, excess nutrients or extreme temperatures can have inhibiting effects, which might result in adverse alterations in food web dynamics and structure due to the loss of sensitive animal and plant species (Odum et al., 1979). In line with Ellis et al. (2017), the empirical data presented in our study demonstrate that subsidy-stress variables such as nutrients and temperature can change in effect direction along their gradients. However, not all the switching cases showed the expected switch in direction for subsidy-stress responses, as the lower and upper gradient cases sometimes showed the same effect direction after the split (e.g. stimulating effect in the full case and inhibiting effect in lower and upper gradients, respectively). This might result from non-linear stressor effects, where more than one switch in stressor direction is present in the full gradient.

Main changes in effect sizes depend on response organism groups

Since we did not find any influence of the sample size on the effect sizes of the individual studies, we can attribute the observed changes in effect sizes to the reduced gradient length. Changes in effect sizes became especially interesting when investigating patterns of single grouping categories. Our findings indicate that along the first stressor gradient (i.e., with increasing first stressor levels), stressor effects on plants decrease, while they increase on benthic invertebrates and even more so on fish. Stressors can disrupt ecological processes governing dynamics of communities (Galic et al., 2018) and following this premise, we interpret the changes in effect sizes to be related to trophic cascading effects between organism groups. Communities are characterised by a network of biotic interrelations among species, which occur within, but also across trophic levels (Bruder et al., 2019; Beauchesne et al., 2021). When interactions between consumers and resources alter communities across more than one link in the food web, this is called a trophic cascade (Kagata and Ohgushi, 2005).

In our analysed cases, the decrease in stressor effects on plants can be an effect of switching stressor importance. For many plant-based metrics, already slight increases in nutrient levels can cause a shift to a new state (Schernewski et al., 2008). With further increasing stress intensity, productivity might still be enhanced, whereas many metrics (e.g. species number, plankton over macrophyte dominance, share of cyanobacteria and chlorophytes) will only change to a minor degree. Animals, in contrast, respond to nutrient enrichment indirectly, e.g. through decreased oxygen concentration at night times or through enhanced food availability that favours few competitive animal species (Diaz and Rosenberg, 2008; Burkholder et al., 2013). Therefore, responses will only be manifested at higher stressor levels (once the plant assemblage has changed to a new state) and will continue with increasing stress levels.

Higher non-linearity in multiple stressor effects for higher trophic levels

The changes in multiple stressor effects indicate that with increasing trophic level, organisms responded to stressors with increasing non-linearity. The changes in effect types, switches in the direction of effects, as well as changes in stressor effect sizes showed the pattern of phytoplankton/benthic flora < benthic invertebrates < fish. Borja et al. (2016) observed a similar pattern when studying the responses of different organism groups to human pressures and management actions: the response of phytoplankton to the changing stressor levels was weak, while benthic invertebrates showed moderate to strong and fish showed strong responses.

Our interpretation is supported by the changes in the explanatory power of models: an increase in the explanatory power can indicate non-linear stressor effects, as the partial stressor gradients better reflect the stressor-effect relationships than the full gradients. Animals, which showed stronger changes in multiple stressor effects, also showed a significantly higher frequency of cases with increasing explanatory power compared to plants. Further, all cases with an increase in explanatory power also showed a change in effect type and/or a switch in stressor direction. The high share of non-linear responses of animal species is in line with observations of Hewitt et al. (2016) and Clark et al. (2021), who also found non-linear responses when analysing land use and climate change impacts on benthic invertebrates.

Non-linear stressor effects can also explain the simultaneous increase in a stressor effect from full to lower and upper partial gradients. In general, we expected the effect size to increase in one gradient part and to decrease in the other, when the stressor effect intensifies or weakens along the first stressor gradient. Increases in both partial gradients might result from non-linear stressor effects, where both partial gradients better reflect the stressor-effect relationships than the full gradient.

Conclusions

Having shown that identified multiple stressor effects are not exclusively inherent to any ecological processes but also depend on how we observe, our study highlights the importance of comprehensive monitoring programmes and adaptive management. Identifying the most prevalent multiple stressor effects is essential for the design of effective mitigation measures, as misguided stressor management can lead to unexpected outcomes and even a worsening of the water bodies' condition (Spears et al., 2021). We have shown that the identified multiple stressor effects can change due to shifts of stressor levels towards the lower or upper stress gradient. As these shifts can be based on the environmental setting and the sampling design, we can draw two important conclusions for management:

i) When based on insufficient data, identified multiple stressor effects in survey-based studies may be incorrect; therefore, monitoring programmes need to be designed to capture the full stressor gradients prevalent in the managed water body.

ii) Changed environmental settings (actual shifts in stressor levels due to stressor mitigation or ecosystem degradation) can affect a change in multiple stressor-effect relationships, thus management actions need to be flexible enough to adapt to them by revising management measures. This especially holds true when management actions address organisms of higher trophic levels, as their responses to changed stressor gradients are more non-linear compared to lower trophic levels.


Discussion

General discussion

Achievements and their relevance

Chapter 1 assesses novel marine monitoring methods regarding their costs and applicability for future monitoring programs (Mack et al., 2020). These include techniques for the *in situ* acquisition of data, such as the Argo Float or Citizen Observation, remote acquisition of data, such as Earth Observation, and techniques for the field or laboratory analysis of data, such as the HydroFIA®pH system and DNA Metabarcoding, respectively. The discussed methods have the potential to significantly improve future monitoring, particularly by facilitating the autonomous collection of real-time data with enhanced spatial and temporal resolution. In addition, methods to monitor ecosystem elements that have not been assessed so far are particularly relevant for emerging stressors such as climate change impacts or underwater noise. Although the study focusses on methods that can fill gaps in Baltic Sea monitoring, all methods are applicable to marine (and in some cases also freshwater) areas worldwide, demonstrating the great impact of the study. Under the light of multiple stressors deteriorating ecosystems worldwide, a comprehensive assessment of the condition of ecosystems is indispensable to pave the road for successful environmental conservation and restoration. After providing environmental managers and decision-makers with an overview on novel methods, it is now up to them to consider those methods and advance monitoring systems as the basis for counteracting global ecosystem degradation.

Chapter 2 examines the combined effects of fine sediment and a novel insecticide, chlorantraniliprole, on organic matter decomposition in streams. Fine sediment and insecticides are common agricultural stressors affecting terrestrial and aquatic ecosystems around the globe and their combined effects on biota are well studied. Chlorantraniliprole, in particular, is a relatively new insecticide and studies examining its interplay with fine sediments and other stressors are lacking. Results from this mesocosm experiment indicate that fine sediment and chlorantraniliprole can inhibit organic matter decomposition and thereby affect important ecosystem functions of surface waters. An interaction of the two stressors was not identified, however, this might rather be due to the study design. By examining a combination of stressors and responses that have not been addressed before, this study gives important insights into the effects of agricultural practices on streams. Understanding the effects of chlorantraniliprole is especially important since it is likely to become more widely used in future agricultural practice due to the increasing ban on neonicotinoid insecticides. Furthermore, most experimental studies address multiple stressor effects on biota, but for a comprehensive understanding of complex stressor effects on ecosystems, also ecosystem functions, such as the organic matter decomposition within streams, need to be studied.

Chapter 3 studies and discusses the role of sample size and stressor gradient length on observed multiple stressors effects (Mack et al., submitted). The finding that both factors play a significant role in the detection of multiple stressor effect types is highly relevant for the scientific community and environmental management, highlighting the need for cautious interpretation of effect types and the need for adaptive management. On the one hand, it calls into question the relevance of the results from previous (meta)analyses that did not take sample size or gradient length into account. On the other hand, it underlines the need to consider these factors in future studies. Observed effect types should therefore not be unquestioningly accepted without an understanding of the underlying ecological mechanisms. In addition, the study presented in *Chapter 3* contributes to the discussion on the use of significance thresholds. Many studies recommend to rethink the use of significance thresholds and focus on effect sizes instead, and this study supports this notion. In general, categorising multiple stressor effects into effect types is very helpful in identifying key stressors to select effective mitigation measures in environmental management. However, it is necessary to constantly review the effects of management measures and eventually revise measures to adapt to changed stressor effects.

Potential and challenges of increasing data volumes

To better understand and predict the interplay and effects of multiple stressors, multiple sources of information need to be combined. The novel methods addressed in *Chapter 1* facilitate the acquisition of a vast pool of environmental data at spatial and temporal scales and resolutions never realised before (Chariton et al., 2015). These observational data provide information on real-world responses to multiple stressors, reflecting whole networks of species interactions (Bruder et al., 2019). Yet, resulting large-scale datasets are usually unbalanced (e.g., spatial and temporal resolution of physical and chemical data is higher than of biological data) and the multitude of different factors and their effects represented in the data can hardly be disentangled (Dafforn et al., 2016). To overcome these hurdles and support the interpretation of large-scale monitoring data, experimental studies addressing specific cause-and-effect relationships remain indispensable, such as the experiment in *Chapter 2*. Although experiments simplify real-world conditions, they are highly valuable in providing insights into interactions of specific stressor combinations and their combined effects on selected response variables (Spears et al., 2021). Furthermore, monitoring and experimental data can be complemented by historical records of environmental variables and expert judgement (Van den Brink et al., 2016). By combining multiple sources of information, their specific disadvantages can be complemented and thus the best possible information content can be extracted from the data. Resulting large data volumes can be used for building ecological models. Models are a widely used tool to understand and predict changes in the environmental condition (Dafforn et al., 2016) as they can be used to directly link human impacts with multiple stressors and changes in the environmental condition. The quality of an ecological model is determined by the quality of data used to build, calibrate and test the model (Dafforn et al., 2016) and thereby, current advances in data acquisition have the potential to greatly enhance model accuracy. This presents a great potential to revolutionise environmental assessment and management in terms of much more precise and faster detection and diagnosis of multiple stressor impacts.

The advances in data collection open up great potential for better understanding of ecological processes, but there are still major gaps to be explored and challenges to overcome. Due to the increase in physical, chemical and biological data, the predictive power of models is continuously increasing. However, modelling the complex interplay of abiotic and biotic ecosystem variables as a basis for ecosystem functions is still a major challenge. Especially quantifying functions over larger spatial and temporal scales is still a difficult, but crucial task for environmental assessment and management (Dafforn et al., 2016). Furthermore, there is a high need for transnational monitoring practices and data storage (Borja et al., 2020). Cooperating monitoring practices across national borders can facilitate a much more effective and cost-efficient acquisition of environmental data, for example by sharing instruments and splitting their costs (as discussed in *Chapter 1;* Mack et al., 2020). Access to acquired data also needs international coordination. To date, environmental data are collected by many individual organisations and thereby stored in many different places and sometimes hard to access (e.g. due to data ownership). This leads to research being slowed down, as it was the case for the study presented in *Chapter 3*, where collecting environmental data was connected to high efforts. There are already projects for transnational data storage, such as the data portal of the International Council for the Exploration of the Sea, where European monitoring data are collected and made available (https://data.ices.dk/view-map). However, collaborative data storage is still insufficient (Borja et al., 2020). Promoting easy and open access to scientific data in order to push research is important, as the great value of high data quantities lies in their collective power (Hampton et al., 2013). To get the best use of globally collected data, data storage and access needs to be better coordinated, requiring institutional collaborations beyond national borders (Borja et al., 2020).

The importance of model selection

Besides the potential increase in model precision due to increasing data volumes, the choice of model plays a key role in environmental research. To study multiple stressor effects and interactions of two co-occurring stressors, many current studies use linear regression models (e.g. Piggott et al., 2015b; Jackson et al., 2016; Ellis et al., 2017; Verbeek et al., 2018; Birk et al., 2020). The approach is common since the identification and interpretation of the single and combined stressor effects is easy and straightforward. Furthermore, the available conceptual frameworks for identifying multiple stressor effects, such as defining effect types, are based on linear techniques. Linear regression is based on the assumption that multiple stressor effects are persistent along the stressors' gradients. It has been known for decades that single stressors can have non-linear effects, such as the unimodal effects of nutrients and temperature, which stimulate plant growth at low levels and inhibit it at high levels (Odum et al., 1979). Likewise, *Chapter 3* demonstrates that the effects of multiple stressors can be non-linear, as the effect types and sizes change with reduced gradient length (Mack et al., submitted). When using linear regression approaches to analyse non-linear (multiple) stressor effects, important stressor effects might be missed. The use of approaches capturing non-linear effects is essential to provide more detailed information about the direction and strength of stressor effects along gradients. Some studies already applied non-linear approaches to identify multiple stressor effects, such as Polynomial Regression (Ellis et al., 2017; Thrush et al., 2008), Boosted Regression Trees (Lemm et al., 2021) or Generalized Additive Models (Pedersen et al., 2019). However, the interpretation of multiple stressor interactions is difficult when using non-linear approaches, as general frameworks are still lacking. In addition, conceptual frameworks to classify stressor interactions are not designed to be applied to non-linear approaches. As such concepts are indispensable for a comparable and unified understanding of stressor effects, re-framing the concepts is necessary when integrating non-linear approaches.

The choice of null models to identify multiple stressor interactions does also have an important, but often neglected influence on study outcomes. Stressor interactions are identified by comparing the joint effect of multiple stressors to a null model that predicts the combined stressor effects without the presence of interactions (= additive effect). When the joint multiple stressor effects deviate from the null model prediction, stressor interactions are identified. Currently, a variety of different null models are used that differ in their underlying assumption on how stressors combine to affect the response variable (Howard and Webster, 2009; Schäfer and Piggott, 2018). This assumption is then reflected in the mathematical definition of the additive effect, e.g. that the effects of the stressors simply add up (simple addition null model) or as the sum of proportional individual effects, distracting their product (multiplicative null model; Howard and Webster, 2009). Thereby, analysing the same dataset with two different null models might result in the identification of two different stressor interactions. This influence of null models is frequently neglected (Griffen et al., 2016) and null models are selected based on statistical convenience rather than knowledge on the mechanisms of potential stressor interactions. Furthermore, Thompson et al. (2018b) argue that the currently used null models often under- or overestimate the occurrence of interactions. As a result, identified interactions may not reflect the actual stressor-effect relationships. Studies addressing this issue propose new null models and emphasise the need to select null models based on a mechanistic understanding of stressor-effect relationships (Griffen et al., 2016; Schäfer and Piggott, 2018; Thompson et al., 2018b), or even the need to stop null model testing as this does not promote the understanding of multiple stressor effects (De Laender, 2018).

Predicting stressor and management effects

Despite decades of research, understanding complex multiple stressor-effect relationships and predicting the impacts of global change to support environmental management is a prevailing challenge in research. In particular future climate change impacts pose a major challenge for management and research, as both the intensity and duration of extreme climate events and their impacts on the environment cannot be reliably predicted so far (Brooks and Crowe, 2019; Easterling et al., 2000; IPCC, 2022). Experimental studies such as the one conducted in *Chapter 2* can give valuable insights into specific stressor-effect relationships by reducing the number of environmental factors included in the study. Based on such insights, variables such as the water body, stressor or response type can allow certain predictions (e.g., that a stressor will not have an effect due to the high dilution potential of the water body or a high tolerance of the response organism). But there is a high number of factors influencing stressor effects on the environment that all need to be identified to be able to accurately predict multiple stressor effects. Factors include not only environmental conditions, such as stressor and response identity, stressor timing and duration, species adaptation or life history stages of response organisms determine stress effect-relationships (Brooks and Crowe, 2019; Lange et al., 2018; Orr et al., 2021; Taherzadeh et al., 2019), but also the sampling strategy (as shown in *Chapter* 3) and statistics underlying the analysis (Howard and Webster, 2009; Schäfer and Piggott, 2018).

So far, there is no approach to capture all these influencing factors in assessing multiple stressor-effect relationships to predict influences on the environment. In this context, the case-specific insights into stressor effects are hardly transferable to other (real-world) cases, so that accurate predictions are hardly conceivable.

Another matter that constrains making predictions is the lack in mechanistic understanding of multiple stressor effects on the environment. The majority of studies are using descriptive approaches to generalise and predict stressor interactions. That is, studies assess the frequency of interactions across different ecosystems and thereby strive to find patterns in order to predict potential stressor interactions in other cases (Birk et al., 2020; Darling and Cote, 2008; Jackson et al., 2016). Such an approach can give hints of potential interactions, but it does not give general insights into the mechanisms by how multiple stressors affect biodiversity or ecosystem functions (De Laender, 2018). To be able to accurately predict stressor effects, a mechanistic understanding of their effects on the environment is needed (Schäfer and Piggott, 2018; De Laender, 2018). Recent studies constructed frameworks for a mechanistic understanding of stressor impacts (De Laender, 2018; Galic et al., 2018; Thompson et al., 2018b). The key of these frameworks is that they have well defined assumptions underlying their predictions, and thereby, deviations from the predictions can give mechanistic insights into stressor effects (De Laender, 2018). In building future research on a mechanistic understanding of stressor-effect relationships, it might be possible to truly predict effects in order to support environmental management towards a better conservation and restoration of aquatic ecosystems.

Future research and management needs

Ecosystem restoration is a central aspect of current governance and research, but successful restoration can be difficult due to a lack in scientific knowledge on recovery pathways. The urgent need to conserve biodiversity and stabilise ecosystem functions is widely acknowledged in global politics. In accordance to this, the current decade (2021–2030) was declared to be the ‘UN Decade of Ecosystem Restoration’. Unfortunately, many restoration efforts yet show little success (Gillis et al., 2017; Spears et al., 2021). There are several reasons for this failure that relate to different sectors, such as missing governance or scientific knowledge (Borja et al., 2020). One reason for failures in restoration measures is that most scientific studies focus on ecosystem degradation, i.e., how increasing stressor levels affect the ecosystem. But for understanding ecosystem recovery, studies on how decreasing stressor levels affect the ecosystem are needed. Since there is a significant lack of such studies, knowledge on the processes governing recovery under real-world conditions is missing. It cannot be estimated to which extent the degradation and recovery pathways match in their underlying mechanisms and thereby, managing ecosystems on the basis of the “reversed” degradation pathway might not end up in a restoration success. For instance, species can adapt to certain stressor levels over time and once this adaptation has occurred, the mitigation of a stressor can itself become a stressor and worsen the condition of a site (Orr et al., 2021, Schäfer and Piggott, 2018). In addition, it cannot be assessed to what extend multiple stressors influence a potential deviation of the recovery from the degradation pathways (Spears et al., 2021).

The necessity for environmental restoration despite scientific knowledge gaps highlights the need for an adaptive environmental management. The condition of the planet is already deteriorated, and the intensity of impairing stressors (such as climate change impacts) are expected to further increase (Spears et al., 2021), which highlights the urgent need for immediate restoration measures. The design of effective mitigation measures based on scientific knowledge is limited due to the lack in the mechanistic understanding of multiple stressor interactions and effects, as well as the lack in studies addressing ecosystem recovery. When dealing with challenges characterised by a high degree of uncertainty, adaptive management is the most important tool in environmental management. The central aspect of adaptive management is to constantly monitor the changes in the condition of the site after applying certain measures (Gregory et al., 2006). Thereby, possible adverse management effects on the specific site can be detected and counteracted by revising the measures (as discussed in *Chapter 3*).

General conclusions

In the light of the deteriorating effects of multiple stressors on global biodiversity and ecosystem functions, ecosystem conservation and restoration is urgently needed. The basis for successful environmental management is a coherent understanding of the relationships between stressors and the environment. Current knowledge on these relationships can be greatly enhanced by the increasing pool of data from environmental monitoring or scientific experiments. The value of these data lies in their collective power and therefore, it is important to share research data and to coordinate data availability beyond institutional and national borders. In hand with the increasing amount of data and the growing number of co-occurring stressors, data analysis to gain insights into stressor-effect relationships is becoming more complex. Since the statistics underlying data analysis strongly determine the findings of research, greater awareness of how model selection, hypothesis testing and the habit of fitting everything into categories' influences obtained results is needed. Therefore, I suggest that each scientific study - from design over implementation to analyses and results interpretation - should be accompanied by a well-trained statistician.

Concerning applied environmental management, I believe it is most important to use adaptive practices. Current monitoring programmes should continuously integrate new technologies that can improve the effectiveness and comprehensiveness of environmental assessment. Management plans for the conservation and restoration of ecosystems under multiple stress should also be adaptive, as the consequences of certain measures cannot yet be fully assessed.

Since decades, scientists have been pointing out the negative consequences of anthropogenic activities for nature. Nevertheless, it took over 30 years and several environmental disasters for these insights to reach the political arena. And yet, there are still never ending discussions and the lack of will to take action, resulting in half-hearted mitigation and adaptation measures to face environmental challenges. It is time to take action now!

For a sustainable and healthy future, environmental conservation as well as climate change mitigation and adaption need to be the core topics in politics. Transnational governance that takes far-reaching measures, even if they might be restrictive, is needed. But also every person is asked to try their utmost to mitigate and adapt to environmental change. As countries of the Global North, the European nations, which have been benefitting from the current system and only moderately affected by the consequences yet, have the social and sustainable responsibility to start to implement these measures.

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Abbreviations

(e)DNA	(environmental) deoxyribonucleic acid
ARMS	Autonomous Reef Monitoring Structure
ASU	Artificial Substrate Unit
b_1	first stressor effect size
b_2	second stressor effect size
b_3	stressor interaction effect size
BSAP	Baltic Sea Action Plan
FUMARI	Future marine assessment and monitoring of the Baltic
G. pulex	*Gammarus pulex*
GLM	generalised linear models
GLMM	generalised linear mixed models
HELCOM	Helsinki Commission
IFC	Imaging Flow Cytometry
IOW	Leibniz-Institut für Ostseeforschung Warnemünde
IPBES	Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services
IUCN	International Union for Conservation of Nature
JRC	Joint Research Centre
L. basale	*Lepidostoma basale*
MSFD	Marine Strategy Framework Directive
MVP	Moving Vessel Profiler
POCIS	Polar Organic Chemical Integrative Sampler
R^2	coefficient of determination
REM	Remote Electronic Monitoring
ROTV	Remotely Operated Towed Vehicle
SIA	Stable Isotope Analysis
UAV	Unmanned Aerial Vehicles
WFD	Water Framework Directive
WWF	World Wide Fund for Nature

Appendices

Appendices

Appendix 1A: Novel marine monitoring methods - Stakeholder surveys

This appendix contains the additional files to Chapter 1: A synthesis of marine monitoring methods with the potential to enhance the status assessment of the Baltic Sea

1. First stakeholder survey

The first stakeholder survey was conducted to get a first estimate on the main gaps in the current Baltic Sea monitoring, and novel methods to fill them. Furthermore, on the base of the replies, the second and more comprehensive survey was designed.

In a FUMARI workshop, a list of the most important stakeholders regarding the marine environmental assessment and its implementation in the Baltic Sea was generated. This list contained ministries and institutions (i.e., Environmental Ministries) in all Baltic countries. The stakeholder survey was sent to this list using the platform survey monkey. The survey was accessible from 24.10. to 05.11.2018.

The survey comprised three questions regarding the gaps in the current Baltic Sea monitoring and novel methods to fill these gaps.

Nine replies were received on the survey (Table A1-1):

- 9 replies (Swedish Agency for Marine and Water Management, SMHI, Finnish Ministry of the Environment (YM), HELCOM, Aarhus University, Klaipeda University, Seanalytics AB).
- Many stakeholders replied that they feel unsure to reply to our survey "because they are not experts", or "not experts in respect to give a holistic view of the entire Baltic monitoring".

  Some replied that gap analyses are ongoing elsewhere.

Table A1-1: Detailed questions and responses

Question 1 **Please list the five most critical gaps of the current Baltic marine monitoring programs with regard to legal requirements. Please list your answers in order of relevance**	
Reply 1	1. There are still gaps in the HELCOM core indicator line-up. 2. The temporal resolution of the monitoring (in situ) in near-coastal island dense archipelago areas is not frequent enough 3. The spatial resolution of the monitoring is too poor to allow for e.g. linkage of water quality to the condition/health of benthic habitats 4. Benthic marine biodiversity is not properly addressed in the monitoring programme, and it has consequences for planning and management of marine areas, and for the Habitats Directive reporting. 5. Marine litter and underwater noise are not monitoring well enough at the moment
Reply 2	1. Monitoring of benthic habitats (other than soft bottom fauna). 2. Structured, fit for purpose, monitoring of species (distribution, abundance...) 3. Opportunistic monitoring (monitor multiple parameters at the same time) to enhance cost effectiveness and efficiency. 4. Monitoring to support MPA management.

Table A1-1 (cont.): Detailed questions and responses

Reply 3	1. Fishing discard ban enforcement 2. Better monitoring of wintering offshore seabirds 3. More focus on indicators of the effects of dangerous substances 4. Continuous monitoring of dissolved oxygen, for model calibration 5. Monitoring of continuous and impulsive noise
Reply 4	1. Extent of benthic habitats habitat quality - in terms of species occurrence on hard bottoms 2. lack in spatial resolution for several parameters (species composition plankton) 3. Introduction of non-indigenous species
Reply 5	1. Lack of taxonomic resolution and taxonomic inconsistency 2. Missing in-situ and remote sensor network for collecting biological data 3. Missing approaches to predict biological patters and processes 4. Missing data (e.g. too sparse spatio-temporal sampling, and absence of trait data to monitor functional status) 5. Missing integration of scientists, monitoring programmes and stakeholders of the biological information generated in the monitoring
Reply 6	1. Monitoring of impacts of harmful substances; 2. Monitoring of marine litter, especially micro-litter and its impacts; 3. Monitoring of impacts of underwater noise; 4. Sufficiently frequent monitoring of water column oxygen conditions as well as carbon system and acidification parameters; 5. Picoplankton and bacterioplankton monitoring.
Reply 7	1. Macrophytobenthos monitoring in the south-eastern Baltic Sea coast and transitional waters (especially in Latvia and Lithuania) is performed relatively seldom (every 3 years), there is a limited budget dedicated for the monitoring and the monitoring sites are not accurately fixed (GPS and a boat drift make position error up to 20-50 m), which makes status evaluation not very clear, especially in very patchy bottoms stretching for 2-4 km along gentle slope till maximum vegetation colonization depth (15-16 m) in the coastal waters. 2. The indicators based on macrophytobenthos for transitional waters have not been successfully tested with pressure gradients in Lithuania, Latvia and Poland. Low relationships between indicators and pressures could be due to low number of samples within a gradient of pressure (e.g. water transparency, chlorophyll a) and/or interaction with other environmental factors such as wave exposure and salinity. 3. There is a lack of indicators for benthic habitats. Several related criteria (species distribution, habitat extent, etc.) have not been developed yet according to the Marine strategy water directive.
Reply 8	1. Too few observations to produce precise indicators 2. Almost no measurements of organic matter 3. Coordination of monitoring activities for eutrophication with higher trophic levels, e.g. fish monitoring 4. Maintaining high quality of monitoring data after consultants take over routine monitoring 5. Harmonisation of monitoring methods for biological parameters across countries or even within countries
Reply 9	1. Funding 2. Spatial coverage 3. Coverage of biological/biodiversity monitoring 4. Low number of intensive stations 5. Few long-term observation stations
Question 2: **Please state, which (if any) novel methods could enhance the Baltic marine monitoring. Please also indicate if you think that this method would improve coverage (e.g. spatial, or in terms of covering important organism groups), cost effectiveness, or reliability.**	
Reply 1	1. EO (Earth Observations) and other remote sensing methods would explode spatial coverage for some basic variables. 2. Automatic sensors and water quality analysers should be more widely used on Freight ships (spatial/temporal coverage), 3. DNA-barcoding may enhance biodiversity monitoring significantly.

Table A1-1 (cont.): Detailed questions and responses

Reply 2	1. Automated systems with sensors + loggers/direct data export would make it possible to overcome the spatial and temporal gaps described in point 2. FI/Baltic Sea states should consider the possibility to join forces in the development of new sensors and automated monitoring systems
Reply 3	Satellite and passive monitoring methods. These allow for higher spatial coverage for lower cost, and can supply several countries with data at once.
Reply 4	Oxygen sensors on gliders. Better use of citizen science for bird data. More amphipod indicator organisms. All would improve data coverage and resolution at little additional expense.
Reply 5	remote sensing & eDNA
Reply 6	In general, I believe we need to promote the use of machines instead of humans to collect data. There are two important advantages in this technology shift. First machines generate much more information; second machine create more consistent information. However, there are many challenges associated with such development, which need to be addressed (e.g. data management and provenance, technological dependencies, etc.). IMHO, methods with potential for use in Baltic monitoring include 1. DNA-based monitoring (incl. metabarcoding, eDNA) 2. Image and sonar-based remote sensing 3. integrated modelling approaches (e.g. combination of mechanistic biophysical models with statistical species distribution models)
Reply 7	Use of autonomous devices for monitoring of pelagic parameters. Most importantly, cost-efficiency will be increased if combine different observational programs (environmental monitoring, operational oceanography, impact monitoring of maritime activities, etc.)
Reply 8	3D acoustic methods could enhance benthic habitat including macrophytobenthos monitoring. However, comprehensive analysis is needed how this method would improve in different aspects. For hard bottom habitat monitoring in a very patchy bottom, development of fixed site monitoring, which could be easily found by divers or ROV and strong enough to withstand very exposed coast to cyclonic waves, may increase the accuracy of measurements.
Reply 9	Scanning FlowCytoMetry for fast and more accurate monitoring of plankton communities. This method is far cheaper than traditional methods and will allow for improved coverage in time and space. DIC, pCO2, pH and total alkalinity measurements. Novel instruments have been developed in recent decades that produce more reliable information on the carbonate system. Autonomous Underwater Vehicles (AUVs) for monitoring benthic vegetation in combination with improved image analysis techniques constitute a more cost-efficient method than today's survey.
Question 3: **Do you think any novel monitoring method could replace an existing one in the near future (1 to 5 years)? If yes, which?**	
Reply 1	Yes/No. I think that the existing empiric data collection probably needs to be kept at the present level, to provide sufficient ground trothing data (of remote sensing data) and as a backup for possible problems that might occur when using data from automated sensor systems. But the data we would get from the new systems would provide a huge improvement in data quality/resolution.
Reply 2	Autonomous gliders should replace CTD via vessel right now, but it takes time to get rid of the RVs
Reply 3	5 years, remote sensing
Reply 4	Nope, I think for the next 5 years we should work towards "integration" of new methods in conventional monitoring programmes. In cases where we see added value of the new methods and redundancy with a conventional method we can phase out the unnecessary method. I believe this may take app. 5-15 years.
Reply 5	I do not believe that novel methods completely replace any existing on in the near future. Instead, the novel methods could increase the reliability and confidence of assessments. And in some cases will reduce the workload spent using classical methods.
Reply 6	It is hard to state if these methods will replace existing ones, but both methods will definitely improve existing monitoring and understanding about changes in the benthic habitats.
Reply 7	FlowCytometry can partly replace phytoplankton microscopy for the quantitative assessment of the plankton community. Novel pH sensors based on colorimetric reactions can replace pH electronic sensors. AUVs with image analysis can replace diver transects.

2. Second stakeholder survey

2.1 Summary:

The second stakeholder survey was conducted to collect detailed stakeholder opinions on the main gaps in Baltic Sea monitoring and methods, which can be used to fill these. Based on the results of the first stakeholder survey, a detailed survey was designed by the FUMARI consortium, to:
a) Specify the shortcomings in the Baltic Sea monitoring regarding the directives MSFD, WFD and BSAP to assess the good status of the Baltic Sea region (including lacks in the regulation by legislation, as well as its implementation).
b) Identify novel methods, which have the potential to supplement and/or replace currently applied methods.
The online survey was designed using Netigate and sent to 42 key stakeholders working in the field of Baltic status assessment (these were identified by the FUMARI project team). Furthermore, the survey was disseminated over various websites and platforms like the websites of the FUMARI project team or in meetings and conferences. The aim was to reach out to as many stakeholders as possible to collect their opinions on this topic. The survey was accessible from 21.03 to 20.05.2019.
We decided to keep the whole survey anonymous, since the first survey indicated that some stakeholders are not willing to give a public statement (since they don't feel like experts or don't have a holistic view on the Baltic monitoring).

2.2 Results:

31 completed survey replies were submitted, most of them from Germany (11) and Sweden (11), followed by Finland (4) and Latvia (2). Estonia, Lithuania and Poland were represented by one reply each. Most of the replies came from stakeholders with their expertise in the Environmental Management working with HELCOM and the MSFD (21) and working with the WFD (7). About half the repliers were working in Baltic Sea research (16).

17 stakeholders replied that "Good status cannot be assessed satisfactory because certain priority areas or pressures in the Baltic Sea marine region are not adequately covered by the existing Descriptors/Quality elements/Baltic Sea Action Plan Objectives" and most of them proposed new thematic categories or stressors that should be observed. Most frequent new thematic categories included dumped munitions, climate change and the damage caused by fishing gear to the sea bottom.

30 stakeholders replied that "the existing indicators used in current Baltic Sea monitoring do not sufficiently cover the assessment of the thematic categories set by existing legislation", 29 of them made suggestions for improvements. The thematic categories mentioned most often were Biodiversity (9), Marine litter (6), Food webs (5) and Sea-floor integrity (5).

Regarding novel methods with the potential to improve Baltic monitoring, stakeholders suggested the Moving Vessel Profiler (MVP), Active Biomonitoring using Blue Mussels, Argo Floats, Gliders, the passive sampler CHEMCATCHER, Unmanned Aerial Vehicles, Earth Observation and DNA barcoding.

Appendix 1B: Novel marine monitoring methods – Method descriptions

This appendix contains the additional files to Chapter 1: A synthesis of marine monitoring methods with the potential to enhance the status assessment of the Baltic Sea

1.1 In situ, research vessel-dependent

1.1.1 Moving Vessel Profiler (MVP)

MSFD Descriptor(s): Eutrophication; Hydrographical conditions

BSAP Objective(s): Clear water; Natural level of algal blooms; Natural oxygen levels

WFD Quality Element(s): Biological quality elements; Hydromorphological quality elements; Physico-chemical quality elements

Quality element(s): Temperature; Salinity; Transparency; Turbidity; Oxygen; Chlorophyll-a

Novel quality element(s): -

Relevant step in monitoring: Field sampling/surveying

Mode of operation: In-situ, research vessel-dependent

Currently applied method(s) replaced/improved: Research vessel-based CTD casts and sampling at fixed stations

The **Moving Vessel Profiler (MVP)** is a free-falling fish, which generates near-vertical profiles of the water column. The fish is attached to a winch on board of a research vessel and operated while the vessel is moving. Once the fish is deployed, it freely descents in the water column and measures data. When it reaches a given depth, it is winched back to the surface and subsequently dropped again for the next profiling cycle (Furlong et al., 2006). The fish falls with a speed of about 3 m/s to a depth between 30 and 3400 m, depending on vessel velocity and profiler model. Profiling frequencies depend on the MVP model, vessel speed and profiling depth. For instance, at the speed of 12 knots, a cycle duration is 1.6 min at 30 m depth and 70 min at 3400 m depth (AML Oceanographic, 2019). The depth of the profiling cycles can be controlled automatically or manually on board and the single cycles can be programmed individually. The measured data are directly transmitted to an on-board computer through an electro-mechanical tow-cable.

For the marine monitoring, the MVP can be operated between stations and therefore increase the number of profiles generated during a monitoring cruise. It generates high-resolution data and therefore provides data on the (sub-)mesoscale. There are two common sensors, which can be mounted on the fish: A sensor to measure water temperature, water pressure, and sound velocity, or a sensor to measure water temperature, water pressure, and salinity. Furthermore, the fish is equipped with a GPS sensor, and additional sensors for the measurement of oxygen and bio-optical measurements have been developed (AML Oceanographic, 2017a; 2017b).

Investment costs:

Very high

400,000 € for personnel training, MVP and sensors.

Monitoring costs:

-

Estimation for one MVP: 10,000 € annually for maintenance and data handling (maintenance ~ 5,000 €; personnel time ~ 80 h/year).

Reliability:

High

Depending on sensors and the indicators being measured. The measurements in the upper water layer cannot be related to a specific water depth, since the upper water level is strongly mixed by the ships' movement.

Environmental impact:

None

No additional ships/vessels are in operation to acquire the data; water-flow-through sampler has no negative impact on the environment or organisms.

Novelty score:

Score 1

Using the MVP, a higher spatial resolution of monitoring data can be achieved. The deployment during a monitoring expedition increases the amount of CTD-profiles without additional vessel operation times. There is no need for stationary vessel time during the measurement, which also saves a lot of time and costs. The mode of operation can be adjusted to specific monitoring interests, defining the intervals and depth of generating profiles.

Limitations:

Moderate

The deployment of the MVP is quite costly in comparison to classical sampling and is dependent on a ship cruise.

Required expertise:

Moderate (trained personnel with specific professional education)

1.1.2 Remotely Operated Towed Vehicle (ROTV)

MSFD Descriptor(s): Eutrophication; Hydrographical conditions

BSAP Objective(s): Clear water; Natural level of algal blooms; Natural oxygen levels

WFD Quality Element(s): Biological quality elements; Hydromorphological quality elements

Quality element(s): Temperature; Salinity; Transparency; Turbidity; Oxygen; Chlorophyll-a; Underwater acoustics

Novel quality element(s): Objects of warfare relicts; Concentrations of explosives and chemical warfare agents in water

Relevant step in monitoring: Field sampling/surveying

Mode of operation: In-situ, research vessel-dependent

Currently applied method(s) replaced/improved: Research vessel-based CTD casts and sampling at fixed stations

The **Remotely Operated Towed Vehicle (ROTV)** is a profiler, which can be operated in three dimensions in the water column. The vehicle is towed to a research vessel, measuring profiles based on its undulating movement within the water column when the vessel is moving. Due to its hydrodynamic shape, it can be operated at high speeds of up to 10 knots. The length of the tow defines the depth of the undulation, which can be up to 360 m with a velocity of up to 1 m/s. The speed of the vessel defines the forward movement of the ROTV. The lateral movement is controlled using a computer on board, reaching 80 m to each side. For real-time data transmission, communication with the on-board computer and energy supply the ROTV is connected to a fibre optic cable (Floeter et al., 2017; MacArtney, 2019).

For the marine monitoring, the ROTV is operated on the vessel between stations and therefore increases the number of profiles generated during a monitoring cruise. It can also be used to obtain high-resolution data in a specific area of interest, since it can be steered in all three dimensions. The ROTV is equipped with a camera and light for the visual control of the vehicle. For measurements, sensors for temperature, salinity, oxygen, currents as well as bio-optical and acoustic properties can be installed. Furthermore, sampling devices can be mounted (MacArtney, 2019). Thus, ROTVs can also be operated for very specific monitoring tasks like the detection and identification of warfare relicts dumped in the sea, and sampling of contaminated water and sediment (Beldowski et al., 2018).

Investment costs:

Very high

350,000 € for personnel training and ROTV (sensors not included).

Monitoring costs:

-

Estimated costs per ROTV: 20,000 € annually for maintenance and data handling for the whole Baltic Sea (maintenance ~ 6,000 €; personnel time ~ 150 h/year).

Reliability:

High

Depending on sensors and the indicators being measured. When deployed correctly, it collects data reliable.

Environmental impact:

None

No additional vessels are in operation to acquire the data, and water-flow-through-sampler has no negative impact on the environment or organisms.

Novelty score:

Score 2

Using the ROTV, a higher spatial resolution of monitoring data can be achieved in comparison to manually sampled CTD-profiles. Due to the three-dimensional control, it can be used to have a close look to transects of interest, for instance, very close to the sea floor or dangerous sites with dumped warfare (Beldowski et al., 2018). Therefore, it can add significantly to the information about the physical and chemical oceanography of these transects.

Limitations:

Moderate

The employment of the ROTV is costly in comparison to classical sampling and is dependent on a ship cruise. It also needs personal operation most of the time. Furthermore, the use of the ROTV to monitor organisms may be limited due to the light, which is necessary to observe the organisms but might influence their behaviour.

Required expertise:

Moderate (trained personnel with specific professional education)

1.1.3 Manta Trawl

MSFD Descriptor(s): Marine litter

BSAP Objective(s): Concentrations of hazardous substances

WFD Quality Element(s): Other pollutants

Quality element(s): Microlitter on the water surface

Novel quality element(s): Microplastic on the water surface

Relevant step in monitoring: Field sampling/surveying

Mode of operation: In-situ, research vessel-dependent

Currently applied method(s) replaced/improved: Research vessel-based grab water sampling

A **Manta Trawl** is a net-based sampling device to collect marine surface microlitter. It resembles an aluminium manta ray around 1.5 m wide, with a mouth opening of about 60 cm. It pulls a net with a usual mesh size of 300 - 333 µm to collect microlitter. The trawl is towed to the side of a research vessel. While the vessel is moving, the Manta is dragged on the surface of the water and collects water with its opening (mouth). The water is filtered through the fine mesh and the litter is stored at the cod end of the net, a removable collecting bag. Two wings keep the trawl in balance with the mouth at about 0.25 m depth in the water. The Manta Trawl can be operated at vessel velocities of 0.5–5 knots. For faster velocities of up to 8 knots, the smaller and lighter high-speed mini trawl can be deployed (Setälä et al., 2016; Tamminga et al., 2018).

For the marine monitoring, the Manta Trawl is deployed while the vessel is moving between monitoring stations. The operation time is around 10 - 30 min, depending on expected microlitter concentration (Tamminga et al., 2018). After recovering the Manta Trawl, the litter is taken from the cod end, fixed and stored. For the quantification of the litter, the trawl is equipped with a flow meter recording the filtered water volume. For further analysis of the collected microplastic, organic material is digested using enzymes and subsequently sorted and quantified using microscopy and spectroscopy (Löder et al., 2017). For a more extensive monitoring of microplastics, the GEMAX Corer offers an efficient method to obtain sediment cores (see method description: GEMAX Corer).

Investment costs:

Low

3,000 € (it is possible to build a Manta Trawl for low costs or borrow it from the "trawl share" program (https://www.5gyres.org/trawl-resources); laboratory equipment for analysis not included.

Monitoring costs (for sampling and analysis of microplastics):

Moderate

55,000 € for annual Baltic Sea-wide monitoring.

Assumption: 400 € for sampling and sample analysis at 3 monitoring stations (consumables for enzymatic digestion ~ 50 €; personnel time for sampling and sample analysis ~ 5 h; Löder et al., 2017; Setälä et al., 2016). When sampling at three sites per WFD coastal water type (414 sites; HELCOM, 2017) once a year, annual monitoring costs are about 55,000 € for the whole Baltic Sea.

Reliability:

High

When the Manta Trawl is correctly assembled, it reliably collects the litter. Microlitter smaller than 300 µm are not sampled, resulting in an underestimation of total concentrations.

Environmental impact:

Low

No additional ships/vessels are in operation, but the subsequent sample treatment is lethal for "bycatch" planktonic organisms.

Novelty score:

Score 2

So far, the monitoring of microplastic is not included in the European monitoring legislations (Kahlert et al., 2019) and the Manta is one of the only methods that has been used for microplastic monitoring worldwide. It enables sampling a large area and therefore covers a water volume sufficient to analyse microlitter present in low concentrations.

Limitations:

Challenging

The operation depends on calm weather and wave conditions to ensure a stable position of the trawl. Particles < 300 µm are not sampled, resulting in an underestimation of total microlitter concentration. Finer nets are not suitable since they easily get clogged. Furthermore, the samples might easily get contaminated with metal and paint particles from the vessel while deployment and recovery of the Manta Trawl.

Required expertise:

Sampling: Low (trained personnel without specific professional education)

Sample analysis: Moderate (trained personnel with specific professional education)

1.1.4 GEMAX Corer

MSFD Descriptor(s): Marine litter

BSAP Objective(s): Concentrations of hazardous substances

WFD Quality Element(s): Other pollutants

Quality element(s): Microlitter in sediments

Novel quality element(s): Microplastic in sediments

Relevant step in monitoring: Field sampling/surveying

Mode of operation: In-situ, research vessel-dependent

Currently applied method(s) replaced/improved: van Veen grab; Nemisto corer

The **GEMAX Corer** is a twin gravity corer to sample the surface of soft sediments. It is deployed from a research vessel, connected to it by a winch on a crane. When released, the corer falls down the water column and vertically cuts into the sediment, driven by its own weight. It is equipped with a closing mechanism to lock the sediment in the system when recovering the corer. The GEMAX Corer is composed of two identical core barrels with a diameter of 90 mm, sampling two replicates. They cut about 50 - 70 cm deep into muddy sediment due to sharpened steel cutters at their ends. The vertical coring is enabled by two fins which hydrodynamically balance the corer and furthermore help closing the core barrels. Back on board, the obtained cores can be sliced into subsamples due to an installed slicer unit (Charrieau et al., 2018, Winterhalter, online).

For the marine monitoring, the GEMAX Corer can be used to sample microlitter, especially microplastic, which is deposited in the sediment. It is deployed from a research vessel, while stopping to conduct measurements at specific monitoring stations. The sampling depth depends on the weight of the corer, the sediment density and the winch speed, which is usually around 1 m/s. Due to its weight, the GEMAX is especially suitable to sample soft mud, which is widespread in the Baltic Sea (HELCOM, 2018). When the GEMAX is back on board, the sediment can be released from the corer barrels in small subsamples due to the slicer unit. Therefore, the shaft is rotated clockwise to release the sediment core into the mounted sub-sampler, which is then cut by a plastic slide (Winterhalter, online). For analysis of microplastic, organic material is digested using enzymes and subsequently sorted and quantified using microscopy and spectroscopy (Löder et al., 2017). For a more extensive monitoring of microplastics, the Manta Trawl offers an efficient method to obtain surface water samples (see method description: Manta Trawl).

Investment costs:

Moderate

20,000 € for a GEMAX Corer including the slicer unit and eight tubes

Monitoring costs (for sampling and sample analysis):

Moderate

70,000 € for annual Baltic Sea-wide monitoring.

Assumption: 500 € for sampling and sample analysis of 3 monitoring stations (consumables for enzymatic digestion ~ 50 €; personnel time for sampling and analysis ~ 7 h). When sampling at three sites per WFD coastal water type (414 sites; HELCOM, 2017) once a year, annual monitoring costs are about 70,000 € for the whole Baltic Sea.

Reliability:

High

The GEMAX Corer obtains sediment cores with a very low level of contamination due to the integrated slicer unit and successful core retrieval is very high in soft sediment. Furthermore, the corer enables sampling of sediment, which is undisturbed, while most other methods, like grabs, mix the sediment due to high turbulence while sampling (Winterhalter, online). The amount of sediment sampled using the GEMAX Corer is lower than many grab methods, but regarding the quantification of microlitter in the sediment this is not problematic, since most of the litter accumulates on the sediment surface.

Environmental impact:

Low

No additional ships/vessels are in operation to acquire the data. Benthic organisms are sampled in addition and the subsequent sample treatment is lethal for them.

Novelty score:

Score 2

So far, the monitoring of microplastic is not included in the European monitoring legislations (Kahlert et al., 2019) and the GEMAX enables a standardised sampling. Microlitter concentration in the sediment is usually relatively low, and the twin corer enables sampling sediment of sufficient quantity for further analysis.

Limitations:

Neglectible

The GEMAX Corer cannot be used in sediment harder than soft mud and the light weight makes it challenging to deploy the corer in rough conditions.

Required expertise:

Sampling: Low (trained personnel without specific professional education)

Sample analysis: Moderate (trained personnel with specific professional education)

1.2 In situ, research vessel-independent

1.2.1 Argo Float

MSFD Descriptor(s): Eutrophication; Hydrographical conditions

BSAP Objective(s): Clear water; Concentrations of nutrients; Natural level of algal blooms; Natural oxygen levels

WFD Quality Element(s): Biological quality elements; Hydromorphological quality elements; Physico-chemical quality elements

Quality element(s): Temperature; Salinity; Transparency; Turbidity; Oxygen; Chlorophyll-a; Bathymetric depth; Underwater acoustics

Novel quality element(s): -

Relevant step in monitoring: Field sampling/surveying

Mode of operation: In-situ, research vessel-independent

Currently applied method(s) replaced/improved: Research vessel-based CTD casts and sampling at fixed stations

The **Argo Float** is a free-floating system, which generates vertical profiles of the water column. Most of the Argos are cylinders of around 180 cm in length, but also spherical models have been developed. They are floating at a set depth, with a maximum of 2,000 m and frequently surface to generate profiles. While they are floating freely, their horizontal range and path are defined by the currents (i.e., passive movement different from Gliders). Argos are moving up and down due to an external oil-filled bladder, which changes the density of the float by expansion and deflation. When surfacing, the Argo measures the vertical profile with a spatial resolution of around 5 m and subsequently transmits the measured data via satellites (Roiha et al., 2018; Siiriä et al., 2018).

For the marine monitoring, Argo Floats can be applied to generate high-resolution vertical profiles of the water column. In general, they profile at 10-day intervals for one year. But depending on monitoring objective, longer or shorter intervals can also be programmed, e.g. generating multiple profiles a day. The floats are equipped with sensors to measure temperature, salinity, and oxygen; newer models also include bio-optical or acoustic sensors. Argo Floats are usually employed in the deep oceans, but they can be easily adjusted for use in the shallow waters of the Baltic Sea (Siiriä et al, 2018). Floats are designed to make about 150 profiling cycles before they are recovered, maintained and deployed again.

Investment costs:

Moderate

30,000 € for personnel training, Argo Float and sensors.

Monitoring costs (for deployment and data analysis):

Low

12,500 € per quality element for annual Baltic Sea-wide monitoring.

Assumptions: Annual maintenance (~ 5,000 €) and personnel time (~ 80 h) result in 10,000 €. For a Baltic Sea-wide monitoring, about 10 Argos need to be deployed, summing up to 100,000 €. Since about eight quality elements are measured, the annual costs to monitor one quality element are 12,500 €.

Reliability:

High

The reliability of Argo measurements is depending on sensors and the indicators being measured, but generally high. For instance, the accuracy of the standard DCT sensor of the Argo Floats is 0.002 °C for temperature, 0.002 practical salinity units for salinity and 0,2 bar for pressure (Sea Bird, 2019). The precision of transmitting the position is around 100 m (depending on number and distribution of satellites). In terms of accuracy in the vertical resolution, Argo float measurements from the upper water layer are expected to be more reliable than research vessel-based measurements, because the vessels cause strong mixing of the water.

Environmental impact:

None

The Argo Float can be deployed and retrieved during regular monitoring cruises which means that no extra ship fuel is needed.

Novelty score:

Score 1

Argo Floats can be deployed to generate a higher spatio-temporal resolution of monitoring. The number of profiles is increased profoundly, since it can provide daily profiles without additional ship operation costs. The mode of operation can be adjusted to specific monitoring interests, defining the intervals to surface, the depth of drifting, or the speed of ascent and descent.

Limitations:

Moderate

It is not possible to program the position of profiling, since the Argos float with the current. Furthermore, the floats need active operator involvement in shallow, well-mixed water bodies like the Baltic Sea (Roiha et al., 2018).

Required expertise:

Moderate (trained personnel with specific professional education)

1.2.2 Glider

MSFD Descriptor(s): Eutrophication; Hydrographical conditions

BSAP Objective(s): Clear water; Concentrations of nutrients; Natural level of algal blooms; Natural oxygen levels

WFD Quality Element(s): Biological quality elements; Physico-chemical quality elements; Hydromorphological quality elements

Quality element(s): Temperature; Salinity; Transparency; Turbidity; Oxygen; pH; Nutrients; Chlorophyll-a; Bathymetric depth; Currents; Turbulence; Underwater acoustics

Novel quality element(s): Warfare relicts

Relevant step in monitoring: Field sampling/surveying

Mode of operation: In-situ, research vessel-independent

Currently applied method(s) replaced/improved: Research vessel-based CTD casts and sampling at fixed stations

Gliders are autonomous underwater vehicles, measuring data to generate profiles at given transects or stations. They are torpedo-shaped and about 180 cm length and move on the surface and within the water column by changing buoyancy due to an oil-filled bladder. With a speed of 20-30 cm/s, they can monitor 30 km/day and have a vertical range of up to 1,500 m. Their sampling frequency is one sample per second, therefore they achieve a high horizontal resolution. Depending on the Glider model, batteries and sensors, they have an average operation time between 20 and 200 days. For data transmission by GPS, they frequently surface and therefore provide near real-time availability of data. Furthermore, individual Gliders can be combined to so-called "fleets" to generate networks of profiles (Brito et al., 2014; Meyer et al., 2016).

For the marine monitoring, Gliders can be applied to generate high-resolution horizontal profiles at a specific transect or vertical profiles at a given station. Depending on the monitoring objective, Gliders can be equipped with sensors to measure temperature, salinity, oxygen, optical properties, currents, nutrients, zoo- and phytoplankton, as well as acoustic sensors and magnetometers (Brito et al., 2014; Meyer et al., 2016). Since they can autonomously move underwater, they can also be operated in ice-covered areas and at harsh conditions. Despite for environmental monitoring purposes, they can also be used for very specific research tasks like the detection of warfare relicts dumped in the sea (Meyer et al., 2016).

Investment costs:

Very high

200,000 € for one Glider line (two Gliders alternating), standard sensors (CTD, oxygen, optical properties) and personnel training (for the whole Baltic Sea, about five Glider lines for 1,000,000 € are needed; estimation based on Rudnick et al., 2012).

Monitoring costs (for deployment and data analysis):

85,000 € per quality element for annual Baltic Sea-wide monitoring.

Assumptions: 200,000 € annually for maintenance, consumables, deployment and personnel when one Glider line is deployed for the year (GROOM 2014), covering about a fifth of the Baltic Sea. For the whole Baltic Sea, 4-5 Glider lines necessary, summing up to 1,000,000 € monitoring costs (estimation based on Rudnick et al., 2012). When twelve quality elements are monitored, this results in 85,000 €.

Reliability:

Problematic

About 40 % of the missions fail due to failures like platform loss or communication defect, but the reliability has increased in recent years and is still increasing (Alenius et al., 2017; Meyer et al., 2016). The accuracy of data acquisition depends on the equipped sensors, but in general, measurements are highly accurate. In terms of accuracy in the vertical resolution, Glider measurements from the upper water layer are expected to be more reliable than research vessel-based measurements, because the vessels cause strong mixing of the water.

Environmental impact:

None

Gliders can be deployed and retrieved during regular monitoring cruises which means that no extra ship fuel is needed.

Novelty score:

Score 1

Gliders can be deployed to enhance the spatio-temporal resolution of monitoring data, since they can autonomously measure at a high frequency.

Limitations:

Challenging

Currently Gliders are mainly deployed from research vessels (reducing the vessel operation time) and not used in autonomous mode. The high rate of failed missions is highly challenging.

Required expertise:

Moderate (trained personnel with specific professional education)

1.2.3 FerryBox

MSFD Descriptor(s): Eutrophication; Hydrographical conditions

BSAP Objective(s): Concentrations of nutrients; Natural level of algal blooms; Natural oxygen levels

WFD Quality Element(s): Biological quality elements; Physico-chemical quality elements; Priority list pollutants; Other pollutants

Quality element(s): Temperature; Salinity; Transparency; Turbidity; Oxygen; pH; Nutrients; Chlorophyll-a; Cyanobacteria; (Zoo)plankton

Novel quality element(s): -

Relevant step in monitoring: Field sampling/surveying

Mode of operation: In-situ, research vessel-independent

Currently applied method(s) replaced/improved: Research vessel-based CTD casts and sampling at fixed stations

A **FerryBox** is an automatic flow-through system for the continuous measurement of water parameters. This system is specially developed for the permanent operation on non-research vessels. While the ship is moving, water enters the FerryBox intake in 3 - 4 m depth. The water flows through the system and passes various sensors, which conduct measurements, and is then released again. The sampling frequency is about 1 sample every 20 seconds, achieving a spatial resolution of about 150-200 m. The software of the FerryBox controls the operation and manages and visualises the data. An integrated communication module enables the remote control and maintenance of the FerryBox, as well as geo-tagged measurements and the data transmission in near-real-time. By an integrated cleaning and anti-fouling system, long-term operation with minimum personnel involvement is possible (EuroGOOS, 2017; Petersen, 2014).

The FerryBox was initially designed to be installed on ferries, which regularly ship their transit routes and volunteer to be so-called "ships-of-opportunity". Therefore, a FerryBox enables the acquisition of long-term time series for the same course, facilitating the monitoring of temporal changes. The standard sensors included in a FerryBox are measuring temperature, salinity, oxygen and turbidity. Various other sensors can be installed to measure multiple parameters like planktonic organisms or nutrients. Furthermore, external analysers and automatic samplers have been developed to be connected to the FerryBox. These enable the sampling and storage of specific water samples or the automatic filtration of, for instance, (zoo)plankton samples for further analyses (Petersen, 2014).

Investment costs:

High

80,000 € for the FerryBox system including sensors (for automatic water sampling 10,000 € and filtration 50,000 € additional).

Monitoring costs (for deployment and data analysis):

Low

24,500 € per quality element for annual Baltic Sea-wide monitoring.

Assumptions: 35,000 € annually for maintenance and data handling per FerryBox transect (maintenance ~ 5,000 €; personnel time ~ 400 h/year). Today, there are seven FerryBox lines in operation (https://www.ferrybox.com/routes_data/routes/baltic_sea/index.php.en), adding up to about 245,000 € annually for the whole Baltic Sea. These can be used to measure about 10 quality elements, thus, costs are 24,500 € annually per element.

Reliability:

High

Data acquisition may fail due to problems with the pump or the sensors, but when maintenance is appropriate, default is negligible. The accuracy of acquired data depends on sensors and the indicators being measured. Compared to traditional research vessel-based samples, correlation for temperature measurements are generally very high (R^2>0.95), for salinity and dissolved oxygen high (R^2~0.69-0.87). Also pH measurements achieve high accuracy (±0.003; Karlson et al., 2016).

Environmental impact:

None

No additional ships/vessels are in operation to acquire the data; water-flow-through-sampler has no negative impact on the environment or organisms.

Novelty score:

Score 1

The routinely monitored routes result in valuable datasets with a high spatio-temporal resolution, recording changes in several water parameters over time.

Limitations:

Neglectible

The calibration and maintenance before and during deployment of the FerryBox is not completely autonomous and needs involvement of trained personnel. Since the intake is in 3 - 4 m depth, only surface water is analysed. Furthermore, the sampling depth and the actual depth of the sampled water cannot be related, since the upper water level is strongly mixed by the ships' movement (Karlson et al., 2016).

Required expertise:

Sampling: Low (trained personnel without specific professional education)

Data handling: Moderate (trained personnel with specific professional education)

1.2.4 Active Biomonitoring with Blue Mussels

MSFD Descriptor(s): Contaminants

BSAP Objective(s): Concentrations of hazardous substances

WFD Quality Element(s): Priority list pollutants; Other pollutants

Quality element(s): Contaminants in water

Novel quality element(s): Explosives and chemical warfare agents in water

Relevant step in monitoring: Field sampling/surveying

Mode of operation: In-situ, research vessel-independent

Currently applied method(s) replaced/improved: Research vessel-based grab water sampling; Water sampling by diving

For **Active Biomonitoring with Blue Mussels**, the bivalves are used as sentinel species in the monitoring of bioavailable pollutants. Therefore, the mussels are translocated to a specific area of interest and, after a certain exposure time, they are recovered to determine their biochemical, physiological and/or organismal response to the ambient water quality. Mussels are filter-feeding sessile organisms that enable monitoring the toxic pollution of a specific location, as they accumulate water chemicals in their tissues (Strehse et al., 2017). Hereby, their soft tissues can be used to determine the time-averaged water pollution, while the shells represent changes over time regarding their whole life span (Schöne et al., 2016).

For the marine monitoring, the Active Biomonitoring with Blue Mussels can be applied in specific locations of interests, e.g. former dumping sites of warfare relicts. Therefore, mussels without former pollution impacts (e.g. from mussel farms), are translocated to the monitoring location. They are placed in cages or nets, which are fixed to a mooring equipped with a lifting body. For assessing the impact of specific pollutants, sets of 15 mussels each are placed along a spatial gradient. For assessing temporal impacts, the mussels can be recovered after different exposure times of 60 - 120 days. For the quantification of aggregated pollutants, gas chromatography - mass spectrometry or liquid-chromatography – mass spectrometry is used. To assess physiological impacts, growth rates and weight gain/loss are calculated (Strehse et al., 2017).

Investment costs:

Low

60 – 1,200 € per mooring, depending on monitoring station. To monitor the 35 current coastal stations for hazardous substances assessment, about 35,000 € need to be invested (for stations, see: http://maps.helcom.fi/website/mapservice/?datasetID=9a958e52-d8fd-4b24-9931-3ab1957ab2e5).

Monitoring costs (for sampling and analysing the stations that are currently used for hazardous substances monitoring):

Low

49,000 € per quality element for annual Baltic Sea-wide monitoring.

Assumptions: 1,400 € per monitoring station (consumables ~ 600 €; personnel time ~ 12 h). The monitoring of all coastal stations, where hazardous substances are currently monitored in the Baltic costs about 49,000 € annually (about 35 stations, see: http://maps.helcom.fi/website/mapservice/?datasetID=9a958e52-d8fd-4b24-9931-3ab1957ab2e5).

Reliability:

High

In comparison to spot-sampling, obtained environmental concentrations are more reliable, since temporal fluctuations are taken into account. The method was shown to reliably and proportionately record changes of bioavailable pollutants occurring in water, but quantification of environmental concentrations is limited due to varying relations between concentrations in water and mussel tissue (Farrington et al., 2016).

Environmental impact:

Moderate

Mussels are actively exposed to pollutants and the subsequent biochemical analysis is lethal for the mussels. Also the moorings may slightly damage the sea floor and divers are needed for the deployment and recovery.

Novelty score:

Score 2

The active biomonitoring using mussels can be used to monitor the occurrence and impact of **bioavailable** pollutants such as warfare relicts. Explosives like TNT and the respective degradation product ADNT or chemical warfare agents like mustard gas (1,4 dithiane) and Clark I (diphenyl arsine, diphenyl arsine oxide) can reliably be detected. Since mussels are sessile organisms, they can be used to monitor specific locations of interest. Furthermore, they are very robust organisms that metabolise pollutants relatively slow while filtering several litres of water a day, offering a tool to detect pollutants in trace concentrations. Since they only accumulate bioavailable pollutants in their tissue and resemble important food sources, entry and bioaccumulation of the respective pollutants in the food web are indicated.

Limitations:

Moderate

In comparison to the chemical analysis of a water sample, only the bioavailable pollutants can be monitored. Mussels can only be deployed in water depths down to 20 m. When the ground is soft and muddy, nets should not be placed on the ground to prevent submergence (Strehse, 2017). Furthermore, the full range of possible contaminant concentrations cannot be determined, since time-averaged concentrations are assessed.

Required expertise:

Sampling: Moderate (trained personnel without specific professional education) using buoys and, when necessary, divers.

1.2.5 Passive Samplers: Chemcatcher and POCIS

MSFD Descriptor(s): Eutrophication; Contaminants

BSAP Objective(s): Concentrations of hazardous substances; Concentrations of nutrients

WFD Quality Element(s): Physico-chemical quality elements; Priority list pollutants; Other pollutants

Quality element(s): Contaminants in water; Nutrients

Novel quality element(s): Explosives and chemical warfare agents in water

Relevant step in monitoring: Field sampling/surveying

Mode of operation: In-situ, research vessel-independent

Currently applied method(s) replaced/improved: Research vessel-based grab water sampling; Water sampling by diving

Passive Samplers are collecting contaminants based on molecular diffusion and sorption to a binding agent (sorbent). In general, the sorbent is placed between two circular membranes, and the chemical composition and selectivity of this disc determines the binding affinity of different chemicals. Several discs are placed in a container with openings for water inlet. The containers are usually made of inert material to limit biofouling. The Passive Samplers are deployed at a specific location and accumulate the contaminants in the surrounding environment over time. After recovery, the contaminants are extracted using a specific solvent, identified and quantified using GC-MS or LC-MS. Thereby, the time-weighted average concentration of contaminants can be determined (Vrana et al., 2005). In this method description, we focus on the devices Chemcatcher (Charriau et al., 2016) and Polar Organic Chemical Integrative Sampler (POCIS; Harman et al., 2012), but depending on monitoring objective, also silicone sheets, semipermeable membrane devices or the diffusive gradients in thin films-technique can be applied. The Passive Samplers can be deployed to detect present contaminants, their temporal trends in concentration levels and their spatial distribution. The type of device should be chosen based on monitoring objective. The Chemcatcher is a universal Passive Sampler, for collecting in-/organic substances of polar or non-polar nature, while the POCIS is selective for hydrophilic organic chemicals. The selectivity of the Chemcatcher is adjusted due to the combination of membranes and Empore discs. The POCIS has two standard configurations for collecting polar organics in general or solely pharmaceuticals. Both samplers work well for the monitoring of dumped munitions and chemical warfare (Belden et al., 2015; Lotufo et al., 2019). The samplers are placed under water, and attached to an anchor and a buoy to stay at a specific height. After a deployment time of 14 days to one month (for the POCIS up to two month), they are recovered for further analysis.

Investment costs:

Low

700 € per Passive Sampler, 1,000 - 3,000 € per deployment system (depending on monitoring station)**.** To monitor the 327 Baltic sites where chemicals warfare materials were found in the past, 1,000,000 € need to be invested.

Monitoring costs:

Moderate

55,000 € per quality element for annual Baltic Sea-wide monitoring.

Assumptions: 500 - 600 € for sampling and sample analysis per monitoring station with three replicates (consumables 130 - 240 €; personnel time ~ 5 h). Monitoring of the two main dumping sites of munitions and chemical warfare agents in the Baltic Sea; the area of the two sites is about 10.000 km^2. When the samplers are deployed with a spatial resolution of 100 km^2, 100 samplers are needed and therefore, costs are about 55,000 € annually. The main dumping sites can be seen here: http://maps.helcom.fi/website/mapservice/?datasetID=9a958e52-d8fd-4b24-9931-3ab1957ab2e5

Reliability:

High

In comparison to spot-sampling, the reliability of obtained contaminant concentrations is much higher, since temporal fluctuations in concentrations are taken into account. Furthermore, water concentrations below the detection limit can be concentrated to measurable levels. The performance of the Chemcatcher and POCIS depend on the family of sampled substances, but they are proven to have a high utility for contaminant monitoring (Charriau et al., 2016; Harman et al., 2012).

Environmental impact:

Low

The anchor might damage the sea floor.

Novelty score:

Score 2

Due to integrated samples, Passive Samplers provide a more representative picture of the long-term environmental conditions than spot-samples. They can be used to assess dumped munitions and warfare agents, which is not done in the currently applied monitoring system of the Baltic Sea.

Limitations:

Neglectible

The deployment is limited to areas without risk of removal or damage by humans, animals, or bad weather events. Furthermore, the full range of possible contaminant concentrations is not determined, since the samplers provide time-averaged concentrations.

Required expertise:

Sampling: Low (trained personnel without specific professional education)

Sample analysis: Moderate (trained personnel with specific professional education)

1.2.6 Artificial Substrates: ARMSs and ASUs

MSFD Descriptor(s): Biodiversity; Non-indigenous species; Eutrophication; Seafloor integrity

BSAP Objective(s): Clear water; Thriving and balanced communities of plants and animals

WFD Quality Element(s): Biological quality elements

Quality element(s): Hard-bottom species

Novel quality element(s): -

Relevant step in monitoring: Field sampling/surveying

Mode of operation: In-situ, research vessel-independent

Currently applied method(s) replaced/improved: Cover estimates and sample collection by divers, either directly or by photography

Artificial Substrates are sampling devices mimicking complex habitats to collect biological communities for further analysis of biodiversity. Artificial Substrates for hard bottom habitats show properties similar to rocky habitats, coral reefs or large algae substrata, and therefore attract colonising organisms like microorganisms, algae and invertebrates, which settle on their surface. To mimic rocky habitats, the Autonomous Reef Monitoring Structure (ARMS) consists of several PVC squares, stacked and connected with stainless steel struts. In contrast, the Artificial Substrate Unit (ASU) is made of four nylon sponges, bound together with cable tie and attached to a stainless-steel stake or ring, to resemble soft corals or sponges (Cahill et al., 2018; Pennesi et al., 2017). The plates, pores and mesh layers provide habitat and substrate for biota (DEVOTES, 2013). After colonisation, the Artificial Substrates are recovered, and the organisms are used for traditional taxonomic and genomic analysis (Cahill et al., 2018; Pearman et al., 2016).

For the marine monitoring, the ARMSs and ASUs are installed on flat and hard substratum on the sea floor. They should be placed at locations where they are exposed to sunlight and currents in about 10-15 m water depth. Furthermore, three replicates with a distance of 2 - 5 m should be deployed for each substrate. The colonisation process can be controlled regularly by taking photographs. ASUs are colonised by mainly macroinvertebrates, ARMSs by microbes, macroalgae and macroinvertebrates (DEVOTES, 2013; Pennesi et al., 2017). After 18 - 36 month, they are recovered, ensuring the retention of all colonising organisms. Therefore, the ARMSs are covered with a 100 µm mesh lined in a crate before dismantling them. The ASUs are gently covered with a bag before they are detached from the ground and recovered. The colonising organisms are removed from the substrates prior to further taxonomic and genomic analyses (DEVOTES, 2013).

Investment costs:

Low

450 / 15 € for building three replicates of ARMS / ASU (DEVOTES, 2013).

Monitoring costs:

Low

36,000 € for annual Baltic Sea-wide monitoring.

Assumptions: 2,500 € for 3 replicates of ARMS and ASU (personnel time ~ 33.5 h: 7 h for installation, 6.5 h for recovery, 20 h for processing the samples for further analysis; DEVOTES, 2013). Monitoring of three sites per subbasin in the Southern Baltic (hard-bottom habitats are not very common in the South) and five sites per WFD coastal water body in the Northern Baltic (hard-bottom habitats more common in the North) add up to 43 stations. ARMSs and ASUs are deployed for three years, thus 36,000 € annually.

Reliability:

High

Using Artificial Substrates increases the reliability in comparison to spot samples, since they collect integrated samples over a long time period, which reflects the benthic communities more consistently. About 20 % of ASUs and 5 % of the ARMSs are broken or lost due to ships anchors or strong waves (DEVOTES, 2013).

Environmental impact:

Low

The Artificial Substrates offer an additional habitat and therefore positive effect for marine colonising organisms and their reproduction. But the subsequent taxonomic and genomic analysis is lethal. Furthermore, the flora and fauna on the surface of ARMS installation is destroyed, but the Artificial Substrates replace the removal of natural habitats from the sea floor.

Novelty score:

Score 2

The use of Artificial Substrates enables the standardised sampling and therefore comparable monitoring data across countries. Compared to the spot-sampling by diving, the Artificial Substrates collect organisms over 2 - 3 years and therefore give a more comprehensive picture of the hard bottom communities, increasing the temporal resolution of the monitoring data.

Limitations:

Moderate

The sampled community might differ from the actual benthic community, resulting in an underrepresentation of some taxonomic groups like macroalgae, while some faunal fractions, such as amphipods, are well sampled. The deployment and recovery are relatively time consuming, since divers need to install and dismantle the Artificial Substrates. Furthermore, they (especially the ASUs) might get damaged or lost due to fishing activities and strong waves during deployment.

Required expertise:

Sampling: Low (trained personnel without specific professional education)

Sample analysis: Moderate (trained personnel with specific professional education)

1.3 Citizen Observations

1.3.1 Citizen Observations

MSFD Descriptor(s): Eutrophication; Hydrographical conditions; Marine litter

BSAP Objective(s): Clear water; Natural level of algal blooms; Natural oxygen levels

WFD Quality Element(s): Biological quality elements; Other pollutants

Quality element(s): Temperature; Salinity; Transparency; Turbidity; Oxygen; pH; Algal blooms; Marine litter; Water level; Macrophytes; Ice cover

Novel quality element(s): Beach litter; Marine litter near shore; Jellyfish occurrence

Relevant step in monitoring: Field sampling/surveying

Mode of operation: Citizen Observations

Currently applied method(s) replaced/improved: (Research vessel/boat based) observations by consultants, employees or experts

In **Citizen Observations**, voluntary observations are made by non-professional observers. Coordinated by researchers, engaged citizens are integrated into science, in this case in environmental observations. There are two different kinds of data collection: Opportunistic data collection means the automatic sensor sampling when the device matches specific requirements, for instance, the recognition of a mobile phone at a certain location. Participatory data collection, which is in the focus of this method description, means the active observation and data transmission by citizens. This participatory approach includes the observation of various environmental phenomena, which are transmitted to specific platforms using the smartphone or computer. These websites are used to coordinate and focus the observational activities. The researchers can design them based on their data needs, specifying places and parameters they need more data for. Citizen Observation results in big data systems, since the observations of millions of citizens lead to large-scale data volumes for subsequent analysis (Palacin-Silva et al., 2016).

For the marine monitoring, different campaigns and programmes have been established on local scale. In the following, some exemplary programs are outlined, which can be implemented to the whole Baltic Sea environment:

To acquire data on transparency and turbidity, citizens are conducting Secchi depth measurements using purchased or homemade discs. The data on Secchi depth, the location and time of observation are then uploaded onto a database and further analysed.

For the enhancement of phytoplankton data, a so-called “algae barometer” has been set up in Finland. In addition to weekly algae observations by researchers, citizens are engaged to estimate the occurrence of phytoplankton and share their observations on the regarding platform.

Several local, national and European wide campaigns were started to collect data on the number of large particles of marine litter near the shore, for instance, “Marine Litter Watch” (https://www.eea.europa.eu/themes/water/europes-seas-and-coasts/assessments/marine-litterwatch) on European level or “SiistiBiitsi” in Finland. Here, single observations on the amount of beach litter are collated. Furthermore, beach cleaning sessions can be organised, including underwater litter removal by recreational divers. In the course of these cleaning sessions, the amount of collected litter is also quantified and reported to the respective websites.

Investment costs:

Low to Moderate

2,500 - 15,000 € to set up, launch publicly and start the database, the Citizen Observer web services and management activities (depending on investments in marketing, training and citizen engagement).

Monitoring costs:

Very low

600 € per quality element for annual Baltic Sea-wide monitoring.

Assumptions: 1,000 – 8,000 € annually for data review and web services (personnel time 10 – 100 h for data review and ~ 10 h for web services). No costs for data acquisition. Taking 8,000 for high-effort, divided by 14 parameters, results in 600 €.

Reliability:

High

Due to the high mass of single observations, reliability can be considered high. Citizen Observations are well correlated with expert observations, but they are biased towards higher values: Citizens do not tend to report, if there is no nuisance occurring.

Environmental impact:

Environmental benefit

Citizens have no negative impact on the environment while observing and in case of beach cleaning sessions for marine litter observations, they have a positive impact by removing litter.

Novelty score:

Score 2

Using Citizen Observations, the spatio-temporal resolution of monitoring data can be enhanced significantly due to the high amount of data. Much more locations are potentially available for the monitoring, including distant areas. Furthermore, the engagement of the citizens may result in a rise of the public awareness on marine ecosystem issues and therefore more environmental friendly lifestyles.

Limitations:

Moderate

The availability of observations is very uncertain without local organizing effort. Some locations might become oversampled and others not sufficiently sampled, as the distribution of active volunteers is not random. The management of Citizen Observation is immature and needs further development: Information services for indicating time and place of required observations are needed.

Required expertise:

Moderate (trained personnel with specific professional education) for setting up and coordinating web services

1.4 Remote sensing

1.4.1 Unmanned Aerial Vehicle (UAV)

MSFD Descriptor(s): Biodiversity; Eutrophication; Commercial fish and shellfish; Hydrographical conditions; Contaminants; Marine litter

BSAP Objective(s): Clear water; Concentrations of hazardous substances; Concentrations of nutrients; Healthy wildlife; Natural level of algal bloom

WFD Quality Element(s): Biological quality elements; Physico-chemical quality elements; Other pollutants

Quality element(s): Temperature; Transparency; Turbidity; Chlorophyll-a; Ice cover; Macrofauna; Mineral oil; Macrolitter

Novel quality element(s): Surface macrolitter

Relevant step in monitoring: Field sampling/surveying

Mode of operation: Remote sensing

Currently applied method(s) replaced/improved: Research vessel-based CTD casts and sampling at fixed stations; Manned aircraft surveys

Unmanned Aerial Vehicles (UAVs), commonly known as "drones", are measurement platforms collecting data while flying over the area of interest. For ecological research, small and light UAVs (<20 kg) are utilised, of which two main types are available: fixed-wing and multi-rotor systems. Depending on type and design, they have differing energy efficiency, flight duration and image stability. In general, UAVs can fly for 15-40 minutes with a speed of 2-25 m/s, covering areas of up to 40 km^2 per flight. For the operation of an UAV, a ground control station and a link for communication and data transmission are required. For the visual operation of the UAV, a coarse resolution video is transmitted in real-time via satellite. Higher resolution or hyperspectral imagery is downloaded back at the ground (Colefax et al., 2018; Setlak et al., 2019).

For the operation in marine and coastal environments, different types of UAVs are deployed. On the open sea, there are higher demands for flight duration and range than in coastal zones, where distances are generally shorter. Furthermore, in the open sea operation the vehicles are usually programmed to autonomously measure data at a given transect or station. In coastal areas, the UAVs are generally remotely controlled by an operator within visual range. Depending on monitoring objective, the UAVs can be equipped with a wide range of sensors, which can be configured separately. Currently, the most used sensor technology are different types of cameras ranging from low-resolution video and photographic imaging to hyperspectral cameras. Another highly used instrument group is the LiDAR (light detection and ranging) sensors, which perform laser scanning (Colefax et al., 2018).

Investment costs:

Low to High

500 € - 100,000 €; platform prices are highly varying.

Monitoring costs:

-

60,000 € annually for monitoring of a 50 ha transect, when visited monthly (Matese et al., 2015).

Reliability:

High

Depending on sensors and the indicators being measured. In terms of accuracy in the vertical resolution, UAV measurements from the water surface are expected to be more reliable than research vessel-based measurements, because the vessels cause strong mixing of the water.

Environmental impact:

None to Low

Especially multirotor systems have relatively high noise emissions.

Novelty score:

Score 1

Compared to research vessel-based sampling and satellite surveys, the use of UAVs generates a higher spatial resolution of data.

Limitations:

Challenging

The operation of UAVs is limited due to their relatively short operating times and civil aviation restrictions, which typically demand an operation within visual range. Environmental conditions like sun glare, turbid water and harsh weather conditions may hinder operation or sight and, thus, data collection (Zeng et al., 2017).

Required expertise:

Moderate (trained personnel with specific professional education)

1.4.2 Earth Observation (EO)

MSFD Descriptor(s): Eutrophication

BSAP Objective(s): Clear water; Natural level of algal blooms

WFD Quality Element(s): Biological quality elements; Physico-chemical quality elements

Quality element(s): Temperature; Salinity; Transparency; Turbidity; Chlorophyll-a, Surface algal blooms

Novel quality element(s): -

Relevant step in monitoring: Field sampling/surveying

Mode of operation: Remote sensing

Currently applied method(s) replaced/improved: Research vessel- and buoy- based manual water sampling and subsequent laboratory analysis; Secchi disk measurement; Temperature measurement using various devices

In this context, **Earth Observation (EO)** means the use of satellites for the remote sensing of biological and physico-chemical properties of the upper water layer. In general, satellites monitor optical parameters of the earth's surface and the atmosphere by observing the sunlight reflected by molecules and particles. Different substances affect the way sunlight is reflected from the ground surface and captured in satellite images. Thus, also the reflection above and within the upper water layer can be recorded to obtain information about its properties. Using EO, satellite images with spatial resolutions of 60 m to 1 km and temporal resolutions of daily to weekly measurements are generated. These data can be analysed using bio-optical models to generate maps of water quality indicators (Attila et al., 2018; Harvey et al., 2015).

Europe-wide satellite missions are performed by the ESA and the NASA, which are offering free access to their obtained data. The deployed satellites measuring water quality parameters are Sentinel-2 carrying a Multi Spectral

Instrument (MSI) and Sentinel-3 carrying an Ocean and Land Cover Instrument (OLCI) and a Sea and Land Surface Temperature Radiometer (SLSTR). These obtained data on wavelengths are first rectified to the specific coordinate system and then converted into water quality indicators. The conversion is generally based on bio-optical modelling (inversion reflectance modelling). These models are developed utilising spectral absorption and scattering properties of the respective substances
surface litter, but these still have to be further developed to be applicable (Anttila et al., 2018; Attila et al., 2018).

Investment costs:

Low - Very high

5,000 € for setting up a tailored data management system for the long term use per WFD coastal waterbody and 600,000 € for the whole Baltic Sea (Attila et al., 2013; 2018).

Monitoring costs:

Moderate

50,000 € per quality element for annual Baltic Sea-wide monitoring (Attila et al., 2013; 2018).

Reliability:

High

About 70% of the satellite images cannot be used due to cloudiness, but this is irrelevant due to the huge number of observations. Depending on indicator monitored, the correlation between manual sampling and EO results is around R^2 = 0.68-0.84, for surface temperature this correlation is very high (R^2 = 0.98; Attila et al., 2018).

Environmental impact:

None

No direct impact from the measurement.

Novelty score:

Score 1

EO offers the possibility to profoundly increase the spatial coverage and temporal resolution of monitoring data, since millions of observations within the assessment period and area are obtained.

Limitations:

Neglectible

The use of satellite images is limited by several meteorological and geographical conditions. These include ice and cloud cover, low water depth, as well as low angles of the sunlight.

Required expertise:

High (high expertise and special skills required)

1.4.3 Remote Electronic Monitoring

MSFD Descriptor(s): Biodiversity; Commercial fish and shellfish; Food webs

BSAP Objective(s): Thriving and balanced communities of plants and animals; Viable populations of species

WFD Quality Element(s): Biological quality elements

Quality element(s): Fish and shellfish; Fisheries bycatch

Novel quality element(s): -

Relevant step in monitoring: Field sampling/surveying

Mode of operation: Remote sensing

Currently applied method(s) replaced/improved: Interviews with fishermen; Patrol vessels; Dockside monitoring and landings; Aerial surveillance; On-board observers; Vessel monitoring systems using satellite communication; Self-reported declarations; Reference fleets

In **Remote Electronic Monitoring (REM)**, video and sensor technology are combined to provide a comprehensive overview on the fishing activity and catch handling on fishing trawlers. Therefore, the REM system includes: a GPS receiver to track position, speed and direction of the vessel; hydraulic pressure and winch rotation sensors to detect fishing activities due to gear usage; a set of digital cameras for observing the fishing and crew activity; a user interface to control and coordinate the devices. Hereby, the GPS and sensors are used to detect the location and time of fishing activity, while the cameras enable the observation of the fishing and therefore detection, identification, and quantification of bycatch, and retained and discarded fish. Data are transmitted in real-time to a ground monitoring station using satellites, except video data, which are stored on portable hard drives until landing (Kindt-Larsen et al., 2012; WWF 2015; 2017).

For the marine monitoring, the REM system can be installed on fishing trawlers to quantify bycatch and drowned water birds and mammals in fishing gear, as well as to determine fishing mortality and stock sizes. The system can record continuously or temporarily when fishing activity occurs, for instance by detecting the usage of the winch. One of the cameras on board is always positioned to view the net and therefore detect bycatch. Other cameras record the catch sorting, discards, and fishery overview. Back on land, these collected data are reviewed to quantify catches and discards and subsequently assess stock sizes and support fisheries management (Bartholomew et al., 2018). The analysis of the REM data can be coupled to Machine Learning to identify and quantify the caught organisms.

Investment costs:

Moderate

13,000 € for the installation of all instruments on one trawler (WWF 2017). For equipping a fleet of 60 trawlers (average size of Baltic countries), 780,000 € need to be invested.

Monitoring costs:

Very high

1,116,000 € per quality element for annual Baltic Sea-wide monitoring.

Assumptions: 4,000 € annually for maintenance and personnel per vessel (maintenance ~ 2,000 €; personnel time ~ 28 h; WWF 2017). All registered trawlers, which are larger than 12 m, are monitored using the REM. There are 558 trawlers in the Baltic Sea (ICES 2018), resulting in 2,232,000 €. Since fishing activity and bycatch are monitored, each quality element costs 1,116,000 €.

Reliability:

High

The detectability, identification and quantification of catch, discards and bycatch is more reliable than conventional methods, since they are not limited by weather conditions, time, or expert knowledge of on-board observers (Bartholomew et al., 2018; WWF 2017).

Environmental impact:

Environmental benefit

No additional ships/vessels are in operation to acquire the data. The practice of fishing might be enhanced, since the surveillance deters non-compliant activities (WWF 2017).

Novelty score:

Score 1

REM was determined to be the most cost-efficient option to monitor fishing activities at the sea, achieving full coverage. In comparison to conventional methods, less personnel time is needed. In addition, the practice of fishing is enhanced, since the surveillance deters non-compliant activities (WWF 2017).

Limitations:

Challenging

The REM system does not gather data on species, which are not commercially fished. Furthermore, it should be installed on all fishing trawlers, otherwise the fishing crews could feel treated unequally and commercial disadvantages might arise. Another impediment is the acceptance by fishermen to be observed (WWF 2017).

Required expertise:

Low (trained personnel without specific professional education)

2. Methods for sample analysis

2.1 Field analysis

2.1.1 HydroFIA®pH

MSFD Descriptor(s): Biodiversity; Eutrophication; Hydrographical conditions

BSAP Objective(s): Natural oxygen levels

WFD Quality Element(s): Physico-chemical quality elements

Quality element(s): pH

Novel quality element(s): -

Relevant step in monitoring: Sample analysis

Mode of operation: Field analysis

Currently applied method(s) replaced/improved: Research vessel-based CTD casts and sampling at fixed stations

The CONTROS **HydroFIA®pH** system is autonomously conducting flow injection analysis (FIA) to determine the pH of water. The system was developed for the continuous long-term measurement of pH of the surface water and is therefore suitable for both the operation on research vessels and non-research vessels like ships-of-opportunity. While the ship is moving, water is flowing through the cube of about half a cubic meter, the pH is measured and the water is released again. The pH measurement with cycles of approximately two minutes is based on an indicator dye, which changes its colour depending on the pH of the water. The indicator is injected into the sample stream and then the mix is passing a flow cell, where it is detected using visible light absorption spectrometry. A second water inlet for regular standard measurements is included and thus, the device is calibration-free. The refilling of reagents is easy and the system automatically cleans by regular acid flushes. Due to this automated design, the system can be deployed long-term with a minimum of personnel involvement (Aßmann et al., 2011; Müller et al., 2018).

For the marine monitoring, the HydroFIA®pH system can be installed on research vessels or ferries shipping regularly transit routes. Deploying them on ferries, long-term time series of the same course can be acquired for pH values, enabling the monitoring of temporal changes in pH and therefore ocean acidification. The indicator dye m-Cresol purple is working on a wide range of pH (7.3 – 8.7) and salinity (0 – 40 practical salinity units), enabling the measurement in marine and brackish waters (Kongsberg, 2018). Furthermore, cross flow filters for the operation in waters with high turbidity and sediment content can be installed in the system. These properties of the indicator enable the operation of the pH measurement system in the environment of the Baltic Sea (Ma et al., 2019). Data are collected and processed by the software from Contros Kongsberg and the system needs an external power supply (Kongsberg, 2018).

Investment costs:

Moderate

10-50,000 €; this is dominated by the product prize; personnel training ~ 8 h

Monitoring costs:

Low

1-10,000 € annual costs per system ~ equally divided in maintenance and consumables

Reliability:

With a precision of ± 0.001 and an accuracy of ± 0.003, the measurements of pH values are highly reliable (Kongsberg, 2018). The measurements are not biased in the presence of high concentrations of dissolved organic matter or hydrogen sulphide (Müller et al., 2018).

Environmental impact:

None

No additional ships/vessels are in operation to acquire the data; water-flow-through-sampler has no negative impact on the environment or organisms.

Novelty score:

Score 1

When deployed on a ferry, the routinely monitored routes result in valuable datasets with a high spatio-temporal resolution, recording changes in pH over time and therefore, monitoring ocean acidification.

Limitations:

Moderate

The initial installation and the quality control of the obtained data are relatively labour intensive. Since the calculation is automatic and not known to the user, it is difficult to correct the data for salinity. Thus, for single sample analysis, the salinity at the sampling site needs to be known. But the systems are mostly used in FerryBox applications and then, it is no problem since the system is fed with salinity.

Required expertise:

Sampling: Low (trained personnel without specific professional education)

Data handling: Moderate (trained personnel with specific professional education)

2.1.2 Imaging Flow Cytometry (IFC) platforms

MSFD Descriptor(s): Biodiversity; Non-indigenous species; Food webs; Eutrophication

BSAP Objective(s): Natural level of algal blooms; No alien species; Thriving and balanced communities of plants and animals

WFD Quality Element(s): Biological Quality Elements; Physico-chemical quality elements

Quality element(s): Phytoplankton; Chlorophyll-a

Novel quality element(s): -

Relevant step in monitoring: Sample analysis

Mode of operation: Field analysis

Currently applied method(s) replaced/improved: Microscopy; High-performance liquid chromatography (HPLC); Absorption analysis

Imaging Flow Cytometry (IFC) platforms are used to comprehensively analyse phytoplankton communities. The instruments combine traditional flow cytometry and automated imaging to analyse and large sample sizes with high speed. Using multiparametric fluorescence and morphology analysis, IFC platforms can be used to measure multiple parameters for each single cell in the sample. Therefore, the sampled cells move along a stream, are excited by lasers and LEDs. Fluorescence or scattering by the individual organisms trigger a flash and a camera and the cells are imaged. Machine learning algorithms and artificial intelligence are used for analysing the images to identify organisms and to measure size etc.

For the marine monitoring, various instruments can be used, depending on monitoring objective. The different IFC instruments include laboratory instruments, instruments that can be included into a FerryBox or autonomous in-situ platforms at fixed stations. Instruments available commercially include the Imaging Flow Cytobot (IFCB); and the CytoSense. Another instrument for imaging phytoplankton is the FlowCam. The IFCB provides autonomous deployment for up to six months, collecting data for: The determination of phytoplankton abundance and composition, as well as size structures and morphology; the estimation of biovolume; the detection of harmful algal blooms; the determination of viability and metabolic activity of the analysed cells. Therefore, IFC platforms can be used to assess biodiversity, non-indigenous species food webs and eutrophication. IFC platforms can also be important components of harmful algal bloom warning and prediction systems and ocean observing systems.

Investment costs:

Very high

120,000 €

Monitoring costs:

Low

estimation; not known in detail, but very low per samples

Reliability:

High

The accuracy of automated image classification is comparable to human experts. Due to the higher capability to measure multiple parameters in a high-throughput mode, reliability of generated data is increased due to the increase in monitoring data.

Environmental impact:

None

No additional ships/vessels are in operation to acquire the data; water-flow-through-sampler has no negative impact on the environment or organisms.

Novelty score:

Score 1

Limitations:

Moderate

Depending on instrument, only specific parameters or species can be detected and analysed. Furthermore, the development of the algorithms for automatic analysis is time consuming and requires specific skills in taxonomic and analytical knowledge. Furthermore, power supply is needed.

Required expertise:

High (high expertise and special skills required)

2.2 Laboratory analysis

2.2.1 DNA Barcoding

MSFD Descriptor(s): Biodiversity; Non-indigenous species; Commercial fish and shellfish; Food webs; Eutrophication; Sea-floor integrity

BSAP Objective(s): No alien species; Viable populations of species; Thriving and balanced communities of plants and animals

WFD Quality Element(s): Biological quality elements

Quality element(s): All biota

Novel quality element(s): New indicator species are made accessible

Relevant step in monitoring: Sample analysis

Mode of operation: Laboratory analysis

Currently applied method(s) replaced/improved: Morphological identification of species

DNA Barcoding is a molecular method to identify species due to the sequence of a marker gene, the barcode. Barcodes are specific parts of the DNA, which include a highly conserved and a variable part. The conserved part is similar between organisms of the same class, allowing the amplification of the DNA. The variable part of the barcode is unique for each species and allows the identification of the organisms to species level. DNA Barcoding is used to identify a single species, while DNA Metabarcoding is performed to analyse whole biological communities in bulk samples. For analysing environmental samples, residual DNA can be extracted from water or sediment rather than an organism, called environmental DNA (eDNA). In all approaches, analysis includes DNA extraction, barcode amplification and sequencing. For species identification, resulting sequences are compared to reference libraries to find matching sequences of known species (Bourlat et al., 2013; Pawlowski et al., 2018).
The DNA-based methods are well advanced for the detection and identification of species, while determining the abundance of species is still rather imprecise for most of the taxonomic classes. DNA Barcoding is convenient for the identification of species that are hard to be identified by their morphological properties, e.g. species that are very small or very similar to other species. Furthermore, all species in a bulk sample or the fixative used to preserve this bulk sample can be identified (DNA bulk sample (fixative) Metabarcoding; Zizka et al., 2019). For a comprehensive detection of all species present in the closer environment, eDNA can be analysed. The species-specific eDNA surveillance enables detecting the presence of a specific target organism, e.g. threatened or invasive species (Kelly et al., 2017).

Investment costs:

Moderate

16,000 € for equipment and personnel training when sequencing externally (personnel training ~ 80 h); for eDNA analysis, an additional laboratory with regarding purity measures is required (additional ~ 11,000 €).

Monitoring costs (for identification of hard-bottom communities):

Low

45,000 € per quality element for annual Baltic Sea-wide monitoring.

Assumptions: 20-60 € for identification of single species (barcoding) and 60-800 € per bulk sample (metabarcoding) when sequencing externally. Here, metabarcoding is used to identify the species sampled using Artificial Substrates (Aylagas et al., 2018). When sampling 3 replicates per southern subbasin (11 subbasins in South, not a lot of hard substrate) and 3 replicates per northern WFD water body (32 water bodies in North, more areas with hard substrate), adding up to 43 sites. Sampling is done using 3x ARMS and 3x ASU per site, therefore 260 samples. For a detailed calculation of metabarcoding costs, see Aylagas et al., 2018.

Using the traditional method of morphological identification, 260 samples cost 110,000 € (when one sample costs 455 €).

Reliability:

High

The identification of species is more accurate than by morphological inspection, also the detection of present species using eDNA is more reliable, since there is no need to visually spot the organisms (Kelly et al., 2017). DNA barcodes can potentially be generated for almost 100 % of the species found at a sampling site with a highly reliable and accurate identification in comparison to morphological identification. However, Barcoding is not reliable in rare cases such as hybridising species or if the barcodes of the species is yet unknown.

Environmental impact:

Low

Barcoding of organismic tissue is lethal for the analysed organisms. Using eDNA has no effect on the environment or organisms.

Novelty score:

Score 1

The spatial and taxonomic resolution of monitoring can be enhanced since the use of DNA Metabarcoding enables the identification of hundreds of individuals at the same time. Identification without taxonomic expertise is enabled and therefore it saves a lot of time for personnel training. Organisms are identified to species level, while classical morphological approaches may be insufficient to differentiate species with similar morphological characteristics or very small organisms. This also enables the use of novel indicator species, which are sensitive to eutrophication or contamination, for instance.

Limitations:

Moderate

Molecular facilities and expertise are needed, and the resulting barcodes do usually not indicate the species abundance (Leese et al., 2018). Furthermore, databases of DNA libraries do not contain the genetic information of all living species. When the libraries lack the relevant information, the organisms cannot be identified using DNA (Meta)barcoding (Pawlowski et al., 2018). However, operational taxonomic units (OTUs) can be used as putative species even when a species assignment is not possible for the respective OTUs. Furthermore, contamination and sample treatment and analysis errors can lead to biased results, especially for eDNA analysis, and therefore sample treatment should follow a strict protocol. Metabarcoding does not reveal abundances / biomass of the organisms and the results are thus not suited for calculating indices required abundance data.

Required expertise:

High (high expertise and special skills required)

2.2.2 Stable Isotope Analysis (SIA)

MSFD Descriptor(s): Food webs; Eutrophication

BSAP Objective(s): Concentrations of nutrients; Thriving and balanced communities of plants and animals

WFD Quality Element(s): Biological Quality Elements; Physico-chemical quality elements

Quality element(s): Nutrients

Novel quality element(s): Food web length and stability

Relevant step in monitoring: Sample analysis

Mode of operation: Laboratory analysis

Currently applied method(s) replaced/improved: Gut contents analysis

Stable Isotope Analysis (SIA) is performed to derive food source links and food web processes. Stable isotopes are variants of a particular chemical element which differ in neutron number and do not spontaneously undergo nuclear decay. Due to thermodynamic and kinetic differences between light and heavy isotopes, they accumulate within different trophic niches (positions within the food web), creating a natural variation in isotopic ratios between species in different niches (Jardine et al., 2017; Michener et al., 2007). The ratio of $^{15}N/^{14}N$ isotopes shows a stepwise enrichment with trophic levels, therefore indicating the position of species within the food web (with primary producers at the first level and predators at level four or higher). Furthermore, small increases in the abundance of ^{15}N indicate pollution by sewage water, since sewage carries higher $^{15}N/^{14}N$ ratios than biologically fixed nitrogen sources. The ratio of $^{13}C/^{12}C$ isotopes varies between primary producers, depending on the photosynthetic pathway and the utilisation of terrestrial or internal carbon sources (Michener et al., 2007). Therefore, $^{13}C/^{12}C$ ratios can be studied to determine whether the diet of an organism is based on pelagic or benthic food sources and the origin of metabolised carbon (Jackson et al., 2011; Layman et al., 2007).

For the marine monitoring, we propose the indicator "Food web length and stability" to monitor food web structures and energy pathways within marine communities. It can be measured comparing the isotopic signatures of fish species representing high-level predators to the isotopic signatures of a planktonic primary consumer. The difference in the $^{15}N/^{14}N$ ratio indicates the length of the food web. In contrast, the $^{13}C/^{12}C$ ratio of the predatory fish species indicates its dominant prey source. Compared to pelagic primary producers, benthic primary producers transfer a higher proportion of ^{13}C into the marine food chain. Changes of the length or the diet between years may indicate instability in the food web. For instance, a reduced length may result from the loss of a trophic level/species by overfishing or eutrophication. For the monitoring of eutrophication, $^{15}N/^{14}N$ ratios present a valuable tracer of time-averaged nutrient loadings and indicate water pollution by sewage. Hereby, primary consumers are analysed, since they reflect the short-term variations in the $^{15}N/^{14}N$ ratios metabolised in primary production (Ziółkowska et al., 2018). For marine sampling protocols for SIA, see Carabel et al., (2006).

Investment costs:

Low or Very high

300,000 € for Isotope Ratio Mass Spectrometer (IRMS), incl. equipment; but usually external analysis, thus, no investment costs necessary.

Monitoring costs (for assessing food web length and stability):

Moderate

51,000 € per quality element for annual Baltic Sea-wide monitoring.

Assumptions: The prize for external analysis of one sample is around 50 €. To determine food web length and stability for one monitoring station requires the analysis of 60 samples (4 species of fish top predators and one primary consumer species; five replicates of each species and three replicates of each organism), summing up to 3,000 €. To monitor food web length and stability for the whole Baltic Sea (each of the 17 sub-basins), the costs are about 51,000 € annually.

Reliability:

High

The reliability of SIA is higher than for traditional microscopic gut analyses, since it gives a time-integrated view on the food web instead of a snapshot. Compared to gut analysis, results from SIA show a more stable indication of the diet of an organism. Gut analysis delivers only a snapshot of the latest prey of an organism. Furthermore, gut analysis is not applicable for very small organisms, where gut dissection and inspection are not possible, or for organisms that crush their food beyond recognition. Precision of the IRMS measurements is high with around 0.01-0.07 ‰ for ^{13}C and 0.05-0.2 ‰ for ^{15}N (Michener et al., 2007).

Environmental impact:

Low

Sample size is small and sampling only once a year.

Novelty score

Score 2

The use of SIA offers the novel indicator “food web length and stability” to assess marine food webs.

Limitations:

Neglectible

Since isotopic values show seasonal variation (Rolff, 2000), SIA should always be conducted at the same time of the year. Sample preparation and the health of the analysed organism can influence the isotopic value. Thus, sample treatment should follow a strict protocol and the physical fitness of the organisms should be assured.

Required expertise:

Moderate (trained personnel with specific professional education) for laboratory analysis

2.2.3 Machine Learning

MSFD Descriptor(s): All

BSAP Objective(s): All

WFD Quality Element(s): All

Quality element(s): All

Novel quality element(s): -

Relevant step in monitoring: Sample analysis

Mode of operation: Computer analysis

Currently applied method(s) replaced/improved: -

In **Machine Learning**, computer systems are trained to autonomously perform specific tasks using algorithms and statistical models. Given the variety of developed algorithms, there is a wide field for possible applications. In general, Machine Learning comprises the following steps: Using a number of real data ("training data"), an algorithm is trained to learn the patterns within the data and apply them autonomously. This trained algorithm is then evaluated using another set of real data ("test data"), indicating the goodness of its predictions. By running multiple training cycles, the reliability of the predictions is increasing. When the algorithm is deemed sufficiently accurate, it can be used to conduct specific tasks (Kelleher et al., 2015).

For the marine monitoring, Machine Learning can be applied for either the analysis of acquired monitoring data or the interpolation of missing monitoring data (Krekoukiotis et al., 2016; Lehikoinen et al., 2019; Williams et al., 2012). For the analysis of acquired monitoring data, a classification algorithm can be trained based on an image recognition system. Photographs of fish and bycatch taken by personnel or cameras installed on a trawler are used to train the algorithm (Williams et al., 2012). After successful training, the algorithm can be used to identify and quantify caught species and bycatch. For the interpolation of missing monitoring data, regression algorithms can be utilized. Existing monitoring data on a specific indicator are used to train the algorithm, which creates statistical connections between the data, and is then able to predict missing data (Srebotnjak et al., 2012).

Investment costs (rating regarding an image recognition system to identify and quantify organisms):

Moderate

15,000 € (3,000 € for a computer, 12,000 € for algorithm development per indicator with required personnel time ~ 1 month).

Monitoring costs (for running one developed algorithm):

Very low

300 € per quality element for annual Baltic Sea-wide monitoring (around 4 h/year to run the algorithm and check for errors).

Reliability:

High

The reliability of Machine Learning depends on the accuracy of the created algorithm and the quality of the available monitoring data. The accuracy of the trained algorithm can be enhanced to a desired level by increasing the number of training steps (Kelleher et al., 2015).

Environmental impact:

None

The computational analysis does not affect organisms or the environment.

Novelty score:

Score 1

Machine Learning can improve the speed and accuracy of monitoring due to automated identification of species or quantification of bycatch.

Limitations:

Moderate

The set-up of an image recognition system is labour intensive. The interpolation of missing data highly depends on the availability of real monitoring data.

Required expertise:

High (high expertise and special skills required) due to the development of an image recognition system

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Appendix 2: Organic matter decomposition in streams

This appendix contains the additional files to Chapter 2: Fine sediment and the insecticide chlorantraniliprole inhibit organic matter decomposition in streams through different pathways

Table A2-1: List of macroinvertebrate species present at the experimental site and their functional feeding groups. Species were collected using kick-sampling (Elbrecht et al., 2016) and identified using metabarcoding

Taxa group	Family	Species	Functional feeding group
Bivalvia	Sphaeriidae	Pisidium caseratum	active filter feeder
Bivalvia	Sphaeriidae	Pisidium subtruncatum	active filter feeder
Oligochaeta	Lumbriculidae	Stylodrilus heringianus	gatherer
Oligochaeta	Lumbricidae	Eiseniella tetraedra	gatherer
Oligochaeta	Tubificidae	Limnodrilus claparedeianus	gatherer
Oligochaeta	Tubificidae	Limnodrilus hoffmeisteri	gatherer
Gastropoda	Planorbidae	Ancylus fluviatilis	grazer
Crustacea	Gammaridae	Gammarus pulex	shredder, gatherer
Coleoptera	Elmidae	Elmis aenea	grazer
Coleoptera	Elmidae	Elmis maugetii	grazer
Coleoptera	Elmidae	Esolus parallelepipedus	grazer
Coleoptera	Hydraenidae	Hydraena gracilis	predator
Coleoptera	Elmidae	Limnius volckmari	grazer
Coleoptera	Gyrinidae	Orectochilus villosus	predator
Coleoptera	Dytiscidae	Oreodytes sanmarkii	predator
Coleoptera	Dytiscidae	Platambus maculatus	predator
Diptera	Chironomidae	Brillia bifida	shredder, gatherer
Diptera	Tabanidae	Chrysops caecutiens	gatherer
Diptera	Chironomidae	Diamesa insignipes	grazer
Diptera	Pediciidae	Dicranota auripontium	predator
Diptera	Pediciidae	Dicranota gracilipes	predator
Diptera	Pediciidae	Dicranota robusta	predator
Chironomidae	Chironomidae	Heterotrissocladius marcidus	gatherer
Diptera	Chironomidae	Macropelopia nebulosa	predator
Diptera	Chironomidae	Macropelopia notata	predator
Diptera	Chironomidae	Micropsectra notescens	gatherer
Diptera	Chironomidae	Microtendipes pedellus	gatherer
Diptera	Chironomidae	Paratrissocladius excerptus	NA
Diptera	Chironomidae	Prodiamesa olivacea	NA
Diptera	Chironomidae	Rheotanytarsus distinctissimus	passive filter feeder
Diptera	Chironomidae	Stempellinella flavidula	NA
Diptera	Chironomidae	Tanytarsus eminulus	gatherer
Diptera	Chironomidae	Zavrelimyia divisa	NA
Ephemeroptera	Baetidae	Baetis rhodani	grazer, gatherer
Ephemeroptera	Baetidae	Baetis scambus	grazer, gatherer
Ephemeroptera	Caenidae	Caenis beskidensis	gatherer
Ephemeroptera	Heptagenidae	Ecdyonurus torrentis	grazer, gatherer
Ephemeroptera	Ephemeridae	Ephemera danica	active filter feeder
Ephemeroptera	Leptophlebiidae	Habroleptoides confusa	gatherer
Ephemeroptera	Ephemerellidae	Torleya major	grazer, gatherer
Heteroptera	Aphelocheiridae	Aphelocheirus aestivalis	predator
Megaloptera	Sialidae	Sialis fuliginosa	predator
Odonata	Cordulegastridae	Cordelugaster boltonii	predator
Plecoptera	Perlodidae	Isoperla grammatica	predator
Plecoptera	Leuctridae	Leuctra hippopus	gatherer, shredder, grazer

Table A2-1 (cont.): List of macroinvertebrate species present at the experimental site and their functional feeding groups. Species were collected using kick-sampling (Elbrecht et al., 2016) and identified using metabarcoding

Taxa group	Family	Species	Functional feeding group
Plecoptera	Leuctridae	Leuctra major	gatherer, shredder, grazer
Plecoptera	Perlodidae	Perlodes microcephalus	predator
Trichoptera	Limnephilidae	Anomalopterygella chauviniana	grazer, shredder
Trichoptera	Limnephilidae	Chaetopteryx villosa	shredder, grazer
Trichoptera	Limnephilidae	Drusus monticola	grazer
Trichoptera	Limnephilidae	Ecclisopteryx dalecarlica	grazer
Trichoptera	Hydropsychidae	Hydropsyche pellucidula	passive filter feeder
Trichoptera	Hydropsychidae	Hydropsyche saxonica	passive filter feeder, predator, grazer
Trichoptera	Hydropsychidae	Hydropsyche siltalai	passive filter feeder
Trichoptera	Lepidostomatinae	Lepidostoma basale	grazer, xylophagous, shredderer
Trichoptera	Polycentropodidae	Polycentropus flavomaculatus	predator
Trichoptera	Rhyacophilidae	Rhyacophila nubila	predator

Table A2-2: Explanation of the functional feeding types found at the experimental site. source: https://www.freshwaterecology.info/fwe_search.php?og=mzb

Functional feeding group	Explanation
grazers	feed on endolithic and epilithic algal tissues, biofilm, partially POM, partially tissues of living plants
xylophagous taxa	feed on woody debris
shredders	feed on fallen leaves, plant tissue, CPOM
gatherers	feed on sedimented FPOM
active filter feeders	feed on suspended FPOM, CPOM; micro prey is whirled; food is actively filtered from the water column
passive filter feeders	feed on suspended FPOM, CPOM, prey; food is filtered from running water, e.g., by nets or specialised mouthparts
predators	feed on prey

Table A2-3: Median algal coverage of the mesocosm for different insecticide treatments in percent

Insecticide concentration	Algal coverage (median ± standard deviation)		
	day 8	day 15	day 20
none	1.8 ± 3.4	0.0 ± 3.0	0.0 ± 2.3
low	2.4 ± 1.8	0.7 ± 2.0	0.0 ± 1.0
medium	17.4 ± 5.2	29.0 ± 5.3	0.6 ± 4.0
high	78.1 ± 5.8	82.6 ± 6.4	64.2 ± 6.3

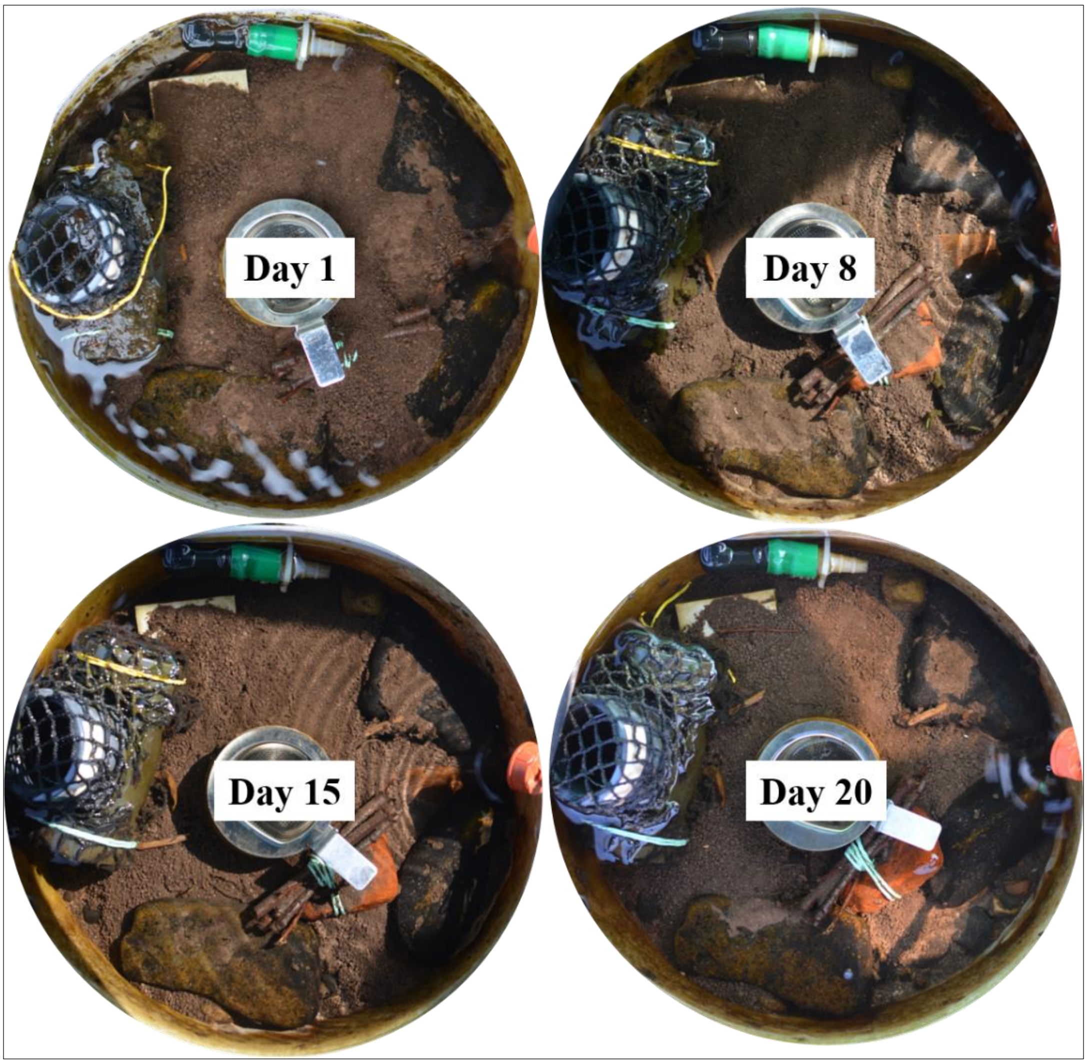

Figure A2-1: Images of exemplary channel 5 in the course of stressor manipulation of 1 until 20 days. The initial fine sediment coverage of 100 % (day 0) shifted throughout the experimental duration.

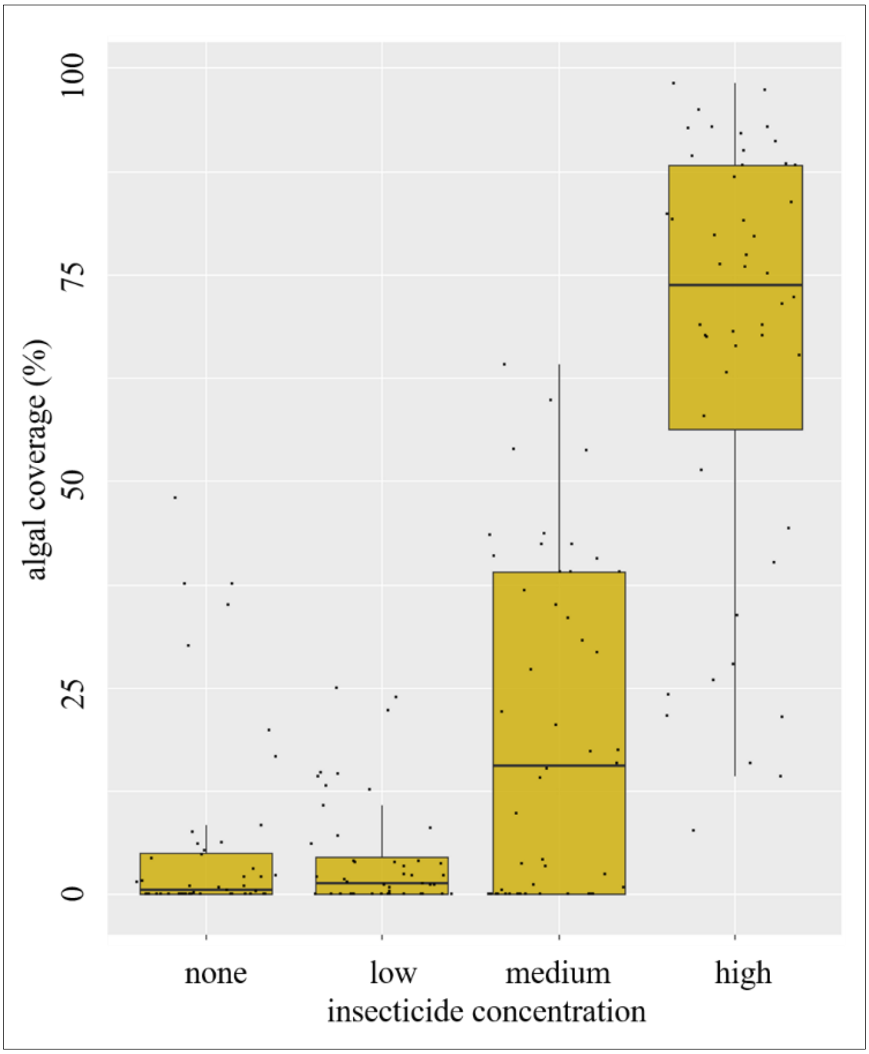

Figure A2-2: % algal cover of the mesocosm for different insecticide treatments. Measurements of days 8, 15 and 20 are combined. For medium and high concentrations algal coverage was significantly higher than for none or low insecticide concentration (Kruskal-Wallis, p =< 2.2e-16).

Appendix 3: Dependence of observed multiple stressor effects

This appendix contains the additional files to Chapter 3: Observed multiple stressor effects depend on sample size and stressor gradient length

Table A3-1: Overview and references on the cases analysed in the study. PP=Phytoplankton, BV=Benthic vegetation, ZP=Zooplankton, BI=Benthic invertebrates, Nut=Nutrient, Therm=Thermal, Chem=Chemical, Morph=Morphological, Hydro=Hydrological, Transit=Transitional

Case IDs	Study name	Water category	Response group					Stressor category							Contact/Reference
			PP	BV	ZP	BI	Fish	Nut	Ther	Chem	Morp	Hydro	Toxic	Light	
001 - 005	Lake Constance, DE (fish)	Lake	x				x	x	x						Jan.Baer@lazbw.bwl.de
007 - 010	Vansjø lake, NO	Lake	x					x	x			x			Couture et al. (2018)
012	Lake monitoring, FI	Lake				x		x				x			Jukka.Aroviita@syke.fi
013	Fish-trait responses in French lakes, FR	Lake					x	x	x						Bouraï et al. (2020)
014 - 016	Northern lakes (phytoplankton), FI, NO, SE, UK	Lake	x					x	x						Birk et al. (2020)
037 - 038	Manuherikia, NZ (invertebrates)	River				x		x			x				Lange et al. (2014)
040 - 044	Odense catchment, DK (invertebrates)	River				x		x	x		x	x			Graeber et al. (2014)
045 - 049	Pinios catchment, GR	River				x		x	x						Stefanidis et al. (2016)
051 - 055	Pomahaka, NZ	River				x		x			x				Ramezani et al. (2016)
056	Ruhr catchment, DE	River				x		x			x				Gieswein et al. (2017)
057 - 063	Second order streams, NZ	River				x		x			x				Matthaei et al. (2006)
073	Odense catchment, DK (fish)	River					x			x		x			Graeber et al. (2014)
074	Odense catchment, DK (macrophytes)	River		x					x			x			Graeber et al. (2014)
075	Lepsämänjoki catchment, FI	River	x					x	x						Rankinen et al. (2019)
079 - 083	Thames catchment, UK	River	x					x	x						Birk et al. (2020)
084 - 088	Agricultural streams, NZ	River				x		x			x				christoph.matthaei@otago.ac.nz
089	Central European upland rivers	River				x					x		x		Birk et al. (2020)
091	EPA monitoring, freshwater, IE (invertebrates)	River				x		x				x			h.feeley@epa.ie

Table A3-1 (cont.): Overview and references on the cases analysed in the study. PP=Phytoplankton, BV=Benthic vegetation, ZP=Zooplankton, BI=Benthic invertebrates, Nut=Nutrient, Therm=Thermal, Chem=Chemical, Morph=Morphological, Hydro=Hydrological, Transit=Transitional

Case IDs	Study name	Water category	Response group					Stressor category							Contact/Reference
			PP	BV	ZP	BI	Fish	Nut	Therm	Chem	Morph	Hydro	Toxic	Light	
093	European very large rivers (invertebrates)	River				x		x			x				Birk et al. (2018b)
094 - 096	Multiple stressors in central European lowland rivers, EU	River				x		x			x				Schinegger et al. (2013)
097	Pesticides effects on invertebrates, FI, FR, DE	River				x		x					x		Schäfer et al. (2013)
098 - 100	Romanian rivers reference modelling, RO	River				x		x					x		Birk et al. (2020)
101 - 106	Sedimentation, NZ (invertebrates)	River				x		x			x				Magbauna et al. (2010)
107 - 108	SPEAR index Sweden, SE	River				x		x			x		x		von der Ohe and Goedkoop (2012)
110	Central European upland rivers (fish)	River					x	x					x		Birk et al. (2020)
111	European very large rivers (fish)	River					x	x			x				Birk et al. (2018a)
112 - 116, 122 - 127	UK investigations (macrophytes)	River		x				x			x				Birk et al. (2020)
117 - 121	EPA monitoring, freshwater, IE (phytobenthos)	River		x				x	x	x		x			B.Kennedy@epa.ie
179	Hong Kong, CN (corals)	Marine				x		x		x					adam.sokolowski@ug.edu.pl
182 - 184	Alicante, ES	Marine		x						x				x	Yolanda.fernandez@ua.es
186 - 195	Hong Kong, CN (phytoplankton)	Marine	x					x	x	x					https://cd.epic.epd.gov.hk/EPICRIVER/marine/
196 - 198	EPA monitoring, marine, IE (invertebrates)	Marine				x		x	x	x					Louise.Healy@Marine.ie
199 - 206	EPA monitoring, marine, IE (phytoplankton)	Marine	x					x	x	x					G.McDermott@epa.ie
236	Kiel, DE (phytoplankton)	Marine	x						x	x					N/A
252	Southampton, US	Marine				x			x	x					Stevens and Gobler (2018)
287 - 288	Gulf of Riga, EE, (fish)	Transit					x		x	x	x	x			Kotta et al. (2018)
292 - 293	Nervion estuary, ES (fish)	Transit					x	x		x					aborja@azti.es

Table A3-1 (cont.): Overview and references on the cases analysed in the study. PP=Phytoplankton, BV=Benthic vegetation, ZP=Zooplankton, BI=Benthic invertebrates, Nut=Nutrient, Therm=Thermal, Chem=Chemical, Morph=Morphological, Hydro=Hydrological, Transit=Transitional

Case IDs	Study name	Water category	Response group					Stressor category							Contact/Reference
			PP	BV	ZP	BI	Fish	Nut	Therm	Chem	Morph	Hydro	Toxic	Light	
294 - 302	Baltic Sea monitoring (phytoplankton)	Transit	x					x	x	x					https://www.ices.dk/data/Pages/default.aspx
304 - 306	Palmico Sound lagoon, US	Transit	x					x	x	x					http://paerllab.web.unc.edu/projects/modmon/
307 - 311	Gulf of Riga, EE (zooplankton)	Transit			x			x	x	x	x				Kotta et al. (2018)
312 - 320	EPA monitoring, Transit, IE (invertebrates)	Transit				x		x	x	x					Louise.Healy@Marine.ie
321 - 325	National Estuarine Dataset, NZ	Transit				x		x			x		x		Berthelsen et al. (2020)
327 - 336	Baltic Sea monitoring (fish)	Transit					x	x	x	x					https://www.ices.dk/data/Pages/default.aspx
339 - 341	EPA monitoring, Transit, IE (phytoplankton)	Transit	x					x	x	x					G.McDermott@epa.ie
342 - 352	National Estuarine Research Reserves monitoring, US	Transit	x					x	x	x					https://coast.noaa.gov/nerrs/

Table A3-2: Multiple stressor effect types of full and partial gradient cases, indicating the frequency of cases showing a specific change in effect type for lower and upper gradient cases separately. There is no significant difference in MS effect types changes between lower and upper partial gradients (chi^2-test; $p > .05$).

Full effect type	Partial effect type	Lower cases	Upper cases
Dominant	Dominant 1st	0.37	0.40
	Dominant 2nd	0.08	0.02
	Additive	0.10	0.02
	Synergistic	0.10	0.11
	Antagonistic	0.02	0.02
	Collapse	0.35	0.43
Additive	Dominant 1st	0.31	0.06
	Dominant 2nd	0.08	0.13
	Additive	0.28	0.34
	Synergistic	0.25	0.09
	Antagonistic	0.03	0.09
	Collapse	0.06	0.28
Synergistic	Dominant 1st	0.27	0.18
	Dominant 2nd	0.04	0.07
	Additive	0.12	0.18
	Synergistic	0.31	0.18
	Antagonistic	0.04	0.04
	Collapse	0.23	0.36
Antagonistic	Dominant 1st	0.04	0.17
	Dominant 2nd	0.00	0.00
	Additive	0.21	0.17
	Synergistic	0.21	0.04
	Antagonistic	0.17	0.22
	Collapse	0.38	0.39

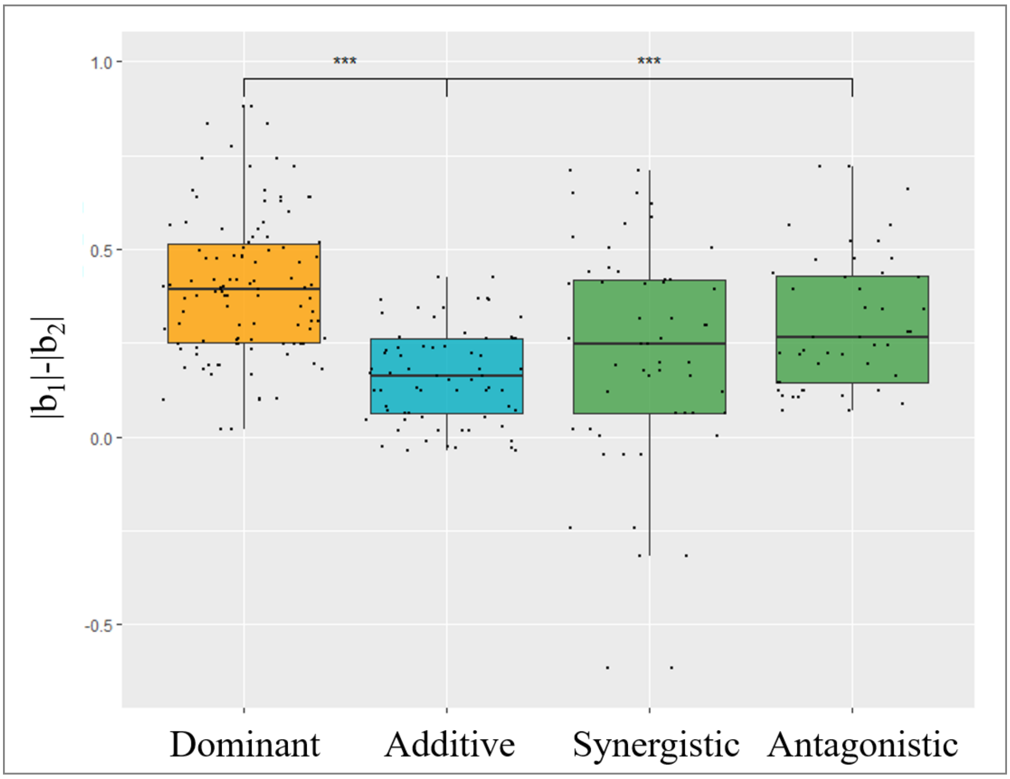

Figure A3-1: The difference in first and second stressor effect sizes for dominant, additive, synergistic and antagonistic full cases. Additive cases show a significantly lower difference than the other cases (Mann-Whitney-U-test; $p < .05$).

Appendix 3 - References

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Declarations

Hiermit erkläre ich, gem. § 7 Abs. (2) d) + f) der Promotionsordnung der Fakultät für Biologie zur Erlangung des Dr. rer. nat., dass ich die vorliegende Dissertation selbständig verfasst und mich keiner anderen als der angegebenen Hilfsmittel bedient, bei der Abfassung der Dissertation nur die angegebenen Hilfsmittel benutzt und alle wörtlich oder inhaltlich übernommenen Stellen als solche gekennzeichnet habe.

Essen, den ________________ ________________________________

Unterschrift der Doktorandin

Hiermit erkläre ich, gem. § 7 Abs. (2) e) + g) der Promotionsordnung der Fakultät für Biologie zur Erlangung des Dr. rer. nat., dass ich keine anderen Promotionen bzw. Promotionsversuche in der Vergangenheit durchgeführt habe und dass diese Arbeit von keiner anderen Fakultät/Fachbereich abgelehnt worden ist.

Essen, den ________________ ________________________________

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Hiermit erkläre ich, gem. § 6 Abs. (2) g) der Promotionsordnung der Fakultät für Biologie zur Erlangung der Dr. rer. nat., dass ich das Arbeitsgebiet, dem das Thema *„Surface waters under multiple stress: monitoring methods, stressor interactions and combined effects"* zuzuordnen ist, in Forschung und Lehre vertrete und den Antrag von Leoni Mack befürworte und die Betreuung auch im Falle eines Weggangs, wenn nicht wichtige Gründe dem entgegenstehen, weiterführen werde.

____Daniel Hering______________________________

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Essen, den __4.7.2022________ Daniel Hering Digital unterschrieben von Daniel Hering Datum: 2022.07.04 15:02:31 +02'00'

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