Quantifying the impacts of multiple stressors on the production of marine benthic resources

by

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Declaration

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Katrin Simone Hilke Schertenleib

Summary

Coastal ecosystems are among the most heavily affected by climate change and anthropogenic activities, which impacts their diversity, productivity and functioning and puts many of the key ecosystem services they provide at risk. Although empirical studies have moved beyond single-stressor-single-species experiments with limited extrapolation potential and have increasingly investigated the cumulative effects of simultaneously occurring multiple stressors, consistent generalities have not yet been identified. Upscaling from controlled experiments to natural ecosystems, therefore, remains an unsolved challenge. Disentangling the independent and cumulative effects of multiple stressors across different levels of biological complexity, revealing the underlying mechanisms and understanding how coastal ecosystems may respond to predicted scenarios of global change is critical to manage and protect our natural capital.

In this thesis, I advance multiple stressor research by applying complementary approaches to quantify the impact of multiple stressors on marine benthic resources and thereby help predict the consequences of expected climate change for coastal habitats. First, I present the newly developed experimental platform QIMS (Quantifying the Impacts of Multiple Stressors) that overcomes some of the shortfalls of previous multiple stressor research (Chapter 2). Second, in a novel empirical study, I investigate the independent and combined effects of moderate ocean warming and acidification on the functioning and production of mussels and algae, considering the effects of interspecific interactions in the presence or absence of the respective other species (Chapter 3). Third, I synthesise monitoring data from Dublin Bay (representative of a typical metropolitan estuary) using conditional inference and a Bayesian Network model and provide alternative system trajectories according to different climate change scenarios. From this new model, I deepen the understanding of the complex linkages between environmental conditions and the diversity and functioning of Dublin Bay to support local decision making and management (Chapter 4).

Empirical tests of independent and cumulative multiple stressor effects require multi-factorial and multi-level designs, as well as high treatment replication to identify stressor interactions and their underlying mechanisms across multiple levels of biological organisation and enable robust data analyses. Many marine multiple stressor mesocosm-based studies to date included a low number of treatments or low replication of treatments or both. The new aquatic mesocosm-based experimental platform QIMS consist of 96 independent replicate units, in which up to three clearly separated factorial treatment levels of temperature and pCO_2/pH each can be precisely manipulated and maintained randomly distributed across all mesocosms. QIMS complements a suite of permanently installed marine mesocosm facilities around the world that simulate ocean warming and/or acidification and facilitates multiple stressor research at an unprecedented level of statistical robustness and fully crossed, multi-level factor combinations.

To investigate the independent and combined effects of moderate ocean warming and acidification on the functioning and production of marine benthic resources, mussels and/or algae were exposed to three levels of temperature (ambient, +0.8 °C, +2 °C) and two levels of pCO_2 (ambient at 450 ppm, elevated at 645 ppm) in a seven-week mesocosm experiment. Additionally, differences in responses according to the presence/absence of the respective other species were assessed (three levels: mussels, algae, mussels and algae). No interactions among any of the 18 experimental treatments ($n = 5$) were identified and no effects of pCO_2 were found. Warming increased mussel mortality and clearance rates, while mussels and algae facilitated each other's production (accumulated biomass, mussel condition index, algal photo physiology) when cultured together instead of separately. Overall mussel mortality was lower when algae were present. These results show the temperature sensitivity of the functioning of key benthic species, while there might be resilience towards moderate ocean acidification. Importantly, the ecological and potential economic benefits of increasing and conserving biodiversity in marine ecosystems is highlighted.

To identify links between environmental and biological variables, and to additionally predict the effects of climate change for Dublin Bay, Ireland, all available monitoring data was synthesised for the first time using conditional inference and a Bayesian Network. The model shows that when silica was limiting during the period from which monitoring data was available, phytoplankton biomass and abundance increased, while benthic invertebrate taxa indicated pristine conditions. When sediment organic content was high, invertebrate taxa richness was high, too, which allowed a greater abundance of wading birds. Having extrapolated warming, precipitation, ocean acidification and sea level rise according to climate change projections, I conclude from the model that climate change will degrade the ecological status of Dublin Bay, particularly affecting wader bird abundance through habitat loss, which emphasises the importance of protecting the remaining habitat.

Through methodological tool development, new empirical insights and a framework to synthesise existing monitoring information, this thesis adds to multiple stressor research opportunities, knowledge and accessibility, particularly in the light of expected climate change. Mesocosm experiments are important to test hypotheses and inform specific management questions, while models can inform holistic ecosystem management strategies or ecosystem maintenance in changing ocean conditions. Additionally, mesocosm experiments can inform or validate mathematical models, while models can highlight data gaps and identify system components that require prioritised management action. For best outcomes, empirical research and modelling should be applied as complementary tools to advance the scientific understanding of a system and thereby facilitate management and planning. Nevertheless, our planet is experiencing both a climate and a biodiversity crisis right now. Adding more detail to known tendencies may be a misleading priority and instead applying and implementing existing scientific knowledge to protect habitats and species, mitigating climate change effects, advancing adaptation to expected effects and making existing information readily available to managers and decision makers may be much more relevant.

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Chapter 1 | General introduction

1.1 Climate change and marine multiple stressor research

Climate change is damaging coastal and marine ecosystems at a larger extent and magnitude than previously estimated, which highlights the urgent demand for prompt climate action and more accurate predictions on how these ecosystems will develop with changing ocean conditions (IPCC, 2022). Coastal ecosystems are among the most heavily affected by anthropogenic activities and are vitally important for many key ecosystem services (Lotze *et al.*, 2006; Halpern *et al.*, 2007; He and Silliman, 2019; Bowler *et al.*, 2020). For example, rocky shores, kelp forests, estuaries and coastal lagoons suffer from increasing, gradual warming and intensifying marine heat waves, acidification, deoxygenation, sea level rise and non-climatic stresses, such as eutrophication, chemical pollution, or exploitation, and as consequence risk irreversible structural changes in community composition, productivity and functioning (He and Silliman, 2019; Smale *et al.*, 2019; Cooley *et al.*, 2022). This undermines their potential to provide food, regulate coastal erosion, recycle nutrients, sequester carbon, support recreational activities and sustain cultural identity as benefits to human society (Cooley *et al.*, 2022). Understanding how these systems respond to predicted global change scenarios is crucial to manage and protect our natural capital (Atkins *et al.*, 2011; Barbier *et al.*, 2011; Gissi *et al.*, 2021).

Impacts of warming on marine ectotherms, including algae, are based on the underlying mechanism of water temperature directly affecting metabolic rates and thereby affecting other physiological processes, such as oxygen consumption, growth or feeding rates (Gillooly *et al.*, 2001; Clarke and Gaston, 2006; Pörtner, 2010; Dell, Pawar and Savage, 2011; Lemoine and Burkepile, 2012). Many of these processes follow thermal performance curves and intensify with warming until an optimum temperature is reached at which metabolic costs are most efficiently covered by the rate of supplied energy, e.g. through consumption and digestion (Lemoine and Burkepile, 2012; Roma *et al.*, 2021). Warming past the optimum temperature unsettles this balance so that costs exceed what can be supplied, hence performance decreases rapidly until critical lethal temperatures are reached (Lemoine and Burkepile, 2012). Various studies suggest that consumption in marine heterotrophs responds faster to temperature change than photosynthesis in marine primary producers, which leads to the risk of overconsumption, rearranged food web structures and standing biomass stock (O’Connor, 2009; O’Connor *et al.*, 2009; Best, Stone and Stachowicz, 2015; Gutow *et al.*, 2016). Habitat-rich ecosystems worldwide such as coral reefs, mussel beds or macroalgae forests all suffer from mass mortality of their key-stone species following extreme heat events or increased grazing, as well as from decreasing population sizes following competition or impaired reproduction (IPCC, 2019; Smale *et al.*, 2019; Veenhof *et al.*, 2022). Hence, warming may alter community compositions and even cause shifts from complex biogenically engineered habitats to degraded algal turf communities and detritus-based systems (Provost *et al.*, 2017; Ullah *et al.*, 2018; IPCC, 2019).

Ocean acidification has been shown to negatively affect physiology, fitness, and resilience to environmental change particularly in calcifying marine species (Findlay *et al.*, 2008; Kroeker *et al.*, 2013; Queirós *et al.*, 2015; Lemasson *et al.*, 2018; Sadler, Lemasson and Knights, 2018). Calcifying species, such as shellfish, respond to ocean acidification with increased metabolic costs, reduced growth, impaired ontogenetic development, reproduction and calcification, which may lead to increased mortality and reduced abundance (Kroeker *et al.*, 2013; Lemasson *et al.*, 2018; Sadler, Lemasson and Knights, 2018; IPCC, 2019; Hoppit and Schmidt, 2022). Fleshy algae, on the other hand, tend to show resistance and may even benefit from acidified conditions in terms of biomass and growth because of increased CO_2 supplies for photosynthesis, especially if additional warming accelerated metabolic rates (Stewart *et al.*, 2013; Mooney-McAuley *et al.*, 2016; Hoppit and Schmidt, 2022). As can be observed in naturally acidified ecosystems, ocean acidification can be expected to simplify communities and facilitate macroalgal overgrowth (Kroeker *et al.*, 2013; IPCC, 2019). Shellfish and macroalgae are important habitat-forming components of coastal marine ecosystems (Mooney-McAuley *et al.*, 2016; Sadler, Lemasson and Knights, 2018), where altered community compositions may profoundly change ecosystem functioning and provisioning (Lemasson *et al.*, 2017; Cooley *et al.*, 2022; Hoppit and Schmidt, 2022).

Publications on marine climate change experiments have increased steadily over recent decades, and single-factor-single-species studies continue to provide the majority of evidence on climate change effects, with a strong focus on the effects of ocean warming and acidification on temperate benthic invertebrates (Wernberg, Smale and Thomsen, 2012; Bass *et al.*, 2021). Temperature effects are pervasive, while the effects of ocean acidification are not ubiquitous and can vary across taxa, populations or even life stages (Wernberg, Smale and Thomsen, 2012; Bowers, 2016; Doney *et al.*, 2020; Hoppit and Schmidt, 2022). The biological mechanisms underlying the effects of ocean acidification are not yet fully understood (Doney *et al.*, 2020 and references therein). More research is needed on the effects and mechanistic pathways of single climate stressors, such as ocean warming and acidification, on marine organisms and more complex ecological communities. Moreover, these environmental stresses rarely occur in isolation and a clear understanding of the cumulative effects of multiple stressors is essential to protect and restore habitat and to predict our capacity to produce bioenergy, food and biomaterials in the future (Côté, Darling and Brown, 2016).

In parallel to the maturing field of climate change research, theoretical frameworks have advanced and refined the identification and classification of interacting effects of multiple stressors to reduce uncertainty around predicting ecological change (e.g. Crain, Kroeker and Halpern, 2008; Piggott, Townsend and Matthaei, 2015; Gunderson, Armstrong and Stillman, 2016; Schäfer and Piggott, 2018; Tekin *et al.*, 2020). This included harmonising terminology across research fields, e.g. defining “stressor” as “any natural or anthropogenic variable that causes a quantifiable change, irrespective of its direction […] in a biological response” (Côté, Darling and Brown, 2016; Orr *et al.*, 2020). When the magnitude of the effect of combined stressors matches the sum of individual effects, accounting for the directionality of individual stressor effects, this is commonly known as an additive effect (Crain, Kroeker and Halpern, 2008). Synergistic interactions exceed the additive effects and antagonistic interactions reduce the cumulative impact (Piggott, Townsend and Matthaei, 2015; Orr *et al.*, 2020). Non-additive effects of multiple stressors are frequently found (Crain, Kroeker and Halpern, 2008; Darling and Côté, 2008), yet the concern that the cumulative effects of multiple stressors are primarily magnified by synergistic interactions has not been consistently identified following several meta-analyses (Crain, Kroeker and Halpern, 2008; Côté, Darling and Brown, 2016; Schäfer and Piggott, 2018 and references therein; Tekin *et al.*, 2020). Applying a multiplicative null model (e.g. for mortality) instead of an additive (e.g. for growth) to predict cumulative effects without interactions, or identifying dominance of single stressors, add to the complexity of multiple stressor theory and the challenge of accounting for multiple stressor interactions in marine ecosystem conservation and management (Folt *et al.*, 1999; Crain, Kroeker and Halpern, 2008; Côté, Darling and Brown, 2016; Schäfer and Piggott, 2018). Additionally, the more stressors are involved, the more outcome options exist for higher order interactions (Orr *et al.*, 2021), i.e. interaction types change and responses to cumulative effects of multiple stressors tend to remain context-dependent (Crain, Kroeker and Halpern, 2008). Additive models serve as mathematical null models for testing interactions in analyses of variance (Folt *et al.*, 1999). Detecting such interactions depends strongly on sample size and stressor gradient length (Mack *et al.*, 2022), however, the large amount of replication needed in ANOVA designs for reliable testing on interactions is rarely implemented, and the appropriate application of ANOVAs to detect interactive effects has recently been questioned in general, which adds to the uncertainty of predicting cumulative effects (Tekin *et al.*, 2020; Burgess, Jackson and Murrell, 2022). In addition to identifying whether response patterns to multiple stressors differ from null models, holistic knowledge of the stressors' mode of actions, the mechanisms underlying their effects, and the importance of natural fluctuations, intensities and relative temporal occurrence patterns of different stressors is required for more accurate predictions of future ecosystems (Crain, Kroeker and Halpern, 2008; Gunderson, Armstrong and Stillman, 2016; Orr, Rillig and Jackson, 2022; Pirotta *et al.*, 2022). Additionally, the consistency or divergence of the cumulative stressor effects across different scales and levels of biological organisation needs to be understood more clearly (Griffen *et al.*, 2016; Gissi *et al.*, 2021).

Marine multiple stressor studies increased in recent decades, in particular those focused on the cumulative effects of climate change, fisheries, shipping and pollution (Gunderson, Armstrong and Stillman, 2016; Bass *et al.*, 2021; Gissi *et al.*, 2021), however, consistent generalities have not yet been identified (Turschwell *et al.*, 2022). A recent systematic review of marine cumulative effect assessments concluded that almost 75 % of all studies showed that climate change effects intensified the impact of non-climatic anthropogenic activities particularly at species level (Gissi *et al.*, 2021). Additionally, there is evidence for interactions between multiple climate change stressors, e.g. species sensitivity to acidification is affected by exposure to additional climate change stressors, such as warming (Doney *et al.*, 2020 and references therein). Despite the wealth of insights into cumulative effects of multiple stressors, upscaling physiological results from empirical, still mainly single species studies to communities and natural ecosystems remains a major challenge of multiple stressor research (Crain, Kroeker and Halpern, 2008; Hoppit and Schmidt, 2022).

Apart from possibly interacting effects of multiple stressors, species' responses depend both on direct effects of the stressors (species level response, e.g. phenology, biology, physiology, genetics, distribution) and on the indirect effects of changes in biotic interactions (community level response, e.g. trophic structures, competition), which complicates upscaling and generalisation attempts further (Crain, Kroeker and Halpern, 2008; Walther, 2010; Legrand *et al.*, 2019; Miranda *et al.*, 2019). Altered species interactions may affect ecological networks as strongly as direct stressor effects (Bruder *et al.*, 2019). As detailed above, warming affects trophic relationships through changing consumer's energy demands or competitive strength. This may profoundly alter community composition and restructure food webs with potential short-term advantages for higher trophic levels but a high risk of long-term depletion of lower trophic levels, all of which changes ecosystem functioning and provisioning (Ullah, Fordham and Nagelkerken, 2021; Cooley *et al.*, 2022; Hoppit and Schmidt, 2022). Similarly, acidification has been found to simplify communities and reduce functional diversity, presumably because of altered growth rates of calcifying and non-calcifying primary producers in addition to changed grazing rates (Kroeker, Gambi and Micheli, 2013; Kroeker, Micheli and Gambi, 2013). Recent research, however, has highlighted the potential of marine macrophytes, such as seaweeds and seagrass, to mitigate negative ocean acidification effects on shellfish by removing CO_2 from the water and increasing pH, thus, acting as a local buffer to acidification (Jiang and Fang, 2021; Jiang *et al.*, 2022; Young *et al.*, 2022).

Manipulative, empirical research to identify the mechanisms behind the independent and combined effects of multiple stressors, including temperature and ocean acidification, and across different levels of biological organisation requires controlled experimental systems that are often impractical or impossible to achieve in the field (Stewart *et al.*, 2013; Sagarin *et al.*, 2016; Yang *et al.*, 2022). Mesocosms have been developed as a tool to conduct experiments that are controlled and repeatable to a degree and can be adapted to contain several levels of biological organisation, thus, some natural complexity (Odum, 1984; Stewart *et al.*, 2013). Such research facilities embody a compromise between small-scale, highly-controlled and easy to replicate laboratory (microcosm) experiments and the complex scope of natural ecosystems studied in field observations (Sagarin *et al.*, 2016; Boyd *et al.*, 2018). While the first are highly valuable to, e.g., identify physiological pathways, they are usually reduced to single components of a whole system and thus reflect limited ecological realism (Stewart *et al.*, 2013; Todgham and Stillman, 2013). Holistic, ecosystem-level evaluations, on the other hand, describe naturally complex conditions, which include much unexplained variability and therefore make it difficult to identify causal and mechanistic relationships (Stewart *et al.*, 2013). Mesocosm experiments include all approaches that combine ecological engineering and comprehensive experimental design to construct artificial model ecosystems for experimentally studying analogous natural ecosystems (Kangas and Adey, 2008). They enable the investigation of biological interactions and natural processes under a range of controlled environmental variables (such as temperature, light intensity, salinity, pH, nutrient concentration) and across different spatial and temporal scales. Realistic biological function and structure are maintained while unexplained variability is reduced by controlling involved functional groups, i.e. biotic complexity, or physical factors such as temperature, light intensity, pH, or resource availability (Giesy and Odum, 1980; Kangas and Adey, 2008). Replication is vital to conduct robust research with a high likelihood of detecting true responses, while minimising unexplained variability and statistical error, but maintaining high ecological realism (Kraufvelin, 1998). Although not as pronounced as in field experiments (Yang *et al.*, 2022), adequate replication is a limitation in many mesocosm studies that needs to be overcome for reliable, statistically robust results and the accurate identification of interacting effects of multiple stressors (Kreyling *et al.*, 2018; Tekin *et al.*, 2020; Burgess, Jackson and Murrell, 2022).

In addition to mechanistic investigations of change in controlled conditions, monitoring long-term environmental and ecological trends in the field is essential to document change of natural ecosystems in a changing climate and under additional anthropogenic stressors. A wealth of monitoring data is available for many regions including EU states that are required to monitor and report the ecological status of their coasts following several legally binding directives (e.g. EU Water Framework Directive and Marine Strategy Framework Directive; European Commission, 2000, 2008). These raw data are often condensed into proxies to assess levels of ecological status for reporting, which limits their suitability for exploratory analysis that tests for relationships among variables, such as species richness and temperature (Voulvoulis, Arpon and Giakoumis, 2017; Feld, Saeedghalati and Hering, 2020). To manage and protect ecosystems effectively, aquatic resource management requires an understanding of the links between environmental conditions and biological communities, the consequences of combined effects of climate change and multiple local or regional stressors, and also predictions of how changing conditions including management decisions may affect communities (Philippart *et al.*, 2011). Consequently, monitoring information must be synthesised and made readily accessible, including extrapolated change according to long-term scenario projections of climate change, to inform effective, anticipatory management action to protect and conserve coastal and marine ecosystems and the services they provide (Feld, Segurado and Gutiérrez-Cánovas, 2016; Gissi *et al.*, 2021). Modelling, e.g. the application of Bayesian Networks, is one way of addressing this need and enhancing ecosystem understanding by integrating complex knowledge, predicting the effects of change, and supporting decision-making (Kelly *et al.*, 2013).

Bayesian Networks (BNs) are a useful modelling tool for linking relevant drivers, pressures and impacts of multiple stressors in complex ecosystems, presenting probabilistic outcomes of desired biodiversity aspects or ecosystem services following certain management choices or climate change scenarios (Uusitalo, 2007). BNs apply conditional inference and calculate the overall probabilities that certain variable conditions, i.e. categorical conditions or data ranges, will be attained. BNs can be used prognostically (given the inputs, what are the outcomes?) or diagnostically (given an output, what were the inputs?). The advantages of BNs as tools to synthesise complex system information include: (i) knowledge from different sources (e.g. monitoring programmes; disciplines such as ecology, hydrology, social science, economics; expert opinion) can be combined; (ii) different types of data (e.g. experimental, monitoring or questionnaire data; quantitative or qualitative) can be incorporated; (iii) uncertain, small or incomplete data sets can be included; and (iv) new information can be added as it becomes available (Uusitalo, 2007; Aguilera *et al.*, 2011; Kelly *et al.*, 2013). The probabilistic BN outputs include explicit information about the uncertainty of data or predictions in a study system, which also identifies knowledge gaps (Chen and Pollino, 2012). Linking existing information from a variety of sources is crucial to deepen a comprehensive understanding of marine systems, tracing pressures back to their origin and predicting the impacts of future climate change so that explicit management actions can be identified.

1.2 Thesis aims and objectives

The overarching aim of this thesis was to quantify the impact of multiple stressors on the production of marine benthic resources, using alternative approaches. Specifically, methodological tool development (Chapter 2), applying a new mesocosm facility for empirical research (Chapter 3), and synthesising monitoring data in a Bayesian Network model (Chapter 4) were combined to enhance multiple stressor research and help predict the consequences of expected climate change for coastal habitats (Figure 1.1).

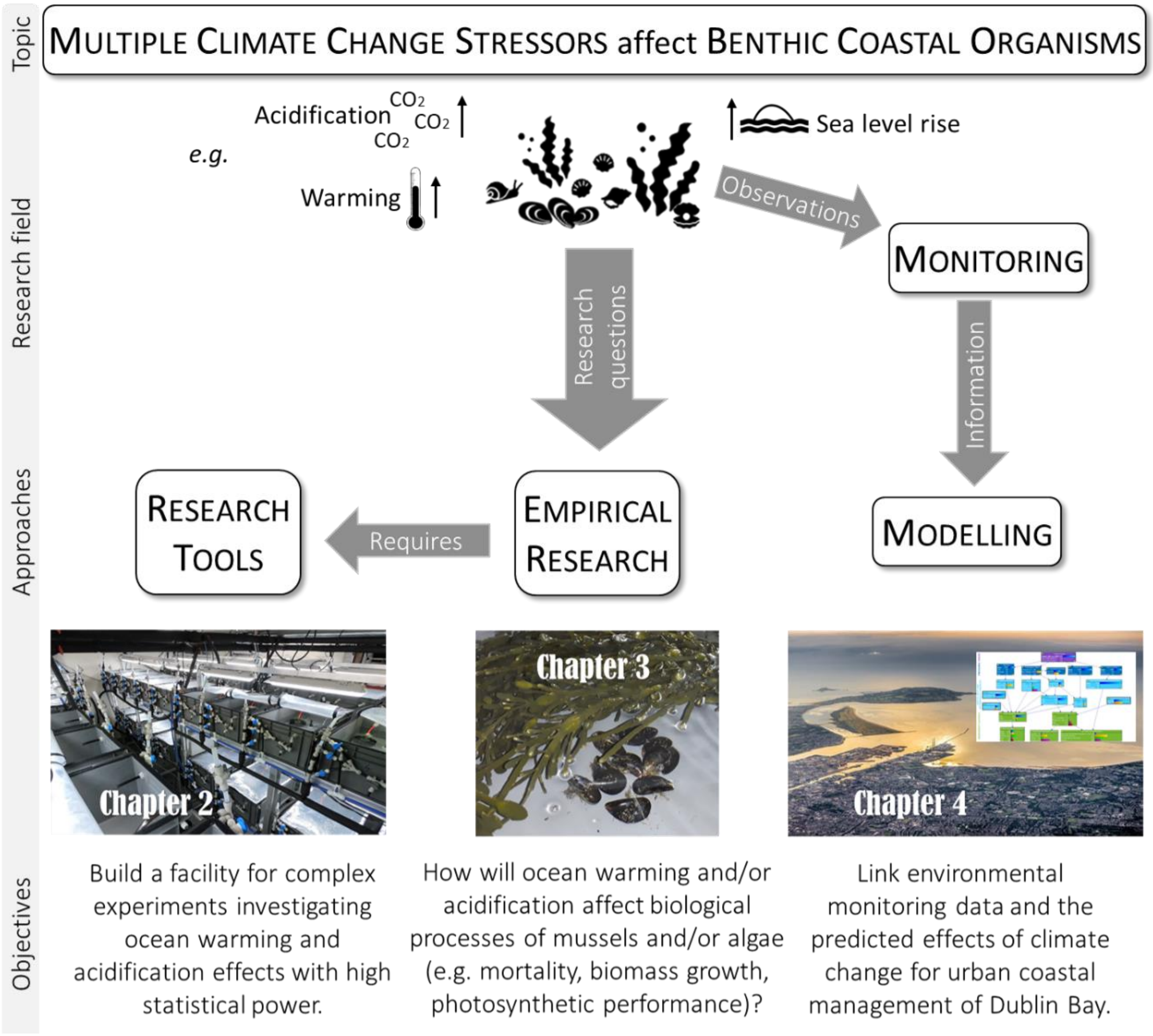

Figure 1.1 Graphical thesis outline with topic and research field investigated, as well as alternative approaches and objectives addressed in the three main chapters of this PhD thesis. Arrows indicate flow of information that this thesis was based on.

1.2.1 Chapter 2

'Quantifying the Impacts of Multiple Stressors' (QIMS) – a new experimental platform for multifactorial experiments with high replication in marine benthic ecosystems

Here, I present a new aquatic mesocosm-based experimental platform: Quantifying the Impacts of Multiple Stressors (QIMS). This new experimental system was designed primarily for marine benthic communities and facilitates nuanced and comprehensive tests of multiple stressors effects and biological interactions using a large quantity of experimental units. It consists of 96 independent replicate mesocosms, allowing for multifactorial and multilevel experimental designs that maintain high replication and provide the necessary statistical power for robust data analyses. I provide evidence of the facility's functionality by demonstrating that clearly separated factorial treatment levels of temperature and pCO_2 can be precisely manipulated and maintained in a fully crossed experimental design for > 40 days. Quantifying the Impacts of Multiple Stressors (QIMS) complements a suite of different marine mesocosm facilities around the world that simulate ocean warming and/or acidification by facilitating marine multiple stressor research at an unprecedented level of statistical robustness and fully crossed treatment combinations.

1.2.2 Chapter 3

Mussels and algae function faster with increasing temperature, resist ocean acidification, and facilitate each other's production

Using an experimental manipulative approach and employing the newly built indoor mesocosm facility QIMS, I advance empirical knowledge on the interactive effects of ocean warming and acidification on local rocky shore species (*Mytilus edulis* and *Saccharina latissima*) considering their biotic interactions. These shellfish and algae are both important habitat engineers and aquaculture species, which is why changes in their abundances, consumption or biomass production may have substantial ecological or economic consequences. Mussels and/or algae were exposed to three levels of temperature (ambient fluctuated naturally from 8.5 to 12.4 °C ± 0.07 [means ± se], stable +0.8 °C and +2 °C intervals) and two levels of pCO_2 (ambient at 450 ppm, elevated at 645 ppm) to test for independent and combined effects of moderate, realistic near-future warming and acidification on a range of biological processes (mussel mortality, biomass, clearance rates and condition index; algal biomass and the photosynthetic performance metrics relative maximum electron transport rate [rETRmax], saturating light intensity [I_k], light harvesting efficiency [α], and maximum quantum yield [F_v/F_m]). Additionally, it was tested whether these responses differed in the presence of the other species (i.e. comparisons of treatments with only mussels present, with only algae and with both mussels and algae), as recent research has highlighted the potential of marine macrophytes to buffer CO_2 effects on calcifying species.

1.2.3 Chapter 4

Linking environmental monitoring data and the predicted effects of climate change for urban coastal management: a case study of Dublin Bay.

Monitoring data of physico-chemical variables, waves, river flow, phytoplankton, benthic invertebrates and wader birds from 2007-2020 was synthesised by applying conditional inference and building a Bayesian Network (BN) that characterises Dublin Bay, which is a typical urban coastal ecosystem. Additionally, the BN provides ecosystem trajectories under different nutrient availability and sediment organic content, as well as a moderate and an extreme predicted climate change scenario and the corresponding effects on biodiversity indicators such as phytoplankton abundance and biomass, benthic invertebrate taxa richness or wader abundance. This modelling type was chosen because data from different sources and types can be combined, even if uncertain or incomplete, and new information can be added as it becomes available. Additionally, the probabilistic outputs include explicit information about the uncertainty of data or predictions in a study system, which also identifies knowledge gaps. The BN thereby supports local management decision-making by deepening the understanding of the complex linkages between environmental conditions and the diversity and functioning of Dublin Bay. Furthermore, it helps to trace changes in biodiversity back to their origin and predict the impacts of future climate change so that explicit management actions can be identified.

Chapter 2 | ‘Quantifying the Impacts of Multiple Stressors’ (QIMS) – a new experimental platform for multifactorial experiments with high replication in marine benthic ecosystems

Authors

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Author contributions

I contributed to the design and especially construction of the QIMS Mesocosm Facility, led and performed tests on the facility’s functionality and wrote the manuscript. Robert Fitzpatrick led the design and implementation of the facility. Nessa O’Connor conceived the idea, secured the funding for the project and provided feedback on the written manuscript.

This work is currently in preparation for submission to the journal *Limnology and Oceanography: Methods*.

2.1 Abstract

1. To predict the ecological consequences of expected global change it is necessary to understand the independent and combined effects of multiple stressors at different levels of biological organisation (from single species to whole ecosystems). Empirical tests of multiple stressors, therefore, require multiple experimental treatments to simultaneously test for effects at various levels of stressor intensity, independently and combined, as well as at different levels of biological organisation. Most multiple stressors studies of marine systems to date were conducted on assembled communities in mesocosms with a low number of treatments or low replication of treatments or both. These limitations remove our ability to: (i) apply robust data analyses; (ii) disentangle or compare single and combined effects of multiple stressors; and (iii) identify mechanisms underpinning biological responses.

2. We present a new aquatic mesocosm-based experimental platform: Quantifying the Impacts of Multiple Stressors (QIMS). This new experimental system was designed primarily for marine benthic communities and facilitates nuanced and comprehensive tests of multiple stressors effects and biological interactions using a large quantity of experimental units. It consists of 96 independent replicate mesocosms, allowing for multifactorial and multilevel experimental designs that maintain high replication. For example, using the three distinct pH levels that are achieved by manipulating CO_2 concentrations in the air supply, and the three water temperature levels provided by a cooling system in a fully crossed design still allows a 10-fold replication of each treatment.

3. We provide evidence of the facility's functionality by demonstrating that clearly separated factorial treatment levels of temperature and pCO_2/pH can be precisely manipulated and maintained in a fully crossed experimental design for at least seven weeks.

4. Quantifying the Impacts of Multiple Stressors (QIMS) complements a suite of permanently installed marine mesocosm facilities that simulate ocean warming and/or acidification and facilitates multiple stressor research at an unprecedented level of statistical robustness and fully crossed, multilevel factor combinations.

2.2 Introduction

Although global change is affecting coastal ecosystems (IPCC, 2022), we still lack information on the independent and combined effects of multiple stressors to predict ecological consequences of expected global change (Crain *et al.*, 2008; Côté *et al.*, 2016). Coastal ecosystems are vitally important for many key ecosystem services, yet they experience or risk serious degradation owing to anthropogenic activities that result in ocean warming and acidification, more frequent and intense extreme weather events, sea level rise, increasing fluctuations in salinity and depletion of oxygen (Harley *et al.*, 2006; Lotze *et al.*, 2006; Halpern *et al.*, 2007; IPCC, 2019, 2022). Only when we can estimate our future ocean's species assemblages, protect and restore habitat, we will be able to accurately predict future provision of ecosystem services, such as our capacity to produce bioenergy, food and biomaterials from marine organisms (e.g. shellfish, seaweed).

Research of recent decades has moved beyond single-stressor-single-species experiments with limited extrapolation potential and has increasingly investigated the cumulative effects of simultaneously occurring multiple stressors, which may be magnified by synergistic interactions (Crain *et al.*, 2008; Wernberg, *et al.*, 2012; Todgham and Stillman, 2013; Côté *et al.*, 2016; Hanson and Walker, 2019). Upscaling from controlled laboratory experiments to natural ecosystems, however, remains challenging because the interactions of combined effects of multiple stressors can differ across levels of biological complexity or trophic order, depend on the number of stressors examined, and on identity, timing and intensity of the combined stressors (Crain *et al.*, 2008; Walther, 2010; Griffen *et al.*, 2016; Wahl *et al.*, 2019).

To comprehensively investigate multiple stressor effects, including their interactions and non-linear responses, high numbers of experimental units are necessary (Kreyling *et al.*, 2018). Yet, trade-offs between experimental complexity (multifactorial experiments with multiple factor levels), the size of experimental units and therefore achievable biological complexity, statistical power and robustness, and replication are common restrictions of global change experimental research because of too few experimental units, apart from limiting logistic and financial feasibility (Stewart *et al.*, 2013; Boyd *et al.*, 2018; Kreyling *et al.*, 2018; Menczelesz *et al.*, 2020). Additionally, many experimental set-ups include nested structures, such as header tanks or waterbaths, or multiple individuals are treated as replicates although housed within the same experimental tank, i.e. pseudoreplication is introduced because treatment replicates lack independence or random interspersal (Hurlbert, 1984; Wernberg *et al.*, 2012; Cornwall and Hurd, 2016). This undermines our ability to accurately predict the effects of multiple stressors. Here, we present a new experimental platform that overcomes these limitations and enables multifactorial, multilevel, and biologically complex experiments with high replication of independent experimental units in benthic marine research.

There are calls both for more factorial experiments investigating for example predicted levels of ocean acidification and warming of plausible future climate scenarios, and for moving to gradient designs in ecological experiments to detect and quantify nonlinear responses, or to extrapolate responses to continuous environmental drivers further (Kreyling *et al.*, 2018; Geraldi *et al.*, 2020). Both approaches require high amounts of experimental units to either disentangle the independent and combined effect of multiple factors at multiple levels, or to cover as many levels as possible along the gradient of an environmental driver to reveal potential non-linearity (Kreyling *et al.*, 2018). Experiments that contain factorial approaches can be analysed with ANOVA (or GLM if required), where explicit tests for interactions among factors will identify non-additive effects of cumulative stressors. Non-linear responses in gradient designs require more sophisticated analyses. Including multiple levels of biological complexity increases the complexity of experimental designs further.

Additionally, adequate replication is indispensable to improve statistical power in factorial designs, or to fit accurate response curves and quantify the amount of variation explained by the environmental driver in gradient designs (Chalcraft, 2019). Furthermore, non-additive interactions in multifactorial experiments, i.e. when the combined effects of multiple stressors are different to the sum of individual effects, can only be detected and identified if either this difference to additive expectations (effect size) or the amount of replication are large (Tekin *et al.*, 2020; Burgess *et al.*, 2022). Too little replication is a major cause of underpowered experiments, which either fail to detect significant effects, or tend to over-estimate the true magnitude of effects and thereby lead to publication bias regarding the true effects of anthropogenic impacts (Yang *et al.*, 2022). At the same time, very high replication may detect statistical interactions beyond biological relevance and thereby waste resources, which is why the minimum biologically relevant effect size should be considered in advance (e.g. the threshold for management action to conserve a threatened species) (Burgess *et al.*, 2022). 'Adequate replication' depends on the variability of the dependent variables, experimental background noise, the stressor's mode of action, or the situational requirements to accurately detect even very small interactive effects, however, the very commonly used 1-4 replicates per categorical treatment are most likely insufficient to detect non-additive effects of interacting environmental drivers (Burgess *et al.*, 2022).

In marine multiple stressor research, the effects of temperature crossed with acidification, salinity or hypoxia are the most widely studied (Gunderson *et al.*, 2016). Warming, which directly affects metabolic rates of marine ectotherms, is closely linked with acidification and changes in the carbon chemistry, which particularly affect photosynthetically active or calcifying species (IPCC, 2019). While much physiological research on single species has been conducted in highly controlled, small-volume microcosms, and recent field work manipulated temperature and pH in tide pools (Sorte and Bracken, 2015; Bracken *et al.*, 2018; Jellison *et al.*, 2022), many ecological, biologically complex global change studies are conducted in mesocosms (Sagarin *et al.*, 2016; Boyd *et al.*, 2018). Mesocosms hold tens to thousands litres of water containing semi-natural assemblages and enable controlled mechanistic investigations (Sagarin *et al.*, 2016; Boyd *et al.*, 2018). Most permanently installed marine mesocosm facilities that simulate ocean warming and/or acidification are limited to < 20 experimental units (Overview of permanently installed marine mesocosms in Table A.1) and, therefore, strongly restrict experimental complexity and replication.

Here, we present the new QIMS (Quantifying the Impacts of Multiple Stressors) Mesocosm Facility that allows nuanced and comprehensive investigation of multiple stressors and biological interactions owing to the large quantity of 96 independent experimental units. Three distinct water temperature levels (ambient, +0.8 °C, +2 °C) and any three pCO_2 aeration levels allow the simulation of predicted Irish end-of-century climate change scenarios and can be randomly allocated to each mesocosm. For example, in Chapter 3 I investigated the effects of near-future warming (3 levels) and acidification (2 levels) on mono- and co-cultures of shellfish and algae. The QIMS Mesocosm Facility presents a valuable tool in marine climate change research to disentangle the individual and combined effects of multiple stressors on the functioning of temperate marine ecosystems in complex, statistically powerful experiments.

2.3 Methods

2.3.1 Mesocosm platform

The QIMS Mesocosm Facility (Figure 2.1) at Trinity College Dublin, Ireland, was purpose-built to simulate a temperate maritime climate typical for the Irish Sea within the NE Atlantic, in current and predicted future conditions. QIMS is an indoors facility that consists of 96 independent experimental units (mesocosms) that can each be individually adjusted to simulate one of three levels of temperature (ambient, +0.8 °C, +2 °C) and one of three pCO_2 levels (ambient and any two, e.g. 550, 650, or 1000 ppm; Figure 2.2). Eleven aluminium racks carry the mesocosms on two levels, with five mesocosms on the top and four on the bottom level.

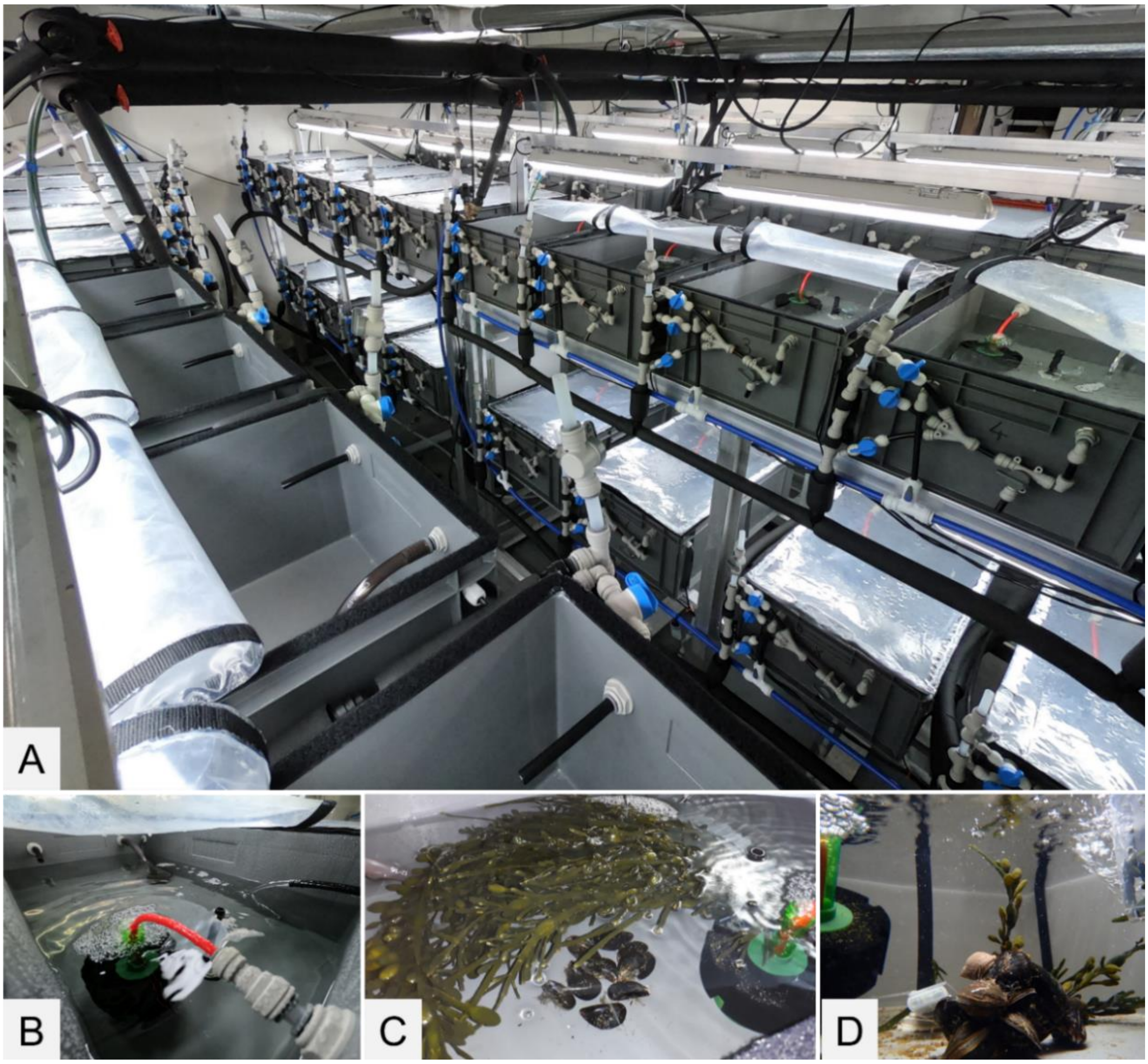

Figure 2.1 Quantifying the Impacts of Multiple Stressors – The QIMS Mesocosm Facility (A). Inside view of a mesocosm (B) and experimental application with biota (C, D).

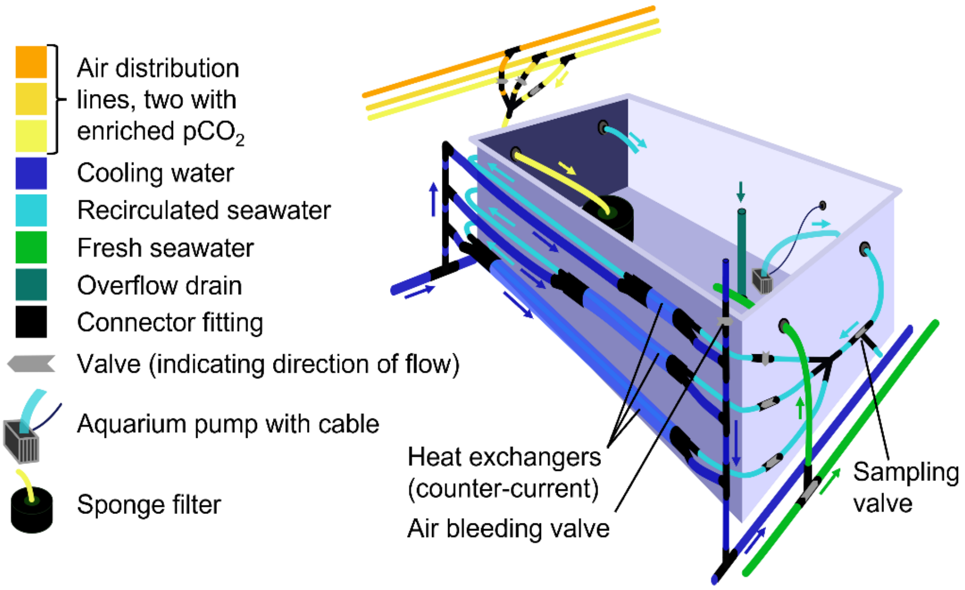

Figure 2.2 Schematic figure illustrating the components of one mesocosm. Arrows indicate the direction of air and water flow.

Each mesocosm is an opaque 40 x 60 x 28 cm (outside dimensions) box (45 L stacking box, Irish Box Company), which is made of inert, food grade polypropylene. Mesocosm are filled with 32 L of seawater collected from 20 m depth at the Irish continental shelf (supplied by Seahorse Aquarium) and can be topped-up as required via individual isolation valves from a 4000-L storage reservoir. The water level in each mesocosm is determined by a removable overflow drain tube made of polyethylene, which is connected to a main floor drain. To ensure homogenous mixing, the water of each mesocosm is continuously recirculated at 130 L/h around the mesocosm, using small aquarium pumps (OptiMax 500, Oase Living Water). The seawater flow returns into each mesocosm at an angle opposite the pumps to maximise mixing and increase surface gas exchange. Mesocosms are aerated at 15.5 mL/min via aquarium sponge filters (PK200 for < 200 L, Xinyou Aquarium) that ensure nutrient cycling by providing a settlement surface for microorganisms such as denitrifying bacteria. The sponge filters also provide mechanical filtration by capturing floating particles via suction caused by the continuous air supply. All water and air lines supplying the individual mesocosms consist of more than 1 km of inert, flexible polyethylene tubing, which is connected by more than 5000 connecting pieces (JG Speedfit Plastic Push-fit Plumbing Fittings). The tubes are opaque to reduce internal biofouling. Lids of transparent polypropylene (Foliarex UV4 Greenhouse Film) are attached to the mesocosms via rims of hook and loop fasteners. In each individual mesocosm, the lids capture the continuously replenished ambient or pCO_2-enriched atmospheres in the 16 L headspaces above the seawater, in addition to preventing mobile organisms, e.g. gastropods, from leaving the mesocosms. Artificial illumination is installed 24 cm above the water surface and provided by 18-watt LED lights with standard artificial daylight colour (Radium LED DAMPPROOF; 6500 Kelvin). 85 % of the provided light penetrates the lids so that 50 ± 4 $\mu mol/m^2s$ (mean ± standard deviation; measured using an underwater quantum meter MQ-510 by apogee instruments) reach the water surface. QIMS is controlled by a professional aquarium computer (GHL, ProfiLux 3), which operates the light timing (e.g. diurnal dark-light-rhythms as required) in addition to monitoring and controlling the cooling and the CO_2 manipulation systems (see below). Additional temperature, conductivity or pH probes can be connected to the computer. Floor fans distributed throughout the facility ensure homogenous air temperature.

2.3.2 Temperature manipulation

To achieve the range of 8 – 16 °C winter and summer median seawater temperatures of Irish coastal waters (O'Boyle *et al.*, 2015) in the QIMS Mesocosm Facility, an independent cooling water circuit individually supplies each rack and each mesocosm in parallel. At each mesocosm, tubes containing the seawater run through the centre of larger cooling tubes. Three parallel-connected beer chillers that include pumps for distribution (MF Refrigeration Ltd, Midi 6 Coil R290 Hydrocarbon Cooler, each with a capacity of 2 kilowatts) continuously cool approximately 2000 L of fresh water at maximum capacity. It is circulated through the facility at a rate of 8200 L/hour via PVC pipelines (50 mm diameter) and, on each rack, polyethylene tubing (15 mm diameter). Balancing valves at the rack returns ensure equal cooling flow in all racks. The chillers are controlled by an integrated interface on which the target temperature of the cooling water can be set. Flow meters and level sensors have been added to the chillers and connected to the aquarium computer for further control and monitoring.

The recirculation set-up of each mesocosm simultaneously functions as a counter-current heat-exchange system that leads two parallel recirculation lines (10 mm diameter) of equal length through cooling tubes (18 mm diameter) of different lengths (Figure 2.2): 64.5 and 45.5 cm long cooling tubes for the coldest of three distinct temperature levels (ambient); 45.5 and 15.5 cm for +0.8 °C; 15.5 cm and no further cooling for +2 °C. Each recirculation line is equipped with an independent shut-off valve, while the cooling lines are continuously flushed to avoid interruptions and therefore stabilise the overall cooling circuit. To avoid or remove air locks that would impair cooling bleeding valves are mounted at the highest point of each mesocosm's cooling system. All lines containing cooling water are insulated against temperature loss to the room.

2.3.3 CO_2 manipulation

Ocean acidification is simulated in the QIMS Mesocosm Facility following best practice (Gattuso *et al.*, 2010): Air is enriched with CO_2 to achieve certain partial pressures (pCO_2) that reflect atmospheric CO_2 concentrations in parts per million (ppm) for different predicted climate change scenarios in 2100 (IPCC, 2014). Via gas exchange at the seawater-atmosphere interface, those atmospheric pCO_2 predictions are expected to decrease ocean pH by 0.05 to 0.3 units (IPCC, 2019).

Two large blower pumps provide air from outside the building to the whole facility. The air is drawn off a manifold through three lines, one of which continues to provide ambient air (450 ppm in Dublin city). The other two lines connect to 3-L CO_2 cylinders that enrich ambient air with selected CO_2 concentrations, e.g. 550 ppm for a strongly mitigated, very low-emission climate change scenario, or 1000 ppm for a business-as-usual, high-emission scenario (IPCC, 2013). Non-return valves at various stages ensure one-directional flows. The air enrichment takes place in 25-L mixing vessels and is continuously monitored by two gas analysers (LI-COR, LI-830; factory-calibrated). The gas analysers are connected to the main aquarium computer, which controls solenoid valves at each CO_2 cylinder and thereby the CO_2 injection. Three colour coded lines distribute the ambient or manipulated air from the mixing vessels to the mesocosms. Each rack is provided with its own take off points (flow rates of 14.5 – 16.5 mL/min) and each mesocosm is fitted with valves to individually select one of the three air supplies. The continuous supply of ambient or CO_2-enriched air is bubbled into the mesocosm water via the sponge filters and captured as a corresponding excess pressure atmosphere in each mesocosm's head space, allowing the seawater to equilibrate accordingly. The continuously replenished excess pressure is released into the room via the hook and rim fasteners.

2.3.4 Proof of concept

pH manipulation at three levels of pCO_2 (ambient [450 ppm], 550 ppm, 1000 ppm) was first tested at room temperature (19 ± 0.1 °C; mean ± se) in 36 mesocosms in 2019. Twelve mesocosms were randomly allocated to each level and left to equilibrate for 24 hours. pH was measured using a handheld multimeter (HACH HQ40d), which was calibrated using certified pH buffers (pH 4, 7 and 10; Lennox Laboratory Supplies). The purpose of this test was to proof the efficiency of the pH manipulation in the QIMS Mesocosm Facility, which is why no biota was added that might confound pH levels through metabolic activity such as respiration.

In a separate, more complex design, two levels of pCO_2 (ambient, 645 ppm) were fully crossed with three levels of temperature (ambient, +0.8 °C, +2 °C) and maintained during a 7-week experiment in 2020, in which all 96 mesocosms were used (Chapter 3). Each abiotic treatment (i.e. abiotic level combination) was replicated 15 times. Additionally, three levels of biota were included: five mesocosms per abiotic treatment contained shellfish, five contained algae, and five contained both shellfish and algae, yielding 18 experimental treatments (Figure 2.3A). One additional mesocosm per abiotic treatment contained only seawater and served to monitor the efficiency and consistency of the abiotic treatment manipulation (Figure 2.3B).

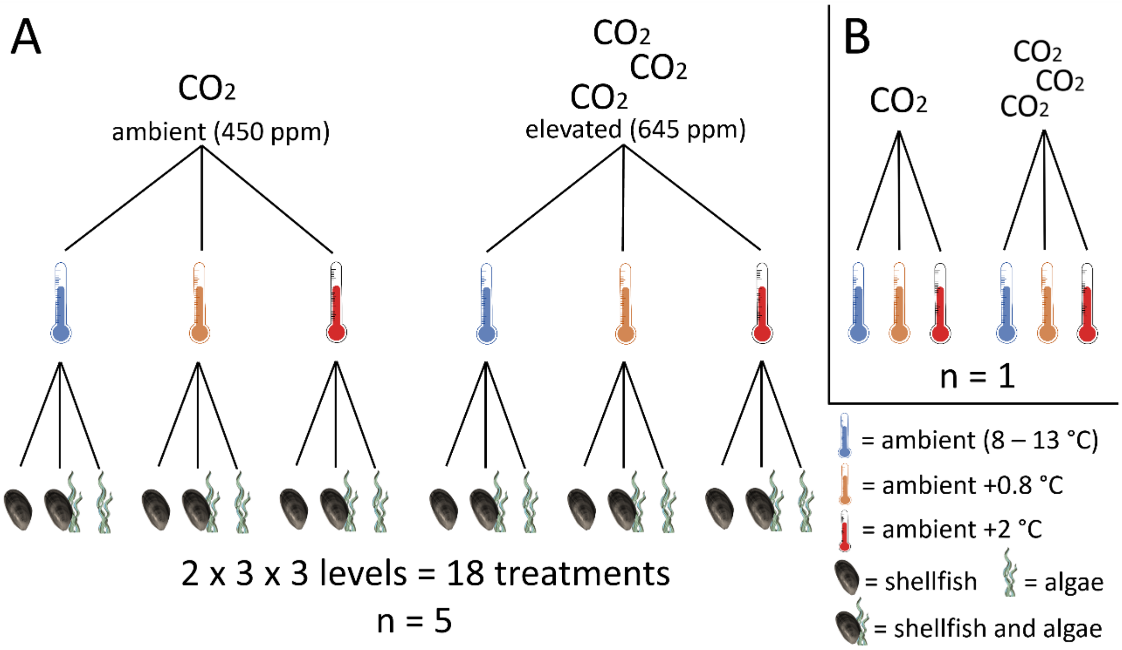

Figure 2.3 Experimental design in A and additional abiotic treatment monitoring in B used to test all 96 mesocosms of the QIMS facility simultaneously.

Mesocosms were acclimatised for a week before abiotic manipulation started. Temperature was logged every 10 minutes in 42 mesocosms using HOBO pendants (type MX2201 and Temperature/Light 64K; seven randomly deployed per treatment; one logger failed) and measured once per week in all mesocosms using a handheld digital thermometer (Fisherbrand Traceable Kangaroo Thermometer; see Figure for variation at each temperature level and corresponding statistical analyses). pCO_2 of CO_2-enriched air was logged every second by the LiCor gas analysers. Room air CO_2 concentrations were monitored every minute for safety and for perturbing influences on the pCO_2 treatments using a rugged visual CO_2 data logger (Driesen-Kern, DK660; factory-calibrated). pH in the blank mesocosms was measured twice weekly. Water was changed after 4 weeks and no pH measurements were taken that week. Measurement equipment to continuously monitor pCO_2 of the ambient air supply, or pH in the mesocosms was not available.

One-way or mixed measures ANOVA were performed to compare means between levels and repeated measurements of individual mesocosms as required. To confirm that assumptions were met, outliers were explored, normality confirmed via qqplots, homogeneity of variances tested using Levene’s Test, and sphericity was tested using the Mauchly’s test of sphericity that is included in the R function anova_test() of the package rstatix version 0.7.2 (Kassambara, 2023). When appropriate, bonferroni-corrected pairwise post-hoc comparisons between levels at each time of measurement were used to confirm that levels remained distinct from each other during repeated sampling. Statistical analyses were conducted in R version 4.2.1 (R Core Team, 2022) using R Studio version 22.07.2 (RStudio Team, 2022) and the package tidyverse version 1.3.2 (Wickham *et al*., 2019).

2.4 Results

Three distinct levels of near-future pCO_2 levels (ANOVA $F_{2,\ 28} = 259.3$; $p < 0.001$; Tukey's Honest Significance Difference post-hocs $p < 0.001$) were simulated in the QIMS Mesocosm Facility in 2019 (Figure 2.4). Data of five mesocosms at 1000 ppm was discarded due to water accumulation in the airlines and consequently insufficient aeration and manipulation.

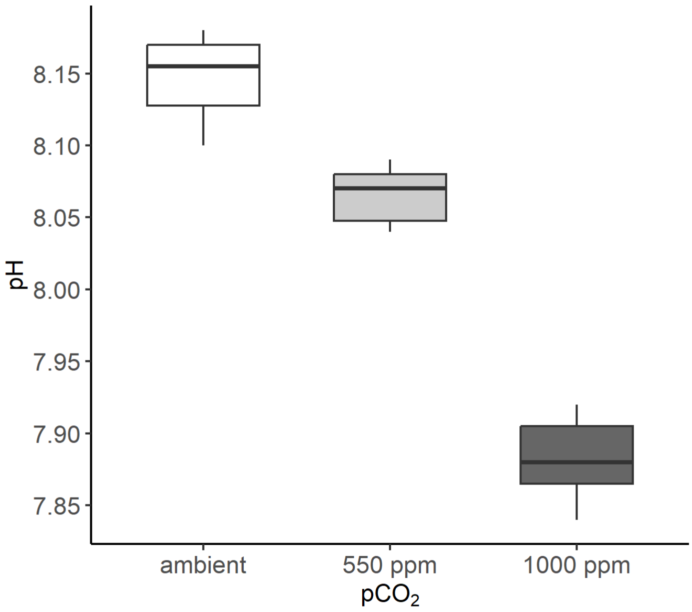

Figure 2.4 Seawater pH in the QIMS Mesocosm Facility at ambient pCO_2 *(white; n = 12), 550 ppm (light grey; n = 12) and 1000 ppm (dark grey; n = 7).*

Ambient temperature in the mesocosms ranged from 8.5 ± 0.07 °C to 12.4 ± 0.07 °C (means ± se), which resembles winter water temperatures of Irish coastal waters (Figure 2.5). Two additional temperature levels at stable +0.8 ± 0.0 °C and +2.0 ± 0.1 °C (means ± se) intervals were implemented after 4-6 hours of warming. All temperature levels fluctuated up to 2 °C per day according to room temperature, which was influenced by outside temperatures and house heating. Time of measurement interacted significantly with the temperature level (mixed measures ANOVA $F_{7,\ 333} = 22.769$; $p < 0.001$), however, bonferroni-corrected pairwise post-hoc comparisons between levels at each time of measurement confirmed that levels remained distinct from each other (Figure A.1; Table A.2).

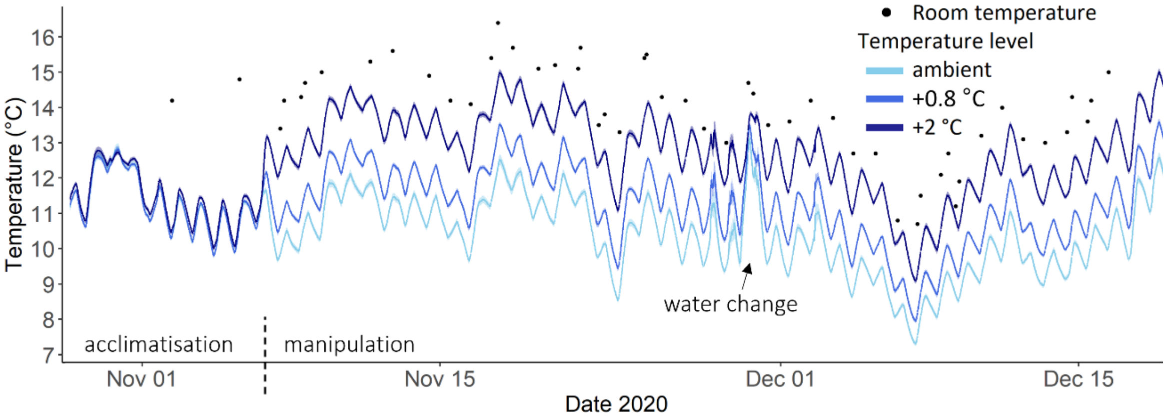

Figure 2.5 Means (lines) and standard errors (shaded areas) of ambient (light blue, n = 13), +0.8 °C (blue, n = 14) and +2 °C (dark blue, n = 14) water temperature in the QIMS Mesocosm Facility during a trial experiment in 2020. Black dots indicate room temperature.

Room pCO_2 was 452 ± 0.12 ppm (mean ± se) and fluctuated daily according to human presence (Figure 2.6). Although human activity caused irregularities in room air CO_2 and the excess pressure atmospheres of each mesocosm were continuously released in the room, room pCO_2 never reached the mean concentration of 645 ± 0.01 ppm (± se) of the elevated pCO_2 level.

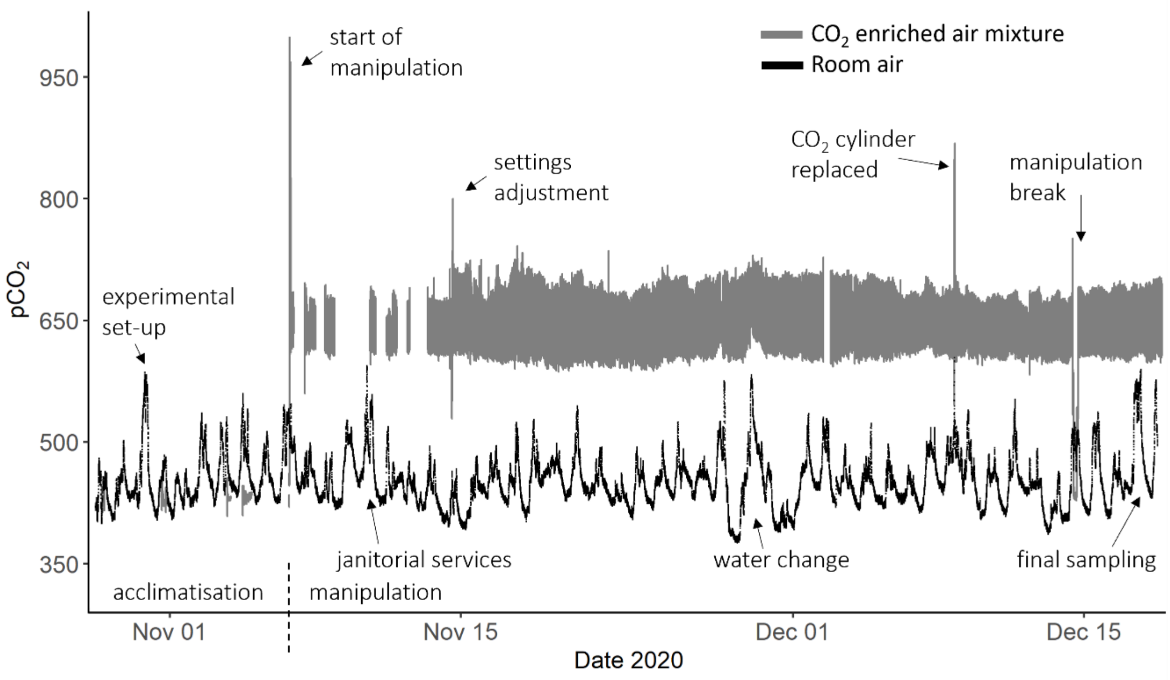

Figure 2.6 pCO_2 (parts per million) of room air (black) and CO_2 enriched air (grey) in the QIMS Mesocosm Facility during an experiment in 2020, including explanations of irregularities. Note that the enriched air pCO_2 was logged per second and fluctuated rapidly according to the CO_2 injections, while room air pCO_2 was logged per minute and fluctuated much slower according to human activity.

pH at ambient and enriched CO_2 concentrations in the blank mesocosms was (mean ± se) 8.09 ± 0.004 and 7.97 ± 0.003 (Figure 2.7). Date of measurement and pCO_2 level did not interact, however each significantly affected pH (mixed measures ANOVA; date: $F_{9,\ 18} = 33.210$, $p < 0.001$;

pCO_2 level: $F_{1,\ 2} = 4720.403$; $p < 0.001$). Bonferroni-corrected pairwise post-hoc comparisons between levels at each time of measurement confirmed that levels remained distinct from each other (Table A.3). The elevated pCO_2 level of 645 ppm was reached in the air mixture within a few minutes of starting the treatment manipulation, while the corresponding pH in the seawater stabilised within 18 hours.

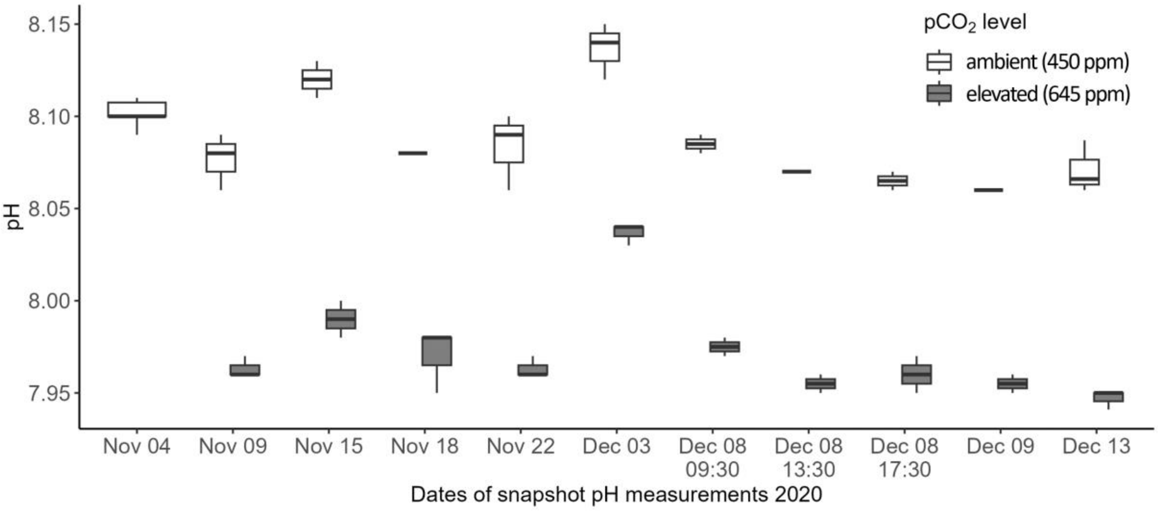

Figure 2.7 pH in mesocosms containing only seawater at ambient pCO_2 (white filling; n = 3, except on Nov 04 [n = 6], Dec 08 and 09 [n = 2]) and 645 ppm (grey filling; n = 3, except on Dec 08 and 09 [n = 2]).

Shellfish and algae added to the remaining mesocosms and their diets influenced the pH (ANOVA $F_{3,\ 72} = 52.882$, $p < 0.001$), however, distinct levels according to the provided pCO_2 concentrations were maintained (Figure 2.8; ANOVA F1,72 = 225.254, $p < 0.001$). Temperature had no effect on pH (ANOVA $F_{2,\ 72} = 1.269$, $p = 0.287$) and no interactions among the factors were found.

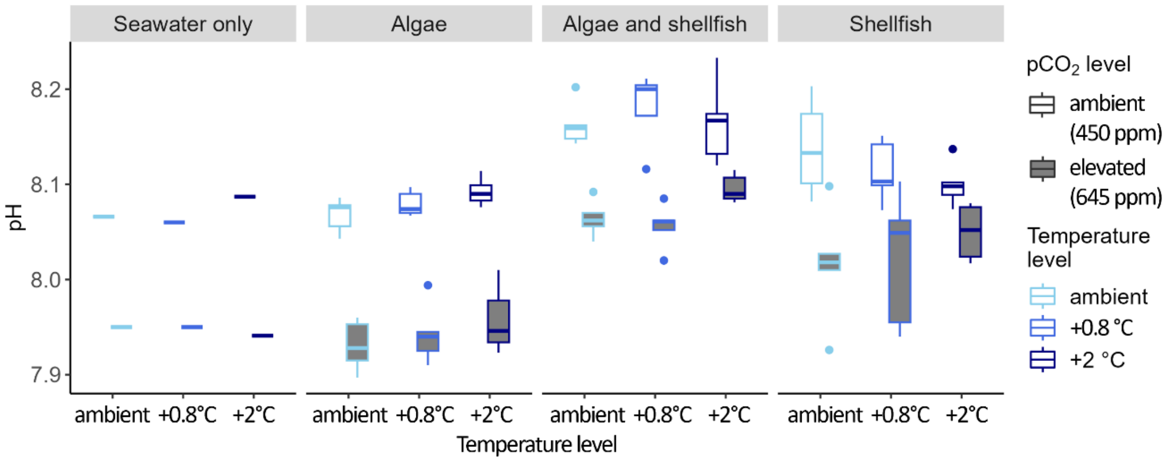

Figure 2.8 pH in mesocosms (n = 1 for seawater only; n = 5 per treatment with biota) containing seawater only, algae and/or shellfish at three levels of temperature (ambient – light blue; +0.8 °C – blue; +2 °C – dark blue) and two levels of pCO_2 (ambient – white filling; 645 ppm –grey filling).

2.5 Discussion

Our test results show that 96 independent 32-L mesocosms can be operated and maintained at up to three different, independent and clearly distinguishable temperature and pCO_2/pH levels in the QIMS Mesocosm Facility. Near-future predicted climate scenarios of up to +2 °C sea surface warming and a decrease of 0.28 pH units at 1000 ppm were achieved independently from anthropogenically influenced room conditions. Biota can be maintained for at least seven weeks as demonstrated in a factorial experiment with 18 treatments and five replicates per treatment (Chapter 3).

QIMS complements a suite of permanently installed marine mesocosm facilities that simulate ocean warming and/or acidification. They all have different research purposes, strengths and limitations (Table A.1). Other facilities are usually limited to < 20 experimental units, which, e.g. in case of the German Kiel Outdoor and especially Indoor Benthocosms, may allow for very elaborate and high-tech infrastructure and a very high degree of controllability (Wahl *et al.*, 2015; Pansch and Hiebenthal, 2019). The Israeli Red Sea Simulator with 80 mesocosms and the wave-action simulating mesocosms of the Queen’s University Belfast (Northern Ireland) with 100 mesocosms are the only other high-replication facilities (Mrowicki and O’Connor, 2015; Bellworthy and Fine, 2018). While both are outdoor facilities, ocean acidification cannot be simulated in the latter. The design and infrastructure of the QIMS Mesocosm Facility resembles the Red Sea Simulator the most, with the clear distinction that the Red Sea Simulator consists of glass aquaria for high availability of natural light, purpose-built for tropical coral reef research. The QIMS Mesocosm Facility targets temperate rocky shore research by, e.g., simulating sturdy rock pools or the shallow rocky subtidal at limited light availability.

Following the test of three pH levels in 2019, water bleeding valves were included in the air distribution lines and aeration problems thereby eliminated. For non-flow-through facilities, such as

QIMS, homogenous and controlled room temperature conditions proved pivotal for targeted water temperature manipulation. The modular nature of QIMS facilitated an inevitable change of location in 2021. The heat-exchanger systems of each mesocosm were updated to increase the temperature intervals between the different levels and achieve 3 °C of warming, which is predicted for an extreme climate change scenario for Irelands East coast in 2100 (Jacob *et al.*, 2014). The new location is still undergoing updates to meet the facility's requirement and final calibration and comprehensive testing of its functionality in the new location and with the improved heat exchangers is on-going. Thorough cleaning between experiments is essential to avoid carry-over effects, e.g. remaining micro fauna and flora, and protocols for chemical cleaning and disinfecting are being developed. The facility could be advanced further by adding, e.g., more automated monitoring to each mesocosm, stronger and dimmable lights, affiliated batch-testing devices for inorganic nutrient analyses and carbon chemistry, by updating the manipulation systems to allow stochastic or controlled natural fluctuations, and by allowing an even wider range of temperature levels to move beyond scenario-based climate change research.

Mesocosm studies are complementary to field studies and allow to identify clear cause-effect relationships and their underlying mechanisms in addition to disentangling interacting effects of multiple stressors that are difficult to tackle in the field. The ecological realism of any mesocosm system is limited, however, by common confounding factors such as initial disturbance through transport and relocation of organisms, wall effects and confined space, lack of lateral and/or vertical exchange, lower light availability and a selected subset of natural communities and trophic levels (Crowe *et al.*, 2012; Sagarin *et al.*, 2016; Boyd *et al.*, 2018). QIMS underlies these limitations as do other mesocosms, and has neither permanent seawater flow-through, nor does it include tidal effects. Nevertheless, because all organisms used in QIMS experiments experience the same constraints, results can reveal meaningful insights in the mechanistic fundamentals of their responses to changing environmental conditions.

QIMS was designed with a focus on disentangling the independent and combined effects of ocean acidification and warming, however, it can be adapted for more general use and a wide range of complex research designs owing to its high number of experimental units. Other environmental variables that do not require plumbing can be manipulated effortlessly in QIMS, e.g. nutrient concentration (addition of fertilizer), salinity (addition of fresh water) or light intensity (e.g. by covering the mesocosm lights with gauze). Regarding ocean acidification and warming effects, relevant research topics include relationships between trophic levels (especially when primary producers or calcifying organisms are involved), or primary producers and calcifying organisms (does photosynthesis buffer acidification effects?), or interspecific interactions between different functional groups and assemblages (ecosystem functioning). Including ocean acidification and warming as well as other stressors, research questions regarding the effects of multiple stressor sequence, intensity, duration, natural variability and considering different perspectives of underlying mechanisms are still understudied and could all be tackled in QIMS (Gunderson *et al.*, 2016; Jackson *et al.*, 2021; Orr *et al.*, 2022). Predictions of ocean acidification all refer to open ocean conditions (IPCC, 2014, 2019), while coastal systems underly substantial temporal and spatial variation that are poorly understood (Duarte *et al.*, 2013), hence investigating how biota influences pH and alkalinity may improve estimating potential future coastal conditions. To better understand the effect of replication on the identification of stressor interactions, experimental facilities such as QIMS with a high amount of experimental units may be useful tools, too (Burgess *et al.*, 2022). Moreover, QIMS is not limited to factorial experimental designs but can also facilitate gradient experiments that are needed to identify ecologically relevant thresholds in stressor intensities or non-linear stressor responses (Hanson and Walker, 2019).

Conclusion

QIMS was designed to quantify the impacts of multiple stressors on marine benthic organisms in fully crossed factorial designs, focussing on but not limited to simulating distinct levels of near-future ocean warming and acidification. Owing to its high number of experimental units, it can be adapted for more general use and a wide range of complex research designs, including multiple possible factors, factor levels and treatments with high replication. Conducting statistically powerful and robust experiments with this many options at hand for experimental manipulation of simultaneously acting multiple stressors, at multiple levels of biological complexity in parallel, will profoundly advance our knowledge about the effects of global change.

Chapter 3 | Mussels and algae function faster with increasing temperature, resist ocean acidification, and facilitate each other's production

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Author contributions

I conceptualised the study, led the applied methodology and investigation, conducted the formal data analysis, created the figures, drafted and revised the manuscript. Tallulah Davey and David Taylor assisted with refining the methodology and conducting the investigation, and Tallulah Davey commented on a first manuscript draft. Nessa O'Connor supervised conceptualising the study and choosing the methodology and provided feedback on the manuscript.

This work is currently in preparation for submission to the journal *Global Change Biology*.

3.1 Abstract

The effects of climate change on coastal biodiversity are a major concern because altered community compositions may change associated rates of ecosystem functioning and service provisioning. While responses of single species or taxa have been studied extensively, it remains challenging to estimate responses to climate change across different levels of biological organisation. Studies that consider the effects of moderate realistic near-future levels of ocean warming and acidification can help identify scope for adaptation and evolution, whereas studies including different levels of biological complexity may reveal opportunities for buffering or facilitation under changing environmental conditions. To test experimentally for independent and combined effects of predicted near-future warming and acidification on key benthic species, we manipulated three levels of temperature (ambient, +0.8 °C, +2 °C) and two levels of pCO_2 (ambient at 450 ppm, elevated at 645 ppm) and quantified their effects on mussels and algae separately and when kept together. Surprisingly, no effects of elevated pCO_2 were identified on mussels or algae, however, warming increased mussel mortality and clearance rates. Moreover, mussels and algae had positive effects on each other's performance (i.e. mussel and algal biomass, mussel condition index, algal relative maximum electron transport rate [rETRmax], saturating light intensity [I_k], and maximum quantum yield [F_v/F_m]) when together compared to treatments with just mussels or just algae. Overall, mussel mortality was lower when algae were also present. Our results show that even moderate warming affected the functioning of coastal benthic species and indicated a level of resistance towards moderate ocean acidification. Importantly, we highlight the ecological and potential economic benefits of conserving biodiversity in marine ecosystems.

3.2 Introduction

The fate of coastal biodiversity is a major concern in changing climate conditions (Pörtner *et al.*, 2021; Cooley *et al.*, 2022; IPCC, 2022). Climate change adds to other anthropogenic drivers of biodiversity change, such as habitat modifications, exploitation or pollution, and can intensify their effects on the distribution, functioning and production of marine species (Bowler *et al.*, 2020; Gissi *et al.*, 2021; O'Hara, Frazier and Halpern, 2021). Additionally, species interactions can be modified by climate change, and climate change effects can be enhanced or mitigated by direct or indirect species interactions, increasing the uncertainty around future coastal biodiversity (Brooks and Crowe, 2018). Coastal regions are particularly exposed to the cumulative effects of multiple stressors, which puts the ecosystem services they provide at risk, e.g. their potential to provide food, regulate coastal erosion, recycle nutrients, sequester carbon, support recreational activities and sustain cultural identity (Bowler *et al.*, 2020; Cooley *et al.*, 2022). Predicting the cumulative impacts of multiple stressors on coastal biodiversity is crucial for managing and protecting vulnerable ecosystem services (Gissi *et al.*, 2021; Keyes *et al.*, 2021; Cooley *et al.*, 2022).

Climate change and its effects on marine benthic species have been studied extensively, yet impacts are highly variable across taxonomic groups and up-scaling results to communities or ecosystems remains challenging (Hoppit and Schmidt, 2022). Warming directly affects metabolic rates and thereby physiological processes along thermal performance curves, on which performance first increases with warming until metabolic costs are most efficiently covered by the rate of supplied energy at an optimum temperature, before this balance is unsettled and performance declines rapidly (Gillooly *et al.*, 2001; Clarke and Gaston, 2006; Pörtner, 2010; Dell, Pawar and Savage, 2011; Lemoine and Burkepile, 2012, Roma *et al.*, 2021). If warming either positively "boosts" or negatively impairs performance of an organism is influenced by the temperature range of the thermal performance curve to which an organism's phenology is acclimatised in its habitat (Vasseur *et al.*, 2014). Calcifying species, such as shellfish, tend to respond to ocean acidification and warming with increased metabolic costs, reduced growth, reproduction and calcification (Lemasson *et al.*, 2018; Sadler, Lemasson and Knights, 2018; Hoppit and Schmidt, 2022). Fleshy algae, on the other hand, tend to show resistance and may even benefit from warmer and acidified conditions because of increased CO_2 supplies for photosynthesis at accelerated metabolic rates (Stewart *et al.*, 2013; Mooney-McAuley *et al.*, 2016; Hoppit and Schmidt, 2022). Other ecosystems dominated by fleshy macroalgae, however, suffer from extreme heat events (Smale *et al.*, 2019), increased grazing, competition or impaired reproduction (Veenhof *et al.*, 2022), which may alter community compositions and even cause shifts from complex macroalgal habitats to degraded algal turf communities (Provost *et al.*, 2017). Shellfish and macroalgae are important habitat-forming components of coastal marine ecosystems (Mooney-McAuley *et al.*, 2016; Sadler, Lemasson and Knights, 2018), where altered community compositions may profoundly change ecosystem functioning and provisioning (Lemasson *et al.*, 2017; Cooley *et al.*, 2022; Hoppit and Schmidt, 2022), thus, understanding their responses to ocean acidification and warming (including interactions with other species closely associated to these biogenic habitats) is highly important. Furthermore, greater understanding is required to predict how species' interactions and ecosystem structures may change in future oceans (Wernberg, Smale and Thomsen, 2012; Hobday *et al.*, 2016; Tagliarolo, Porri and Scharler, 2018).

Recent research has highlighted the potential of marine macrophytes, such as seaweeds and seagrass, to mitigate negative ocean acidification effects on shellfish by removing CO_2 from the water and increasing pH, thus, acting as a local buffer to acidification (Jiang and Fang, 2021; Jiang *et al.*, 2022; Young *et al.*, 2022). In addition to increasing overall mean pH with increasing macrophyte biomass, diurnal pH fluctuations become more pronounced, which offers shellfish (e.g. mussels, oysters) temporal refugia from acidification stress that can be used for increased calcification activity (Wahl *et al.*, 2018; Ricart *et al.*, 2021). Integrated multi-trophic aquaculture, which was first established to utilise surplus organic and inorganic nutrients produced by cultivated finfish through co-cultivation with detritivores, filter feeding shellfish and macroalgae (Mooney-McAuley *et al.*, 2016), has now recognised the commercial potential of co-culturing shellfish and seaweeds to facilitate shellfish production (Hamilton *et al.*, 2022). Seaweed aquaculture is an important industry with growing biomass demands for bioenergy purposes and for products in the food, pharmaceutical and fertiliser industries, producing ca. 35 million tonnes of algal biomass annually (Mooney-McAuley *et al.*, 2016; FAO, 2022). Approximately 18 million tonnes of marine molluscs, mainly bivalves, are produced per year, which represents half of coastal and marine animal aquaculture (FAO, 2022). The impact of climate change on aquaculture production is largely unknown and theoretical predictions of potential effects, blue economy benefits or biodiversity contributions vary tremendously (Gubbins, Bricknell and Service, 2013; Forbes *et al.*, 2022; Theuerkauf *et al.*, 2022; Troell *et al.*, 2022; Hengjie *et al.*, 2023). Therefore, investigating and disentangling the mechanisms behind the combined effects of ocean acidification and warming on co-cultures of shellfish and seaweed, including adaptation and mitigation potential towards climate change, is not only of ecological, but also of commercial interest (Duarte *et al.*, 2017; Jiang and Fang, 2021; Young *et al.*, 2022).

Much research has been conducted on single species and the effects of ocean acidification and warming treatments based on or exceeding the worst case scenarios of IPCC climate change predictions (Navarro *et al.*, 2016; Geraldi *et al.*, 2020; Knights *et al.*, 2020; Armstrong *et al.*, 2022; Zhang *et al.*, 2022). These studies improve our understanding of population dynamics and the structure of communities under extreme predicted future conditions (Rall *et al.*, 2010; Lemoine and Burkepile, 2012). Incorporating more moderate near-future experimental treatments, however, is also required to identify how species and ecosystems may respond within the next few decades or in moderate climate change scenarios by 2100, and to identify their potential for adaptation (Geraldi *et al.*, 2020).

In the current study, we tested empirically for effects of increased temperature and/or ocean acidification on blue mussels (*Mytilus edulis*) and sugar kelp sporophytes (*Saccharina latissima*) grown together and separately with a fully factorial experimental design. Temperature (ambient, +0.8 °C, +2 °C) and pCO_2 (ambient, 450 ppm; elevated, 645 ppm) were manipulated based on predicted sea surface temperature and atmospheric pCO_2 of the Irish Sea under a moderate climate change scenario by the year 2100, or sooner in the case of a more extreme scenario (IPCC, 2013, 2014; Jacob *et al.*, 2014). We quantified effects of these abiotic experimental treatments (temperature, pCO_2) on several proxies for the functioning of mussels and algae including: mussel mortality, mussel biomass, mussel condition index, clearance rates, shell and byssus strength of the mussels, as well as algal biomass and photosynthetic performance (i.e. relative maximum electron transport rate [rETRmax], saturating light intensity [I_k], light harvesting efficiency [α], and maximum quantum yield [F_v/F_m]) of the algae. Additionally, we tested whether the functioning of mussels and algae differed in the presence or absence of each other. Because our experiment took place in late autumn and early winter, i.e. at the lower end of the temperature range naturally encountered by our study organisms per year, we hypothesised that mussels would respond to warming with increases in all processes quantified, except mortality, which we expected to decrease. Simultaneously, we expected acidification to have a negative effect. Additionally, we hypothesised that the presence of the algae would strengthen positive effects of warming, and act as a local buffer to mitigate negative effects of acidification. Furthermore, we hypothesised that predicted warming, ocean acidification and the presence of mussels will have positive effects on the productivity and photosynthetic performance of the algae. As a consequence, we expected total accumulated biomass of mussels and algae to be greater in the treatments where they were together compared to the sums of biomass of treatments containing just mussels or just algae.

3.3 Methods

3.3.1 Experimental design

The experiment comprised of two analogous sets of treatments to test simultaneously both the effects of the abiotic factors ocean warming and acidification on mussels or algae separately, in addition to testing for effects of mussels and algae on each other (Figure 3.1). Specifically, we tested whether the presence of a second functional group (mussels or algae) would alter the effects of the abiotic factors on either group (mussels or algae). Water temperature was manipulated at three levels: ambient, ambient +0.8 °C, and ambient +2 °C. pCO_2 was manipulated at two levels to simulate ocean acidification with the corresponding pH: ambient (450 ppm) and elevated (645 ppm). Each experimental set consisted of twelve treatments, of which six overlapped. In total, 18 treatments ($n = 5$) were distributed randomly across 90 mesocosms and the factorial design enabled the testing of the independent and combined effects of all factors and their respective levels. The warmed and acidified treatments simulated predicted conditions in Ireland in a moderate climate change scenario in the year 2100, or conditions that might prevail in a few decades on the way to an end-century extreme business-as-usual scenario (IPCC, 2014; Jacob *et al.*, 2014).

Six additional mesocosms that contained only seawater but no species were also established to monitor variation in the abiotic conditions manipulated in the experimental set-up at each combination of temperature and pCO_2 levels. All species were collected from the region and acclimatised to laboratory conditions at ambient temperature and pCO_2 for one week before temperature and pCO_2 manipulations started (Kong *et al.*, 2019). Response variables were taken after 6-7 weeks at manipulated abiotic conditions.

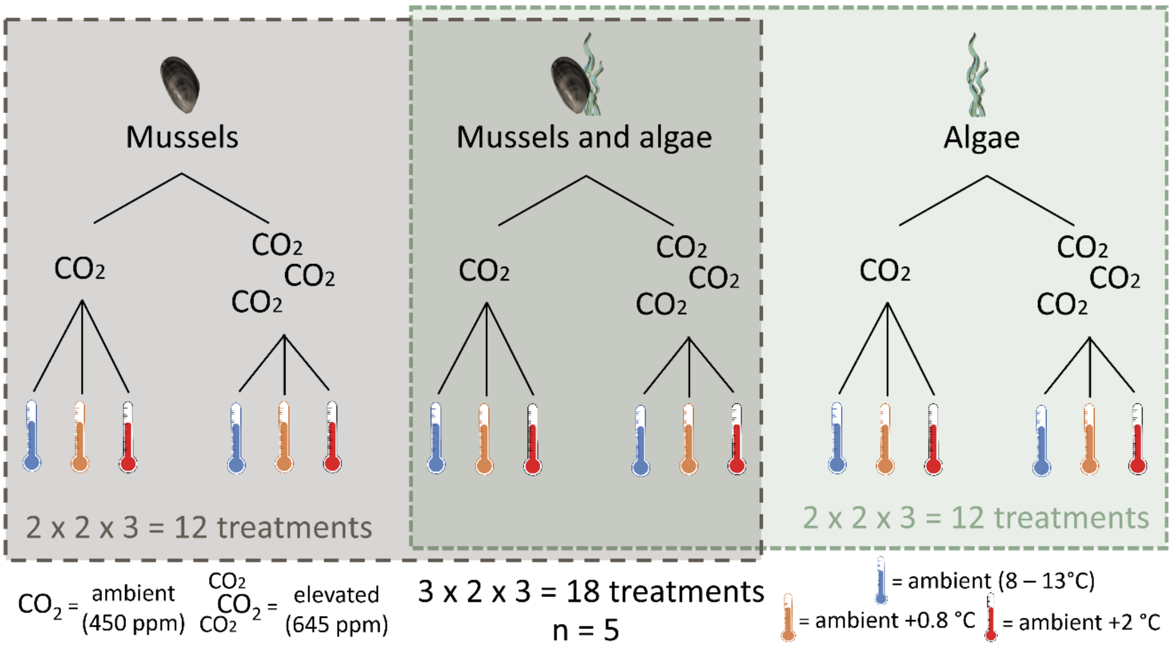

Figure 3.1 Experimental design for testing the effects of ocean warming and acidification on mussels or algae separately, in addition to testing for effects of mussels and algae on each other.

3.3.2 Experimental set-up

The experiment was carried out using the experimental mesocosm platform QIMS (Quantifying the Impacts of Multiple Stressors; Chapter 2) at Trinity College Dublin, Ireland, from 28/10/2020 to 18/12/2020. Salinity of the supplied seawater was 34.4 and illumination was set to a 10:14 hour light:dark cycle, reflecting average Irish autumn daylight conditions. Oxygen levels were measured weekly and remained saturated > 8.2 mg/L. Halfway through the experiment, the water was drained and replaced with fresh seawater to maintain high water quality. During this process, mussel faeces and biofilm were removed from the mesocosm bottoms. Mussels and algae, which can both occur in the intertidal in the wild, were disturbed as little as possible, however, exposure to air for approximately 60 minutes was unavoidable.

3.3.3 Temperature manipulation

Temperature was manipulated and monitored as detailed in Chapter 2 to achieve three distinct levels. Mesocosm temperature fluctuated in parallel with room temperature up to 2 °C per day. Room temperature decreased owing to outside temperatures over the course of the experiment and the coldest ('ambient') temperature level ranged between 8.5 °C ± 0.07 and 12.4 °C ± 0.07 (means ± se). The medium temperature level was warmer by 0.8 °C ± 0.0 and the warmest level by 2.0 °C ± 0.1 (means ± se).

3.3.4 pCO$_2$ manipulation and pH

pCO$_2$ was manipulated as described in Chapter 2 to achieve two levels of pH. Ambient pCO$_2$ was 452 ± 0.12 ppm, while elevated pCO$_2$ was 645 ± 0.04 ppm (mean ± se). The corresponding pH in the blank mesocosms (without biota) was (mean ± se) 8.09 ± 0.004 at ambient pCO_2 and 7.97 ± 0.003 at elevated pCO_2.

3.3.5 Study organisms

One-year-old mussel spat (*Mytilus edulis*) was obtained from seeded rope at 1 m depth in Killary Fjord (Killary Fjord Shellfish; http://killaryfjordshellfish.com/who.html) in October 2020 (*in situ* water temperature 11.2 °C, pH 8.24) and kept moist and cool for transport until put in 13 °C aerated seawater in Trinity College Dublin. After five days, 3.01 g ± 0 (mean ± se) of live mussel spat wet biomass, made up of 30-40 mussels with an average individual wet biomass of 0.096 mg ± 0.001 (mean ± se) and shell lengths between 7 and 16.5 mm, were assigned randomly to mesocosms. In the mesocosms, the mussels were placed on garden mesh tiles (15 x 15 cm polypropylene with 7 mm single mesh diameter), 2 cm above the bottom. The tiles were fastened with a cable tie close to the recirculation return to ensure that they were exposed to similar hydrodynamic conditions. Mussels that fell off the mesh were gently placed on top again at the start of the experiment. After two weeks, all had attached either to the mesh or to other mussels. Empty mesh was similarly deployed in all other mesocosms.

Mussels were fed daily with a concentrated marine microalgal mix (Reed Mariculture Shellfish Diet 1800) according to recommended feeding instructions (0.7 ml Shellfish Diet per gram live spat), to ensure starvation was not the underlying cause for any effects possibly seen in response variables (Thomsen *et al.*, 2013). The total amount of Shellfish Diet per mesocosm during the first week of the experiment was 2 ml, which was increased to 2.5 ml in week two and three, and to 2.75 ml in week four, anticipating growth of the mussels. After week 3.5 the mussels no longer cleared the water, possibly because of mortality, thus, the food dosage was reduced to 1.7 ml daily per mesocosm containing mussels until the end of the experiment.

Algal sporophytes were obtained on seeded aquaculture string from the Queen's University Marine Laboratory (QML) in Portaferry. The string had been sprayed with a *Saccharina latissima* (kelp, brown macroalgae) gametophyte culture and was grown following standard operation procedure hatchery conditions (Gorman, 2014) for eleven weeks prior to addition to the mesocosms. Both in the mesocosms and in the hatchery, the kelp sporophytes grew slowly and at low density compared to previous trials, possibly compromised following reduced care in the hatchery during the 2020 Covid-19 pandemic lockdowns (e.g. hatchery room lights had been kept switched off, instead of adding to the culture light, reducing light availability for the juvenile kelp). As a result, kelp individuals were microscopic when seeded string was deployed into the mesocosms but were expected to grow rapidly.

In the treatments that included algae, 65 cm of seeded string were weighed and randomly assigned to one of the 60 respective mesocosms. To establish this experimental treatment, the seeded string was wrapped around 60 cm long polypropylene header ropes (10 mm diameter), which were fastened

1 cm below the water surface diagonally to the bottom area after soaking in seawater for two days. Once a week, a freshly prepared solution of premixed f2 powder (Varicon Aqua, 0.5 ml f2 solution L^{-1} seawater) was added to the mesocosms with seeded string because we expected the kelp to grow and deplete the nutrients over a week.

3.3.6 Data collection

Mussel mortality

Every two to four days, the mesocosms were checked for dead mussels, which were identified by their shells gaping open and no closing response to physical stimulation, and often by lack of attachment. Mussels that died during the acclimatisation period were replaced prior to the temperature and pCO_2 manipulation, using similar-sized live individuals that had been kept at similar lab conditions. After abiotic manipulations had started, dead mussels were removed and recorded to quantify mortality. The total amount of dead mussels per mesocosm was used for data analyses. Mortality was independent of mussel size (Appendix B.1), which enabled averaging and standardising mussel response variables that were measured per mesocosm (e.g. clearance rate or final biomass) according to individuals.

Mussel biomass and condition index

The mussels were retrieved on their mesh tiles from the mesocosms after 51 days to estimate wet biomass, which was then standardised by the number of alive mussels. Accumulated biomass per mussel was calculated as the difference between initial and final mean individual wet biomass (mg). Total accumulated mussel biomass per mesocosm was calculated by multiplying the accumulated biomass per mussel with the number of alive mussels. To calculate the condition index, a subsample of five mussels of similar shell length was selected per mesocosm and dissected into shells and flesh, which were then weighed and dried at 80 °C until dry weight remained constant. Following Lucas & Beninger (1985), the condition index was calculated to assess the bivalves' physiological state, as the shell is a product of cumulative growth while the flesh represents recent metabolic activity that may be reduced under stress:

$$Condition\ Index = \frac{Dry\ weight_{Flesh}}{Dry\ weight_{Shell}} * 100 = [\%]$$

Mussel clearance rates

Clearance rate samples were taken on the 35th and 36th day of manipulated temperature and pCO_2 conditions. On the previous day, the mussels had received a reduced feeding dosage of 1 ml shellfish diet per mesocosm. To measure clearance rates, mussels were given 1.7 ml Shellfish Diet per mesocosm. After allowing 10 minutes for homogenous dispersal, the first of three 50 ml samples was drawn from each mesocosm to determine the initial cell concentration (T0). Two (T1) and 17 hours (T2) after feeding, additional water samples were taken. The samples were stored at 4 °C and stirred before four coulter counter cell counts were conducted from a 20 ml subsample (Beckman

Coulter Counter Z Series, aperture 100 µm, Kd: 59.29, sampling volume: 0.5 ml, count of particles between 6 and 19 µm). Each first count was discarded (to ensure the coulter counter tubes were flushed and only contained the current sample) and the mean taken from the subsequent three counts.

Clearance rates were calculated following Coughlan (1969):

$$Clearance\ rate = \frac{M}{n * t} * \ln\left(\frac{conc_{T0}}{conc_{T1}}\right) = [\frac{L}{mussel * h}]$$

where M is the volume of the suspension, n is the number of animals per mesocosm, $conc_{T0}$ and $conc_{T1}$ are the concentrations of the suspension at the start (T0) and after time t. Samples from the mesocosms that contained no species and were used for monitoring the manipulated abiotic factors confirmed low background particle load and continuous counting accuracy throughout the measurements.

Shell strength

To analyse shell strength, the force needed to break them was determined using a materials testing machine for compression tests (ZwickRoell zwickiline Z2.5) at the Department of Mechanical, Manufacturing & Biomedical Engineering, Trinity College Dublin. Three mussels of each mesocosm were collected on the last day of the experiment and stored for five days at 4 °C before they were dissected into shell valves and flesh. Visibly intact right shell valves were placed individually into the machine with the shell valve openings lying flat on the machine in similar orientation (Mackenzie *et al.*, 2014). A load cell of 20 N was used for smaller shells and 200 N for the largest. Force was applied from the top at a speed of 200 mm/min until shell failure occurred. The force applied was logged at 0.01 s intervals using testXpert II software (ZwickRoell) that determined the maximum applied force (F_{max}). 60 shells broke visibly during dissection and were discarded, and 19 tested shells were excluded from analysis because they cracked multiple times instead of showing one clear failure event. A total of 101 shell breaking tests were available for data analysis, with shells from all but five (different treatments) of the 60 mesocosms and seven to eleven shells per treatment. Shell length (Appendix B.1) was used to standardise F_{max} and the average applied force per mm (N/mm) was calculated for each mesocosm and used for data analysis.

Byssus strength

After recording wet biomass, mussels were cut off the mesh tiles at the byssus stem, leaving the byssus threads as intact as possible. Areas where the byssus threads clearly belonged to one single mussel were marked and the mesh tiles were returned to their mesocosms for intermediate storage. Four days later, the mesh tiles were individually fastened to a materials testing machine for traction tests (ZwickRoell zwickiline Z2.5; load cell: 20 N; Department of Mechanical, Manufacturing & Biomedical Engineering, Trinity College Dublin) in the centre below the byssus cluster and parallel to the bottom of the machine. A plastic-wrapped wire was fed centrally through the cluster so that the cluster stem was positioned in a bend of the wire, with approximately equal amounts of byssus threads on each side (exact numbers could not be determined). The wire ends were clamped into the top machine end, which pulled the wire vertically away from the mesh at 5 mm/min (Bouhlel *et al.*, 2017), exerting traction on the byssus cluster until all the byssus threads on one side of the wire ruptured. All tests were conducted in air. The applied force was logged at 0.1 s intervals using testXpert II software (ZwickRoell), recording the succession and magnitude of applied tensile force and load drops over time. In adult *Mytilus californianus*, large drops in loads can be assigned to single threads breaking and the sum of individual load drops exceeds the maximum force applied to a whole byssus cluster (Bell and Gosline, 1996). The many and delicate byssus threads of the juvenile *M. edulis* used in this study did not allow to link load drops to individual observed thread ruptures. Histograms of all load drops that were recorded in a test showed, however, that load drops < 0.01 N occurred more than five times as often as the next force range in the histogram. This indicates the background noise of the tests and justifies the load drop limits considered for analysis as > 0.01 N. The average load drop > 0.01 N was used as a proxy for byssus strength. A total of 43 tests were conducted on byssus clusters from 34 mesh tiles. Test results from the same mesh tile were averaged and data of three random mesocosms per treatment were used for analysis, except for the two acidified mussel-only treatments at increased temperatures, for which only two mesh tiles were available for byssus strength testing.

Algal biomass

The kelp sporophytes on the seeded strings grew unexpected slowly, presumably because of unusual hatchery conditions due to pandemic lockdowns (see above). Over time we observed that microphytobenthos (primarily benthic diatoms and, to a lesser extent, green algae) grew on the seeded string, resulting in a microscopic, mixed algal assemblage on the seeded strings. Instead of quantifying biomass of individuals of kelp at the end of the experiment as planned, the total accumulated biomass of algae associated with seeded string was quantified. Final abundance of kelp individuals, which had reached up to 1.4 mm in length at the end of the experiment, were counted using a dissection microscope.

Photosynthetic performance

Photosynthetic performance of the algae present in the mesocosms was tested using a pulse amplitude modulation (PAM) fluorometer (DIVING-PAM-II, Walz) on the 39th day at manipulated abiotic conditions. A leaf clip (DIVING-LC, Walz GmbH) was connected to the seeded string to eliminate light and ensure consistent spacing of the fiber optic with the algae. Rapid light curves (RLCs) were taken on ambient-light acclimated algae to assess differences in potential photosynthetic performance. Prior to starting the RLCs, tissue was quasi-dark adapted for a few seconds to allow re-oxidization of the primary electron acceptor (Schreiber, 2004; Randall *et al.*, 2019). Relative electron transport rates were determined at steps of increasing actinic light intensity, from which the DIVING-PAM-II built-in software calculated the relative maximum electron transport rate, i.e. photosynthetic capacity rETRmax, as well as the saturating light intensity I_k, and the initial slope of the RLC, i.e. the light harvesting efficiency α (Randall *et al.*, 2019). After 15 minutes of dark acclimation, a different section of the seeded string was then used to measure the maximum quantum yield F_v/F_m, calculated as $\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m}$ where F_m represents the maximal fluorescence after a saturating light pulse and F_0 the steady-state fluorescence under weak initial illumination before the light pulse (Miranda *et al.*, 2019). The higher the yield, the more suitable the conditions (Bilger, Schreiber and Bock, 1995).

Total accumulated biomass

Total accumulated biomass for mussels and algae that were kept in the same mesocosm was calculated by adding the accumulated algal biomass to the accumulated mussel biomass. To compare between the total accumulated biomass of mussels and algae that had been kept together and the sum of accumulated biomass of mussels kept on their own and algae kept on their own in the same abiotic treatments, each possible combination of accumulated biomass per mesocosm of mussels kept on their own (n = 5) and algae kept on their own (n = 5) was calculated (n = 25).

3.3.7 Data analyses

To test hypotheses, three-way Analyses of Variance (ANOVAs) were performed using temperature (three levels), pCO_2 (two levels) and the presence of a second functional group (two levels; i.e. mussels vs. mussels and algae; or algae vs. mussels and algae) as fixed orthogonal factors and including all possible interactions (i.e. temperature*pCO_2*FunctionalGroups). Data of rETRmax and I_k were log transformed to meet the assumptions of ANOVA. Normality of errors was confirmed by plotting histograms of the residuals and applying Shapiro-Wilks-tests. Homogeneity of variances was tested using Levene's tests and by plotting the residuals as a function of the fitted values. To test for autocorrelation in the residuals, Durbin-Watson tests were conducted, and the presence of influential data points was assessed using Cook's distance. Data of mussel shell strength and mussel byssus strength were slightly unbalanced, hence ANOVA type 3 sums of squares were considered. When the ANOVA indicated differences between more than two treatment levels, Tukey's Honest Significance Differences were calculated as post-hoc tests. To assess whether the final amount of kelp sporophytes differed among treatments, a generalised linear model of the family quasipoisson was applied. To test for differences in total accumulated biomass when mussels and algae were kept together and the sums of accumulated mussel and algal biomass when kept separately, the sums of all possible biomass combinations of mesocosms that had only mussels and only algae were calculated for each abiotic treatment (temperature levels crossed with pCO_2 levels), which yielded 25 samples per abiotic treatment. Variances were inhomogeneous between these groups and the corresponding five samples of mesocosms in which mussels and algae had been kept together at the same abiotic treatments, which is why the Scheirer-Ray-Hare test was used for analysis, followed by the Dunn test for post-hoc comparisons (Mangiafico, 2016).

All statistical analyses and data visualisation were conducted in R version 4.2.1 (R Core Team, 2022) using R Studio version 2022.07.2 (R Studio Team, 2022) and the packages tidyverse version 1.3.2 (Wickham *et al.*, 2019), car version 3.1.1 (Fox and Weisberg, 2019), Hmisc version 4.7-2 (Harrell Jr, 2022), rcompanion version 2.4.34 (Mangiafico, 2023), and FSA version 0.9.5 (Ogle *et al.*, 2023). Byssus strength test files were prepared and the average load drop > 0.01 N calculated in Microsoft Excel.

3.4 Results

3.4.1 Mussel responses

No interactions were identified among any of the factors on mussel response variables. We did find an effect of temperature on mussel mortality ($F_{2, 48} = 6.382$; $p = 0.004$) and post-hoc tests indicated significant differences ($p = 0.002$) between the ambient and the warmest (+2 °C) temperature level (Figure 3.2). pCO_2 had no effect on mussel mortality ($F_{1, 48} = 0.37$; $p = 0.549$). Fewer mussels died when algae were present ($F_{1, 48} = 8.403$; $p = 0.006$).

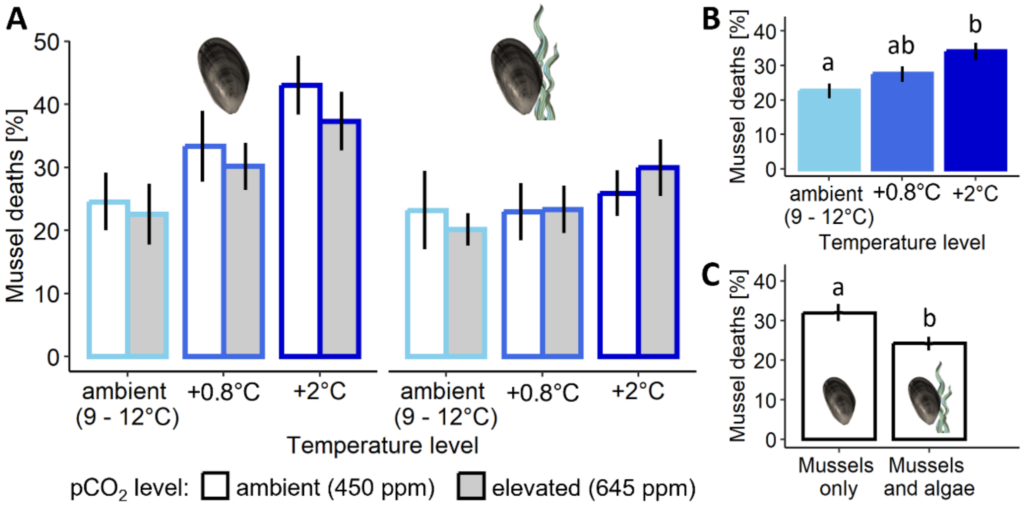

Figure 3.2 Mean (± standard error) mussel deaths in A (full data set) at ambient (light blue), +0.8 °C (blue) and +2 °C (dark blue) temperature and at ambient (white bars) and elevated (grey bars) pCO_2, in the absence (left panel in A) or presence (right panel in A) of algae, $n = 5$; in B at different temperatures (significant main effect); and in C without and with algae (significant main effect). Significant differences among groups of means are indicated by lower case letters ($p < 0.01$).

Temperature had no effect on mussel biomass ($F_{2, 48} = 2.398$; $p = 0.102$), nor on the condition index ($F_{2, 48} = 0.086$; $p = 0.917$), nor did pCO_2 ($F_{1, 48} = 0.821$, $p = 0.369$ and $F_{1, 48} = 0.093$; $p = 0.862$; Figure 3.3). The presence of algae, however, had a positive effect on mussel biomass ($F_{1, 48} = 22.073$; $p < 0.001$) and condition index ($F_{1, 48} = 18.437$; $p < 0.001$; Figure 3.3). Mussel biomass almost doubled in the presence of algae compared to treatments without algae and mussel condition index was almost 20 % greater in the presence of algae compared to their absence (Figure 3.3A).

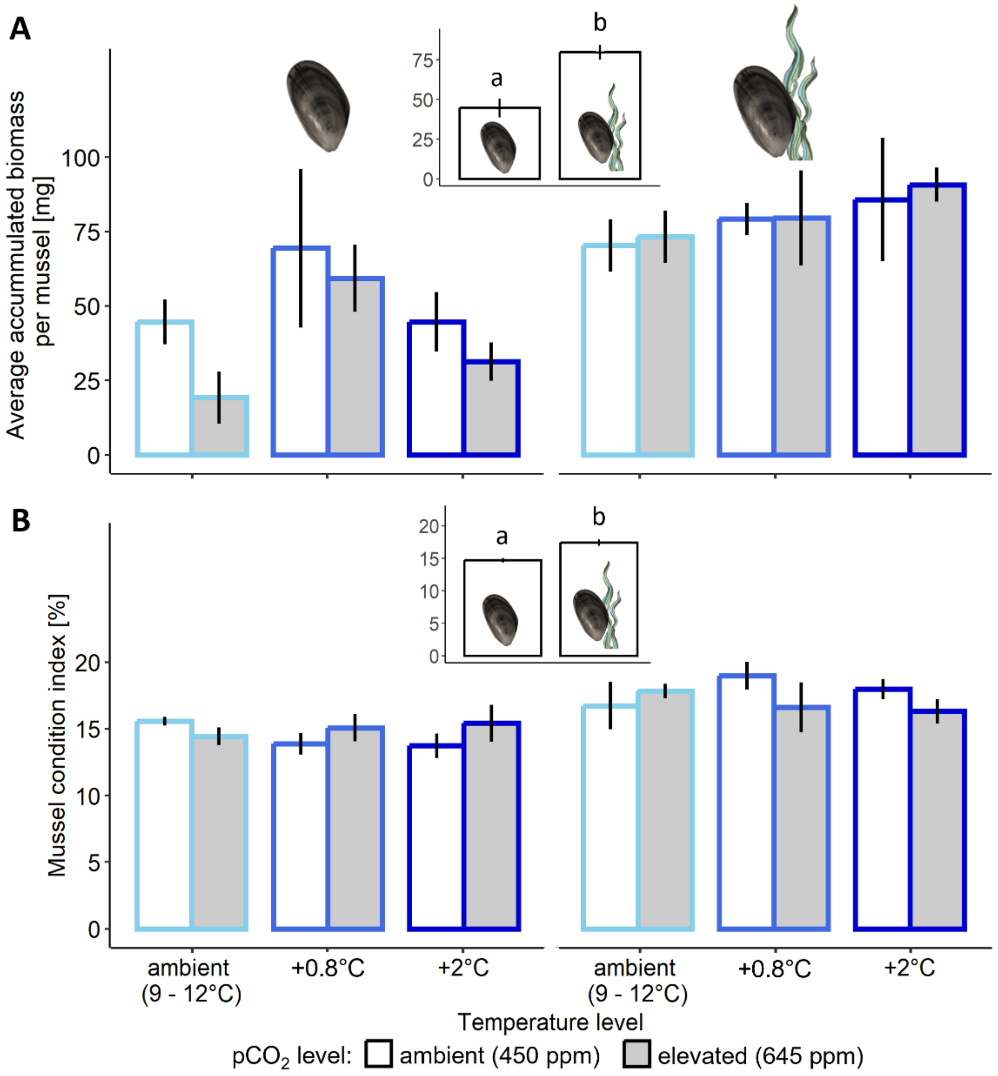

Figure 3.3. Mean (± standard error) accumulated biomass per mussel in A and condition index of mussels in B at ambient (light blue), +0.8 °C (blue) and +2 °C (dark blue) temperature, and at ambient (white bars) and elevated (grey bars) pCO$_2$, in the absence (left panel) or presence (right panel) of algae, n = 5. Insets show significant differences among groups of means without and with algae, also indicated by lower case letters ($p < 0.001$).

No differences among treatments were found in clearance rates after 2 hours of feeding (temperature: $F_{2, 48} = 2.213$, $p = 0.120$; pCO$_2$: $F_{1, 48} = 3.156$, $p = 0.082$; algae: $F_{1, 48} = 0.750$, $p = 0.391$), however, after 17 hours, a significant effect of temperature on mussel clearance rates was identified ($F_{2, 48} = 3.556$; $p = 0.036$), with an almost 40 % greater rate in the warmest temperature level compared to the ambient temperature level (Figure 3.4). No effects of pCO$_2$ ($F_{1, 48} = 0.708$; $p = 0.404$) nor the presence of algae ($F_{1, 48} = 1.870$; $p = 0.178$) were identified after 17 hours.

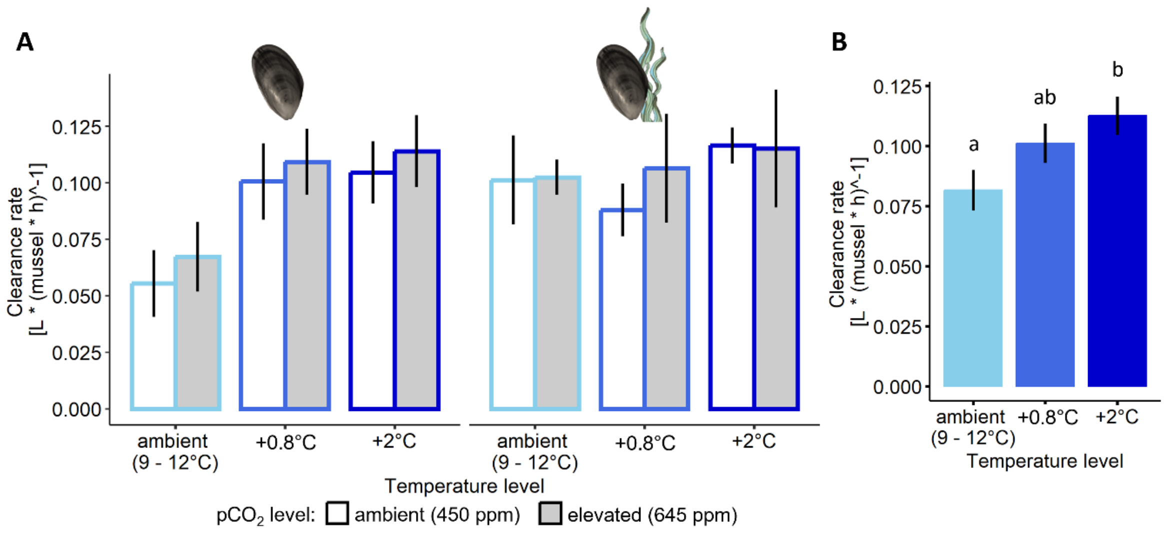

Figure 3.4. Mean (± standard error) mussel clearance rates in A (full data set) at ambient (light blue), +0.8 °C (blue) and +2 °C (dark blue) temperature, and at ambient (white bars) and elevated (grey bars) pCO_2, in the absence (left panel) or presence (right panel) of algae, n = 5; and in B (significant main effect) at different temperatures with significant differences among groups of means indicated by lower case letters ($p < 0.05$).

No effects of temperature, pCO_2 nor the presence/absence of algae on mussel shell strength (temperature: $F_{2, 43} = 0.454$, $p = 0.638$; pCO_2: $F_{1, 43} = 0.000$, $p = 0.995$; algae: $F_{1, 43} = 0.774$, $p = 0.384$) or byssus strength (temperature: $F_{2, 22} = 0.058$, $p = 0.944$; pCO_2: $F_{1, 22} = 0.685$, $p = 0.417$; algae: $F_{1, 22} = 0.201$, $p = 0.658$) were found.

3.4.2 Algal responses

No interactions were identified among any of the factors on algal response variables. Neither temperature ($F_{2, 48} = 0.014$; $p = 0.986$) nor pCO_2 ($F_{1, 48} = 0.734$; $p = 0.396$) affected the accumulated biomass of algae in the mesocosms. In the presence of mussels, however, 20 % more biomass accumulated than in the absence of mussels ($F_{1, 48} = 17.156$; $p < 0.001$; Figure 3.5).

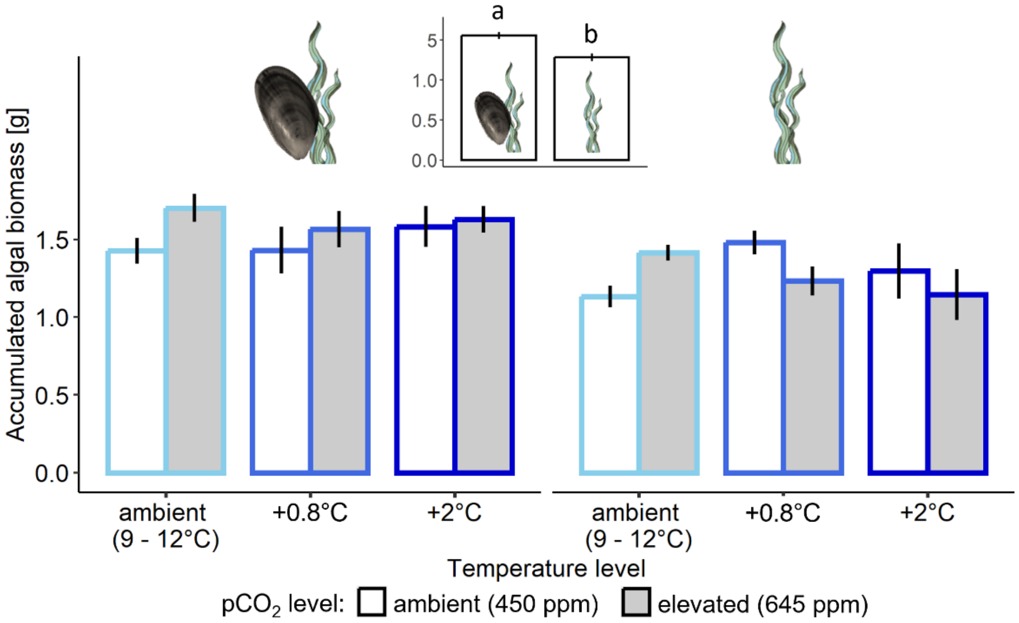

Figure 3.5. Mean (± standard error) accumulated biomass of algae at ambient (light blue), +0.8 °C (blue) and +2 °C (dark blue) temperature, and at ambient (white bars) and elevated (grey bars) pCO_2, in the presence (left panel) or absence (right panel) of mussels, n = 5 per bar. Inset shows significant differences among groups of means with and without mussels, also indicated by lower case letters ($p < 0.001$).

At the end of the experiment, no effects of temperature ($F_{2, 29} = 5.161$; $p = 0.527$) nor pCO_2 ($F_{1, 29} = 0.000$; $p = 1.000$) on the abundance of kelp individuals were identified, however, sporophytes could only be counted in the treatments without mussels.

Results of the rapid light curves revealed no effects of temperature nor pCO_2 on the relative maximum electron transport rate rETRmax (temperature: $F_{2, 48} = 0.324$; $p = 0.725$; pCO_2: $F_{1, 48} = 2.130$; $p = 0.151$) or on the saturating light intensity I_k (temperature: $F_{2, 48} = 1.459$; $p = 0.243$; pCO_2: $F_{1, 48} = 0.774$; $p = 0.383$) of algae in these treatments (Figure 3.6). The presence of mussels, however, significantly affected rETRmax ($F_{1, 48} = 10.107$; $p = 0.003$) and I_k ($F_{1, 48} = 31.966$; $p < 0.001$; Figure 3.6). rETRmax increased by 30 % in the presence of mussels and I_k by 45 %. The light harvesting efficiency α did not show any differences among experimental treatments (temperature: $F_{2, 48} = 1.400$, $p = 0.257$; pCO_2: $F_{1, 48} = 2.790$, $p = 0.101$; mussels: $F_{1, 48} = 3.234$, $p = 0.078$).

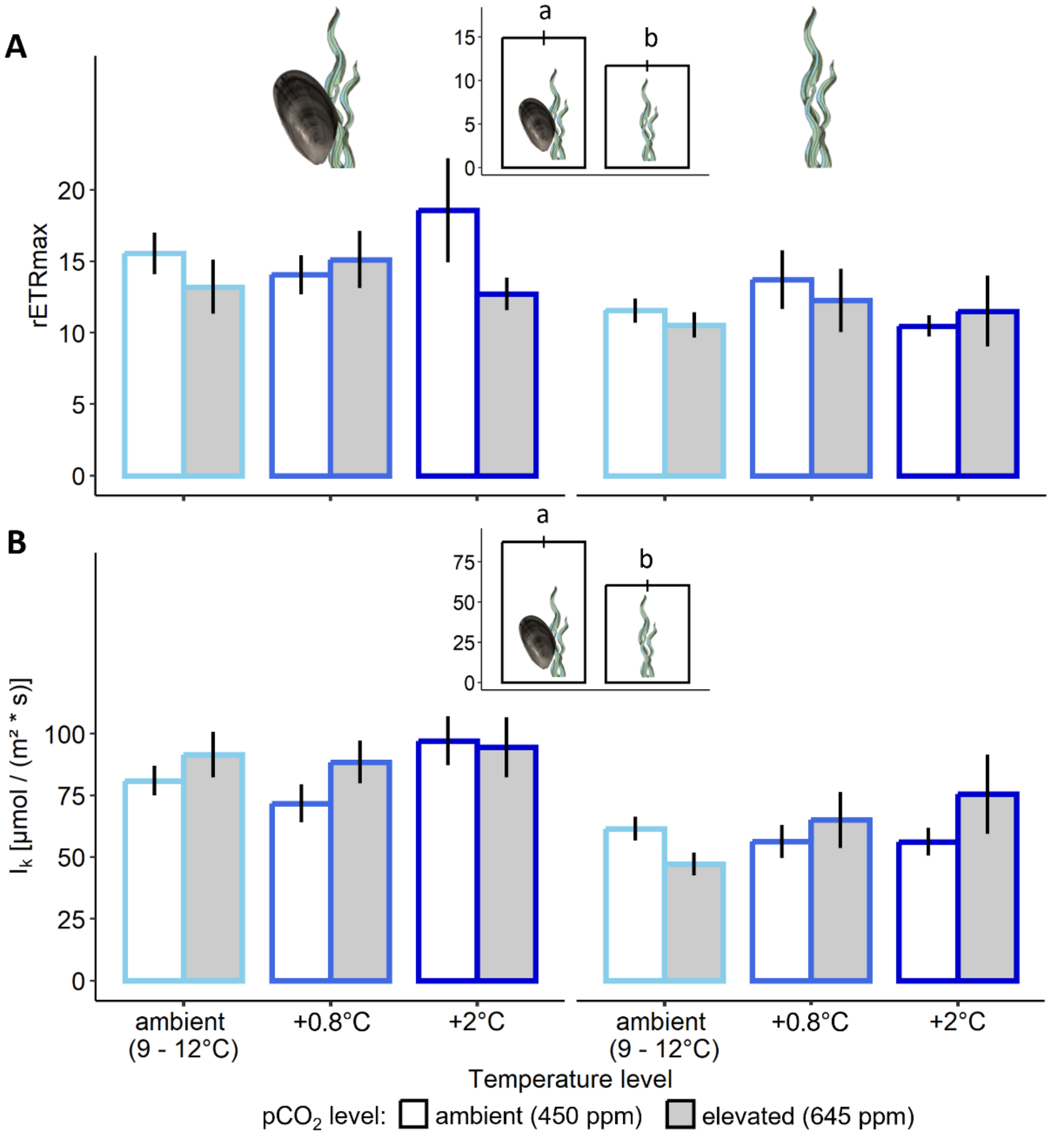

Figure 3.6. Mean (± standard error) relative maximum electron transport rate in A and saturating light intensity in B at ambient (light blue), +0.8 °C (blue) and +2 °C (dark blue) temperature, and at ambient (white bars) and elevated (grey bars) pCO_2, in the presence (left panel) or absence (right panel) of mussels; n = 5. Insets show significant differences among groups of means with and without mussels, also indicated by lower case letters ($p < 0.001$).

The maximum quantum yield F_v/F_m was significantly affected by temperature ($F_{2, 48} = 3.261$; $p = 0.047$), but not by pCO_2 ($F_{1, 48} = 0.972$; $p = 0.329$) nor the presence or absence of mussels ($F_{1, 48} = 1.602$; $p = 0.212$). Post hoc tests were inconclusive (Table B.1) but we can suggest tentatively that the medium temperature level differed from the ambient and the warmest level (Figure 3.7).

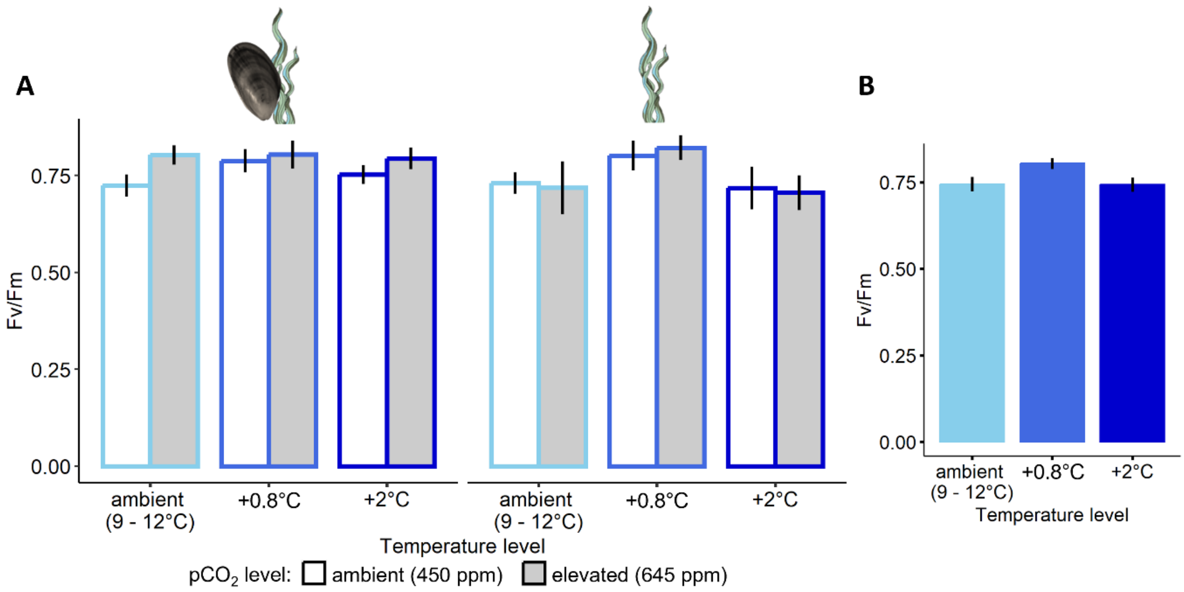

Figure 3.7. Mean (± standard error) maximum quantum yield of algae in A (full data set) at ambient (light blue), +0.8 °C (blue) and +2 °C (dark blue) temperature, and at ambient (white bars) and elevated (grey bars) pCO$_2$, in the presence (left panel) or absence (right panel) of mussels, n = 5; and in B (significant main effect) as groups of means at different temperatures.

3.4.3 Total accumulated biomass

No factors interacted when comparing the total accumulated biomass of mussels and algae from the treatments where they were kept together to the sums of biomass from the treatments that contained just mussels or just algae. If mussels and algae were kept separately or together significantly affected the total accumulated biomass ($F_{3, 228}$ = 135.902; $p < 0.001$), which was 54 % higher when kept together than the sums when kept separately (Figure 3.8). Temperature had an effect on the total accumulated biomass ($F_{2, 228}$ = 16.314; $p < 0.001$) and post-hoc tests indicated significant differences between the ambient and the medium (+0.8 °C; $p = 0.003$) and the medium and the warmest (+2 °C; $p < 0.001$) temperature level. pCO$_2$ had no effect on the total accumulated biomass. Differences in total accumulated biomass were mainly driven by mussels (Figure 3.5; Figure 3.8).

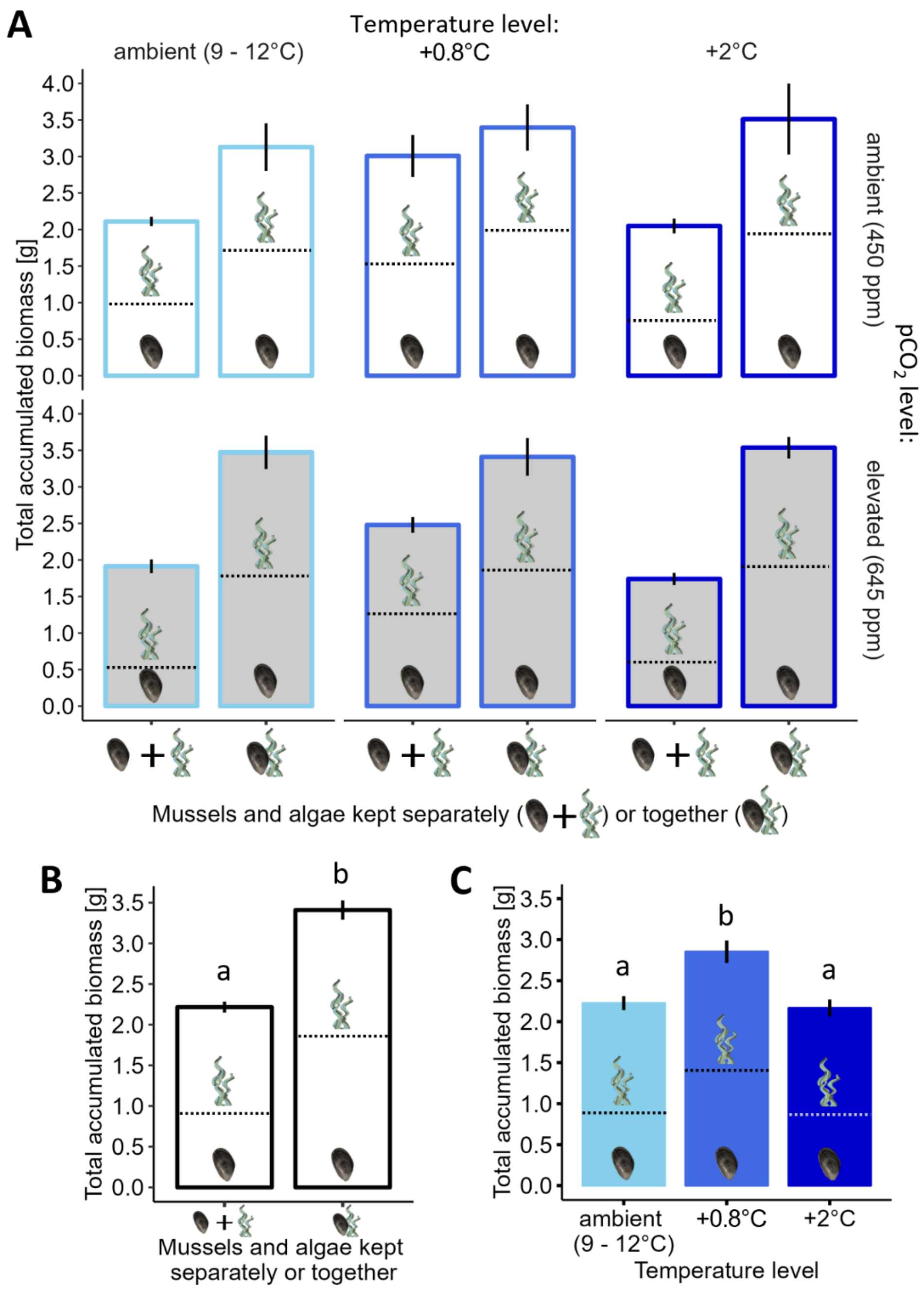

Figure 3.8 Mean (± standard error) total accumulated biomass per mesocosm in A (full data set) for the sum of mussel and algal biomass kept in separate treatments (n = 25) compared to the total accumulated biomass of mussels and algae kept together (n = 5). Proportional contributions of mussels or algae to total biomass accumulation are indicated by dashed lines. The panels show means at ambient (light blue), +0.8 °C (blue) and +2 °C (dark blue) temperature, the rows at ambient (white bars) and elevated (grey bars) pCO_2. Significant main effects of functional group (mussels and algae kept separately vs. together) in B and of temperature in C with significant differences among groups of means indicated by lower case letters ($p < 0.05$).

3.5 Discussion

Understanding how species interact when exposed to multiple stressors of climate change is crucial to estimate future ecosystem structure and the provisioning of ecosystem services. Here, we show that moderate predicted warming, but not ocean acidification, affected the functioning of mussels and algae and that culturing them together enhanced both their performances compared to treatments that contained only mussels or only algae.

Mussel clearance rates were higher at warmer temperatures as we hypothesised, but so was mussel mortality contrary to our expectations and although the experimental temperatures were at the lower range end of what the mussels would experience seasonally in the wild. This indicates that the mussels were not able to maintain their fitness despite increasing their energy intake. Average accumulated biomass of individual mussels and condition index were not affected by temperature. The fact that marine ectotherms, including bivalves and algae, adapt their metabolic rates and directly related traits, such as feeding rates, according to the prevailing water temperature within their thermal range is well understood (e.g. Kittner and Riisgård, 2005; Pörtner, 2010; Gao *et al.*, 2019; Roma *et al.*, 2021). When food supply is high, biomass in *M. edulis* usually increases accordingly, while the condition index can decrease in response to warming when food is limiting (Thomsen *et al.*, 2013; Mackenzie *et al.*, 2014). When mussels died, the relative availability of food increased for the remaining mussels, which may have sufficed to compensate increased energy demands at higher temperatures, e.g. required for byssus production, and may explain the absence of temperature effects on individual biomass and condition index (Roberts and Carrington, 2023).

The absence of a pCO_2 effect on mussel responses may be because coastal ecosystems experience large daily and seasonal fluctuations in pCO_2 and respective pH conditions (Duarte *et al.*, 2013; Vargas *et al.*, 2017, Fernández, Leal and Henríquez, 2019). When the mussel spat used in our experiment was collected in October 2020, a snapshot measurement of in-situ pH of 8.24 was taken, i.e. 0.15 logarithmic units higher than in the ambient treatment in the presented experiment, which is a greater interval than between the ambient and elevated pCO_2 level applied. Long-term, continuous seasonal monitoring data of pH of Killary Fjord is not available. Between 2007 and 2009, however, summer pH ranged from 7.87 to 8.30 (O'Boyle *et al.*, 2013), reflecting the influences of groundwater from surrounding calcerous limestone, nutrient inputs and biological activity (Duarte *et al.*, 2013; McGrath *et al.*, 2016). Therefore, the experimental pCO_2 level of 645 ppm with an associated decrease in pH of 0.1 units compared to the ambient level of 450 ppm, or pH 8.09, respectively, lies within the range that the mussels experience and are likely adapted to in their natural habitats (Melzner *et al.*, 2013; O'Boyle *et al.*, 2013; Thomsen *et al.*, 2013). It is currently unknown if natural carbon chemistry fluctuations will simply shift according to future mean background acidification, or if fluctuations will become more extreme. The effects of both possibilities should be included in future acidification research, given that increased temperature variation may be more harmful to ectotherms than increased mean temperature (Vasseur *et al.*, 2014; Pansch and Hiebenthal, 2019).

Furthermore, biological processes, such as growth and calcification, in juvenile *M. edulis* are mainly driven by food abundance, and are not impacted by pCO_2 levels of up to 3350 ppm when food supply is high (Melzner *et al.*, 2011; Thomsen *et al.*, 2013). Similar to our results, increased clearance rates were found in individual juvenile *Mytilus chilensis* at +4 °C of warming, whereas acidified conditions of 700 ppm did not have any effects compared to ambient (380 ppm) conditions yet (only highly acidified conditions at 1000 ppm reduced the clearance rates independent of temperature; Navarro *et al.*, 2016).

There was no effect of any of the experimental treatments on shell strength. Other studies found evidence of shell dissolution in morphometric analyses and/or weakened shell strength in crushing tests after 6 – 9 months of exposure to strong acidification (> 2400 ppm or > -0.4 pH units) when mussels were kept at poor food supply (Melzner *et al.*, 2011; Mackenzie *et al.*, 2014). New shell material grown under acidified conditions has repeatedly proven to be more brittle in its microstructure, thus mechanically weaker than shells grown in ambient conditions (Fitzer, Vittert, *et al.*, 2015; Fitzer, Zhu, *et al.*, 2015), especially when food supply was low (Melzner *et al.*, 2011). Our results show that 1-year-old spat can resist shell degradation under moderate ocean acidification for at least 6 weeks when kept at a favourable food supply.

During this study, byssal thread production was not affected by any of the experimental treatments. Previous studies suggested that byssus strength is highly size-dependent and that the attachment strength of juvenile life stages may be less affected by ocean acidification than that of larger adults (Clements and George, 2022). Although it is possible that our experiment would have revealed effects when considering size-effects and with higher replication, the absence of any effects of all applied treatments aligns with previous findings that the quality and quantity of byssus production is generally prioritised over other energy expenditure, such as growth, in mussels in stressful conditions despite high energetic costs (Roberts and Carrington, 2023).

Similar to the pattern found in mussels, algal energy uptake as F_v/F_m depended on temperature but not pCO_2, and there was no temperature effect on accumulated algal biomass. This may indicate that either the maximum quantum yield potential was not fully exploited, or that other cellular maintenance was prioritised over biomass and growth despite favourable temperatures.

Total accumulated biomass of mussels and algae combined was significantly higher at +0.8 °C than at ambient or the warmest (+2 °C) temperature. The proportional contributions of mussels to total accumulated biomass and the absence of a temperature effect on accumulated algal biomass suggest that differences in total accumulated biomass were driven by the mussels. Mussel clearance rates but also mortality were significantly higher at +2 °C than at ambient temperature, indicating an increase with temperature. At the medium temperature level of +0.8 °C warming, mussels apparently still managed to cover their increased energy demand and, therefore, died less often than at the highest temperature level of +2 °C warming, resulting in the highest total accumulated biomass. While total accumulated biomass at +2 °C resembled that of ambient temperature, the underlying mussel population contained fewer individuals. This highlights that small intervals of warming between 0.8 and 2 °C, even at the lower range end of seasonally experienced temperatures, can have noticeable effects on community composition, which may have profound ecological consequences.

A key finding of this study was that the presence of algae enhanced mussel performance by decreasing mussel mortality and increasing mussel biomass and condition index, confirming our hypotheses. Concurrently, the presence of mussels also enhanced algal performance by increasing algal biomass and strengthening photosynthetic adaptation (relative maximum electron transport rate rETRmax and the saturating light intensity I_k). This mutual facilitation is exemplified in particular by comparing the total accumulated biomass from treatments where both mussels and algae were present to the sums of biomass from treatments of only mussels and only algae: the total biomass of mussels and algae grown together exceeded the sums of biomass from treatments in which only mussels and only algae were grown by up to 54 %. Positive feedbacks between microphytobenthos and blue mussels have been comprehensively reviewed by Andriana *et al.* (2021): Benthic diatoms usually dominate photosynthetically active biofilms and not only contribute substantially to producing extracellular polymeric substances that surround the biofilms and bind particles together, but also play an important role as resuspended food source for higher trophic levels, e.g. blue mussels (Kang *et al.*, 2006; Evrard *et al.*, 2012; Andriana *et al.*, 2021). In turn, the mussels enrich the biofilms with nutrients by depositing ammonium, faeces and pseudofaeces, which fuels benthic primary production and diatom growth, and eventually benefits mussels in sandy habitats by, e.g., increasing sediment surface stability and sedimentation (Lindström Swanberg, 1991; Andriana *et al.*, 2021). In the present study, it remains unclear if mussel mortality decreased in the presence of algae because of additional food through resuspended microphytobenthos, or rather by an improved environment for decomposing microorganisms that metabolised toxic waste products like ammonia and, therefore, maintained a better water quality. The microalgae in the treatments with mussels and algae were fed both with f2 medium and through mussel faeces and pseudofaeces, which increased biomass accumulation compared to treatments in which mussels were absent. Simultaneously, however, the microalgae in these treatments seem to have outcompeted the juvenile kelp sporophytes, presumably owing to too fast and/or dense growth. Our results of algal photo physiology align with Rugiu *et al.* (2020) who exposed 1-year-old *Saccharina latissima* to mussel farm effluent and found increased rETRmax and I_k (but no effect on the light harvesting efficiency α) compared to control treatments. To detect interacting effects among the applied factors, higher replication may have been needed (Burgess, Jackson and Murrell, 2022).

Conclusion

Our study showed that short-term metabolic processes related to energy intake (mussel clearance rates or algal maximum quantum yield) increased with moderately elevated temperature, while moderately elevated pCO_2 did not have any effect. Simultaneously, mussel mortality increased with warming, resulting in highest total accumulated biomass at the medium applied temperature level of

+0.8 °C. We show that, depending on species’ positions on their thermal performance curves and in their thermal ranges, future marine communities in a warmed ocean are likely to undergo severe changes in their structure and functioning, even if changes in temperature are 0.8 – 2 °C and may seem small. The tolerance of mussels towards elevated pCO_2 and lowered pH when food is abundant highlights the importance of considering ecosystem dynamics and trophic interactions under global change. Furthermore, considering that 645 ppm CO_2 did not impact the performance of juvenile mussels negatively (yet) and that marine organisms will be exposed to more frequent and more intense environmental fluctuation extremes leaves potential for cross-generational adaptation to more acidic conditions in the future.

Mussels and algae mutually facilitated their performance, overall productivity and energy management (mussel condition index, both mussel and algal biomass, algal electron transport rate and light sensitivity). Considering that the total biomass of mussels and algae grown together substantially exceeded the biomass sums of treatments in which only mussels and only algae were grown and that mussel mortality was significantly reduced when algae were present, increasing and conserving biodiversity in marine ecosystems or aquaculture may provide noticeable ecological or economic benefits.

Chapter 4 | Linking environmental monitoring data and the predicted effects of climate change for urban coastal management: a case study of Dublin Bay.

Authors

Katrin Schertenleib, Michael Bruen, Tasman P. Crowe, Nessa E. O'Connor

Author contributions

I conceptualised the study together with Michael Bruen and Tasman Crowe. I chose the data analysis approach (with input from Michael Bruen), conducted the formal analysis, implemented the model, created the figures, led the validation process, wrote the first draft and revised the manuscript. All co-authors commented on the manuscript. Nessa O'Connor acquired the funding and supervised the overall progress of this work.

A version of this manuscript was published in the *Journal of Sea Research* in December 2023 (Schertenleib *et al.*, 2023).

4.1 Abstract

Environmental managers of coastal regions must consider the combined effects of climate change and multiple other stressors simultaneously. While routine monitoring programmes exist, this information is usually summarised as a pre-defined metric or index for ecological status classification without integrating analyses of biological and environmental monitoring data in a format that is more useful for managers. We present a framework using conditional inference tree analyses and Bayesian Network methodology that synthesises monitoring data, identifies links between environmental and biological variables, and predicts the effects of climate change for Dublin Bay (Ireland). The ecological quality status of phytoplankton biomass was usually high but degraded when silica became limiting. Sediment organic content was positively related to benthic invertebrate richness and the abundance of wading birds, although invertebrate communities were most indicative of pristine conditions when sediment organic content was low. Importantly, climate change simulations showed that the ecological status of Dublin Bay will decline, particularly affecting wading bird abundance through habitat loss, which highlights the importance of removing other stressors from the ecosystem.

4.2 Introduction

Coastal ecosystems experience increasing anthropogenic pressures, which affect their biodiversity and associated rates of ecosystem functioning (Lotze *et al.*, 2006; Halpern *et al.*, 2007; He and Silliman, 2019; O'Hara, Frazier and Halpern, 2021). Resource exploitation, marine pollution including eutrophication and harmful chemicals, or physical modifications are among the human-driven processes that impact marine ecosystems, particularly in urban areas (Todd *et al.*, 2019). Consequences include resource reduction, habitat modifications or loss, altered hydrodynamics, or altered species interactions, fitness, reproduction and development, which may lead to loss of foundation species, changes in biodiversity, and productivity (Todd *et al.*, 2019). In turn, human societies face the repercussions of lost or overexploited food sources or raw materials, reduced water quality, algal blooms, erosion, or degraded cultural, recreational or aesthetic value (Atkins *et al.*, 2011). The remedies of these take time, are of uncertain success and costly (Bayraktarov *et al.*, 2015; Duarte *et al.*, 2020). On top of these rather direct anthropogenic pressures, climate change undermines marine biodiversity and ecosystem functioning through warming, acidifying and deoxygenating seawater, causing sea level rise and more frequent weather extremes (Hewitt, Ellis and Thrush, 2016; IPCC, 2022; Matthews and Wynes, 2022). Results are mass mortality events, species or habitat loss, range shifts, changes in ecosystem structure and phenology, or altered species interactions, all of which threaten human food security, coastal settlements and infrastructure, tourism-related industries, water quality, human well-being and health (IPCC, 2019, 2022). Representative Concentration Pathways (RCPs) of increasing greenhouse gas concentrations have been developed to model future climate scenarios and estimate potential ecosystem trajectories, including RCP 4.5 and 8.5 illustrating moderate and extreme climate change, respectively (van Vuuren *et al.*, 2011; IPCC, 2013). Even though the importance of biodiversity conservation, climate change mitigation and adaptation has been recognised with increasing urgency, effective action and societal transformation emerge slowly (Matthews and Wynes, 2022). This may partly be due to the complexity of the problems to solve, the uncertainty about how to tackle the combined effects of multiple stressors, and the uncertainty of whether management interventions will achieve desired outcomes (Cardinale *et al.*, 2012; Côté, Darling and Brown, 2016; Matthews and Wynes, 2022; Moore and Schindler, 2022; Seddon, 2022).

Various monitoring programmes try to capture environmental and ecological trends and regularly report their descriptive findings as summary metrics or indices. For instance, the Water Framework Directive (WFD) of the European Union is a statutory example that defines common principles for monitoring and taking action to achieve or maintain ‘good ecological status’ in all European water bodies (European Commission, 2000). Extensive monitoring activities are routinely reported, however, when information is condensed into proxies for levels of ecological status, it can no longer be used to identify relationships with typical environmental conditions (Voulvoulis *et al.*, 2017; Feld *et al.*, 2020). There are few robust frameworks that integrate the biological and environmental data to characterise and help manage coastal ecosystems for a range of outcomes (Feld, Segurado and Gutiérrez-Cánovas, 2016). To manage and protect aquatic environments effectively, however, aquatic resource management requires an understanding of the links between environmental conditions and biological communities, and also predictions of how changing conditions may affect communities (Philippart *et al.*, 2011). For example, how will the diversity of communities or abundance of key species respond to predicted climate change and what are the possible responses to different management options?

Modelling can integrate complex knowledge of a system and the application of Bayesian Networks (BNs) is one approach to enhance system understanding, predict the effects of change, and support decision-making (Kelly *et al.*, 2013). BNs are useful for linking potential drivers, pressures and impacts of multiple stressors in complex ecosystems, presenting probabilistic outcomes of desired biodiversity aspects or ecosystem services following specific management choices or climate change scenarios (Uusitalo, 2007). Such models are illustrated as an acyclic conceptual influence diagram that contains nodes (variables) and links (relationships) between them in a directed cause-effect structure, which is easy to interpret and modify by users and stakeholders. Nodes consist of multiple states, i.e. categorical conditions or data ranges, and the links between the nodes represent conditional, probabilistic (Bayesian) relationships (Kelly *et al.*, 2013). BNs apply conditional inference and calculate the overall probabilities that certain variable conditions will be attained. BNs can be used prognostically (given the inputs, what are the outcomes?) or diagnostically (given an output, what were the inputs?), however, no concept of time or circular connections such as ecological feedback loops can be included, restricting BNs to static, conditional snapshots of the study system. The advantages of BNs as tools to synthesise complex system information include: (i) knowledge from different sources (e.g. monitoring programmes; disciplines such as ecology, hydrology, social science, economics; expert opinion) can be combined; (ii) different types of data (e.g. experimental, monitoring or questionnaire data; quantitative or qualitative) can be incorporated; (iii) uncertain, small or incomplete data sets can be included; and (iv) new information can be added as it becomes available (Uusitalo, 2007; Aguilera *et al.*, 2011; Kelly *et al.*, 2013). The probabilistic BN outputs include explicit information about the uncertainty of data or predictions in a study system, which also identifies knowledge gaps (Chen and Pollino, 2012).

Dublin Bay, Ireland, is a temperate estuarine ecosystem bordering a metropolitan area and is thus subjected to many common anthropogenic stressors. Therefore, its management requires informed decision-making to be effective. Apart from the exposure to industrial pressures, including agricultural residue and nutrient discharges from rivers and Dublin City's wastewater treatment plant, industrial and heavy metal pollution, port and industrial activities and their associate risk of pulses of pollution, Dublin Bay is extensively used for recreational activities, both on sea and on land (Brooks *et al.*, 2016; Cabana *et al.*, 2020). All these pressures, in addition to climate change, impact the system by changing the abiotic conditions (e.g. water temperature, salinity, nutrient concentration, or pollutant levels), which in turn affect biodiversity (e.g. migrating bird population) and ecosystem functioning (e.g. biochemical oxygen demands, nutrient cycling or productivity rates). Biodiversity and biochemical processes in Dublin Bay provide important ecosystem services, for instance water quality regulation, cultural and aesthetic value, or the opportunity to observe wildlife, which all benefit Dublin as a liveable city and tourist destination (Brooks *et al.*, 2016; Cabana *et al.*, 2020).

Acknowledging the high ecological value of rare and internationally important habitats and species of wildlife, Dublin Bay and its multiple Special Protection Areas (SPAs) and Special Areas of Conservation (SACs) have been designated as UNESCO Biosphere (Dublin Bay Biosphere Partnership, 2017). This highlights the importance of effective conservation management and pressure mitigation to maintain or restore favourable conservation condition. In Dublin Bay, SPA and SAC conservation objectives include maintaining a stable or increasing area of the habitat type 'mudflats and sandflats not covered by seawater at low tide', and stable or increasing long-term population trends of waterbirds such as brent goose, redshank, curlew or bar-tailed godwit (NPWS, 2013a, 2013b, 2015a, 2015b). However, after designation, there is a lack of regular monitoring and assessment if or how the status of these features changes, which undermines effective conservation management.

We present a framework in which we synthesise environmental monitoring data from Dublin Bay in Ireland using conditional inference tree analyses and the Bayesian Network (BN) methodology to support local management decision-making by deepening the understanding of the complex linkages between environmental conditions and the diversity and functioning of Dublin Bay. Additionally, key physico-chemical variables, and their possible effects on biological variables, were extrapolated according to climate change projections to provide ecosystem trajectories under different climate change scenarios (IPCC, 2013, 2019). Nutrient conditions, sediment organic content and climate change scenarios were linked with physico-chemical intermediate variables, wave, and river flow data to model effects on phytoplankton, benthic invertebrates and waders as socio-economically relevant biodiversity indicators.

Our data analysis was based on the following broad hypotheses:

1. Weather and discharges into Dublin Bay affect physico-chemical variables (e.g. nutrient concentrations may increase with sewage discharge, water temperature increases with warmer weather, salinity decreases with precipitation and high river discharge loads, water agitation increases in windy conditions).
2. Physico-chemical variables affect phytoplankton and benthic invertebrates (e.g. phytoplankton biomass increases with temperature; Platt *et al.*, 1987).
3. Phytoplankton growth increases pH and the concentration of dissolved oxygen through photosynthesis (Provoost *et al.*, 2010; Jakobsen *et al.*, 2015).
4. High dissolved oxygen concentrations, pH, and sediment organic content are beneficial to benthic invertebrates.
5. The availability of benthic invertebrates and of habitat area positively affect the abundance of waders.
6. Climate change will affect biodiversity through changes in physico-chemical variables (e.g. warming will increase phytoplankton abundance, but loss of intertidal areas will reduce wader abundance).

4.3 Materials and Methods

To characterise Dublin Bay, a BN was built from available monitoring data following an established iterative process (Jakeman *et al*., 2006; Marcot *et al.*, 2006; Chen and Pollino, 2012; Harris *et al.*, 2017), which included: (i) conceptualising Dublin Bay as a study system; (ii) defining the model structure by selecting variables and specifying links between them; (iii) discretizing continuous variables into categorical states; and (iv) populating conditional probability tables. The geographical area covered by the BN includes transitional waters of the River Tolka, North Bull Island and Liffey Estuary and the coastal waters of Dublin Bay (EPA, 2022; Figure 4.1).

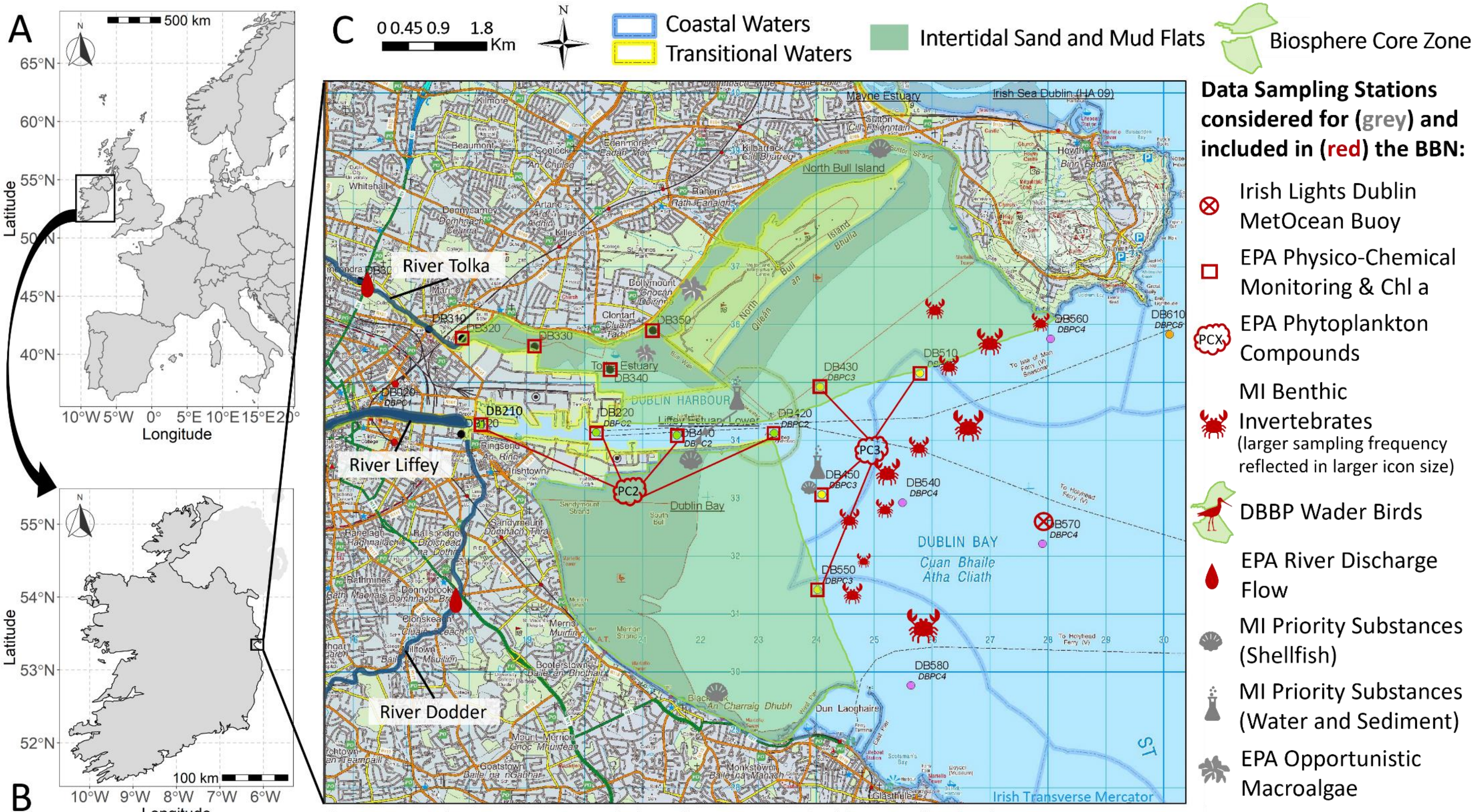

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Figure 4.1 A, B Dublin Bay in Ireland, C sampling stations from which monitoring data was provided for the BBN. EPA: Environmental Protection Agency Ireland; MI: Marine Institute Ireland: DBBP: Dublin Bay Bird Project.

Dublin Bay was conceptualised holistically by applying the DPSIR framework (Drivers – Pressures – State Change – Impact – Response [Atkins *et al.*, 2011]). We identified natural (e.g. tidal water exchange with the Irish Sea) and anthropogenic drivers (e.g. climate change, urbanisation, agriculture in the hinterland) that exerted pressures on the bay (e.g. input of organic particulate matter, or warming), which changed the state of abiotic environmental variables (e.g. sediment organic content, or water temperature) and thereby had the potential to affect various ecological processes and related biodiversity indicators (e.g. phytoplankton biomass, wader abundance).

The model structure, i.e. the variables represented as nodes in the network and the conditional links among them, was drawn from previous studies (e.g. Wilson, 2005; Brooks *et al.*, 2016; Cabana *et al.*, 2020), available monitoring data, climate change projections (IPCC, 2013, 2019), and conditional inference tree (ctree) data analysis (Hothorn, Hornik and Zeileis, 2006). Data were obtained from nine monitoring programmes (Figure 4.1; for details see Table C.1). Variables were excluded if ctree data analysis (details below) did not reveal significant relationships to other variables (Figure 4.1, Appendix C.2). For some potentially relevant biodiversity groups (e.g. fish, marine mammals) or processes (e.g. subtidal blooms of filamentous brown algae and associated beach fouling), no data was available (Appendix C.2). Climate change projections were incorporated to compare the current state of Dublin Bay with predicted conditions following RCPs 4.5 and 8.5. for the period 2081-2100 (IPCC, 2013, 2019).

When defining the variable states, i.e. the discrete and mutually exclusive categories or data ranges in which a variable occurs and into which continuous data must be discretised to be incorporated into a BN, we adopted existing thresholds where applicable (e.g. predictions of the Intergovernmental Panel on Climate Change, or classifications of the EU WFD). Conditional probability tables were filled following data analysis.

Data analyses were conducted with R Studio version 1.4.1106 (RStudio Team, 2021) and R version 4.1.0 (R Core Team, 2021), using the function *ctree* of the package partykit (Hothorn and Zeileis, 2015), and the libcoin (Hothorn, 2021) and tidyverse (Wickham *et al.*, 2019) packages. The GeNIe Modeler version 3.0.6518.0 (BayesFusion, 2021) was used to implement the Bayesian Network.

4.3.1 BN structure

The BN comprises 20 variables (Figure 4.2), including four ‘input nodes’, (defined as variables that are not affected by any other variable): (i) ‘climate change’ to compare between the current state of Dublin Bay and predicted conditions following RCPs 4.5 and 8.5. for the period 2081-2100 (IPCC, 2013, 2019); (ii) nutrient ratio of dissolved inorganic nitrogen to phosphate (DIN:PO_4); (iii) nutrient ratio of phosphate to silicate dioxide (PO_4:SiO_2); and (iv) sediment organic content. Only climate change can be classified as a driver, whereas the other three input variables reflect abiotic environmental states. For those, quantitative information on the underlying pressures and drivers that produced them was unavailable (e.g. agricultural runoffs or tidal water input from the Irish Sea). The node climate change is linked to more explicit pressures, such as warming, ocean acidification, changes to precipitation patterns and sea level rise. These, in turn, are linked to the intermediate abiotic nodes water temperature, pH, salinity, water agitation, the ecological quality status (EQS) of oxygenation as defined in the WFD, and the areal extent of intertidal sand and mud flats. These abiotic nodes are linked to the condition of five biodiversity indicators, which are the 'output nodes' and include: (i) EQS of phytoplankton biomass; (ii) EQS of phytoplankton abundance; (iii) benthic invertebrate taxa richness; (iv) benthic invertebrate disturbance sensitivity; and (v) the abundance of bar-tailed godwits (*Limosa lapponica lapponica*), which are representative of key wading bird populations.

Bar-tailed godwits were chosen as a final biodiversity indicator in the BN because they are exclusively tied to the intertidal zone, and use all of Dublin Bay's intertidal area independent of tidal phase as their home range (Granadeiro *et al.*, 2006; Tierney *et al.*, 2017). The species is considered 'Near Threatened' in the IUCN Red List (Wetlands International, 2022) and amber-listed as bird of conservation concern in Ireland (Colhoun and Cummins, 2013), with the decline of available intertidal habitat posing a major threat to these waders (Tierney *et al.*, 2017). Bar-tailed godwits occur in Dublin Bay at nationally important numbers (> 150 birds) during nine months of the year (Tierney *et al.*, 2017) and peak counts can meet the threshold of international importance, which reflects 1 % (> 1500 birds) of the biogeographic population of Northern and Western Europe (Wetlands International, 2022).

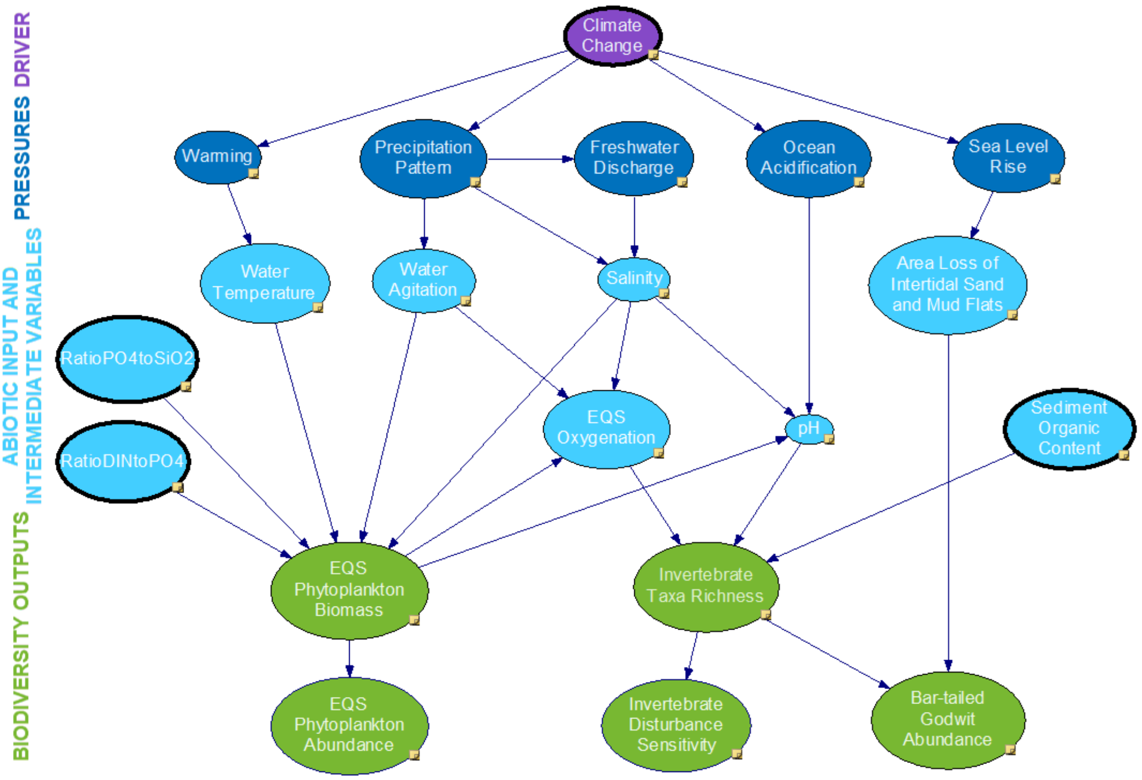

Figure 4.2 Bayesian Network of environmental variables and biodiversity of Dublin Bay, including drivers (purple), pressures (dark blue), abiotic variables (light blue) and biodiversity outputs (green). The four input nodes have bold outlines.

4.3.2 Data analysis

Temporal and spatial resolution, sampling dates and sampling locations differed between the available monitoring data sets (Table C.1) but were matched as closely as possible when merging them for data analysis (Appendix C.3). Data obtained from different sampling locations and at different dates were treated as independent observations considering that water residence time in Dublin Bay is < 3.4 days (O’Boyle *et al.*, 2015), which is less than the minimum temporal resolution of the analysed data, and that the water column is constantly mixed due to inputs from various directions and sources, including tidal hydrodynamics.

We used open-ocean projections of ocean acidification (IPCC, 2014, 2019) as best available estimates, being aware that coastal dynamics and developments under climate change are largely unclear and are influenced by a complex multitude of drivers (Duarte *et al.*, 2013).

Only data of the photosynthetically productive period from March to September 2007-2020 were considered when modelling physico-chemical variables (the input nodes PO_4:SiO_2 and DIN:PO_4, and the intermediate variables water temperature, salinity, EQS oxygenation, pH) to accommodate existing thresholds on the ecological status of certain water conditions (EPA Ireland, 2006) and to remove confounding effects of seasonal sampling bias (O’Boyle *et al.*, 2015; Ní Longphuirt, Mockler, *et al.*, 2016). A preliminary investigation confirmed that the single annual winter samples represented a different set of characteristics to the three annual summer samples and should be modelled separately.

The variable ‘water agitation’ was included as a proxy for weather conditions (cloud cover, light intensity), expecting a calm seawater surface during good weather periods and an agitated to turbulent seawater surface during poor(er) weather conditions. Our statistical analysis confirmed that turbulent conditions increased dissolved oxygen concentrations, probably because of increased mixing at the water-atmosphere boundary, and additionally decreased primary productivity, presumably because of higher disturbance levels or reduced light availability in more cloudy conditions. Water agitation was calculated as the ratio of wave height to wave length, which reflects wave steepness and indicates the likelihood that waves will break or shoal (Masselink, Hughes and Knight, 2011). Wave length was estimated from wave period using an equation for wave celerity at intermediate water depth (Masselink, Hughes and Knight, 2011).

To include information on phytoplankton community structure beyond changes in biomass, concurrent changes in phytoplankton abundance were investigated. Similarly, ‘Invertebrate Disturbance Sensitivity’ was included as a variable in the BN as an indicator for subtidal benthic soft-bottom invertebrate community composition. It is based on the strong correlation ($p < 0.001$; Pearson’s $r = -0.64$) of taxa richness with the percentage of taxa that was assigned the Ecological Group I according to AMBI (AZTI’s Marine Biotic Index [Borja *et al*., 2000]). The Ecological Group I contains indicator taxa and those sensitive to organic enrichment. The less species of Group I were present in the invertebrate samples, the more taxa of Group II were found, which are described as indifferent, followed by taxa of Group III, which are considered as tolerant to organic enrichment. The remaining groups IV and V contain (second-order) opportunistic species, which were present in the samples at less than 10, or 3 %, respectively. Low invertebrate taxa richness correlated strongly with a high percentage of species indicative of pristine conditions, i.e. sensitive instead of tolerant to enrichment. Somewhat counterintuitively, a decrease in richness of these particular taxa is recorded as status improvement in the BN because it is correlates with the presence of the sensitive species that are indicative of pristine conditions.

Currently, Dublin Bay has 15 km^2 intertidal sand and mud flat habitats, which extend almost 3 km at their widest and are dominated by well-aerated sands apart from muddy habitats in the estuaries and Bull Island lagoon (NPWS, 2015b).When estimating climate change effects of sea level rise on the area of intertidal sand and mud flats, we assumed the persistence of the current urban, artificial embanking of Dublin’s coast lines. This will prevent coastal retreat and cause ‘coastal squeeze’ instead of the gain of compensating intertidal areas with rising sea levels, and will ultimately cause the loss of intertidal sand and mud flat habitats (Pontee, 2013).

When modelling the effects of intertidal area loss on bar-tailed godwit abundance according to sea level rise predicted for RCP 4.5 or 8.5, we maintained the abundance thresholds for national and international importance that refer to the current bird population. We acknowledge that if the waders cannot establish themselves elsewhere in the bay due to coastal squeeze, the overall bird population size might decline (Iwamura *et al.*, 2013), which would lead to adjusted thresholds of counts that reflect national and international importance. To estimate future bar-tailed godwit abundance in Dublin Bay, we applied an equation that describes the general relationship between estuary area and wader abundance in New Zealand (Whelan *et al.*, 2003):

$$ln(maximum\ abundance) = 0.6647 * ln(area) - 4.457$$

According to the estimated area loss under the climate change projections RCP 4.5 and 8.5, future bar-tailed godwit populations may be reduced to 89 %, or 82 %, respectively, compared to current maximum abundance. Applying a similar equation that characterises average peak counts of bar-tailed godwits in eastern England as a function of estuary size (average peak count = 0.0789 * estuary size in ha + 13.158 (Prater, 1981)) resulted in an even more drastic population decline to 85 %, or 77 %, respectively.

4.3.3 Defining the node states and discretising continuous variables

A total of 63 variable states were defined, with 2-5 states per variable (Table 4.1; Figure C.1). Existing thresholds were adopted where applicable: RCPs 4.5 and 8.5 and the magnitude of the corresponding projected changes for the period 2081-2100 (IPCC, 2013, 2019; Jacob *et al.*, 2014) informed the states of warming, ocean acidification, precipitation pattern, sea level rise and the associated area loss of intertidal sand and mud flats (decrease – no change – little to strong increase). Thresholds defined by the EU WFD to classify the status of water quality in transitional and coastal waters were used to characterise the EQS of phytoplankton biomass, of phytoplankton abundance, and of water oxygenation (high – good – moderate – poor – bad). Thresholds at which bar-tailed godwits are considered to occur at numbers of national or international importance at a site defined by the Ramsar Convention and applied by Birdwatch Ireland were applied to discretise wader abundance apart from absence or presence. For pH and invertebrate disturbance sensitivity, i.e. the percentage of invertebrate taxa that are assigned to the Ecological Group I according to AMBI, no previous thresholds existed, which is why data ranges were determined following visual analysis of histograms (Feld, Saeedghalati and Hering, 2020). All remaining continuous variables were discretised into states using ctree analysis (Appendix C.5), which applies tree-structured regression models (Hothorn, Hornik and Zeileis, 2006). This type of analysis partitions child node data (the case-specific response variable) according to regression-based break points in the parent node data (the case-specific influencing variable(s)). For example, modelling ctree for chlorophyll a concentration as a response of salinity, water temperature and the nutrient ratios DIN:PO_4 and PO_4:SiO_2 (already applying the previously identified states of those influencing variables) identified child node data chunks, from which the distribution of the respective data across the child node states was extracted (Figure 4.3). Note that the identified thresholds between the states of the nutrient ratios DIN:PO_4 and PO_4:SiO_2 are purely data-based and do not relate to the Redfield ratio (Redfield, 1958).

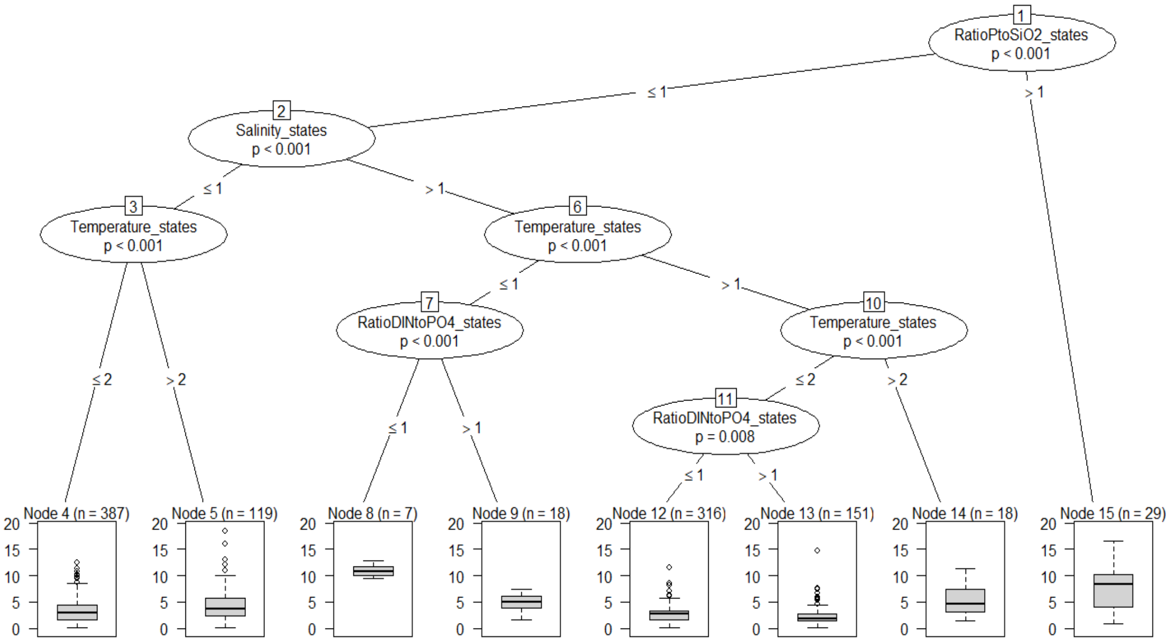

Figure 4.3 Conditional inference tree example of modelling chlorophyll a concentration as a response of salinity, water temperature and the nutrient ratios DIN:PO$_4$ and PO$_4$:SiO$_2$, applying the previously identified states of those influencing variables. Significant break points in the predictor variables were determined by the model and the response variable data were partitioned accordingly. End node size of the data partitions and the data distribution of the response variable in the form of boxplots is depicted at the bottom of the graph.

In three cases, data analysis required to add states of “unknown” data relationships to account for limited overlaps of different data sets or for parent node state combinations that could not be matched with child node data. For the node water agitation only five years of data were available (2015-2019), while for its child nodes EQS Phytoplankton Biomass and EQS Oxygenation and their other parent nodes 15 years of data were available (2007-2020). The effects of the nutrient ratios on the EQS of phytoplankton biomass disappeared when the data set was reduced to the overlapping time period, which is why we analysed the monitoring periods separately. The BN calculates the overall probabilities of occurrence of the child node states and accounts automatically for whether the water agitation is known in the parent node or not, i.e. proportionally scales the information. If water agitation is unknown, then the information in the child node states depends exclusively on the other parent nodes. A state of “unknown” node condition was also added both to invertebrate taxa richness and disturbance sensitivity. The reason in this case was that not all parent node state combinations could be matched with invertebrate taxa richness data, and, because both variables originated from the same, coinciding data set, no invertebrate disturbance sensitivity data could be matched with “unknown” invertebrate taxa richness. At the same time, the state of “unknown” invertebrate taxa richness accommodated the fact that, similar to the node water agitation and its child nodes, the child node bar-tailed godwit abundance had been sampled during more years than invertebrate data were available.

Table 4.1 Summary of all nodes, states, state ranges and units, underlying data and sources in the Bayesian Network of environmental variables and biodiversity of Dublin Bay.

Node	States	State range	Unit	Underlying data	Data source
Climate change (input node)	Period_2007_to_2020 Year_2100_RCP4_5 Year_2100_RCP8_5	most recent information; 2081-2100; 2081-2100		Relative Concentration Pathways 4.5 and 8.5 for the year 2100	IPCC, 2013, 2014, 2019
RatioDINtoPO$_4$ (input node)	DIN_limited PO$_4$_limited	≤ 10.525 > 10.525	ratio	DIN : PO$_4$	Environmental Protection Agency of Ireland physico-chemical monitoring[1] 2007-2020
RatioPtoSiO$_2$ (input node)	PO$_4$-limited SiO$_2$-limited	≤ 1.02 > 1.02	ratio	PO$_4$: SiO$_2$	Environmental Protection Agency of Ireland physico-chemical monitoring[1] 2007-2020
Sediment Organic Content (input node)	Low High	≤ 0.819 > 0.819	%	Loss of ignition	Marine Institute benthic invertebrate monitoring in Dublin Bay[2] 2014-2020
Warming	None Little Moderate High	0 1 2 3.5	°C	Projected change in water temperature for the period 2081-2100 relative to 1971-2000	Jacob *et al.*, 2014
Water Temperature	Cool Medium Warm	≤ 10.79 > 10.79, ≤ 17.17 > 17.17	°C	Sea surface temperature	Environmental Protection Agency of Ireland physico-chemical monitoring[1] 2007-2020
Ocean Acidification	None Some Substantially	0 -0.093 -0.315	pH units	Projected change in pH for the period 2081-2100 relative to 1971-2000	IPCC, 2014, 2019

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[1] The Environment Protection Agency Ireland maintains the database right.

[2] The Marine Institute Ireland maintains the database right and granted a revocable, non-exclusive, non-transferable license to the authors to use the data for the permitted purpose of informing the BN

pH	Low	< 7.95	pH units	pH	Environmental Protection Agency of Ireland physico-chemical monitoring[1] 2007-2020
	Medium	≥ 7.95; < 8.15			
	High	≥ 8.15			
Freshwater Discharge	Low	≤ 1.546	m^3/s	Daily mean flow of the rivers Dodder and Tolka	Environmental Protection Agency HydroNet[3] 2007-2020
	High	> 1.546			
Precipitation Pattern	Decrease_15	-15	%	Projected regional, seasonal change of precipitation for the period 2071-2100 compared to 1971-2000	Jacob *et al.*, 2014
	No_Change	0			
	Increase_15	+15			
Water Agitation	Calm	≤ 0.039	ratio	Wave height : wave length	Irish Lights Dublin Met Ocean weather buoy[4] 2015-2019
	Agitated	≤ 0.062			
	Turbulent	> 0.062			
	Unknown	NA			
Salinity	Brackish	≤ 32.97	salinity units	Salinity	Environmental Protection Agency of Ireland physico-chemical monitoring[1] 2007-2020
	Marine	> 32.97			
EQS Oxygenation	High	High-UL: 95%ile <120; 95%ile < 115-120 High-LL: 95%ile > 80; 95%ile > 80-85	%	Dissolved oxygen saturation (range between upper (UL) and lower limit (LL), considering salinity: first value applies between 0-17 salinity, followed by linear interpolation between second value at salinity 17 and third value at salinity of 35)	Environmental Protection Agency of Ireland physico-chemical monitoring[1] 2007-2020
	Good	Good-UL: 120 < 95%ile < 130; 115-120 < 95%ile < 120-130 Good-LL: 80 > 95%ile > 70; 80-85 > 95%ile > 70-80			
	Moderate_to_Bad	MtB-UL: 95%ile > 130; 95%ile > 120-130 MtB-LL: 95%ile < 70; 95%ile < 70-80			
Sea Level Rise	None	0	m	Projected regional mean sea level rise for the year 2100 relative to 1981-2000	Palmer *et al.*, 2018
	Some	0.45			
	Substantially	0.7			

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[3] Environmental Protection Agency Ireland HydroNet data on river flow in Dublin Bay, licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0).

[4] Commissioners of Irish Lights MetOcean data on wave height and period in Dublin Bay, licensed under a Creative Commons Attribution 4.0 International Licence (http://creativecommons.org/licenses/by/4.0/).

Area loss of Intertidal Sand and Mud Flats	None Some Substantially	0 16.36 25.45	%	Change in areal extent of intertidal sand and mud flats according to projected mean sea level rise	Area between low and high water lines calculated using digital maps by Geological Survey Ireland[5] and the mean tidal range of 2.75 m (Mansfield, 1992)
EQS Phytoplankton Biomass (biodiversity output)	High Good Moderate_to_Bad	median <5.0 and 90%ile <10.0 median <10.0 and 90%ile <20.0 median >10.0 and 90%ile >20.0	µg/L	Chlorophyll a concentration; state ranges following Cusack *et al.*, 2008	Environmental Protection Agency of Ireland physico-chemical monitoring[1] 2007-2020
EQS Phytoplankton Abundance (biodiversity output)	High Good Moderate Poor Bad	High: max ≤ 12000 & med ≤ 8000 Good: max ≤ 12000 & med > 8000 max ≤ 34750 & med ≤ 8000 Moderate: max ≤ 34750 & med > 8000 max ≤ 250000 & med ≤ 8000 Poor: max ≤ 250000 & med > 8000 max ≤ 308000 & med ≤ 8000 Bad: max ≤ 308000 & med > 8000 max >308000 & med ≤ 8000	cell counts per litre	Taxa cell counts; state ranges according to both the median taxa cell count threshold established in unpolluted reference conditions (8000 cells/l) and the maximum taxa cell count thresholds established for abundance class boundaries in Irish estuarine waters (Ní Longphuirt *et al.*, 2019).	Environmental Protection Agency of Ireland phytoplankton monitoring[1] 2007-2020
Invertebrate Taxa Richness (biodiversity output)	Low Low_to_Medium Medium_to_High High Unknown	≤ 17 > 17; ≤ 30 > 30; ≤ 37 > 37 NA	S (taxa richness)	Number of taxa of marine subtidal benthic soft-bottom invertebrates from 0.1 m^2 surface area day grab samples, sieved through a 1 mm mesh	Marine Institute benthic invertebrate monitoring[2] 2014-2020

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[5] Geological Survey of Ireland Irish Public Sector Data on intertidal sand and mud flat areas, licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) licence (http://creativecommons.org/licenses/by/4.0/).

Invertebrate Disturbance Sensitivity (biodiversity output)	Sensitive	> 66	%	Percentage of taxa that was assigned the Ecological Group I according to AMBI (AZTI's Marine Biotic Index [Borja *et al.*, 2000]) and is sensitive to organic enrichment	Marine Institute benthic invertebrate monitoring[2] 2014-2020
	Indifferent	> 33; ≤ 66			
	Tolerant	≤ 33			
	Unknown	NA			
Bar-tailed Godwit Abundance (biodiversity output)	Bay-wide		counts	Monthly low-tide bird counts at a subsite	Dublin Bay Birds[6] Project 2014-2020
	International	≥ 880			
	Importance Likely	≥ 150; < 880			
	Nationally Important				
	Present below	< 150; > 0			
	Thresholds	0			
	Absent				

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[6] Managed by BirdWatch Ireland and funded by Dublin Port Company.

4.3.4 Linking nodes and filling the conditional probability tables

Nodes were linked in the BN when ctree analysis indicated significant ($p < 0.05$) partitioning of child node data according to parent node states, and, therefore, significant underlying regression models in the respective data. Separate analyses were run for all child nodes, i.e. all except for the input nodes, resulting in 32 links (Appendix C.6). Ctree analysis automatically accounts for non-linear relationships and interactions between variables when it partitions continuous variables into discrete ranges.

After defining the node states and identifying statistically significant links, the conditional probability table (i.e. the full crossing of the child node states with all possible parent node states, or state combinations of multiple parent nodes) of each node was filled according to the frequency at which data of a certain child node state occurred under specific parent node conditions. 946 conditional probabilities were quantified.

No predicted probability distributions exist for the states of the climate change pressures sea level rise and ocean acidification, which is why we linked the predicted magnitude of change with 100 % probability of occurrence to the respective climate change scenario. While pH data from recent years were available in the child node of ocean acidification, no probability distribution could be extrapolated for the states of the loss of intertidal sand and mud flat habitats associated with sea level rise, thus, we linked the estimated loss with 100 % probability of occurrence to the respective sea level rise scenario.

pH and dissolved oxygen conditions that can be expected in an extreme climate change scenario (following RCP 8.5) have not yet been captured with current monitoring, which is why their effects on invertebrate taxa richness and disturbance sensitivity could not be estimated. Consequently, the corresponding change in wader abundance was estimated exclusively according to the effects of sea level rise and the subsequent loss of intertidal habitat.

4.3.5 Operating the BN

To run the BN, a climate change state must be selected manually by the operator of the model (or the output node results will be a mix of current data and extrapolations to future conditions), while it is optional to specify the states of the other input nodes. If no other node state is selected, the model incorporates the general probability of occurrence of all node states based on available monitoring data, and this is the only alternative to specifying a node state at 100 % probability of occurrence for running the BN.

The fully parametrised BN can be used in two ways: First, it can serve to investigate changes in the probability distributions of the output node states according to specific selections of input node states. However, not all possible output node states are targeted by changes to the input states because of too little changes caused in intermediate nodes. So alternatively, specific node connections in the internal network and the effects of selected intermediate node states can be explored individually to gain a deeper understanding of the processes unfolding in Dublin Bay.

We ran the model with all possible combinations of input node states, including their general probabilities of occurrence, and present the changes in probability distributions of biodiversity output nodes relative to general current climate conditions in this paper. Probability distributions of invertebrate taxa richness and invertebrate disturbance sensitivity states were scaled proportionally to the amount of available data. Changes in probability distributions of less than 3 % were ignored due to high uncertainty (Kelly-Quinn *et al.*, 2022). Note that changes in the conditional probabilities only reflect the probability that a variable will occur at a certain state, not that the variable itself will increase or decrease by the same percentage (Kelly-Quinn *et al.*, 2022).

4.3.6 Heuristic validation

To test whether qualitative model performance (network structure and direction of conditional relationships) aligned with expert opinion, a survey with independent experts was conducted. A digital survey was completed by nine environmental scientists with knowledge of Dublin Bay, where participants were asked to predict relationships among nodes in the BN (Appendix C.7). Responses were weighted according to the experts' own expertise ratings and transformed into a score (0-1) for consent and disagreement among experts, which was then compared to the model performance. The results were presented to the experts in a 2-hour online workshop, during which points of disagreement among the experts and divergence of expert expectation and model performance were discussed.

4.4 Results

4.4.1 Model outcomes

We built a BN with climate change scenarios, the ratios of DIN:PO_4 and PO_4:SiO_2, and sediment organic content as input nodes, and the WFD EQS of phytoplankton biomass and abundance, the taxa richness of benthic, subtidal invertebrates and their disturbance sensitivity, and the abundance of the waders bar-tailed godwits as output nodes. The probability of occurrence of output node states degraded with increasing climate change relative to general current climate conditions, except for invertebrate taxa richness and disturbance sensitivity that improved when sediment organic content was low (Figure 4.4, Table 4.2).

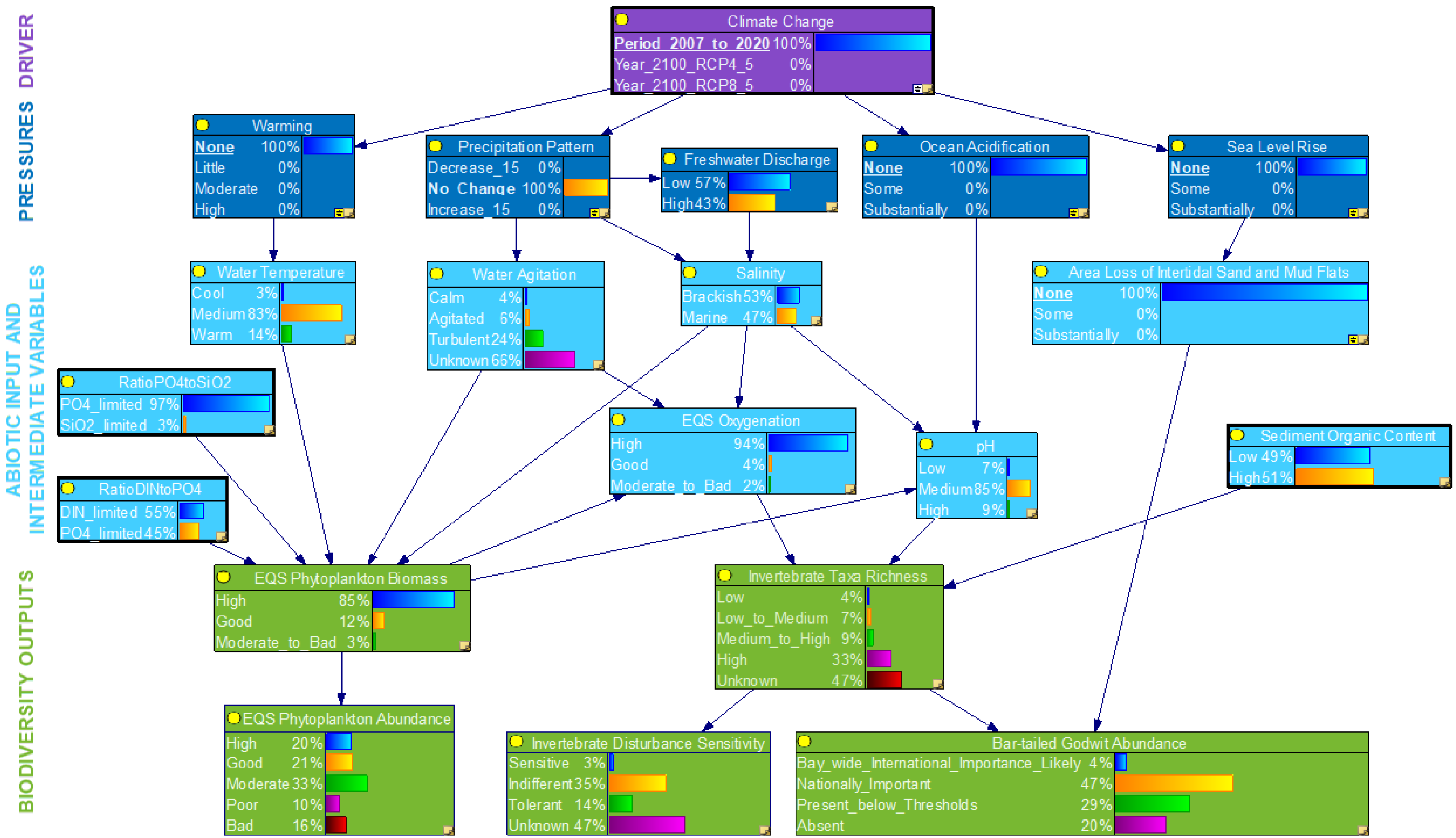


Figure 4.4 Bayesian Network of environmental variables and biodiversity of Dublin Bay, node states, and conditional links among them showing the general frequency distributions of the node states as % representing available monitoring data from the years 2007-2020. Drivers (purple), pressures (dark blue), abiotic variables (light blue) and biodiversity outputs (green) are listed from top to bottom. Input nodes have bold outlines.

Table 4.2 Conditional probabilities of output node state occurrence with fully crossed input node state combinations from the Bayesian Network of environmental variables and biodiversity of Dublin Bay. General reference conditions from 2007-2020 are highlighted in bold on violet background, with light violet (no change, i.e. < 3 %), shades of green (improvements) or blue (degradation) illustrate the magnitude of change in probability distributions relative to reference conditions. Colour intensity increases with magnitude of change. States of 'Unknown' data depict incomplete data overlap between available response and predictor variable data. Remaining node states were scaled proportionally to sum up to 100 %. Phytoplankton was not linked to sediment organic content and no invertebrate data were available to be extrapolated to RCP 8.5 conditions.

Sediment Organic Content		general			general			general			Low			Low			Low			High			High			High		
Ratio PO_4:SiO_2		general			PO_4-limited			SiO_2-limited			general			PO_4-limited			SiO_2-limited			general			PO_4-limited			SiO_2-limited		
Climate Condition		**Current***	RCP 4.5**	RCP 8.5**	Current	RCP 4.5	RCP 8.5	Current	RCP 4.5	RCP 8.5	Current	RCP 4.5	RCP 8.5	Current	RCP 4.5	RCP 8.5	Current	RCP 4.5	RCP 8.5	Current	RCP 4.5	RCP 8.5	Current	RCP 4.5	RCP 8.5	Current	RCP 4.5	RCP 8.5
EQS Phytoplankton Biomass	High	**85**	-11	-14	+1	-10	-13	-35	-39	-40																		
	Good	**12**	+8	+10	0	+7	+10	+13	+15	+16																		
	Moderate to Bad	**3**	+3	+4	-1	+3	+3	+22	+24	+24																		
EQS Phytoplankton Abundance	High	**20**	-1	-1	0	-1	-1	-4	-4	-4																		
	Good	**21**	+1	+1	0	+1	+1	+1	+1	+1																		
	Moderate	**33**	0	0	0	0	0	+1	+1	+1																		
	Poor	**10**	0	0	0	0	0	+1	+1	+1																		
	Bad	**16**	0	0	0	0	0	+1	+1	+1																		
Invertebrate Taxa Richness	Low	**8**	-5		0	-5		+1	-5		+17	+42		+17	+42		+21	+42		-4	-8		-4	-8		-2	-8	
	Low to Medium	**13**	-3		0	-3		+2	-4		+28	-13		+28	-13		+28	-13		-4	-4		-4	-4		-3	-4	
	Medium to High	**17**	-1		0	-4		+2	+2		0	-17		0	-17		-5	-17		+1	-1		0	-1		+3	+3	
	High	**62**	+9		0	+12		-5	+7		-45	-12		-45	-12		-44	-12		+7	+13		+8	+13		+2	+9	
	Unknown (absolute)	***47***	*69*	*100*	*47*	*69*	*100*	*46*	*68*	*100*	*88*	*96*	*100*	*88*	*96*	*100*	*83*	*94*	*100*	*9*	*43*	*100*	*9*	*44*	*100*	*11*	*44*	*100*
Invertebrate Disturbance Sensitivity	Sensitive	**6**	-3		0	-3		1	-3		+11	+19		+11	+19		+12	+11		-2	-4		-2	-3		0	-4	
	Indifferent	**66**	+2		0	+2		1	+2		0	-16		0	-16		+4	0		+1	+1		+1	+1		0	+2	
	Tolerant	**28**	+1		0	+1		-2	+1		-11	-3		-11	-3		-16	-11		+1	+3		+1	+2		0	+2	
	Unknown (absolute)	***47***	*69*	*100*	*47*	*69*	*100*	*46*	*69*	*100*	*88*	*96*	*100*	*88*	*96*	*100*	*83*	*94*	*100*	*9*	*43*	*100*	*9*	*43*	*100*	*11*	*44*	*100*
Bar-tailed Godwit Abundance	Bay-wide International Importance likely	**4**	-1	-3	0	-1	-3	0	-1	-3	-1	-1	-3	-1	-1	-3	-1	-1	-3	+1	-1	-3	+1	-1	-3	+1	-1	-3
	National Importance	**47**	-2	-3	0	-2	-3	-1	-2	-3	-2	-4	-3	-2	-4	-3	-2	-4	-3	+1	-1	-3	+1	-1	-3	+1	-1	-3
	Present below thresholds	**29**	+3	+6	0	+3	+6	+1	+3	+6	+3	+5	+6	+3	+5	+6	+3	+5	+6	-2	+2	+6	-2	+2	+6	-2	+2	+6
	Absent	**20**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Colour code:

Current general probability distribution without specifications [%]

No change (i.e. < 3 %) relative to current general distribution

Improvement (+)	3-5 %	6-10 %	11-20 %	21-50 %
Degradation (-)	3-5 %	6-10 %	11-20 %	21-50 %

*period 2007-2020 **climate change projections for the year 2100

EQS Phytoplankton Biomass

Under current climate and nutrient conditions, the EQS of phytoplankton biomass in Dublin Bay from 2007-2020 was classified as 'High' in 85 % of observations (Figure 4.4). The ratio of DIN:PO_4 affected the EQS of phytoplankton biomass significantly in certain conditions (Figure 4.3), however, these conditions did not appear when the full BN was run with climate change as overall input node. When the ratio of PO_4:SiO_2 was set to SiO_2 limitation, a 'High' quality output state of the system was 35 % less likely, while the probability of attaining a 'Good' or 'Moderate to Bad' EQS increased by 13 and 22 % respectively, i.e. these less favourable states became 2-7 times more likely compared to how often they prevailed in 2007-2020 (Table 4.2). With increasing severity of climate change projections, the model indicated worsening shifts of up to 14 % probability from a 'High' EQS to a 'Good' and, at a lesser extent, 'Moderate to Bad' status (Table 4.2). Effectively, these less favourable conditions were predicted to be twice as likely than during recent years. PO_4 limitation in the PO_4:SiO_2 ratio mitigated this effect slightly (Table 4.2). When SiO_2 limitation was specified in addition to climate change scenarios, attaining a ‘Good’ or ‘Moderate to Bad’ status became equally likely at 27 or 28 % probability, this suggests that a ‘Moderate to Bad’ status may become eight times more likely (Table 4.2).

EQS Phytoplankton Abundance

A moderate EQS of phytoplankton abundance was most common in Dublin Bay during 2007-2020, while ‘Good’ or ‘High’ status occurred more often than ‘Poor’ or ‘Bad’ (Figure 4.4). Our model indicated that the EQS of phytoplankton abundance may degrade under SiO_2-limited conditions, however, it was not affected by climate change (Table 4.2).

Invertebrate Taxa Richness and Disturbance Sensitivity

Invertebrate taxa richness was 'High' at 62 % of the available observations (Figure 4.4) but became 5 % less likely under SiO_2-limitation (Table 4.2). When sediment organic content was 'Low', the probability of state occurrence shifted from 'High' taxa richness to 'Low to Medium' (+28 %) and 'Low' (+17 %), with the latter effectively becoming three times as likely as during recent years (Table 4.2). When sediment organic content was ‘High’, this led to an opposite shift of 5 % under additional SiO_2-limitation from the states of lower to high(er) taxa richness, and 8 % under general or PO_4-limited nutrient conditions (Table 4.2). Despite the lack of data for RCP 8.5, the model shows that the probability to attain 'High' taxa richness increased by 9 % under climate change scenario RCP 4.5 and general sediment organic content, 7 % when SiO_2 and 12 % when PO_4 was limiting (Table 4.2). Under 'High' sediment organic content, the shifts in the probability distribution under scenario RCP 4.5 almost doubled compared to those observed under current conditions (Table 4.2). When sediment organic content was specified as 'Low' then 'Low' taxa richness was estimated to become six times more likely under RCP 4.5 conditions compared to what was observed in current data (Table 4.2). Whenever low taxa richness became more likely, the species sensitive to organic enrichment occurred 3-4 times more likely than observed during recent years, while high taxa richness was associated with higher proportions of species indifferent or tolerant to organic enrichment (Table 4.2).

Abundance of bar-tailed godwits

Bar-tailed godwits occurred at numbers of national importance in 47 % of the observations, were present below this threshold in 29 % of the occasions, and absent 20 % of the time (Figure 4.4). In 4 % of the observations, the threshold for international importance was met (Figure 4.4), which translates into ten events during the seven years of available data. This is very close to the twelve occasions derived from the raw data (independent of the data handling required for the BN) and indicates that the model estimates for when the threshold of international importance will be met may be conservative. In both climate change scenarios, nationally important numbers were estimated to occur 2-3 % less often in the probability distribution, while presence at numbers below the current threshold for national importance increased by 3-6 % (Table 4.2). Following RCP 8.5 predictions, our model indicated that the abundance of bar-tailed godwits may exceed the threshold of international importance in only 1 % of the observed probability distribution, meaning a reduction of 75 % to only one occasion every 2-3 years (Table 4.2).

The probability of attaining bar-tailed godwit abundances above the nationally or internationally important thresholds decreased when sediment organic content was ‘Low’, while the probability of the waders being present below any threshold increased by 3 % in current and by 5 % in predicted RCP 4.5 climate conditions (Table 4.2). 'High' sediment organic content did not affect the probability distribution of wader abundance (Table 4.2). The effects of nutrient conditions on phytoplankton biomass did not pass through the trophic levels and had no effect on the abundance of bar-tailed godwits (Table 4.2). Seasonal absence of these waders was similar in all modelling scenarios (Table 4.2).

Quantified uncertainty in the BN

All modelled changes in the EQS of phytoplankton biomass can be interpreted with high confidence, because they involve at least 10 % of change in the probability distribution (Kelly-Quinn *et al.*, 2022; (Table 4.2). The same applies to most of the climate change predictions for invertebrate taxa richness and to all changes that can be expected in conditions of low sediment organic content, including the corresponding shifts in the invertebrate disturbance sensitivity. In contrast, the very even probability distribution of the five states of the EQS phytoplankton abundance in current conditions and changes according to SiO_2 limitation, as well as the small changes (< 5 %) in the probability distribution of bar-tailed godwit abundances under low sediment organic content or in a moderate climate change scenario need to be interpreted with caution (Table 4.2). All remaining modelled changes in probability distributions are 5-10 % and should be interpreted at an intermediate level of uncertainty (Table 4.2).

4.4.2 Expert survey and workshop

In five of ten predicted relationships in the expert survey conducted to help with model validation (Appendix C.7), expert expectations aligned with the effects indicated by the BN (Table 4.3). In two cases (effect of the EQS phytoplankton biomass on the EQS oxygenation; effect of SiO_2-limitation on the EQS phytoplankton biomass) fewer experts scored 0.03 points higher deviating from model predictions than the majority of experts whose expectations aligned with the BN (Table 4.3). Not all experts answered all questions.

Table 4.3 Weighted mean scores of expert opinions on predicted relationships between BN variables compared to BN performance. Behind each score, the number of experts on which the score is based is given in brackets. The highest score per relationship is highlighted in bold.

One-factorial relationships: Effect of…	**Alignment with BN model**	**Opposite effect expected**	**No relationship expected**
PO_4- vs. SiO_2-limitation on EQS Phytoplankton Biomass	0.67 (3)	**0.70** (2)	NA
DIN- vs. PO_4-limitation on EQS Phytoplankton Biomass	0.40 (1)	**0.58** (5)	NA
Water Temperature on EQS Phytoplankton Biomass (summary of two survey questions)	0.32 (6)	**0.64 (6)**	0.57 (4)
Water Agitation on EQS Phytoplankton Biomass	**0.65 (4)**	0.60 (1)	NA
the EQS Phytoplankton Biomass on pH (two questions)	**0.62 (7)**	0.53 (4)	NA
the EQS Phytoplankton Biomass on EQS Oxygenation (summary of two questions)	0.65 (9)	0.35 (2)	**0.68 (5)**
pH on Invertebrate Taxa Richness	**0.80 (1)**	0.40 (1)	0.70 (2)
EQS Oxygenation on Invertebrate Taxa Richness	**1.00 (1)**	0.60 (2)	0.80 (1)
Invertebrate Taxa Richness on waders	**0.55 (4)**	NA	NA
Relative importance of the PO_4:SiO_2 ratio compared to the DIN:PO_4 ratio on EQS Phytoplankton Biomass	0.13 (1)	**0.88 (7)**	NA

Follow-up discussions during the expert workshop clarified expectations on magnitude of measurable change in oxygenation, and increased awareness on rare (3 % of observations 2007-2020) but nonetheless occurring SiO_2-limitation in Dublin Bay (Figure 4.4). Experts disagreed among each other regarding a predictable relationship between water temperature and the EQS of phytoplankton biomass, however, those disagreeing with the model rated their expertise highest (Table 4.3). Acceptance of the model performance was reached acknowledging that the BN was parametrised according to coinciding monitoring data instead of empirical cause-effect relationships, i.e. phytoplankton growth can be nutrient-limited despite suitable water temperatures. Asked how the EQS phytoplankton biomass would respond when Dublin Bay changed from DIN-limited to PO_4-limited, one expert agreed with low confidence with the BN, expecting no change. Five experts agreed with each other that the EQS would change, four of which expected an improvement, while one expected degradation. Multiple experts pointed out that Dublin Bay is supplied constantly with nitrogen inputs from river discharges, and diurnally with tidal inputs of phosphorous from the Irish Sea, although many experts imagined long-lasting, stable nutrient conditions in a closed system when answering the question. The BN indication of no change was accepted given the constant flushing of Dublin Bay with nitrogen and phosphorus, which also led to agreement regarding the final survey question on the relative importance of the PO_4:SiO_2 ratio compared to the DIN:PO_4 ratio in Dublin Bay. The workshop concluded in heuristic consent and no amendments to the BN were required.

4.5 Discussion

The BN predicts an overall degradation in ecological status of Dublin Bay under the intermediate and extreme (business as usual) scenarios of predicted climate change, which is illustrated by a reduction in four of five proxies for ecological health and biodiversity. Specifically, the WFD EQS of phytoplankton biomass (high status will be 14-17 % less likely), invertebrate taxa richness (low taxa richness will be 62% less likely), invertebrate disturbance sensitivity (will be 50 % less likely to show pristine conditions), and the abundance of bar-tailed godwits (75 % decline of presence at international importance) will all be lower. The EQS phytoplankton abundance was not affected by climate change, but by SiO_2 limitation.

The ratio of DIN:PO_4 did not affect the biodiversity indicators included in the BN, contrary to intuitive initial expert expectation. DIN limitation occurred in 55 % of the observations and PO_4-limitation in the remaining 45 %, illustrating the alternating states of low and high tides with a periodic pulse supply of PO_4 from the Irish Sea and continuous supply of riverine DIN inputs (O'Higgins and Wilson, 2005; O'Boyle *et al.*, 2015). Owing to the short residence time of water in the well-flushed Dublin Bay, phytoplankton biomass tends to be low compared to the amount of available DIN and PO_4, which indicates export of both nutrients and phytoplankton into the Irish Sea rather than retention within the Bay (O'Boyle *et al.*, 2015). This highlights the potential implications for Dublin Bay should its residence time increase due to climate change. During recent decades, management efforts in Irish river catchments (e.g. reducing fertiliser applications, improving farming practices) substantially reduced phosphorus levels as phosphorus is considered the limiting nutrient in freshwater systems (O'Boyle *et al.*, 2016). Nitrogen loadings have decreased to a lesser extent, causing elevated DIN:PO_4 ratios that may cause shifts in phytoplankton community compositions or opportunistic green macroalgae blooms in estuaries with strong marine influences, which are usually DIN-limited (Ní Longphuirt, O'Boyle, *et al.*, 2016; O'Boyle *et al.*, 2016). Macroalgal blooms regularly occur in Dublin's Tolka Estuary, which is considered at risk of eutrophication due to the proximity to the urban waste water treatment plant (Jeffrey *et al.*, 1995; Ní Longphuirt, Mockler, *et al.*, 2016). Additionally, the remineralisation of settled particulate matter inputs from the Irish Sea facilitates macroalgal blooms in Dublin Bay and is considered to be the main cause of the (not monitored) subtidal *Ectocarpus* mats that get washed up on Dublin’s beaches in autumn and indicate eutrophic conditions (Jeffrey, Madden and Rafferty, 1993; Jeffrey *et al.*, 1995; Jennings and Jeffrey, 2005; Wilson, Rybarczyck and Elkaim, 2007). Reducing nitrogen loads considerably might reduce opportunistic macroalgae blooms, which may be of particular interest if macroalgae in Dublin Bay will show similar increased growth as phytoplankton biomass under expected climate change.

Phytoplankton production in Dublin Bay is currently phosphorous limited (97 % of the observations from 2007-2020) and increases in phytoplankton biomass and a corresponding degradation of the EQS phytoplankton biomass can be expected with high certainty under SiO_2-limitation. SiO_2-limitation impairs the growth of diatoms, which are a major food source for primary consumers such as zooplankton, filter feeders and fish (Officer and Ryther, 1980; Zhang *et al.*, 2020). When diatom growth is limited, flagellates can dominate phytoplankton communities, which may show in higher cell abundance (Howarth and Marino, 2006). Flagellate communities persist longer because they are grazed less heavily, and can introduce nuisances such as shellfish poisoning, discoloured and malodorous water, or hypoxia to an ecosystem (Officer and Ryther, 1980). Reduced grazing decreases the flux of organic matter from the water column to the sediment (Howarth and Marino, 2006), which may explain why low invertebrate taxa richness and higher percentages of invertebrate species that are characteristic of pristine conditions became more likely under SiO_2-limitation. While the tidal exchange with the Irish Sea cannot be managed locally, river and sewage treatment discharge, which are main sources of SiO_2 in coastal systems, hold opportunities for management action to maintain a high EQS of phytoplankton biomass (Zhang *et al.*, 2020). These include maintaining a phosphorous-limited PO_4:SiO_2 ratio in effluents, or considering the implications that regulating the flow of Dublin’s largest river Liffey through up-stream reservoirs may have on the nutrient balance in Dublin Bay.

Invertebrate taxa richness was high in 62 % of observations from 2007-2020 and became even more likely when sediment organic content was high and PO_4 limiting. Conversely, low invertebrate taxa richness was most likely to occur when SiO_2 was limiting, and especially when sediment organic content was low. Deposition, decomposition and bioturbation of particulate matter drive sediment organic content, which was almost equally often low (49 %) or high (51 %) from 2007-2020, and potentially even productivity and food web structuring in Dublin Bay (Wilson, Brennan and Murray, 2002). Even though the tidally-driven input of particulate matter from the Irish Sea has been quantified as ten times more than the combined discharges from rivers and the sewage treatment plant, the latter is considered to fuel localised eutrophication through wind- or wave-driven dynamics (Wilson, Brennan and Murray, 2002).

Our data analysis identified a strong negative correlation between invertebrate taxa richness and the percentage of taxa that are indicative of pristine conditions (invertebrate disturbance sensitivity) as opposed to species that are indifferent or tolerant to organic enrichment. Increasing taxa richness in combination with an increasing amount of tolerant or even opportunistic species has been found previously after ongoing disturbance through organic enrichment (Culhane *et al.*, 2019). It is thought that this was because the more sensitive species persisted temporarily before they were replaced by more indifferent and tolerant species. Following the intermediate disturbance theory, high taxa richness may reflect a continuously changing species composition caused by higher rates of disturbance than rates of recovery, which temporarily prevents that competitive exclusion leads to prolonged coexistence (Connell, 1978; Tanentzap, Lee and Schulz, 2013; Huston, 2014). Increased diversity has also been attributed to higher productivity, which can be caused by increased resource availability through moderate organic enrichment (Mittelbach *et al.*, 2001; Abdelrhman and Cicchetti, 2012; Huston, 2014). Consequently, although higher biodiversity is commonly considered as desirable, the community composition should be assessed when evaluating ecosystem functioning or health. Quick changes in taxa richness following disturbance are usually not reflected by summarising indices such as the Infaunal Quality Index (IQI) that is applied in WFD monitoring to indicate the ecological status of invertebrate communities (Borja *et al.*, 2009; Culhane *et al.*, 2019). This is consistent with the absence of significant links between predictor variables and invertebrate metrics in our analyses, except when we used taxa richness.

Our BN estimated increasing invertebrate taxa richness in the moderate climate change scenario, primarily caused by changes in the probability distributions of pH and oxygenation states. Climate change and particularly sea level rise, with its influence on intertidal habitats, have the potential to cause substantial changes in benthic macro invertebrate communities (Fujii, 2012). These communities are pivotal to intertidal, estuarine and coastal food webs because they support higher trophic levels such as larger crustaceans, fishes and birds (Fujii, 2012). Sea level rise may cause coastal squeeze, which not only causes intertidal habitat loss but may also alter sediment deposition and particle size distribution, the vertical shore profile, the salinity gradient between freshwater and marine conditions, and mixing conditions (Fujii, 2012). All of these consequences may affect intertidal benthic communities and prey availability for higher trophic levels, which aligns with the effect of invertebrate taxa richness on wader abundance as higher trophic level in our BN.

According to our data analysis, benthic invertebrate taxa richness and disturbance sensitivity were positively linked with bar-tailed godwit abundance, even though the only available invertebrate data originated from subtidal instead of intertidal communities. In the Wadden Sea, bar-tailed godwits regularly prey on polychaetes such as *Arenicola marina*, *Hediste diversicolor*, *Nephtys hombergii*, *Scoloplos armiger*, and *Lanice conchilega*, in addition to bivalves such as *Macoma balthica* and crustaceans such as *Carcinus maenas* (Scheiffarth, 2001). Five of these species belong to the AMBI Ecological Group III, which is tolerant to disturbance such as organic enrichment, and the remaining two species belong to group II (Phillips *et al.*, 2014). These ecological groups were more common in Dublin Bay when taxa richness was high, which explains the positive effect of high taxa richness on bar-tailed godwit abundance. High invertebrate taxa richness mitigated negative effects of moderate climate change on bar-tailed godwit abundance, while low invertebrate taxa richness had an enhancing effect, probably reflecting the absence or presence of preferred invertebrate prey species.

Regardless of benthic invertebrate taxa richness, a major threat to migrating wader populations such as bar-tailed godwits, especially those exclusively tied to the sandy and muddy intertidal areas, is habitat loss (Fujii, 2012). Bar-tailed godwits perform extreme annual migrations and as site-faithful waders heavily rely on few staging sites (Battley *et al.*, 2012). In Dublin Bay, bar-tailed godwits are only one of > 50 migratory waterbird and seabird species, of which 23 occur at nationally important numbers including three at internationally important numbers, and annual peak counts of up to 35 000 birds can be observed during low tide (Tierney *et al.*, 2017). Most birds spend the winter in Dublin Bay but high numbers in spring and autumn show the importance of the site for migratory passage stops (Tierney *et al.*, 2017).

Dublin Bay is an urban marine ecosystem and its shorelines are almost exclusively artificially embanked to protect settlements and infrastructure from storm floods and sea level rise (Brooks *et al.*, 2016). Current high tides wash up against these embankments, or even wash over them during storm floods. Sea level rise will lead to coastal squeeze and decrease the current intertidal area by up to 25 % (Table 4.2). Establishing new resting and foraging areas to compensate for those lost to sea level rise, e.g. by enabling coastal retreat locally, is one option of complying with the current SPA conservation objectives to maintain wader abundances in Dublin Bay. As this would put urban infrastructure at risk and is therefore not practical, a crucial measure to conserve Dublin Bay as an important area for waders and to comply as closely as possible with the UNESCO Biosphere conservation objectives is protecting the remaining habitat. Dublin's shorelines, however, are among the most heavily used in Ireland, both for land-based activities and for water sports (Brooks *et al.*, 2016). Already at the current intertidal areal extent waterbirds are frequently disturbed by human recreational and leisure activities, which causes the birds to move and interrupts their foraging or resting at the cost of energy expenditure (Tierney *et al.*, 2017). Our BN indicates that climate change, including sea level rise, will reduce wader abundance, putting the status of Dublin Bay as an internationally important staging site for far-migrating waders such as the bar-tailed godwits at risk. Consequently, management needs to consider potential conflicts of interest in the both ecologically and culturally important shorelines and intertidal areas, and find solutions on how to balance the recreational demands of society with existential requirements of local wildlife (Granadeiro *et al.*, 2006).

In summary, SiO_2 limitation degraded the EQS phytoplankton biomass but enhanced low invertebrate taxa richness and higher percentages of species indicative of pristine conditions. At the same time, this caused increasing occurrence probabilities of lower wader abundance, presumably caused by a change in prey availability. High sediment organic content favoured higher invertebrate taxa richness and species that are indifferent or tolerant to organic enrichment, therefore no associated change occurred in the probability distribution of wader abundance. Increasing climate change enhanced the shift of probabilities towards high invertebrate taxa richness and dominance of species indifferent or tolerant to disturbance, however, habitat loss due to sea level rise dominated the negative effect of climate change on wader abundance.

Conclusion

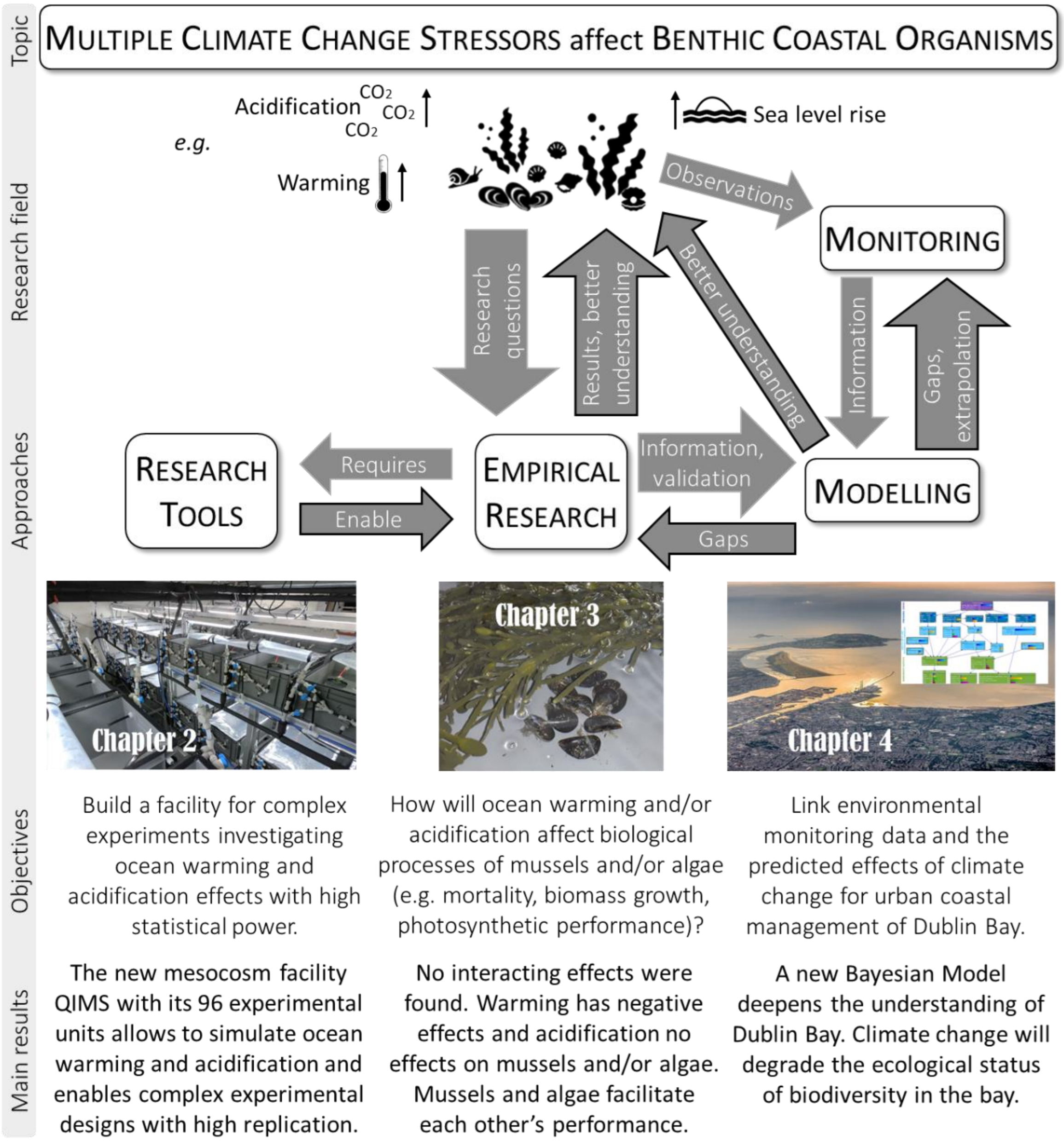
If management actions in Dublin Bay were targeted to maintain or increase wader populations under current climate conditions, then increased invertebrate taxa richness and higher organic content in the sediment would be needed. However, this would oppose efforts of improving the EQS of phytoplankton and the occurrence of macroalgae blooms in Dublin Bay. Methods of achieving the best, or at least the most acceptable, balance between these can be explored using the BN model. Conditions prevailing under climate change RCPs 4.5 and 8.5 will affect both fauna and phytoplankton EQS negatively with increasing intensity, which emphasizes the urgent need for effective climate change mitigation. As long as no solution can be found to compensate for habitat loss due to sea level rise, local management should strongly consider how to best protect the remaining habitat of the at-risk biodiversity in Dublin Bay and should also consider habitats at other sites along the coast.

This study shows how Bayesian Networks are a useful tool to move beyond direct predictor-response analyses and assess how a multitude of interacting variables in a complex system influence each other and how the system may respond to various combinations of driving input variables. The BN presented in this study highlights the value of linking existing information from a variety of sources in a way that deepens the understanding of a system, provides context-dependent trajectories, informs reasoning, and facilitates discussion and management decisions on a local level. At the same time, parametrising the model revealed data gaps and the need for concerted and coordinated monitoring programmes. While large quantities of monitoring data exist and are continuously compiled, they are rarely directly compatible across space and time (Dafforn *et al.*, 2016). What is more, even though the WFD launched extensive national monitoring programmes, much more concerted monitoring efforts would be necessary, both in temporal resolution and in terms of additional variables, to capture the full range of ecological processes even in the short physical retention time of water in Dublin Bay and across seasonal, ecologically successive patterns. Additionally, there still is a huge lack of quantitative information regarding, e.g., suspended material and deposition, chemical pollution and effects of ship traffic, littering, subtidal macroalgae blooms, intertidal invertebrates, or disturbance of waders through recreational activities including dog-walking. Most importantly, linking the data of monitoring programmes that are conducted in parallel is crucial to deepen a comprehensive understanding of marine systems like Dublin Bay, and tracing pressures back to their origin so explicit management levers can be identified.

Chapter 5 | Synthesis

5.1 Overview and key findings

The overall aim of this thesis was to advance the knowledge and understanding of the impact of multiple stressors on marine benthic organisms by targeting different sources of uncertainty regarding the fate of these species in a changing climate. Specifically, I addressed knowledge gaps and limitations owing to: inadequate experimental facilities; a lack of empirical data to determine the role of interspecific interactions on responses to ocean acidification and/or warming; and the paucity of current synthesis and accessibility of monitoring data. First, a new mesocosm-based experimental platform was developed to overcome the typical limitations in marine multiple stressor experimental designs, i.e. allow for greater replication of more experimental treatments (Chapter 2). Subsequently, this experimental facility was employed and its functionality was tested by quantifying the independent and cumulative effects of ocean warming and acidification on the functioning of a benthic filter feeder and a primary producer on their own and together, with a fully crossed experimental design to test for all possible interactions (Chapter 3). Finally, available monitoring data for Dublin Bay was synthesised and made more accessible to local managers by building a Bayesian Network on the links between environmental variables and biodiversity, including estimated effects of projected climate change scenarios (Chapter 4). Applying the three approaches presented in this thesis – methodological tool development, empirical research, and modelling – exemplifies how existing gaps in multiple stressor research can be tackled from different angles to eventually predict the consequences of expected climate change for coastal habitats more accurately (Figure 5.1).



Build a facility for complex experiments investigating ocean warming and acidification effects with high statistical power.

How will ocean warming and/or acidification affect biological processes of mussels and/or algae (e.g. mortality, biomass growth, photosynthetic performance)?

Link environmental monitoring data and the predicted effects of climate change for urban coastal management of Dublin Bay.

The new mesocosm facility QIMS with its 96 experimental units allows to simulate ocean warming and acidification and enables complex experimental designs with high replication.

No interacting effects were found. Warming has negative effects and acidification no effects on mussels and/or algae. Mussels and algae facilitate each other's performance.

A new Bayesian Model deepens the understanding of Dublin Bay. Climate change will degrade the ecological status of biodiversity in the bay.

Figure 5.1 Graphical thesis outline with topic and research field investigated, as well as alternative approaches and objectives addressed in the three main chapters of this PhD thesis, extended from Figure 1.1 and now including main results. Arrows indicate flow of information, those with black outline summarise contributions of this PhD thesis to the field of research.

Elaborate experimental platforms, such as QIMS presented in Chapter 2, allow sophisticated, mechanistic studies targeting a range of research questions regarding the effects of multiple stressors and biological interactions in multifactorial or gradient experiments, including the consideration of necessary and/or research on sufficient replication for statistical robustness. Too little replication has limited the detection of significant effects, while simultaneously causing the over-estimation of effect sizes, which resulted in a biased perception regarding the true effects of anthropogenic impacts (Yang *et al.*, 2022). QIMS is only the third of a suite of specialised, permanently installed marine mesocosm facilities that simulate ocean warming and/or acidification and offer a high number of experimental units (80-100 instead of commonly 12 in other facilities) for advanced experimental complexity and replication. It is currently the only such facility in which both ocean warming and acidification can be simulated for temperate rocky shore research. Despite the limited realism of mesocosm facilities (Crowe *et al.*, 2012; Boyd *et al.*, 2018), QIMS offers unprecedented opportunities for disentangling the independent and combined effects of multiple stressors across different levels of biological complexity on biologic functioning, production and diversity, or for identifying non-linear responses in gradient designs. Including ocean acidification and warming as well as other stressors, research questions regarding the effects of stressor sequence, intensity, duration, natural variability and considering different perspectives of underlying mechanisms are still understudied and could all be addressed using QIMS (Gunderson *et al.*, 2016; Jackson *et al.*, 2021; Orr *et al.*, 2022). Revealing underlying mechanisms or identifying general tendencies in biotic responses to global change will greatly improve our ability to scale up experimental results to natural ecosystems and protect, restore and manage our natural capital.

In Chapter 3 I show how warming affects the functioning of marine ectotherms, using a mesocosm experiment investigating the effects of moderate ocean warming and acidification on the functioning and production of a calcifying filter feeder and a primary producer cultured separately and together. Surprisingly, acidification had no effect on either group, contrary to the expectations of impaired calcification and improved primary production (Hoppit and Schmidt, 2022). We found clear evidence, however, that mussels and algae had positive effects on each other's performance, which highlights the ecological benefits of conserving marine biodiversity. Mussel mortality and clearance rates both increased with warming, which indicates that already at low levels of warming metabolic costs may scale faster than what can be compensated by increased energy supply (Lemoine and Burkepile, 2012). At the same time, total accumulated biomass was highest at the medium temperature level, reflecting especially higher survival than in the warmest treatment. These effects of moderate warming may have substantial consequences both for direct trophic relationships between marine ectotherm species, and also for the community structure and composition of marine ecosystems under climate warming. The absence of effects of moderately elevated pCO_2 on either mussels and algae in my experiment and the recent reports that macrophytes may buffer acidification effects on shellfish in their proximity (Wahl *et al.*, 2018; Jiang and Fang, 2021; Ricart *et al.*, 2021; Young *et al.*, 2022) highlight possible temporal refugia for calcifiers from acidification stress that may increase the chances for adaptation to more acidified ocean conditions. Interestingly, independent of water temperature and pCO_2, both the algae and the mussels benefited from each other's presence, e.g. by showing increased biomass, which may have been facilitated by nutrient enrichment through mussel excretions, improved nutrient recycling and, therefore, water quality through the algae, and algal exudates that may have served as additional food source for the mussels (Andriana *et al.*, 2021). These findings emphasise that benthic communities may rely on delicate dependencies and balances between species and trophic groups, and that conserving biodiversity is crucial to stabilise, maintain and protect benthic ecosystem functioning and service provision (Solan *et al.*, 2020; Chen, 2021).

Clear synthesis of complex information is needed to effectively manage ecosystems. Integrated analyses of biological and environmental monitoring data, including alternative trajectories of an ecosystem according to different possible climate change scenarios, is indispensable for managing aquatic ecosystems effectively and in an anticipatory approach for a range of outcomes (Boyd *et al.*, 2018). Food security, the protection of coastal infrastructure, water quality, tourism, human well-being and health, as well as biodiversity conservation in a changing climate depend on a deep understanding of the effects of multiple stressors and biotic interactions in the ecosystem of interest, possible outcomes of alternative management interventions, and predictions of how climate change may affect biological communities (Philippart *et al.*, 2011; Feld, Segurado and Gutiérrez-Cánovas, 2016; Cooley *et al.*, 2022). Robust frameworks, however, that synthesise this information and make it readily accessible for managers and decision makers are rare (Feld, Segurado and Gutiérrez-Cánovas, 2016). The Bayesian Network (BN) of Dublin Bay presented in Chapter 4 of this thesis is one approach for filling this gap. It links environmental variables with biodiversity indicators using conditional inference and presents probabilistic outcomes of desired biodiversity aspects following specific substance inputs to the system (nutrient ratios, sediment organic content) and climate change scenarios, including transparent information about the uncertainty of data or predictions in the system (Uusitalo, 2007; Chen and Pollino, 2012). In the case of Dublin Bay, the BN revealed the negative effects on the ecological status of Dublin Bay that can be expected with increasing climate change, e.g. phytoplankton nuisances may become more likely, while the abundance of charismatic wading birds will probably decline. The model therefore highlighted the importance of carefully managing local stressors in Dublin Bay that are likely to further affect biodiversity, e.g. regulating nutrient inputs to control phytoplankton communities, or resolutely protecting intertidal habitat to conserve waders, if biodiversity conservation is the aim. What is more, analysing environmental and biological monitoring data in an integrated way identifies knowledge gaps regarding the availability of data in the first place or on the relationships between variables, which can then be addressed by refined monitoring efforts or empirical research. For example, compiling data for the Dublin Bay BN was limited because of low resolution monitoring of hazardous substances in water, sediment and biota, or missing data on subtidal macroalgae blooms. If monitoring efforts were increased and sampling streamlined across space and time to facilitate the analysis of how the consequences of a changing variable feeds through the whole ecosystem instead of only descriptively documenting the isolated trend of change, it would allow much more nuanced and informed management action in addition to helping prioritise management action. Additionally, a knowledge gap of the expected effects of extreme climate change on benthic invertebrate taxa richness and disturbance sensitivity was identified, which exemplifies uncertainty around the effects of climate change on higher trophic levels that prey on benthic invertebrates until this gap is filled, for example in a mesocosm study.

Addressing the question “How can we better predict the effects of multiple stressors of climate change on marine benthic resources?”, this thesis shows clearly how different approaches can all generate valuable pieces of information. For best outcomes, however, the different available scientific approaches (e.g. monitoring; field observations; empirical research, such as mesocosm studies or manipulative field studies; modelling) should be applied as complementary tools to advance the scientific understanding of a system and thereby facilitate management and planning (Boyd *et al.*, 2018; Gissi *et al.*, 2021). Regular monitoring can document long-term change in natural systems and can, similar to field observations, draw attention to specific patterns of change that require deeper understanding for future management (Boyd *et al.*, 2018). Mesocosm experiments are important to test hypotheses and inform specific management questions, e.g. how to increase aquaculture yield most efficiently, while models can inform holistic ecosystem management strategies, e.g. the restoration of keystone species by providing their required habitat characteristics, or ecosystem maintenance in changing ocean conditions. Furthermore, mesocosm studies are complementary to field studies and allow us to identify clear cause-effect relationships and their underlying mechanisms, in addition to disentangling interacting effects of multiple stressors that are difficult to tackle in the field because of confounding factors and often low statistical power (Boyd *et al.*, 2018; Yang *et al.*, 2022). Field studies, on the other hand, are not restricted by the limited realism of mesocosms, but instead simultaneously reflect all environmental and biotic processes in natural systems and are, therefore, needed to verify conclusions and predictions about how communities and ecosystems may develop in changing environmental conditions (Crain, Kroeker and Halpern, 2008). Models, such as BNs, can combine, extrapolate, predict, or condense data as desired while transparently communicating uncertainty. These models can also be used to highlight knowledge gaps, which can then be addressed in empirical studies, and identify system components that require prioritised management action (Gissi *et al.*, 2021; Pirotta *et al.*, 2022). Empirical research can additionally inform model parameterisations and can even validate completed models. Similar to literature reviews or meta-analyses that qualitatively or quantitatively synthesise research on specific questions to identify general trends, models can synthesise vast amounts of information and their findings can, therefore, be communicated more readily to non-science stakeholders, managers and decision makers than independent, usually context-dependent primary research results.

5.2 Future work

Regarding ocean acidification and warming effects, relevant unsolved ecological research questions include how such cumulative stressors affect relationships between trophic levels (especially when calcifying organisms are involved), or primary producers and calcifying organisms (does photosynthesis buffer acidification effects?), or interspecific interactions between different functional groups and assemblages (ecosystem functioning). Priority should be given to understand how key species, such as ecosystem engineers, processes and ecosystems that have been identified as either very vulnerable or resilient to environmental change respond to the cumulative effects of multiple stressors (Riebesell and Gattuso, 2015). Protecting and restoring biodiversity directly contributes to climate change mitigation and best outcomes are achieved when management measures account for both biodiversity conservation and climate change mitigation or adaptation (Pörtner *et al.*, 2021).

The best currently available predictions of future ocean acidification all refer to open ocean conditions (IPCC, 2014, 2019), while carbon chemistry in coastal systems underlies substantial temporal and spatial variation that are poorly understood (Duarte *et al.*, 2013). Seawater alkalinity and pH show strong local fluctuations owing to biological processes (e.g. photosynthesis and respiration, see Cornwall *et al.*, 2013; Wahl *et al.*, 2018; Lowe, Bos and Ruesink, 2019), and investigating how biota influences pH and alkalinity may improve estimating potential future coastal conditions.

Environmental stresses on ecosystems rarely occur in isolation, and they often fluctuate naturally following diurnal, lunar or seasonal patterns, climate dynamics or weather events, or biological activity, however, such dynamics have rarely been considered in multiple stressor research (Pansch and Hiebenthal, 2019; Jackson, Pawar and Woodward, 2021). In addition to general trends in global change, such as gradual ocean acidification or warming (IPCC, 2014, 2022), environmental variability is expected to increase, for example including more frequent and intense precipitation or heatwave events (Hobday *et al.*, 2016; IPCC, 2022). The phenological decoupling of interacting processes, such as phytoplankton and zooplankton blooms (Stenseth *et al.*, 2002; Winder and Schindler, 2004), has highlighted the importance of considering the effects of temporal shifts on the occurrence of certain environmental conditions on biotic responses (Walther, 2010). Similarly, the frequency of fluctuation around a warming mean has been found to affect mussel growth (Pansch and Hiebenthal, 2019) and increased variation may be more harmful for terrestrial ectotherms than increased mean temperature (Vasseur *et al.*, 2014). Naturally fluctuating co-occurring stressors may peak in or out of phase, which in addition to stressor magnitude and frequency of occurrence, is likely to affect cumulative responses (Gunderson, Armstrong and Stillman, 2016; Pansch and Hiebenthal, 2019). Interacting stressor effects are likely when the stressors occur simultaneously, or when a physiological response following the first stressor still persists when a second stressor is added to the system (Gunderson, Armstrong and Stillman, 2016). There is a substantial lack of research investigating how duration and frequency of stressor intensity peaks, the sequential, overlapping, or simultaneous occurrence of stressors, and the recovery intervals between stress periods affect biological responses across multiple levels of organisation (Gunderson, Armstrong and Stillman, 2016; Pansch and Hiebenthal, 2019; Jackson, Pawar and Woodward, 2021).

Additionally, depending on their size, longevity and metabolic rates, organisms operate at different timescales, i.e. the same stress event may span different amounts of affected species' life times, different life stages, or even multiple generations (Jackson, Pawar and Woodward, 2021). This is likely to affect responses at different levels of biological organisation, including adaptation to and within- or cross-generation carry-over effects of previously experienced stress (Pansch *et al.*, 2018; Jackson, Pawar and Woodward, 2021; Donelan, Ogburn and Breitburg, 2022). Conducting more long-term and/or multi-generational studies may indicate the potential and the time possibly needed for adaptation of marine organisms to environmental change (Sunday *et al.*, 2014; Riebesell and Gattuso, 2015; Jackson, Pawar and Woodward, 2021; Orr *et al.*, 2022).

Regarding experimental design, the more factors are included in an experiment, the more complex the design becomes and the more challenging the interpretation and presentation of results, which may undermine how informative such studies are (Boyd *et al.*, 2018). Collapsed (one main factor with many levels, all other stressors collapsed into a second combined factor) and reduced (single stressor treatments and a treatment of all stressors combined, omitting lower-order interactions) factorial designs have been proposed as a possible solution to reduce the amount of treatments that require replication, while maintaining high statistical power and creating informative results (Boyd *et al.*, 2018). Testing the effect of different levels of replication on the identification of stressor interaction will bring additional clarity to designing efficient experiments (Burgess, Jackson and Murrell, 2022).

To advance the conservation of marine ecosystems and inform efficient management action, it may be meaningful to conduct experiments on currently impacted ecosystems and the removal of local anthropogenic stressors in changing climate conditions instead of assessing responses to added stress (Côté, Darling and Brown, 2016). Removing local stress, e.g. eutrophication or resource extraction, from climate refuges or habitats that are known to suffer already from changing climate conditions may benefit marine biodiversity more and more long-term than first empirically identifying all possible stressor interactions (Côté, Darling and Brown, 2016; Pirotta *et al.*, 2022). Nevertheless, when populations are already adapted to stressed conditions, the removal of stressors can have varying consequences and, therefore, should be based on a mechanistic understanding of the stressor effects on the respective organisms (Orr *et al.*, 2022).

Finally, to effectively drive aquatic management, and to advance adaptation strategies and mitigation policies to face changing environmental conditions, available ecological knowledge needs to be communicated efficiently, clearly and easily accessible to stakeholders, managers and decision makers (Boyd *et al.*, 2018). When communicating existing information and when developing new research questions, the stakeholders' awareness, interests, needs and values that underlie their willingness and acceptance of implementing adequate solutions need to be considered (Boyd *et al.*, 2018; Pirotta *et al.*, 2022). Guidelines to improve and facilitate knowledge transfer at the science-policy or the science-society interface may be crucial to limit climate change and help human society to act more sustainably (Folke *et al.*, 2021).

5.3 Conclusion

Human vulnerability risks, projected adverse impacts, biodiversity losses and ecosystem damages escalate with worsening climate change, calling for prompt mitigation and adaptation actions (IPCC, 2022). Empirical research, such as mesocosm experiments, with statistically powerful sophisticated set-ups and designs can be useful to disentangle the independent and combined effects of multiple stressors, including biotic interactions and underlying mechanisms, to test hypotheses of specific management questions, and to inform, complement or validate models. Models can be useful to condense and extrapolate large amounts of data according to climate change predictions, thereby making the results of research more readily accessible and communicable. Methodological advancement, knowledge advancement and knowledge synthesis complement each other to gain a holistic understanding of the natural world and the ways it may change in response to multiple anthropogenic stressors (Boyd *et al.*, 2018).

Our planet, however, is experiencing both a climate and a biodiversity crisis right now (Pörtner *et al.*, 2021; IPCC, 2022). A substantial amount of research is predicting devastating effects of climate change to natural ecosystems, putting human livelihood that vitally depends on our natural capital at considerable risk (Cooley *et al.*, 2022; IPCC, 2022). To protect our ecosystems, habitats and species, we must mitigate climate change effects and stop the processes driving climate change. We must act before it is too late, e.g. by advancing adaptation to expected effects and making existing information readily available to managers and decision makers, is essential to minimise damage (Hayden and Mahin, 2022). Adding more detail to known tendencies may be a misleading priority given the excessive CO_2 (equivalent) emission pathway human society is still pursuing (Matthews and Wynes, 2022). There will always be open research questions to investigate and some uncertainty regarding the future (Orr *et al.*, 2021). Given the undeniable crises that already threaten the existence of humans and other living creatures, however, focussing our scientific and management efforts on synthesising existing ecological information, e.g. using BNs, and making it accessible for decision makers so they can apply it accordingly in prompt, adequate action is more urgent than ever.

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Appendix A | Supplementary Material for Chapter 2

A.1 Overview of permanently installed marine mesocosms

Table A.1 Permanently installed marine mesocosms (non-exhaustive), including their strengths, limitations and capacities.

Facility name	Location	Research focus	Strengths	Maximum # temperature levels	Maximum # acidification levels	Exp. units	Volume per unit (L)	In-/ outdoors/ in-situ	Limitations	Reference
QIMS – Quantifying the Impacts of Multiple Stressors	TCD, Dublin, Ireland	independent and combined stressor effects	high number of treatments and replication	3 (ambient, +1 °C, + 2°C)	3 (ambient - any two more)	96	32	indoors	Max. 24 treatments with 4 replicates; relatively small units; no open flow-through	
KIB – Kiel Indoor Benthocosms	Geomar, Kiel, Germany	environmental variability	highly controllable (various steady or fluctuating settings); open flow-through possible	12 (any)	5 (any)	12 (subunits possible)	600	indoors	Max. 12 treatments with 1 replicate	Pansch & Hiebenthal 2019

KOB – Kiel Outdoor Benthocosms	Geomar, Kiel, Germany	near natural climate change experiments	treatments are offsets to natural in-situ fluctuations (dynamic nominal value); open flow-through possible; wave generation possible	12 (any)	12 (ambient; any elevation)	6-12	3000	outdoors	Max. 12 treatments with 1 replicate	Wahl et al. 2015
KOSMOS – Kiel Off-Shore Mesocosms for future Ocean Simulations	Geomar, Kiel, Germany	Biogeochemistry and ecology of plankton communities	large; admits water stratification at natural light conditions	1 (ambient)	9 (ambient; any elevation)	9	50 000 – 75 000	in-situ	Max. 9 treatments with 1 replicate; susceptible to wave action	Riebesell *et al.* 2013
Planktotrons	ICBM, Wilhelmshaven, Germany	aquatic biodiversity and food webs	inhibited wall growth (increases experimental duration); water stratification	12 (any between 5°C and 35°C)	1 (ambient)	12	600	indoors	Max. 12 treatments with 1 replicate; no open flow-through	Gall *et al.* 2017

Sylt Benthic Mesocosm Facility	AWI, Sylt, Germany	climate change experiments in marine ecology	tide and current simulations; open flow-through possible	12 (any)	12 (ambient; any elevation)	12	1800	outdoors	Max. 12 treatments with 1 replicate; cannot be operated < -5 °C	Pansch *et al.* 2016
	QUB, Portaferry, UK	Wave action and temperature effects on rock pool communities	open flow-through; wave effects; capacity for many treatments and high replication	2 (ambient, +2°C)	1 (ambient)	100	43	outdoors	relatively small units	Mrowicki & O'Connor 2015
PML-IMAS – PML Intertidal Mesocosm Acidification System	PML, Plymouth, UK	biological responses to potential future conditions	semi-diurnal tidal cycle; temperature follows average monthly SST	2 (ambient, +2°C)	3 (any)	20	700	indoors	Max. 5 treatments with 4 replicates; no open flow-through	Queiros *et al.* 2015

National Mesocosm Centre	UiB, Bergen, Norway	pelagic ecosystem CO_2 enrichment; carbon cycling	large; admits water stratification at natural light conditions; haloclines possible	1 (ambient)	3 (any)	9	27 000	in-situ	Max. 3 treatments with 3 replicates; no open flow-through	Schulz *et al.* 2008
MedSeA – Mediterranean Sea Acidification under changing climate	Corsica, France	OA effects on plankton communities	large; admits water stratification at natural light conditions	1 (ambient)	6 (any elevation)	9	50 000	in-situ	Max. 6 treatments with 1 replicate; no open flow-through	Gazeau *et al.* 2017
	HCMR, Crete, Greece	OAW effects on planktonic communities in the Mediterranean Sea	relatively large volume; ultra-oligotrophic environment; potential for more, smaller units	2 (ambient, +3°C)	2 (ambient, elevated)	12	3000	outdoors	Max 4 treatments with 3 replicates; no open flow-through	Krasakopoulou *et al.* 2015

RSS – Red Sea Simulator	IUI, Eilat, Israel	long-term study of coral reef organisms responding to climate change scenarios	open flow-through; natural in-situ fluctuations; periodic rotation of tank locations during experiments; automated PAM (photosynthesis) assessments; capacity for many treatments and high replication	6 (any between 12°C and 40°C)	4 (ambient, any elevation)	80	40	outdoors	Max. 24 treatments with 3 replicates; relatively small units	Bellworthy & Fine 2018
	HIMB, Hawaii, USA	OA effects on coral reef ecology	rapid open flow-through; natural in situ fluctuations	example of 2 (ambient, +2°C)	example of 2 (ambient, -0.3 pH units)	12 (subunits possible)	500	outdoors	capacity, i.e. treatment and replication options unclear	Jokiel *et al.* 2014

	Coral Vivo, Porto Seguro, Brazil	climate change (mesocosms) and ecotoxicology (48 connected 1-L aquaria)	mesocosm treatments are offsets to natural in-situ fluctuations, metal contaminant treatments can be added in the aquaria; open flow-through	4 (ambient, any up to +6°C)	4 (ambient, any elevation)	16	130	outdoors	Max. 4 mesocosm treatments with 4 replicates	Duarte *et al.* 2015
	NMSC, Coffs Harbour, Australia	climate change effects on ecology	open flow-through	2 (ambient, +3°C)	2 (ambient, any elevation)	20; or 12	230; or 1100	outdoors	Max. 4 treatments with 3-5 replicates	Miranda *et al.* 2019; Provost *et al.* 2017
MESO – Marine Experiments for a Sustainable Outcome	SARDI, West Beach, Australia	predicting ecological consequences of climate change	open flow-through; allowing natural variability	15 (any between 0 °C and 35°C)	2 (ambient, any elevation)	15	2300	outdoors	e.g. 5 treatments with 3 replicates	Falkenberg *et al.* 2016
	SIMS, Sydney Harbour, Australia	climate change experiments in marine ecology	Open flow through	2 (ambient, +3°C)	2	36	52	outdoors	pCO_2-manipulated atmosphere not captured	Cole *et al.* 2021

A.2 Variation at each temperature level in the QIMS Mesocosm Facility in 2020

On eight occasions, temperature measurements of all three levels were taken in all mesocosms (Figure A.1, exept Nov 03, Dec 08 and Dec 09 when only two leves were measured). A mixed measures ANOVA was used to compare means between the different temperature levels (between-subject factors) and repeated measurements of the individual mesocosms (within-subject factors). Assumptions were met, i.e. outliers explored, normality confirmed via qqplots, homogeneity of variances tested using Levene's Test, and sphericity was tested using the Mauchly's test of spherisity that is included in the R function anova_test() of the package rstatix version 0.7.2 (Kassambara, 2023). Time of measurement interacted significantly with the temperature level ($F_{7,\ 333} = 22.769$; $p < 0.001$) and bonferroni-corrected pairwise post-hoc comparisons between levels at each time of measurement confirmed that levels remained distinct from each other (Table A.2).

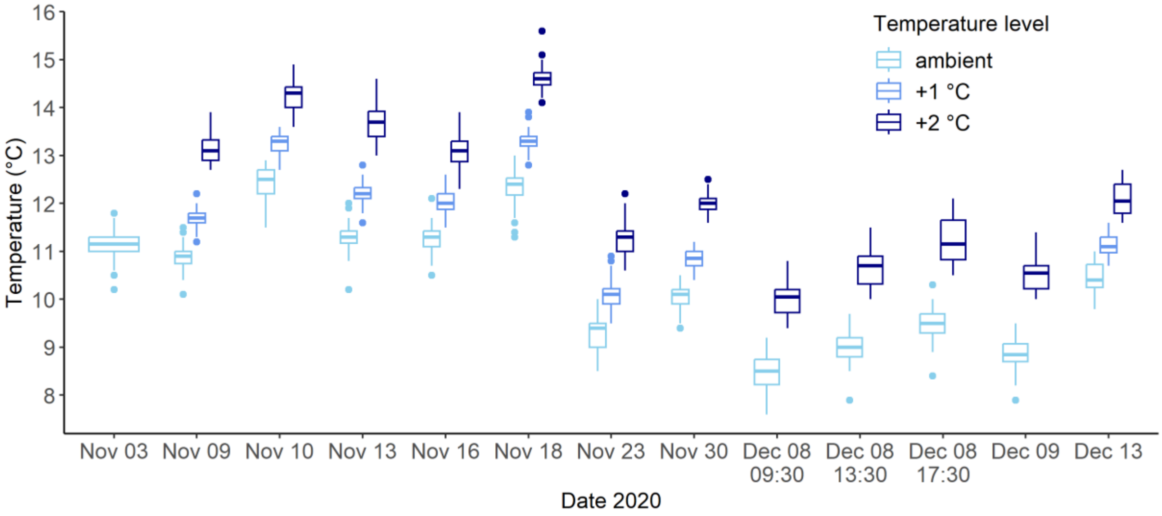


Figure A.1 Snapshot water temperature measurements in the QIMS Mesocosm Facility during acclimatisation (Nov 3; n = 96) and at the ambient (light blue; n = 32), +1 °C (blue; n = 32) and +2 °C (dark blue; n = 32) temperature levels.

Table A.2 Post-hoc pairwise t-tests between temperature levels on different days of measurement during the 2020 QIMS testing period.

Date of measurement	Level 1	Level 2	n1	n2	P adjusted
Nov 09	Ta	T+	32	32	< 0.001
Nov 09	Ta	T++	32	32	< 0.001
Nov 09	T+	T++	32	32	< 0.001
Nov 10	Ta	T+	32	32	< 0.001
Nov 10	Ta	T++	32	32	< 0.001

Nov 10	T+	T++	32	32	< 0.001
Nov 13	Ta	T+	32	32	< 0.001
Nov 13	Ta	T++	32	32	< 0.001
Nov 13	T+	T++	32	32	< 0.001
Nov 16	Ta	T+	32	32	< 0.001
Nov 16	Ta	T++	32	32	< 0.001
Nov 16	T+	T++	32	32	< 0.001
Nov 18	Ta	T+	32	32	< 0.001
Nov 18	Ta	T++	32	32	< 0.001
Nov 18	T+	T++	32	32	< 0.001
Nov 23	Ta	T+	32	32	< 0.001
Nov 23	Ta	T++	32	32	< 0.001
Nov 23	T+	T++	32	32	< 0.001
Nov 30	Ta	T+	32	32	< 0.001
Nov 30	Ta	T++	32	32	< 0.001
Nov 30	T+	T++	32	32	< 0.001
Dec 13	Ta	T+	32	32	< 0.001
Dec 13	Ta	T++	32	32	< 0.001
Dec 13	T+	T++	32	32	< 0.001

A.3 Distinct pH levels at ambient and 645 ppm pCO_2

Table A.3 Post-hoc pairwise t-tests between pCO_2 levels on different days of measurement during the 2020 QIMS testing period.

Date of measurement	Level 1	Level 2	n1	n2	P adjusted
Nov 09	Ca	C+	3	3	< 0.001
Nov 15	Ca	C+	3	3	< 0.001
Nov 18	Ca	C+	3	3	< 0.001
Nov 22	Ca	C+	3	3	< 0.001
Dec 03	Ca	C+	3	3	< 0.001
Dec 08 9:30	Ca	C+	2	2	< 0.01
Dec 08 13:30	Ca	C+	2	2	< 0.01
Dec 08 17:30	Ca	C+	2	2	< 0.05
Dec 09	Ca	C+	2	2	< 0.01
Dec 13	Ca	C+	3	3	< 0.001

Appendix B | Supplementary Material for Chapter 3

B.1 Shell length determination and size-independent mortality

At the start and the end of the experiment, the mussels from each mesocosm were photographed on graph paper for subsequent shell length determination. Initial and final individual mussel shell lengths were determined from a random half of the mesocosms (*n* = 30) by measuring the maximum anterior-posterior axes (Seed, 1968) using image analysis (ImageJ 1.53q (Rasband, 1997). Mussel lengths were comparable within photos but not across photos because the camera was readjusted several times when photos were taken. As a consequence, no absolute length growth rates could be determined. However, Kolmogorov Smirnov tests confirmed that in 29 of the 30 examined mesocosms the lengths distributions remained the same throughout the experiment, i.e. mussel mortality was independent of size (Table B.2). Accordingly, mussel individuals of a mesocosm were treated as similar on average and clearance rate samples that were taken before mussel retrieval at the end of the experiment were standardised by the number of alive mussels per mesocosm.

Shell lengths of mussels used in shell strength tests were determined separately with the same methodology and without camera readjustments to ensure comparability of the lengths.

B.2 Supplemental Tables

Table B.1 TukeyHSD test on the effect of temperature (3 levels: Ta – ambient, T+ – ambient +1 °C, T++ – ambient + 2°C) on the maximum quantum yield F_v/F_m.

	Diff	Lower 95% CI	Upper 95% CI	*P (adj)*
T++-T+	-0.062	-0.128	0.005	0.074
Ta-T+	-0.060	-0.126	0.007	0.085
Ta-T++	0.002	-0.065	0.068	0.998

Table B.2 Two-sample Kolmogorov-Smirnov tests comparing the initial and final mussel length distributions in 30 mesocosms

Mesocosm ID	D	*P*
B1	0.200	0.723
B6	0.217	0.559
B9	0.179	0.812
C1	0.400	0.048
C4	0.110	0.998
C5	0.140	0.952
E2	0.133	0.962
E6	0.125	0.970
E9	0.173	0.807
F3	0.153	0.906
F4	0.242	0.358
F6	0.110	0.998
G1	0.122	0.984
G8	0.162	0.964
G9	0.230	0.571
H1	0.163	0.736
H3	0.259	0.249
H7	0.138	0.989
H8	0.186	0.761
I1	0.130	0.957
I4	0.212	0.585
I5	0.236	0.600
J2	0.167	0.893
J4	0.264	0.453
J5	0.125	0.980
J7	0.137	0.973
J9	0.176	0.862
K3	0.250	0.441
K5	0.208	0.436
K9	0.208	0.637

Appendix C | Supplementary Material for Chapter 4

C.1 Data provided for, analysed and included in the Bayesian Network on environmental variables and biodiversity of Dublin Bay

Table C.1 Data provided by different monitoring programmes and institutions that was analysed and included (highlighted in bold) in the Bayesian Network on environmental variables and biodiversity of Dublin Bay.

Data, provider and monitoring programme	Variables analysed for and included in (bold) BN	Time period and temporal resolution	Sampling stations
Physico-chemical monitoring data gathered by the Environmental Protection Agency Ireland (EPA) as part of the national Water Framework Directive monitoring programme for Transitional and Coastal Waters. The Environment Protection Agency maintains the database right.	**Salinity; Temperature [°C]; pH; DO_Saturation [% sat]; chl_a [mg/m^3]; DIN:PO_4; PO_4:SiO_2** (; Secchi [m]; BOD [mg/L O_2]; TON [mg/L N]; NH_3 [mg/L N]; PO_4 [µg/L P]; SiO_2 [µg/L]; Station number; Survey date [dd/mm/yyyy]; Water depth [m]; Sample depth [m])	14 years (26/2/2007 - 10/09/2020); 3-4 seasonal samplings (1 winter, 3 summer) per sampling station per year, either at low or high tide. When tidal schedule allowed, second sample (return) was taken on the same day at the other tidal extreme.	12: 4 each in the Liffey Estuary Lower, the Tolka Estuary, and Dublin Bay
Irish Lights Dublin MetOcean Buoy; Commissioners of Irish Lights, licensed under a Creative Commons Attribution 4.0 International Licence (http://creativecommons.org/licenses/by/4.0/).	**WaveHeight [m]; WavePeriod [s]** (Licensed under the Creative Commons Attribution 4.0 International Licence (http://creativecommons.org/licenses/by/4.0/))	5 years (28/1/2015 - 28/1/2020); 3-6-minute intervals	1: central mouth of Dublin Bay
Phytoplankton monitoring data gathered by the Environmental Protection Agency as part of the national Water Framework Directive monitoring programme for Transitional and Coastal Waters. The Environment Protection Agency maintains the database right.	**235 taxa [cells/L]**	13 years (2007-2019); at least one sample per winter and per summer, occasionally additional samples in late summer	2: EPA stations as in physico-chemical parameters are combined to "PhytoComp" stations. Relevant for BN are Liffey Estuary Lower (PhytoComp2) and central bay (PhytoComp3)

Benthic Invertebrate monitoring and Infaunal Quality Index (IQI) assessment by the Marine Institute. The Marine Institute Ireland maintains the database right and granted a revocable, non-exclusive, non-transferable license to the authors to use the data for the permitted purpose of informing the BN.	**Loss of ignition (LOI) [%]**; Species counts per replicate grab; Total abundance; **Species richness**; Simpson's Diversity Index; Functional AMBI index (AZTI's Marine Biotic Index [Borja *et al.*, 2000]); percentage distribution of Ecological Groups; IQI	6 years (2014-2020 ex. 2018); annually in January	5 -8 subtidal sites; summarised to five areas for the presented analysis
River Flow data by HydroNet, Environmental Protection Agency Ireland, licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0)	Date, **Daily Mean Flow (m^3/s)**	14 years (2007-2020); daily	2: Dodder (Waldrons Bridge), Tolka (Botanical Gardens)
Bar-tailed godwit counts recorded by the Dublin Bay Birds Project, managed by BirdWatch Ireland, and funded by Dublin Port Company	**Low tide counts**	7 years (2013-2020); monthly counts per subsite	3: Multiple subsites in Dublin Bay, summarised to North, Mid, South for the presented analysis
Priority substances in shellfish monitored by the Marine Institute. The Marine Institute Ireland maintains the database right and granted a revocable, non-exclusive, non-transferable license to the authors to use the data for the permitted purpose of informing the BN.	(Heavy) metals and (hazardous) priority substances [mg/kg]	19 years (2000-2019) in Sutton, nine years in Liffey Estuary and South Dublin Bay; annually 1-3 samples collected between September and December	3: Sutton, Liffey Estuary Lower, Dublin Bay Station 2 (Blackrock)
Priority substances in water monitored by the Marine Institute. The Marine Institute Ireland maintains the database right and granted a revocable, non-exclusive, non-transferable license to the authors to use the data for the permitted purpose of informing the BN.	(Heavy) metals and (hazardous) priority substances [µg/L]	2 years (2014; 2020); monthly samples	2: Liffey Estuary Lower, Dublin Bay Station 2

Priority substances in sediment monitored by the Marine Institute. The Marine Institute Ireland maintains the database right and granted a revocable, non-exclusive, non-transferable license to the authors to use the data for the permitted purpose of informing the BN.	(Heavy) metals and (hazardous) priority substances [µg/L]	3 years (2015-2017); annual samples	2: Liffey Estuary Lower (each year), Dublin Bay Station 2 (only 2015)
Opportunistic macroalgae monitoring data gathered by the Environmental Protection Agency as part of the national Water Framework Directive monitoring programme for Transitional and Coastal Waters. The Environment Protection Agency maintains the database right.	cover [%] biomass [g/m^2] Presence (1) or absence (0) of entrained algae	10 (2007-2010, 2012-2017); annual samples	1: Bull Island South Lagoon; 2-4 patches per year

C.2 Excluded variables and data gaps

The structure of the BN presented in this paper consists only of a selection of the variables that were initially considered for inclusion. In this section we outline which variables were excluded after investigation from the model even if monitoring data were available, and for which environmental and biotic variables we identified major data gaps that limit our understanding of the system.

Contrary to the rivers Dodder and Tolka, there was no access to flow data from Dublin’s main river Liffey, which is regulated by reservoirs upstream, hence freshwater discharge from the Liffey is not included in the BN.

The Water Framework Directive requires the monitoring and reduction of a range of toxic chemical pollutants that are classified as priority substances, of which some are additionally ranked as ‘hazardous’, i.e. persistent and likely to accumulate in organic tissue (European Commission, 2000 and amendements since). Hazardous and priority substances remain in the water column only for short time periods, but they may accummulate in the sediment and in marine organisms such as shellfish. Hence, adverse effects of pollutants in biota may only be seen after some lag period during which the concentration of certain pollutants accumulates. Even though the concentrations of a large range of substances are determined in regular monitoring, thresholds above which adverse effects may occur have only been identified to a limited degree, are often still under investigation, and inconsistent in their units (OSPAR Coordinated Environmental Monitoring Programmes (CEMP) or European Environmental Objectives for Surface Waters). We assembled current thresholds for (hazardous) priority substances in water, sediment and biota from national and international legislation and conventions and aggregated available monitoring data according to whether no detected priority substance exceeded a threshold of concern, < 10 %, or ≥ 10% of detected priority substances exceeded a threshold of concern.

Priority substances (including heavy metals and organic pollutants) intertidal shellfish tissue (*Mytilus edulis*) have been sampled in Dublin Bay since 2000. Hazardous substances in water, however, were sampled monthly in 2014 and 2020, while those in sediment were only sampled annually in 2015-2017. Replication of overlapping data was too little for meaningful analysis. Similarly, no statistically significant relationship was found between priority substances detected in intertidal shellfish tissue and the few available concurrent subtidal invertebrate metrics.

Even though some data on biochemical oxygen demand were available, they were excluded from the model for multiple reasons: First, the resolution was less than for the other physico-chemical monitoring data and when merging the data with the invertebrate data, only five data points remained; second, most values were close to the limit of quantification, which also changed from 1 mg/l O_2 to 0.5 mg/l O_2 in 2013; third, only three of 582 available data points were not in "High" WFD water quality status; fourth, the data that could be matched in location and time with other variables did not show any significant links.

Water transparency or light availability was excluded from the model for two reasons: The available Secchi measurements were biased towards bad visibility and thus not representative because at 12 % of the observations Secchi depth was limited by the visible sea floor due to tidal differences in water depth. Data on turbidity or suspended sediment does not exist, however, it would be a valuable addition to the BN model. Nevertheless, calculating the ratio of mixing depth to photic depth (O'Boyle *et al.*, 2015) showed that phytoplankton growth in Dublin Bay was never light limited during the period 2007-2020.

No direct links were found between ambient nutrient concentrations and chlorophyll a. This is reasonable considering that ambient nutrient conditions are the sum of what remained after previous nutrient concentrations were transformed into biomass during photosynthesis and of what was washed into or out of the bay. The very strong correlations between ambient nutrient concentrations and phytoplankton biomass that can be found in closed systems such as lakes (Phillips *et al.*, 2008) are very unlikely to occur in an open system such as Dublin Bay, in which the water body is mostly replaced each tidal cycle, or after 3.4 days at the latest. Additionally, Dublin Bay receives constantly fluctuating inputs from rivers and sewage plant discharge, and is in constant open exchange with the Irish Sea, with the latter accounting for more than twice the load of diffuse, dissolved nutrient inputs compared to terrestrial, diffuse loadings (Wilson, 2005). O'Boyle *et al.* 2015 explicitly examined the factors that affect phytoplankton growth in Irish estuaries and coastal waters and concluded that in the cases of the Tolka Estuary and the Liffey Estuary Lower very short water retention times primarily control chlorophyll concentrations and prevent the accummulation of phytoplankton biomass despite high nutrient loads. Vice versa, primary production does not alter nutrient concentrations noticeably, which indicates that the supply of nutrients is not limiting, i.e. exceeds the demand (O'Higgins and Wilson, 2005). For the far eastern parts of Dublin Bay that are subjected to strong exchange with the Irish Sea, the availability of nitrogen and silica was identified as the main limiting factor (O'Boyle *et al.*, 2015).

Nevertheless, while the hydrodynamics in Dublin Bay constrain phytoplankton growth, the nutrient enriched transitional waterbodies might benefit benthic macroalgae (Wilson, Rybarczyck and Elkaim, 2007; O'Boyle *et al.*, 2015). Intertidal opportunistic green algae macroalgae growth is monitored annually by the EPA in the Bull Island South Lagoon and parts of the Tolka Basin, but no accompanying physico-chemical parameters are recorded that could explain the growth. Probably due to the lack of temporal and spatial overlap of the macroalgae data and the physico-chemical water monitoring, we did not find significant links between the variables and, hence, excluded opportunistic green macroalgae from the BN.

Regular, substantial beach fouling caused by detached, filamentous fronds of the subtidal brown algae *Ectocarpus sp.*, especially in autumn, has been observed in Dublin Bay (Jeffrey, Madden and Rafferty, 1993; pers. observation from the project team; various news paper articles). However, no monitoring of such events and the conditions facilitating them exists. Both the intertidal opportunistic green macroalgae and the subtidal *Ectocarpus sp*. Growth are believed to rely on remineralisation of nutrients from particulate matter through sediment infauna and periods of warm temperatures and low wave energy (Jeffrey, Madden and Rafferty, 1993; Jeffrey *et al.*, 1995; Jennings and Jeffrey, 2005; Wilson, Rybarczyck and Elkaim, 2007). Even though particulate inputs have often anecdotally been attributed to riverine and wastewater treatment discharge, Wilson *et al.* 2002 found these inputs to be negligible compared to tidal particulate inputs. Again, no regular monitoring of these variables exists, despite their importance to processes in the bay.

Similarly, rocky shore macrophytes and angiosperms (e.g. the very vulnerable intertidal seagrass beds) are not monitored in Dublin Bay, despite the requirements stated by the WFD. Drafts of monitoring protocols exist and are being developed further (Cusack *et al.*, 2008), but regular sampling has not been implemented yet. No intertidal monitoring has been put in place, except for opportunistic macroalgae surveys in the Bull Island lagoon and waterbird surveys, no estuary and coastal fish monitoring has been established, and marine mammals are only recorded opportunistically.

C.3 Methods for combining data from different sources of different spatial or temporal resolution

Subtidal benthic soft-bottom invertebrates were sampled annually in January, but no physico-chemical water monitoring data existed for this time. Hence, physico-chemical variables from the previous primary production growing period were linked to invertebrate data sampled the following winter, assuming that the sampled winter communities would still reflect growth and reproduction conditions from the previous summer. The physico-chemical monitoring data were summarised per year and sampling station, retaining the maximum states of temperature, dissolved oxygen (DO) and chlorophyll a concentration. The maximum, i.e. highest, pH states were retained, too, because then medium and high pH states were maintained among the summarised observations, as opposed to only the medium pH state and one observation with low pH state if we had chosen to retain the lowest occurring pH state. To preserve the influences of freshwater, the minimum states of salinity were retained. Invertebrate data were linked to the five furthest East located physico-chemical monitoring sampling stations (Figure 4.1) and were averaged when data of multiple invertebrate sampling stations were assigned to the same physico-chemical sampling station.

If parent node data was available at a higher temporal or spatial resolution than child node data, then the links between the variables were informed by reducing the parent node data to the highest compatible resolution (keeping as many aligned sampling stations as possible and the smallest joint time unit). In the cases where more parent node states existed than could be matched with the data of a certain child node (but were relevant in links to other child nodes), the corresponding child node state probability distributions were filled with equal probabilities to reflect the high uncertainty. If much more child node data was available than parent node data (e.g. five years of wave buoy data vs. 14 years of dissolved oxygen monitoring), a parent node state ‘no_data’ was established. This allowed us to include both the true probability distribution of the child node data for which no parent node data was available, as well as the probability distribution according to parent node state combinations that were actually informed by data.

C.4 Supplemental Figure

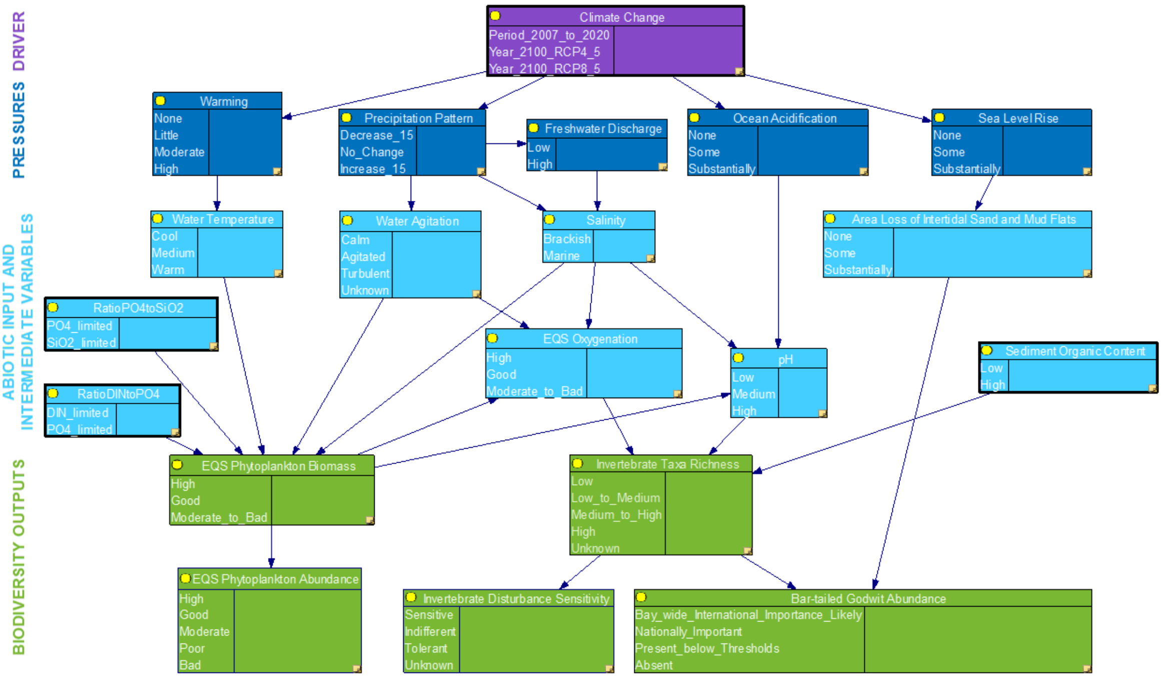


Figure C.1 Bayesian Network of environmental variables and biodiversity of Dublin Bay showing all included nodes, their respective states, and the conditional links between them. Drivers (purple), pressures (dark blue), abiotic variables (light blue) and biodiversity outputs (green) are listed from top to bottom. The four input nodes (top, left and right) are highlighted by bold node outlines.

C.5 Details on how conditional inference tree analyses were applied

When multiple break points of a parent node were identified in the same ctree model, the intervals between them and the size of the resulting data blocks of the child node were considered before adopting the final break point(s) so as to keep the number of states per node as low as possible and the final data blocks as large as possible. If threshold values were very close (e.g. the ratio of DIN:PO_4 was split at 10.39 and 10.66 when modelling its effect on chlorophyll a as a proxy for phytoplankton biomass) we ran additional ctrees to confirm if applying the mean value as break point (e.g. 10.525 in the case of the DIN:PO_4 ratio) maintained the response variable data partitioning. If the data of the same parent node were split at different thresholds when modelling different child nodes as response variables, we modelled additional ctrees in which we applied the single or combined thresholds, or their mean, to determine the best break points for both relationships. The 'best break point(s)' maintained the partitioning of the response variable data as closely as possible to the previously determined single threshold values and resulted in the overall lowest p-values of the compared ctrees. Priority was given to break points that were based on a larger data set, given that different amounts of data were available for different variables.

C.6 Details on linking nodes, i.e. modelling relationships between variables

To manage the increasing complexity of the BN, only ctree models that contained all possible parent nodes of a child node were considered, no one-factorial relationships if a child node had multiple parent nodes. Potentially influencing variables (parent nodes) that were first included in the full model but did not significantly affect the response variable (child node) in combination with the other parent nodes, were consecutively removed from the analysis (e.g., no significant effect of temperature on dissolved oxygen was identified through ctree analysis, hence the potential link between temperature and dissolved oxygen, which had been included initially based on ecological knowledge of the project team, was removed from the BN). Removing redundant variables from the ctree models increased the significant influences of the remaining relevant predictor variables (p-values decreased). One exception was made in the case of invertebrate taxa richness, where influences of pH, DO and sediment organic content were only significant in one-factorial models, instead of in combination. When exploring the relationships between temperature, chlorophyll a concentration, DO, salinity, and sediment organic content with a variety of metrics that characterise the invertebrate samples (the infaunal quality index IQI, taxa richness, total abundance, Simpson's Diversity Index, or the percentage of species that was allocated to a certain functional ecological group according to AMBI (AZTI's Marine Biotic Index [Borja *et al.*, 2000])), only taxa richness was significantly influenced by pH, DO, and sediment organic content. These relationships, however, were only significant in one-factorial models, not in combination. Temperature and chlorophyll a concentration did not affect any of the invertebrate metrics. Hence, we parameterised the conditional probability table of taxa richness by manually calculating the probabilities of occurrence of invertebrate taxa richness states according to the fully crossed conditions of the parent node states of pH, DO and sediment organic content.

To estimate the effect of invertebrate taxa richness on bar-tailed godwit abundance, monthly bar-tailed godwit counts were summed per year. Once significantly influencing thresholds in the invertebrate taxa richness had been determined, parameterising the BN depended on the proportions at which monthly wader counts of the respective years exceeded the threshold of international importance, met or remained under the thresholds of national importance, or were absent. The replication of counts aggregated over the entire bay were too few to find significant links with the invertebrate data, so counts from three subsites of Dublin Bay (North, Mid, South) were assessed separately. The threshold for abundance of international importance, however, was only met at whole bay level. From comparing subsite counts in months during which bar-tailed godwits were present at internationally important numbers we concluded that if > 880 birds were counted in one of the subsites during one month, the sum of all subsite counts would very likely (73.5 %) exceed the threshold of international importance.

C.7 Expert survey

“Section 1: Environmental impacts on biodiversity in Dublin Bay

Hello and welcome to this survey on how changes in environmental variables are linked to biodiversity in the marine realms of Dublin Bay.

This survey is part of the marine Dublin Bay case study of Land2Sea, an international EU/EPA-funded project that investigates the impacts of human and global change associated pressures on freshwater and marine biodiversity and ecosystem processes that are critical for nature's contributions to people.

BACKGROUND:
The aim of this survey is to gauge how well our quantitative Bayesian Network (BN) aligns with expert expectations when considering links between pressures, environmental variables and biodiversity in the marine parts of Dublin Bay. The model was built by analysing environmental monitoring data from 2007-2020 and extrapolating results according to local climate change predictions. Continuous variables, e.g. temperature, had to be discretised into ranges – called 'states' – to be incorporated in the BN. Those variable states were defined according to existing classifications of the Water Framework Directive (WFD), or through conditional inference tree data analysis. Please note that only monitoring data of the productive period from March to September has been considered for the abiotic variables.

INSTRUCTIONS:
All questions in this survey target your experience from recent years, NOT what you would expect under climate change at the end of the century. Bayesian statistics work with probabilities of occurrence that always sum up to 100 %. So when the question is which changes you expect in the variable states, please consider a redistribution of 100 % probability. This means if you expect one of the states to be 'less likely', another state needs to become 'more likely' to balance the change. Similarly, 'a lot more likely' should be balanced by one other state that becomes 'a lot less likely' or two other states that each become 'less likely'. To make this more intuitive, we added the values -2 to +2 to the answer options - the sum of all ticks in an answer must balance out to 0!

Please contact Katrin Schertenleib (schertek@tcd.ie) if you have any questions.

The estimated time for completion of this survey is 20 minutes. It consists of 4 sections and a total of 12 scenarios of change.

Please make sure to click 'submit' at the end of the survey or your answers will not be saved.

Thank you very much! We appreciate your participation in this survey and thank you very much for offering your time and expert knowledge!

Please enter a valid email address ______________________

Section 2: Influences on Phytoplankton (1/4)

What change do you expect in the following scenarios of nutrient limitations and weather conditions, based on your experience of recent years?

Please note that the response variable is the Ecological Quality Status (EQS) for Phytoplankton Biomass according to the WFD. It is based on the Chlorophyll a concentration (the ranges are given in the answer options) and basically the EQS is lower the more Chl a is present.

In each scenario, please think of a shift in the distribution of probability of occurrence of the states, i.e. if one state becomes more likely, another must become less likely - the sum of all options ticked must be 0!

Scenario 1: When Dublin Bay changes from PO4-limited to SiO2-limited, which change in the EQS of Phytoplankton Biomass would you expect to see in the system? (The sum of all options ticked must be 0!) Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
High Status (Chl a <5 µg/L)	☐	☐	☐	☐	☐
Good Status (Chl a <10 µg/L)	☐	☐	☐	☐	☐
Moderate to Bad Status (Chl a >10 µg/L)	☐	☐	☐	☐	☐

Comments regarding Scenario 1: ________________

How do you rate your expertise regarding Scenario 1?

	1	2	3	4	5	
Poor	☐	☐	☐	☐	☐	High

Scenario 2: When Dublin Bay changes from DIN-limited to PO4-limited, which change in the EQS of Phytoplankton Biomass would you expect to see in the system? (The sum of all options ticked must be 0!) Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
High Status (Chl a <5 µg/L)	☐	☐	☐	☐	☐
Good Status (Chl a <10 µg/L)	☐	☐	☐	☐	☐
Moderate to Bad Status (Chl a >10 µg/L)	☐	☐	☐	☐	☐

Comments regarding Scenario 2: ________________

How do you rate your expertise regarding Scenario 2?

	1	2	3	4	5	
Poor	☐	☐	☐	☐	☐	High

Which ratio (PO4:SiO2 or DIN:PO4) do you expect to affect the EQS of Phytoplankton Biomass more?

PO_4:SiO_2 ☐

DIN:PO_4 ☐

Scenario 3a: When water temperature increases from 'Cool' (≤ 11 °C) to 'Medium' (> 11, ≤ 17 °C) during March-September, which change in the EQS of Phytoplankton Biomass would you expect to see in the system? (Please think of a shift in the distribution of probability of occurrence of the states here, i.e. if one state becomes more likely, another must become less likely - the sum of all options ticked must be 0!) Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
High Status (Chl a <5 µg/L)	☐	☐	☐	☐	☐
Good Status (Chl a <10 µg/L)	☐	☐	☐	☐	☐
Moderate to Bad Status (Chl a >10 µg/L)	☐	☐	☐	☐	☐

Scenario 3b: When water temperature increases from 'Medium' (> 11, ≤ 17 °C) to 'Warm' (> 17 °C) during March-September, which change in the EQS of Phytoplankton Biomass would you expect to see in the system? (The sum of all options ticked must be 0!) Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
High Status (Chl a <5 µg/L)	☐	☐	☐	☐	☐
Good Status (Chl a <10 µg/L)	☐	☐	☐	☐	☐
Moderate to Bad Status (Chl a >10 µg/L)	☐	☐	☐	☐	☐

Comments regarding Scenario 3: ________________

How do you rate your expertise regarding Scenario 3?

	1	2	3	4	5	
Poor	☐	☐	☐	☐	☐	High

Scenario 4: 'Water Agitation' (the ratio of wave height to wave length) is a proxy for weather conditions (temperature, cloud cover, light intensity) with a calm seawater surface during good weather periods and an agitated (choppy) seawater surface during poor(er) weather conditions. When the seawater surface was calm and changed to become choppy, which change in the EQS of Phytoplankton Biomass would you expect to see in the system? (Please think of a shift in the distribution of probability of occurrence of the states here, i.e. if one state becomes more likely, another must become less likely - the sum of all options ticked must be 0!) Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
High Status (Chl a <5 µg/L)	☐	☐	☐	☐	☐
Good Status (Chl a <10 µg/L)	☐	☐	☐	☐	☐
Moderate to Bad Status (Chl a >10 µg/L)	☐	☐	☐	☐	☐

Comments regarding Scenario 4: ________________

How do you rate your expertise regarding Scenario 4?

	1	2	3	4	5	
Poor	☐	☐	☐	☐	☐	High

General comments regarding Section 2 ‘Influences on Phytoplankton’: ________________

Section 3: Influences of Phytoplankton

In coastal ecosystems, primary producers such as phytoplankton modify pH and dissolved oxygen. What changes in pH and oxygenation do you expect when the EQS of Phytoplankton Biomass changes, based on your experience of recent years? (The EQS of Phytoplankton Biomass is lower the more Chl a is present.)

In each scenario, please think of a shift in the distribution of probability of occurrence of the states, i.e. if one state becomes more likely, another must become less likely - the sum of all options ticked must be 0!

Scenario 5a: When the EQS of Phytoplankton Biomass changes from 'High' (Chl a <5 µg/L) to 'Good' (Chl a <10 µg/L), which change in the states of pH would you expect to see in the system? (The sum of all options ticked must be 0!) Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
Low (< 7.95)	☐	☐	☐	☐	☐
Medium (≥ 7.95; < 8.15)	☐	☐	☐	☐	☐
High (≥ 8.15)	☐	☐	☐	☐	☐

Scenario 5b: When the EQS of Phytoplankton Biomass changes from 'Good' (Chl a <10 µg/L) to 'Moderate to Bad' (Chl a >10 µg/L), which change in the states of pH would you expect to see in the system? (The sum of all options ticked must be 0!) Mark only one box per row.

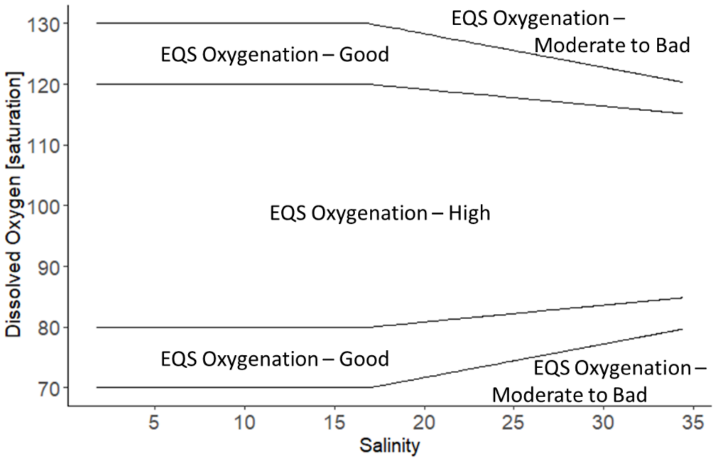
	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
Low (< 7.95)	☐	☐	☐	☐	☐
Medium (≥ 7.95; < 8.15)	☐	☐	☐	☐	☐
High (≥ 8.15)	☐	☐	☐	☐	☐

Comments regarding Scenario 5: ________________

How do you rate your expertise regarding Scenario 5?

	1	2	3	4	5	
Poor	☐	☐	☐	☐	☐	High

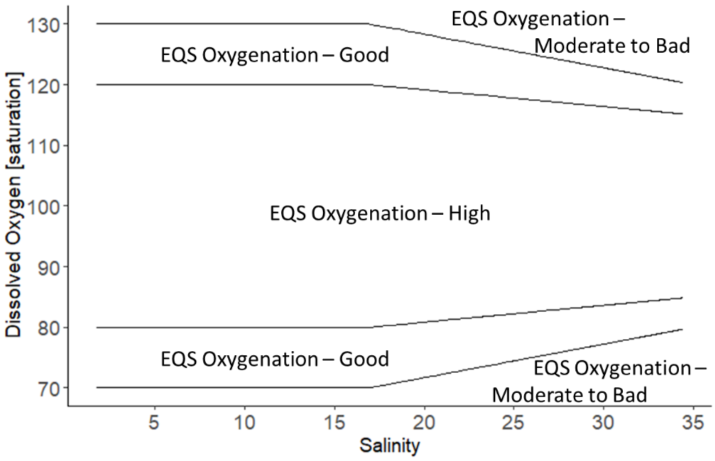
Scenario 6a: When the EQS of Phytoplankton Biomass changes from 'High' (Chl a <5 µg/L) to 'Good' (Chl a <10 µg/L), which change in the EQS of Oxygenation would you expect to see in the system? The EQS of Oxygenation is salinity-dependent as shown in the graph below. (Please think of a shift in the distribution of probability of occurrence of the states here, i.e. if one state becomes more likely, another must become less likely - the sum of all options ticked must be 0!)



Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
High Oxygenation Status	☐	☐	☐	☐	☐
Good Oxygenation Status	☐	☐	☐	☐	☐
Moderate to Bad Oxygenation Status	☐	☐	☐	☐	☐

Scenario 6b: When the EQS of Phytoplankton Biomass changes from 'Good' (Chl a <10 µg/L) to 'Moderate to Bad' (Chl a >10 µg/L), which change in the EQS of Oxygenation would you expect to see in the system? The EQS of Oxygenation is salinity-dependent as shown in the graph below. (The sum of all options ticked must be 0!)



Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
High Oxygenation Status	☐	☐	☐	☐	☐
Good Oxygenation Status	☐	☐	☐	☐	☐
Moderate to Bad Oxygenation Status	☐	☐	☐	☐	☐

Comments regarding Scenario 6: ________________

How do you rate your expertise regarding Scenario 6?

	1	2	3	4	5	
Poor	☐	☐	☐	☐	☐	High

General comments regarding Section 3 'Influences of Phytoplankton': ________________

Section 4: Influences on Invertebrate Taxa Richness

What change in subtidal benthic Invertebrate Taxa Richness (not abundance!) do you expect in the following scenarios of changing pH or Oxygenation, based on your experience of recent years? Please note that low taxa richness was found to highly correlate with species assemblages that – according to AMBI ecological functional group classification – contain mostly species that are sensitive to organic enrichment, while higher taxa richness correlated strongly with assemblages that contained mostly species that are classified as indifferent or tolerant towards organic enrichment.

Please think of a shift in the distribution of probability of occurrence of the states here, i.e. if one state becomes more likely, another must become less likely - the sum of all options ticked must be 0!

Scenario 7: When the status of pH changes from 'High' (≥ 8.15) to 'Medium' (≥ 7.95; < 8.15), which change in the states of subtidal benthic Invertebrate Taxa Richness would you expect to see in the system? (The sum of all options ticked must be 0!) Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
Low (i.e ≤ 17 taxa)	☐	☐	☐	☐	☐
Low to Medium (i.e. > 17; ≤ 30 taxa)	☐	☐	☐	☐	☐
Medium to High (i.e. > 30; ≤ 37 taxa)	☐	☐	☐	☐	☐
High (i.e. > 37 taxa)	☐	☐	☐	☐	☐

Comments regarding Scenario 7: ________________

How do you rate your expertise regarding Scenario 7?

	1	2	3	4	5	
Poor	☐	☐	☐	☐	☐	High

Scenario 8: When the EQS of water Oxygenation changes from 'High' to 'Good', which change in the states of subtidal benthic Invertebrate Taxa Richness would you expect to see in the system? (The sum of all options ticked must be 0!) Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
Low (i.e ≤ 17 taxa)	☐	☐	☐	☐	☐
Low to Medium (i.e. > 17; ≤ 30 taxa)	☐	☐	☐	☐	☐
Medium to High (i.e. > 30; ≤ 37 taxa)	☐	☐	☐	☐	☐
High (i.e. > 37 taxa)	☐	☐	☐	☐	☐

Comments regarding Scenario 8: ________________

How do you rate your expertise regarding Scenario 8?

	1	2	3	4	5	
Poor	☐	☐	☐	☐	☐	High

General comments regarding Section 4 'Influences on Invertebrate Taxa Richness': ________________

Section 5: Influences on Wader Abundance

What change in Wader Abundance as represented by bar-tailed godwits do you expect when Invertebrate Taxa Richness changes, based on your experience of recent years?

Please think of a shift in the distribution of probability of occurrence of the states here, i.e. if one state becomes more likely, another must become less likely - the sum of all options ticked must be 0!

Scenario 9: When Invertebrate Taxa Richness increases from 'Low' to 'High', which change in the monthly abundance of bar-tailed godwits would you expect to see in Dublin Bay? (The sum of all options ticked must be 0!)

Note: 450 birds is the bay-wide threshold for national importance in our model - 150 birds at a site is the actual threshold for nationally important counts of this species and has been considered at sub-site-level (North, Mid and South Dublin Bay) in our analysis. The threshold for international importance of 1500 birds has only been reached at bay level during the 7 years of available data.

Mark only one box per row.

	A lot less likely (-2)	Less likely (-1)	No change (0)	More likely (+1)	A lot more likely (+2)
International importance	☐	☐	☐	☐	☐
National importance	☐	☐	☐	☐	☐
Presence below thresholds	☐	☐	☐	☐	☐
Absence	☐	☐	☐	☐	☐

Comments regarding Scenario 9: ________________

How do you rate your expertise regarding Scenario 9?

	1	2	3	4	5	
Poor	☐	☐	☐	☐	☐	High

General comments regarding Section 5 'Influences on Wader Abundance': ________________

Section 6: Almost done!

Are you interested in participating in a follow-up online workshop on the results of the model?

The aims of the workshop will be to demonstrate the model, discuss its results including the estimated effects of climate change projections, and to answer questions that might have come up during this survey. It will take place in the coming months and we will keep it as short as possible (1-3h) to not take up too much of your time!

Yes ☐

No ☐

Any final remarks? ________________

Thank you very much! Please click on "submit" to save your responses.”