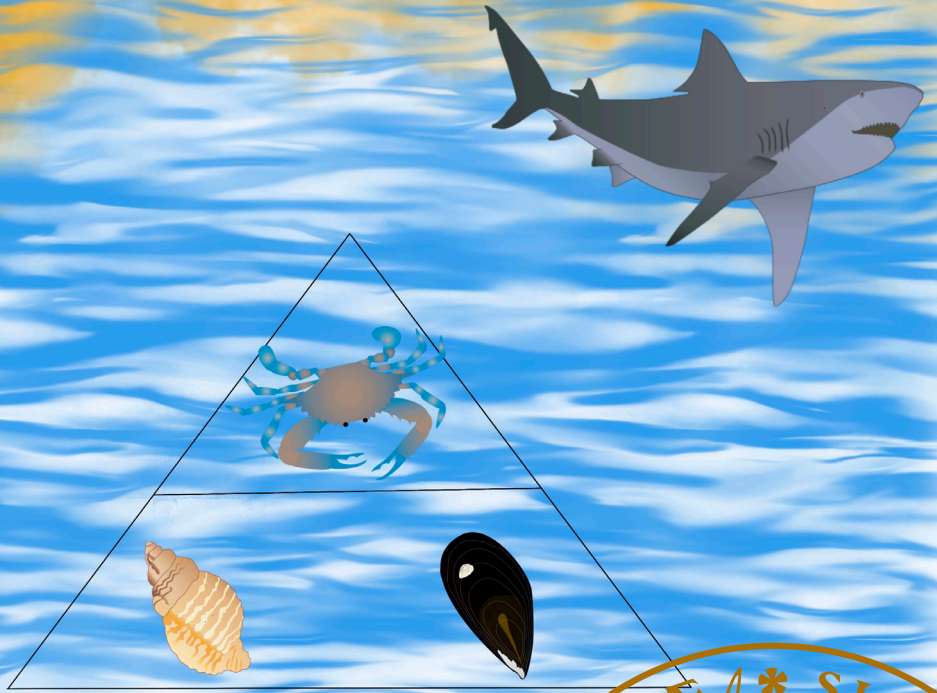


# Climate change effects on marine species across trophic levels

NAN HU

DEPARTMENT OF BIOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY





Climate change effects on marine species across trophic levels



# Climate change effects on marine species across trophic levels

Nan Hu



**LUND**  
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Science, Lund University, Sweden.  
To be defended in the Blue Hall, Ecology Building, Sölvegatan 37, Lund, Sweden  
on Friday 28<sup>th</sup> of October 2022 at 9:00.

*Faculty opponent*  
Prof. Henrik Pavia,

Department of Marine Sciences, University of Gothenburg

<b>Organization</b> LUND UNIVERSITY Department of Biology, Aquatic Ecology	<b>Document name</b> Doctoral dissertation	
	<b>Date of issue</b> 28 <sup>th</sup> October 2022	
	<b>Sponsoring organization</b> China Scholarship Council	
<b>Title and subtitle</b> Climate change effects on marine species across trophic levels		
<b>Abstract</b> <p>Climate change and anthropogenic activities are producing a range of new selection pressures, both abiotic and biotic, on marine organisms. While there are numerous studies that have investigated the response of individual marine organisms to climate change, few studies have focused on differences in organismal responses across trophic levels. Such trophic differences in response to climate change may disrupt ecological interactions and thereby threaten marine ecosystem function. In addition, predation is known as a strong driver that impacts individuals and populations. Despite this, we still do not have a comprehensive understanding of how different trophic levels respond to climate change stressors, predation and their combined effects in marine ecosystems.</p> <p>The main focus of this thesis is to identify whether marine trophic levels respond differently to climatic stressors and predation. To explore these questions, I have used a combination of traditional mesocosm experiments, together with a statistical method called meta-analysis. I initiated the research by study the responses of marine gastropods at two trophic levels to ocean acidification and predation using long-term mesocosm experiments together with a gastropod-specific meta-analyses. I focused on the amount of phenotypic plasticity in morphological traits of snails when exposed to the two stressors. In order to generalise and test these assumptions among a greater number of marine taxa, I used the meta-analysis approach to investigate the effects of ocean acidification and warming, as well as their combined effects on four marine trophic levels. Finally, to study the individual and combined effects of ocean acidification and predation with respect to inducible defences, I again applied a mesocosm experiment and used blue mussels as a model species.</p> <p>By using long-term mesocosm experiments and the gastropod-specific meta-analysis on marine gastropods from two trophic levels, I showed that these trophic levels varied in their responses to both ocean acidification and predation. Gastropods at lower trophic levels exhibited greater phenotypic plasticity against predation, while those from higher trophic levels showed stronger tolerance to ocean acidification. Next, by using a meta-analysis, including a large number of species and taxa, examining the effects of ocean acidification and warming, I revealed that top-predators and primary producers were most tolerant to ocean acidification compared to other trophic levels. Herbivores on the other hand, were the most vulnerable trophic level against abiotic stress. Again, using the meta-analysis approach, but this time incorporating only factorial experimental data that included the interactive effects of ocean acidification and ocean warming, I showed that higher trophic levels again were the most tolerant trophic level, and herbivores being most sensitive, with respect to the combined effect of the two stressors. Contrary to previous discussions in the literature concerning multiple climate-related stressors, antagonistic and additive effects occurred most frequently, while synergistic effects were less common and which decreased with increasing trophic rank. Finally, by conducting a fully-factorial experiment using blue mussels, I found that mussels with previous experience contact with predator has developed greater inducible defences than ones without previous experience. However, levels of ocean acidification may mask predator cues, or obstruct shell material, and consequently disrupt blue mussels inducible defence from crab predation.</p>		
<b>Key words</b> Trophic levels, predation, gastropods, mussels, ocean acidification, ocean warming, interactive effects, interaction types		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		<b>Language</b> English
<b>ISSN</b> and key title		<b>ISBN:</b> 978-91-8039-403-1 (print) 978-91-8039-404-8 (pdf)
Recipient's notes	<b>Number of pages</b>	Price
	Security classification	

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature 胡南 (Nan Hu)

Date 2022-09-19

# Climate change effects on marine species across trophic levels

Nan Hu



**LUND**  
UNIVERSITY

Cover by Nan Hu

Copyright Nan Hu

Paper 1 © by the Authors (Published by Wiley Open Access)

Paper 2 © by the Authors (Published by Elsevier)

Paper 3 © by the Authors (Manuscript unpublished)

Paper 4 © by the Authors (Manuscript unpublished)

Faculty of Science  
Department of Biology

ISBN 978-91-8039-403-1 (print)

ISBN 978-91-8039-404-8 (pdf)

Printed in Sweden by Media-Tryck, Lund University  
Lund 2022



Media-Tryck is a Nordic Swan Ecolabel  
certified provider of printed material.  
Read more about our environmental  
work at [www.mediatryck.lu.se](http://www.mediatryck.lu.se)

**MADE IN SWEDEN** 



# Table of Contents

List of papers .....	8
Author contributions.....	9
Abstract .....	10
Popular science summary .....	12
<b>Introduction.....</b>	<b>14</b>
Climatic changes in the ocean and their potential effects on marine species ...	16
Ocean acidification .....	17
Ocean warming .....	17
Organism responses to stressors.....	19
The role of previous experience .....	21
Multiple stressor interactions .....	22
Trophic differences in response to stressors .....	23
Study systems and approaches .....	24
Marine molluscs used in long-term mesocosm experiments .....	24
Meta-analysis .....	25
<b>Aims of the thesis.....</b>	<b>26</b>
<b>Methods.....</b>	<b>27</b>
<b>Main results.....</b>	<b>30</b>
Marine gastropods at different trophic levels respond to stressors differently...	30
Climate change effects across trophic levels .....	32
Individual and interactive effect of ocean acidification and predation.....	34
Individual and interactive effect of ocean acidification and warming .....	36
<b>Conclusions and future perspectives .....</b>	<b>38</b>
<b>References .....</b>	<b>39</b>
<b>Acknowledgements .....</b>	<b>48</b>

## List of papers

This thesis is based on the following papers, referred to by their roman numerals:

- I. Hu, N., Brönmark, C., Bourdeau, P.E. and Hollander, J. 2022. Marine gastropods at higher trophic level show stronger tolerance to ocean acidification. *Oikos*, 9: e08890.
- II. Hu, N., Bourdeau, P.E., Harlos, C., Liu, Y. and Hollander, J. 2022. Meta-analysis reveals variance in tolerance to climate change across marine trophic levels. *Science of the Total Environment*, 827:154244.
- III. Hu, N., Hollander, J., Brönmark, C. and Persson A. 2022. Ocean acidification and predation risk, in isolation and in combination, show strong effects on marine mussels. Manuscript.
- IV. Hu, N., Bourdeau, P.E. and Hollander, J. 2022. Marine trophic levels vary in magnitude of responses and interaction types to individual and combined effects of ocean acidification and warming. Manuscript.

## Author contributions

- I. NH, JH and PEB conceived and led the study. NH and JH performed the field collection. NH carried out the experiments, analysed the data and drafted the first version of manuscript. CB helped to plan the study. All authors contributed significantly to revisions and the publication.
- II. NH, JH and PEB conceptualized and planned the study. NH, CH and YL collected data. NH performed the statistical analysis and drafted the first version of the manuscript. All authors edited, read and approved the manuscript.
- III. NH, JH, CB and AP conceived and designed the experiments. NH conducted the experiments and analysed the results. NH wrote the first version of the manuscript with contributions from all co-authors. All authors edited, read and approved the manuscript.
- IV. NH, JH and PEB conceived and planned the study. NH searched, screened publications, extracted and analysed the data and led the writing of the first draft of the manuscript with contributions from all co-authors. All authors read and edited the manuscript and provided guidance and revisions.

List of authors: Nan Hu (NH), Johan Hollander (JH), Paul E. Bourdeau (PEB), Christer Brönmark (CB), Anders Persson (AP), Christian Harlos (CH), and Ying Liu (YL).

## Abstract

Climate change and anthropogenic activities are producing a range of new selection pressures, both abiotic and biotic, on marine organisms. While there are numerous studies that have investigated the response of individual marine organisms to climate change, few studies have focused on differences in organismal responses across trophic levels. Such trophic differences in response to climate change may disrupt ecological interactions and thereby threaten marine ecosystem function. In addition, predation is known as a strong driver that impacts individuals and populations. Despite this, we still do not have a comprehensive understanding of how different trophic levels respond to climate change stressors, predation and their combined effects in marine ecosystems.

The main focus of this thesis is to identify whether marine trophic levels respond differently to climatic stressors and predation. To explore these questions, I have used a combination of traditional mesocosm experiments, together with a statistical method called meta-analysis. I initiated the research by study the responses of marine gastropods at two trophic levels to ocean acidification and predation using long-term mesocosm experiments together with a gastropod-specific meta-analyses. I focused on the amount of phenotypic plasticity in morphological traits of snails when exposed to the two stressors. In order to generalise and test these assumptions among a greater number of marine taxa, I used the meta-analysis approach to investigate the effects of ocean acidification and warming, as well as their combined effects on four marine trophic levels. Finally, to study the individual and combined effects of ocean acidification and predation with respect to inducible defences, I again applied a mesocosm experiment and used blue mussels as a model species.

By using long-term mesocosm experiments and the gastropod-specific meta-analysis on marine gastropods from two trophic levels, I showed that these trophic levels varied in their responses to both ocean acidification and predation. Gastropods at lower trophic levels exhibited greater phenotypic plasticity against predation, while those from higher trophic levels showed stronger tolerance to ocean acidification. Next, by using a meta-analysis, including a large number of species and taxa, examining the effects of ocean acidification and warming, I revealed that top-predators and primary producers were most tolerant to ocean acidification compared to other trophic levels. Herbivores on the other hand, were the most vulnerable trophic level against abiotic stress. Again, using the meta-analysis approach, but this time incorporating only factorial experimental data that included the interactive effects of ocean acidification and ocean warming, I showed that higher trophic levels again were the most tolerant trophic level, and herbivores being most sensitive, with respect to the combined effect of the two stressors. Contrary to previous discussions in the literature concerning multiple climate-related stressors,

antagonistic and additive effects occurred most frequently, while synergistic effects were less common and which decreased with increasing trophic rank. Finally, by conducting a fully-factorial experiment using blue mussels, I found that mussels with previous experience contact with predator has developed greater inducible defences than ones without previous experience. However, levels of ocean acidification may mask predator cues, or obstruct shell material, and consequently disrupt blue mussels inducible defence from crab predation.

In summary, marine trophic levels respond differently to both biotic and climatic stressors. Higher trophic levels, together with primary producers, were often more robust against abiotic stress and may therefore be better prepared for future oceans compare species from lower trophic levels. These results may provide vital information for: implementing effective climate change mitigation, to understand which stressors to act on, and when and where to intervene for prioritizing conservation actions.

## Popular science summary

Human activity has been expelling a large amount of carbon dioxide (CO<sub>2</sub>) into the atmosphere. This rising CO<sub>2</sub> leads to increased sea surface temperature via the greenhouse effect, and when CO<sub>2</sub> enters seawater, it increases dissolved CO<sub>2</sub>, bicarbonate and hydrogen ions concentrations. The hydrogen ions produced in this process lower the pH of seawater, and result in a process called ocean acidification. Ocean acidification can have strong negative impacts on species such as snails, barnacles, and other calcifying organisms, which may be forced to build thinner and more brittle shells or exoskeletons under acidic conditions. With less protection from calcified structures, organisms may become more vulnerable to predation under climate change. In addition, increasing ocean temperatures is another consequence of climate change, and particularly extreme temperatures are thought to be the cause of e.g. coral bleaching that may result in reduced growth rates, reproductivity, and sometimes death. Such increases in seawater temperature and acidity can have significant effects on many marine organisms. Thus, in the future ocean, marine organisms will be challenged by multiple threats coming from both abiotic (i.e. climatic) and biotic (i.e. predation) stress, which may interact with one another.

All marine organisms are embedded in food webs that contain different food chains. Food webs are maintained by the flow of energy from primary producers at the base of the food web through primary and secondary consumers, all the way up to apex-predators that reside at the top of the food web. The position of the organisms in the food web is defined by the trophic level, and in this thesis, I have classified four trophic levels (primary producers, herbivores, meso-predators, and top-predators). However, as primary producers use photosynthesis to gain energy, herbivores graze primary producers, and predators are carnivores, their conditions (eat or be eaten) and environment are fundamentally different. Thus, trophic levels may respond to climate change and predation differently due to differences in selection pressure and evolutionary history, mobility, body size, and the ability to regulate physiological process. With a rapidly changing climate, the knowledge about how species, populations, and ecosystems will respond is key for management actions and effective governance; few studies however have looked into whether marine species at different trophic levels will respond to stressors similarly or differently.

In my theses, I studied the effects of trophic levels on tolerance to climate-related stressors by using experiments on various marine species together with a statistical method called meta-analyses. I focused on marine gastropods because they have representative species from multiple trophic levels, and are easy to collect and cultivate in a laboratory environment. For example, some snails are grazers representing herbivores where the main food source is algae or biofilm growing on stones and rocks. However, among these herbivore snails, it is common to find their shells at the shore with small holes on the shell, which are likely from predation

attacks. These predators are carnivorous snail species representing meso-predators that are a trophic level above herbivorous. By comparing the morphology and growth traits of snails after exposing the grazing and carnivore snails to low pH (a proxy of ocean acidification) and crab cues (mimicking predation) for 120 days, I found that grazing snails were more responsive to the risk of predation, while carnivorous snails were more robust to ocean acidification. In combination with these experiments, I collected data from 247 peer-reviewed articles focused on the effects of ocean acidification and warming on many different marine species. Using these data, I ran statistical models, which showed that there are differences in tolerance to acidification and warming among trophic levels. It turned out that herbivores are the most vulnerable trophic level in response to ocean acidification, while higher trophic levels were less sensitive. However, in the natural world, stressors like ocean acidification and ocean warming are not exposed in isolation but rather in a combination, affecting marine organisms simultaneously. When data were analyzed in respect to combined stressors, again using the meta-analysis approach, I found consistent results, where higher trophic levels demonstrated the greatest tolerance and herbivores were the most sensitive trophic level. In addition to these questions, I also focused on the interplay between ocean acidification and predation and the fact that calcifying species have difficulty building thicker shells at lower pH levels, and consequently can be more vulnerable to shell crushing predators. Interestingly however, in southern Sweden, there are geographic differences in the extent of contact experience between blue mussels and their predator, the green crab. Blue mussels on the west coast have always been in contact with the green crab, whereas on the east coast the predatory crab is absent. With these fortunate conditions, in an experimental point of view, makes blue mussels in southern Sweden a suitable study organism for investigating the effect of predation risk and ocean acidification in isolation and combination, and whether the historical contact to predators can help to develop greater inducible defences. It was shown that crab cues influenced mussels' ability to build stronger shells on the west coast, where mussels have previous experience with crab predation. However, differences between the west and east coast mussels disappeared when mussels from both groups were exposed to low pH. This could be because predation cues are less detectable in a low pH environment, or calcium carbonate is less available for building material of the shell.

In summary, my thesis shows that different marine trophic levels respond to climate change and predation differently. Since such trophic differences will fundamentally alter trophic interactions, there is a risk that future marine communities will be disrupted by climate change and pose a great risk on marine food webs if not managed effectively.

# Introduction

Marine ecosystems are maintained by the flow of energy among different trophic levels from primary producers at the base of food webs to intermediate consumers, apex predators, pathogens, and finally back again through detrital and decomposition processes (Lindeman 1942). Consequently, marine communities are biological networks in which the performance of one species is tightly directly or indirectly correlated with that of other species via a variety of biological interactions, such as predation, competition, facilitation, mutualism. The aggregate effect of these interactions within and among trophic levels constitutes ecosystem function, which in turn provides ecosystem services that society depends upon, such as aquaculture and fisheries production, recreation, and water purification.

There is widespread consensus among climate scientists today that climate change due to growing human pressures are having profound and diverse consequences for marine ecosystems (Doney et al. 2012, IPCC 2014). In addition, the rates of changes in recent decades have been rapid and may exceed the potential tolerances of many marine organisms to adapt, and a common view is that numerous marine organisms will fail to counteract the rate and magnitude of climate change (Hoffmann and Sgrò 2011, Barnosky et al. 2011, Bellard et al. 2012). There is pronounced evidence from different ecosystems that species respond individualistically to climate change stressors (Petchey et al. 1999, Parmesan et al. 1999, Thomas et al. 2001, Jellison et al. 2016). Such differential species responses will inevitably disrupt species interactions. Moreover, particularly strong disruption to a community is likely if the average sensitivity of species differs among trophic levels because trophic interactions will be fundamentally altered (Voigt et al. 2003). Environmental stress models have hypothesized that trophic levels vary in response to different stressors including environmental stressors and biotic stressors (e.g. predation and competition) (Menge and Sutherland 1976, 1987). The relative importance of different stressors to a specific trophic level is predictable along environmental stress gradients that range from benign to harsh habitats (Menge and Sutherland 1987). Yet, this trophic differential to abiotic and/or biotic stress has not been investigated or demonstrated for marine ecosystems, partially due to the great difficulty and expense of long-term studies of multispecies and trophic groups.

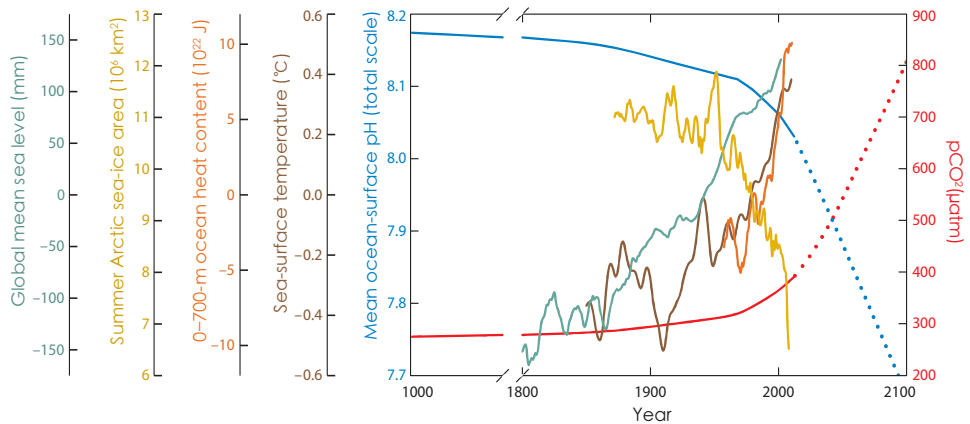
The overarching aim of this thesis is to increase our understanding on whether and how marine trophic levels respond differently to climatic and biotic stressors. Before further specifying the research questions, I here give a brief overview of



environmental changes in the ocean, a general description of strategies used by organisms to respond to stressors, a general statement of the interactive effects of multiple stressors, and the overall research progress about trophic level differences in the marine environment.

# Climatic changes in the ocean and their potential effects on marine species

The ocean is the largest habitat on Earth. However, rising atmospheric carbon dioxide (CO<sub>2</sub>) caused by human activities are rapidly altering the ocean chemistry and environment at an unprecedented rate (Doney et al. 2012, Poloczanska et al. 2013). Recent assessments of multi-stressor effects show that more than half of the ocean has been experiencing an increase in cumulative human impact, mainly driven by increasing climate stressors, such as acidity/pH, temperature, mean sea level, and polar sea-ice area (Fig 1; Doney et al. 2012, Reusch 2014, Nagelkerken and Connell 2015a, Halpern et al. 2015).



**Figure 1.** Changes in global mean sea level (teal line) (Jevrejeva et al. 2008), summer Arctic sea-ice area (yellow line) (Walsh and Chapman 2001), 0-700-m ocean heat content (orange line) (Levitus et al. 2009), sea-surface temperature (brown line) (Petit et al. 1999) (Adapted from Doney et al. 2012).

## **Ocean acidification**

Ocean acidification is a predictable consequence of rising atmospheric carbon dioxide (Doney et al. 2009). As a direct result of dissolution of CO<sub>2</sub>, ocean waters reduce pH and reduce calcium carbonate (CaCO<sub>3</sub>) saturation (Gattuso and Buddemeier 2000). Under a “business-as-usual” scenario, this process of ocean acidification will continue and accelerate with increasing CO<sub>2</sub> emissions (IPCC 2014). Many marine organisms, from phytoplankton to fish, are sensitive to lower calcium carbonate and pH (reviewed by Kroeker et al. 2010). However, experimental evidence has indicated that responses of marine organisms might vary (Kroeker et al. 2010). For example, primary producers, such as macroalgae, seagrasses and phytoplankton, might benefit from elevated CO<sub>2</sub> because CO<sub>2</sub> could act as a resource for the photosynthesis process (Riebesell and Tortell 2011, Harley et al. 2012, Nagelkerken and Connell 2015, Connell et al. 2017). However, the lower availability of bicarbonate ions along with increasing concentration of carbon dioxide can reduce growth of calcifying organisms including molluscs, echinoderms, calcifying macroalgae, reef-building corals, and calcifying unicellular plankton (reviewed by Kroeker et al. 2010). In addition, changes in ocean acidification likely led to higher body fluid *p*CO<sub>2</sub> in animals, which may result in acid-base balance disturbances (Miles et al. 2007). High trophic level species with highly mobile and developed intracellular/extracellular pH regulatory mechanisms may be more tolerant to ocean acidification (Kroeker et al. 2010). However, Melzner et al. (2009) argued that larger animals with large volumes have to spend more energy expenditure to maintain pH gradients, which can lead to reallocation of energy for other functions, such as growth and reproduction.

## **Ocean warming**

Rising atmospheric greenhouse gas concentrations have increased global average temperatures by ~0.2 °C per decade over the past 30 years (Hansen et al. 2006). More than 80% of the heat has been absorbed by the ocean, which will continually drive global ocean surface temperatures to rise by an average of (± SD) 3.7 ± 0.7 °C by the end of this century (Poloczanska et al. 2013, Bopp et al. 2013). Warming can fundamentally impact biological processes simply because molecular kinetic energy is very sensitive in response to elevated temperature (Hochachka and Somero 2002). Moderate increases in temperature have direct impact on the metabolic rate of ectothermic marine organisms, such as molluscs, with consequences for growth, reproduction, distributions, abundances, as well as phenology (Peck 2002, Pörtner 2006, Pörtner et al. 2007, Convey et al. 2009, Poloczanska et al. 2013, Llopiz et al. 2014, Thackeray et al. 2016, Pecl et al. 2017). On the other hand, warmer temperatures may also raise the respiratory demand, potentially reducing aerobic scope for activity (e.g. feeding, predator avoidance, digestion), with consequences allocating less energy for growth and reproduction (Pörtner and Knust 2007). With

regarding to moderate temperature increase, organisms tend to acclimate and/or adapt to local conditions. Yet, beyond species thresholds, acclimatization may fail, with the consequence of reduced fitness, and with the risk that mortality increases. In this case, populations would decline and local extinction may occur (Hochachka and Somero 2002). However, ocean warming may benefit some species or populations, such as algae and seagrass (Connell et al. 2017), due to greater availability of food or nutrients (Doney et al. 2012), reduced maintenance costs (e.g. energy used for respiration, acid-base balance), or reduced competition or predation.

## Organism responses to stressors

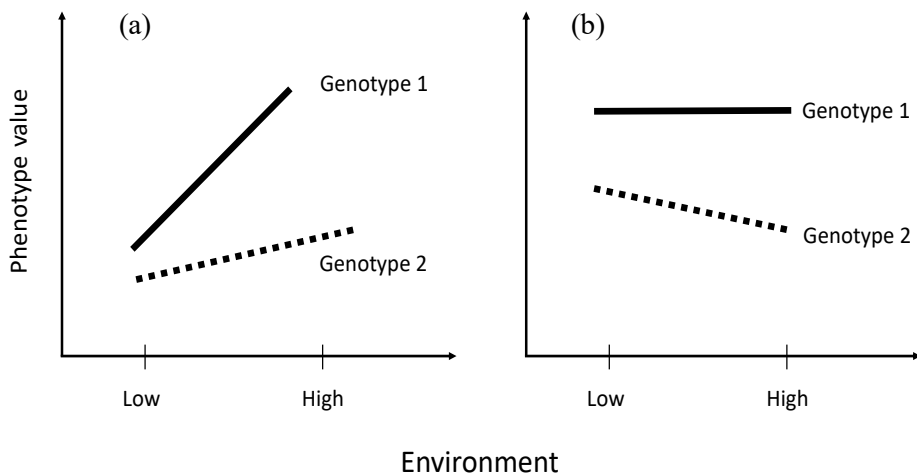
In ecological research, the term “stressor” has frequently been expected as detrimental (Folt et al. 1999). However, what is stressful or detrimental to one species is likely to be beneficial to another, either directly or via species interaction. For example, high  $p\text{CO}_2$  that is generally detrimental to calcifying species such as coral and molluscs (Kroeker et al. 2010) can be, as food resources, beneficial to primary producers (Connell et al. 2017). Therefore, stressors, in this thesis, are defined as a variable that, natural or anthropogenic, exceeds its range of normal variation and affects (whether negatively or positively) individual taxa, community composition, or ecosystem functioning relative to a reference condition (Crain et al. 2008).

Organisms can respond to climatic stressors in several ways; they can move to new areas that become available within their threshold, or they can acclimate and/or adapt their physiology or behaviour to extend their thresholds (Donelson et al. 2019). However, because spatial gradients in  $p\text{CO}_2$  and temperature are relatively low and unstructured, most species are less likely to find refuge through migration (Perry et al. 2005, Kelly and Hofmann 2013). Evolutionary adaptation, therefore, will be a fundamentally important response to these widespread future changes (Sunday et al. 2014).

Phenotypic plasticity has been identified as a fundamental phenomenon that allows organisms to maintain confronting with environmental change and give populations the time to adapt to climate change (Chevin et al. 2010). Phenotypic plasticity is broadly defined as the adjustment of phenotypic values of genotypes without genetic changes (Fig 2; Pigliucci 2001). Phenotypic plasticity is thought to be particularly important for long-lived species, but it is likely to be a critical component of adaptive responses for most species (Munday et al. 2013). A growing body of work in a wide range of marine taxa has addressed plasticity in behaviour, morphology, and/or physiology in response to both biotic and abiotic stressors. For example, marine gastropods can increase their shell thickness and mass when exposed to predation cues (Palmer 1990, Trussell and Nicklin 2002, Hollander et al. 2006, Bourdeau 2009). Similarly, intertidal species can adapt to a highly variable coastal environment via exhibiting considerable thermal plasticity (Somero 2005).

However, there is a special form of phenotypic plasticity termed phenotypic buffering (i.e., the maintenance of fitness-related phenotypes in response to environmental stress; Waddington 1942, Bradshaw 1965), which essentially reflects the ability of acclimation (Sunday et al. 2014) and is equivalent to environmental tolerance (Reusch 2014). Classical phenotypic plasticity selects those genotypes that are favoured and can adaptively adjust their phenotype to rapidly take advantage of novel conditions, which have a positive slope reaction norm line (Fig. 2). In contrast, phenotypic buffering selects those genotypes for enhanced tolerance, and

the reaction norm would essentially be a slightly negative or flat line (Schlichting and Pigliucci 1998). The flatter the reaction norm, the greater phenotypic buffering and tolerance. In other words, phenotypic buffering or tolerance is the ability of organisms to maintain rather than adjust a trait or functioning in response to stressors. Key examples are the increased expression of shock proteins to maintain proper cellular metabolism as a response to heat stress (Sørensen et al. 2003, Császár et al. 2010, Bergmann et al. 2010). Phenotypic buffering is expected to be employed by organisms in response to climatic stressors (Reusch 2014).



**Figure 2.** A conceptual model describing two types of plasticity by which the functional phenotype might be different or similar across a stressful environment. (a) Phenotypic plasticity is illustrated with a non-zero slope reaction norms of the phenotypic value for both underlying and observed traits, and where the magnitude of phenotypic plasticity determines the steepness of the slope (genotype 1 > genotype 2). (b) Phenotypic buffering, genotype 1 shows no change in a functional phenotype of interest despite underlying changes in gene expression, metabolic rates, or energy allocation, while genotype 2 has a negative change (less tolerance). In this case, genotype 1 shows greater phenotypic buffering compare genotype 2. (Adapted from Reusch 2014).

## **The role of previous experience**

The ability of species to respond to new environments are dependent of previous experience (Hollander and Bourdeau 2016, Fox et al. 2019). Previous environments impose selection, causing the adaptation of populations to the mean and variation in conditions experienced. It is often thought that species that have experienced environmental fluctuations will be more likely to have higher capacity to respond phenotypically to future change. For example, marine snails in the genus *Nucella* differed in their magnitude of phenotypic plasticity depending on their distribution along the shore (Bourdeau 2009). Lower-shore species that exposed to crabs for longer periods of time exhibited greater increases in shell thickening and growth than upper-shore species. However, most marine organisms may not have past experience of climate change induced environments. In this case, reaction norms should have random and erratic shapes in climatic environments, owing to the absence of past selection in these environments (Ghalambor et al. 2007). It is, therefore, difficult to predict in general whether and how much phenotypic plasticity and/or buffering will be adaptive in novel climatic environments (Bonamour et al. 2019).

## Multiple stressor interactions

Despite the fact that studies on single-stressor and single-species remain the norm, organisms never live in an environment in which stressors act separately. Interactions among multiple stressors, where the effects of one is dependent on the magnitude of another, are very common across ecosystems (Crain et al. 2008, Dieleman et al. 2012, Jackson et al. 2021). These interactions may lead to non-additive responses, where the combined effects are more or less than the addition of individual effects (synergistic or antagonistic, respectively), which highly limits our ability to predict interaction outcomes (Crain et al. 2008, Piggott et al. 2015). For example, ocean acidification may lower species' upper thermal lethal limits, which is more likely to lead to detrimental warming effects suggesting a synergistic interaction between ocean acidification and warming (Walther et al. 2009). Consequently, multiple stressors can interact in complex ways, amplifying or dampening the direct effects of stressors on species.

A critical goal of modern ecology is a quantitative understanding of how multiple drivers will interact (Harley et al. 2006, Darling and Côté 2008). If stressors interact synergistically or antagonistically, additive models will underestimate or overestimate the ecological impacts of multiple stressors. In contrast, if multiple stressors act individually, predictions based on additive expectations will be suitable (Sala et al. 2000, Halpern et al. 2015). A few recent highly cited syntheses found that climatic stressors mostly act synergistically in marine and coastal ecosystems (Crain et al. 2008, Harvey et al. 2013, Przeslawski et al. 2015). However, other studies demonstrated that additive effects accounting for the majority of interactions (Strain et al. 2014, Jin et al. 2019). Disparities between the interaction types among the above syntheses suggest that interaction types may be specific depending to stressor types and magnitudes, biological response variables (i.e., traits) and ecological variables (species, population, ecosystem).

Climatic stressors interact not only within the environmental milieu, but also with biotic stressors such as predation. For instance, a marine mollusc may suffer from reduced calcification due to elevated  $p\text{CO}_2$  but may at the same time increase their calcification rate in response to predation threat (Fitzer et al. 2015a), which would create an antagonistic interaction effect. On the other hand, ocean acidification may also induce a rounder and more globular shell shape of mussels, i.e. the same shell shape expected as an induced defence in response to shell-crushing crabs. Here, the two stressors would accordingly result in a morphological change in the same direction and thus increase prey survival rate in the presence of predators (Fitzer et al. 2015). Thus, climatic stressors and biotic stressors may additively, synergistically, or antagonistically disturb the same physiological pathways among individuals with vital consequences for how prey accommodate stressors (Rosenblatt and Schmitz 2014). Unfortunately, our understanding concerning multiple-stressor effects between climatic and biotic stressors is currently lacking.



## Trophic differences in response to stressors

Environmental stress models have been influential for decades in marine community ecology (Menge and Sutherland 1976, 1987). These models suggest that the relative importance of environmental stressors, competition, and predation varies predictably with trophic position. For example, predation has less effect on higher trophic levels than basal levels (Hairston et al. 1960, Menge and Sutherland 1976). In contrast, these models have hypothesized that higher trophic levels may be more tolerant to environmental stressors, such as high temperature or low salinity than lower trophic levels (Menge and Sutherland 1987, Menge and Olson 1990). Further, previous meta-analysis studies suggest that competition and predation have different magnitude of effects among trophic levels (Gurevitch et al. 2000). Although all of these models and syntheses suggest that trophic levels may respond differently to stressors, studies tend to focus on local stressors rather than climatic stressors such as ocean acidification and warming.

Only a few previous studies have explicitly examined climatic sensitivity of trophic levels (e.g. Petchey et al. 1999, Voigt et al. 2003, Thackeray et al. 2016). The results from these studies vary significantly in terms of which trophic level was more vulnerable to climate change, mainly because these studies focused on dissimilar ecosystems or taxa. For example, Petchey et al. (1999) found that herbivores and top-predators in the aquatic microbial communities had the highest extinction risk in response to warming, while primary producers were positively affected. Voigt et al. (2003) focused on a grassland ecosystem, and found that trophic sensitivity to global warming increased by trophic position and that the top trophic level was the most sensitive. Thackeray et al. (2016) used 10,003 phenological data sets including both terrestrial and aquatic species to examine the relative impact of climate change on species at different trophic levels, and showed that secondary consumers (the highest trophic level in their classification) showed consistently lower climate sensitivity than other groups. Previous work has also shown that marine pelagic trophic levels respond to climate change differently in phenology (Edwards and Richardson 2004) than in benthic systems. Best et al. (2015) found that warming had positive effects on the feeding rates and population growth of higher trophic levels, but no effect on primary producers. Although the direction and magnitude of climate sensitivity may vary markedly among marine trophic levels, identifying systematic trophic differences could have substantial socio-ecological implications. Such knowledge would afford some predictability of future ecological impacts and help to identify species or trophic levels that can represent effective sentinels of climate impact, facilitating the progress of indicators and estimates of vulnerability for conservation and national adaptation programmes (Williams et al. 2008, Walpole et al. 2009, Butchart et al. 2010).

## Study systems and approaches

In my thesis work, I studied the trophic differences in response to stressors using a combination of two approaches: 1) long-term mesocosm experiments and 2) meta-analysis. The mesocosm experiments focused on the marine molluscs (snails and mussels), which face different stressors including climate change and predation. The meta-analyses contain large number of marine species at different trophic levels from primary producers to top-predators for the purpose of generalisation. By using these approaches, I examine whether marine trophic levels respond differently to stressors and if there is a systematic pattern.

### **Marine molluscs used in long-term mesocosm experiments**

Gastropods play important roles in the maintenance of ecosystem health and trophic dynamics in coastal and marine ecosystems from the intertidal to subtidal zones (Poore et al. 2012). Marine gastropods have previously been demonstrated to show plastic phenotypic responses to predator cues (e.g. Vermeij and Currey 1980, Palmer 1990, Trussell and Nicklin 2002, Hollander et al. 2006, Bourdeau 2011), as well as climatic stressors (e.g. Marshall and McQuaid 2011, Leung et al. 2017). Predator cues have been shown to increase shell thickness and alter shell shape that reduce predation susceptibility (Trussell and Nicklin 2002, Hollander et al. 2006). For climatic stressors, gastropods tend to produce less soluble shells by changing carbonate polymorphs or regulating the content of magnesium ions in calcite as strategies to minimize shell dissolution in response to ocean acidification (Leung et al. 2017). Another study has demonstrated that intertidal snails could respond to high temperature via phenotypic buffering in metabolism, down-regulating their metabolic costs by exploiting strategies of metabolic depression (Marshall and McQuaid 2011). This, together with the fact that marine gastropods have representative species from multiple trophic levels, make them a suitable group of organisms for addressing the scientific questions in my thesis.

Similarly, to marine gastropods, marine mussels often show phenotypic plasticity in their shell morphology, such as thicker and heavier shells by increasing calcification rate in response to predation risk (Leonard et al. 1999, Manríquez et al. 2021). Climatic stressors such as ocean acidification can negatively impact shell calcification and internal shell dissolution in mussels (Fitzer et al. 2015b). Thus, for calcifying mussels, ocean acidification may interact with the expression of plastic defence traits against predation and negatively affect survival rate. In this regard, mussels are a suitable group for examining the interactive effects between predation and ocean acidification. In addition, in the absence of predation threat, an anti-predator response may be lost within a few generations (e.g. Blumstein et al. 2004, Hudson et al. 2017), and conversely, individuals experiencing novel predators may immediately alter anti-predator behaviour (Hawkins et al. 2008, West et al. 2018).

Due to differences in salinity tolerance, the geographical distributions of our study species, blue mussels and green crabs, do not overlap completely. Along the brackish coast of southern Sweden there is a slight increase in salinity from east to west and blue mussels are abundant along this entire gradient. However, green crabs (and other mussel predators such as sea stars) are only present along the west coast. Thus, there are geographic differences in the extent of contact experience between blue mussels and green crabs; mussels on the west coast have been in contact with this crab for centuries, whereas mussels on the east coast have not had such contact. Such geographic differences allow me to use blue mussels as a model organism to investigate the role of previous experience (co-evolution) in response to predation and climate-related stressors.

## **Meta-analysis**

Meta-analysis is the quantitative, scientific synthesis of research findings, contributing to the development of evidence-based practice and the resolution of seemingly contradictory research outcomes (Gurevitch et al. 2018). Meta-analysis is a powerful statistical tool for the summarization of large sets of data, but requires a consistent measure of effect size for testing general hypotheses. Meta-analysis provides summary effect sizes with each data set weighted according to some measure of its importance, with more weight given to large studies with precise effect estimates and less to small studies with imprecise effect estimates. Generally, each study is weighted in inverse proportion to the variance of its effect. The effect size of  $\ln RR$  (natural logarithm of response ratio) characterizes the log proportional change in the response variable between control and experimental treatment, which is a more powerful and less biased effect size than other effect size, such as standard mean difference (SMD or Hedges'  $d$ ) and standardized mean difference with heteroscedasticity (SMDH) (Yang et al. 2022). It can be back-transformed using to show the proportion changes in the experimental treatment relative to the control. The criteria for meta-analysis is therefore that the retrieved studies need to have verified the quantity of change in the studied area, standard deviation and sample size, with specific control and experimental groups. As meta-analysis uses research results, there are within- and between-study non-independence that may arise from multiple response variables from the same group of organisms, multiple experimental treatments being compared to the same control, or some studies conducted by the same group of researchers, and so on (Koricheva et al. 2013). Luckily, those potential non-independency issues can be accommodated by using random effects models with sampling variance-covariance matrices (Noble et al. 2017).

# Aims of the thesis

In this thesis, I have aimed to investigate whether marine species at different trophic levels respond to stressors differently. I examined their ecological and physiological responses to predation cues and climatic stressors (i.e., ocean acidification and warming). I have also studied the interactions between predation and ocean acidification effects on blue mussels, and tested the role of previous experience. I also investigated whether marine trophic levels differently respond to combined effects of ocean acidification and warming. Specifically, I have addressed the following questions:

- (1) Whether marine snails from different trophic levels (i.e., herbivores and carnivores) exhibit different magnitudes of phenotypic plasticity in response to ocean acidification and predation? (**paper I**)
- (2) How do ocean acidification and ocean warming individually affect marine species at different trophic levels (i.e., primary producers, herbivores, meso-predators, and top-predators)? (**paper II**)
- (3) How do crab cues and ocean acidification affect blue mussels in isolation and combination; does the historical contact with stressors matter? (**paper III**)
- (4) Do marine trophic levels respond differently to interactive effects between ocean acidification and ocean warming? (**paper IV**)

# Methods

In **paper I**, I tested whether marine snails from different trophic levels exhibited different magnitudes of phenotypic plasticity in response to lower pH and crab cues. I expected that snails from lower trophic levels would show greater phenotypic plasticity in response to crab cues, while snails from higher trophic levels would exhibit greater phenotypic buffering (i.e., stronger tolerance) under lower pH (as a proxy of ocean acidification).

Snails from two trophic level were used for the mesocosm experiments: the grazers *Littorina saxatilis* and *Littorina fabalis*, and a meso-predator *Nucella lapillus*. Snails were caught on the coast and then acclimated to laboratory conditions for four weeks before the start of the experiments. All three species were exposed to either crab cues, low pH, or a control environment without the two stressors. The lower pH was maintained by bubbling CO<sub>2</sub>. During the experiments, crabs and *N. lapillus* were fed mussels once a week. *L. fabalis* were fed macroalgae. The experiments lasted 120 days, from 18 June to 17 October 2018.

After the experiment, I measured a number of morphological variables known to be associated with susceptibility to crab predation and ocean acidification (Palmer 1990, Trussell and Nicklin 2002, Bourdeau 2009, Duquette et al. 2017). I measured the total shell length and wet body weight of live snails. Then, I measured the shell weight after removing the soft body parts. I also calculated the ratio of shell weight and shell length as a proxy for shell thickness. Soft tissue mass was calculated by subtracting shell mass from the total wet body weight. To compare the magnitude of change induced by crab cues or low pH for each of three snail species, I calculated the effect size for: the tissue mass, shell thickness, shell mass, and shell strength as the logarithm of the response ratio (lnRR).

I also included a meta-analysis in **paper I** to test the hypotheses among additional gastropod species from the two trophic levels. I searched the literature for both field and laboratory experiments testing the effects of ocean acidification and predation by using ISI Web of Science. Studies that met the selection criteria were used to extract data. Effect sizes then were calculated for each experiment and categorical random effect models were fitted and heterogeneity tests were performed. I also tested publication bias using two methods: funnel plots and the Egger's regression (Viechtbauer 2010).

In **paper II**, I used multi-level meta-analyses that included 1278 unique observations to investigate whether marine species from four trophic levels varied in response to ocean acidification and warming, and to examine whether calcifying and non-calcifying species respond differently to ocean acidification and warming. I expected that primary producers and top-predators would be tolerant to stressors and herbivores, particularly calcifying species, would be sensitive to ocean acidification.

The data were collected from 247 peer-reviewed publications on Web of Science and Scopus databases after selecting with a series of criteria, which contained 1278 unique observations. I calculated the log response ratio for each observation using modified sample sizes to deal with dependence of sampling errors due to shared controls. Then I ran categorical multi-level random effects models with sampling variance-covariance matrices accounting for dependence due to shared organisms. All analyses were conducted using the R package “*metafor*”.

In **paper III**, the aim was to investigate how ocean acidification and predation risk from predatory crabs, individually and in combination, affect inducible defenses and growth of mussels *Mytilus edulis*. In addition, I tested whether the historical context affect the response to the stressors. I expected that 1) experienced mussels would respond more strongly to crab cues; 2) ocean acidification would disrupt the inducible defenses of mussels to crab predation.; and 3) crab cues and ocean acidification would interact antagonistically.

Juvenile blue mussels were collected from east (crab naïve) and west coast (crab experienced) and transported to laboratory facilities at Lund University. Mussels were maintained 3-4 weeks in the lab before the experiments to allow for acclimation. A full factorial design was implemented, including three factors with two levels each: pH (control at pH 8.1, the pH offset of  $-0.3$  at 7.8), mussel population (crab naïve east coast versus crab experienced west coast), and crab cue (presence, absence). For the low pH treatment, the required pH level concentration was maintained by bubbling CO<sub>2</sub> using a gas flow meter/controller. Before the experiment, mussels were individually marked with nail polish of different colors at the base of the shell (Wong et al. 2012). During the experiment, mussels were fed daily with a mix of the algae. Crabs were fed adult mussels. The experiment lasted ~120 days, from June to October 2020. After the experiment, I measured morphological and growth traits including shell length, wet body weight, shell weight, shell thickness and shell strength. I also calculated soft tissue mass by subtracting shell weight from the wet body weight. I ran three-factor nested ANCOVA models for each trait using different covariates. I calculated the effect size of Hedge’s *d* to classify interaction types between low pH and predation for each trait.

**Paper IV** examined how ocean acidification and warming interactively affected different marine trophic levels. I expected that higher trophic levels would be more

tolerant that lower trophic levels and have less proportion of synergistic interactions (which was largely viewed as more detrimental) in combined stressor situations. In addition, a previous study has shown that discontinuous distribution of trophic position was only evident among lower trophic levels (i.e., primary producer and herbivore), whereas, above the herbivore trophic level, food webs are better characterized as a tangled web where trophic position are continuous (Thompson et al. 2007). For this reason, I merged the “meso-predator” and “top-predator” levels into one level as the “predator”. I presented results for both merged and unmerged of meso and top-predators.

I searched papers on the ISI Web of Science databases with different combinations of key words. Studies had to be fully factorial experiments that incorporated the mean effect of control, individual ocean acidification, warming, and their combination. Then, data were extracted from the papers that met my selecting criteria. I calculated individual and main effect sizes of individual and combined effects of ocean acidification and warming following Morris et al. (2007). Individual effect sizes were used to classify interaction types and main effect sizes, which provide a more realistic measure of a stressor’s effect were applied to multi-level models.

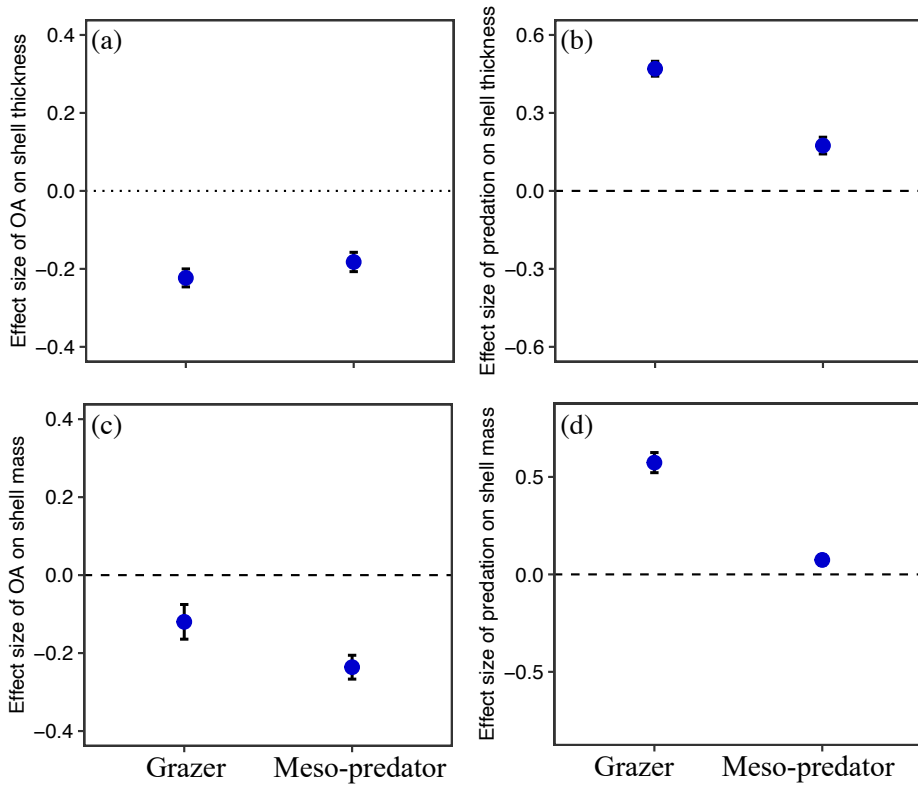
# Main results

## Marine gastropods at different trophic levels respond to stressors differently

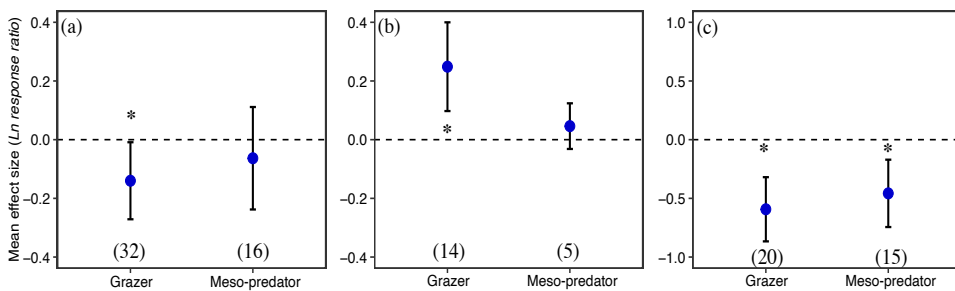
In **paper I**, I found that gastropods at different trophic levels respond differently to lower pH and crab cues. Grazer snails exhibited greater phenotypic plasticity in shell thickness and mass as inducible defenses in response to crab cues (Fig. 3b, c). The meso-predator snail *Nucella lapillus* showed greater phenotypic buffering in shell thickness (Fig. 3a) when exposed to lower pH. The meta-analysis supported these findings and revealed that meso-predatory gastropods showed stronger phenotypic buffering than grazing gastropods (Fig. 4a), as the overall mean effect sizes of meso-predatory gastropods were less negative than grazers. However, grazers had a more positive effect size in calcification compared to meso-predators in response to predation, suggesting greater phenotypic plasticity (Fig. 4b).

These results demonstrated differential tolerance to climate-related environmental stress between trophic levels. Snails from both higher and lower trophic levels, showed adaptive phenotypic plasticity to crab cues by producing thicker and stronger shells, which is consistent with previous findings (Palmer 1990, Hollander et al. 2006, Bourdeau 2009, Brönmark et al. 2011). However, in the field of plasticity research, few if any studies have truly evaluated the actual magnitude of plasticity against predation among species from different trophic levels. Although marine gastropods of both grazers and meso-predators demonstrated plastic developmental strategies against crab predation, the grazers in this study displayed a significantly greater response to predator cues. This result is in line with the assumptions of some consumer-stress models in which selection from predation should be higher at the base of the food web (Menge and Sutherland 1976, Sih et al. 1985)





**Figure 3.** Mean effect sizes (± 95% CI) of ocean acidification and predation cue on (a, b) shell thickness, and (c, d) shell mass of mesopredator *Nucella lapillus* and grazing snails *Littorina fabalis* and *Littorina saxatilis*.



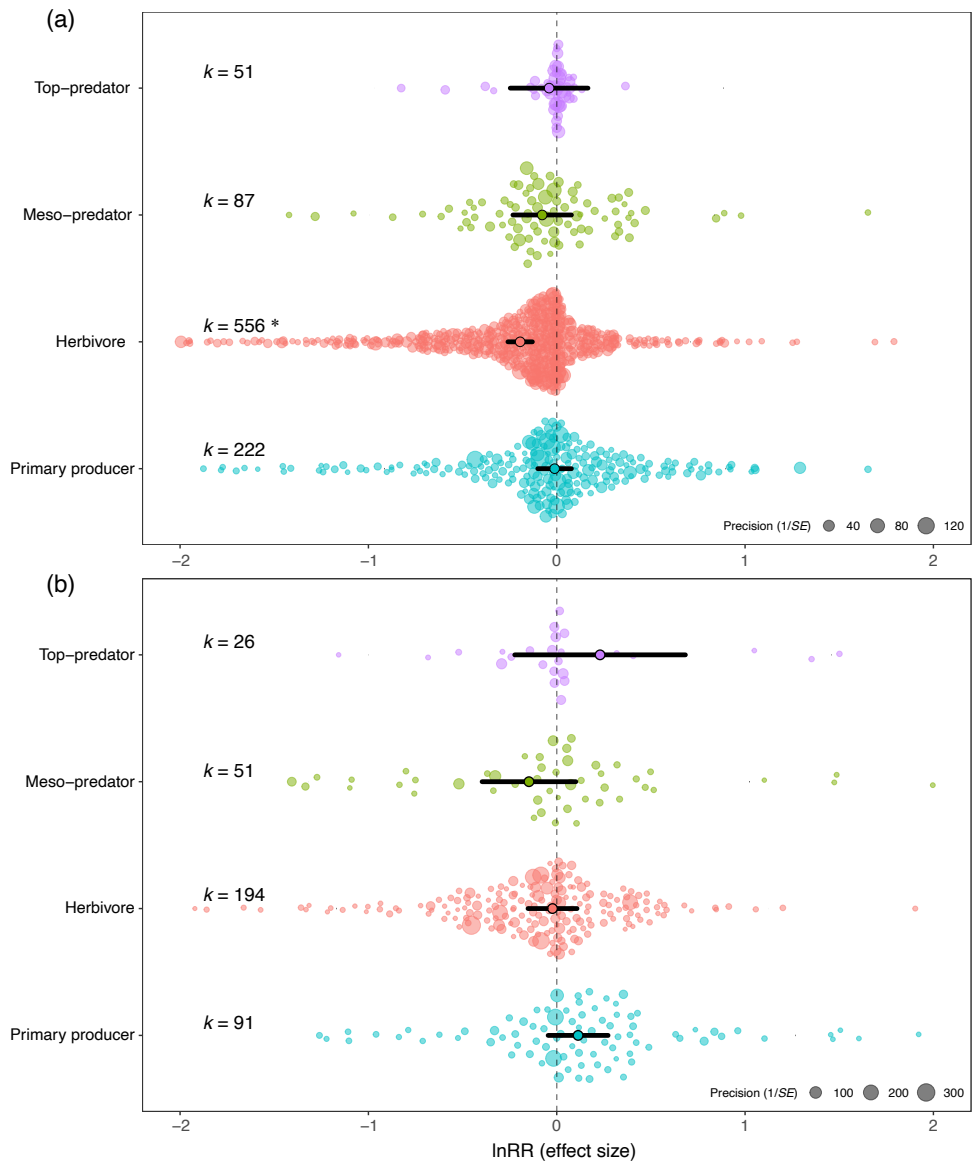
**Figure 4.** Mean effect size response from (a) ocean acidification and (b) predation effects on shell thickness in mesopredators and grazing snails, together with predator cue effects on (c) life-history traits. Effect sizes together with 95% confidence intervals are shown for each trophic level. The number of studies is shown in parentheses, and an asterisk (\*) indicate a significant effect ( $P < 0.05$ ).

## Climate change effects across trophic levels

Although in **Paper I** found that snails at higher trophic levels showed greater phenotypic buffering (more tolerant) to ocean acidification, it only included one taxon with two trophic levels. **Paper II**, used a meta-analysis, contained four trophic levels, including 1278 experiments, with 236 different marine species from 18 different phyla. The result showed that herbivores were significantly negatively affected by ocean acidification, while primary producers and top-predators were negligibly affected by ocean acidification (Fig. 5a). While meso-predators showed a more negative mean effect size than herbivores, the high degree of variation among species and studies in this group made the effect non-significant (Fig. 5b). Although non-significant, primary producers and top-predators exhibited positive effect sizes, even though top-predators showed greater variation in their responses (Fig. 5b).

The previous studies on trophic differences in tolerance to climate related stressors vary to a large extent (e.g. Petchey et al. 1999, Voigt et al. 2003, Thackeray et al. 2016). Petchey et al. (1999) for example, found that top-predators had the highest extinction risk, while primary producers significantly increased with warming. Our work is partly in line with their results because primary producers have a positive effect size in response to warming. Voigt et al. (2003) studied a grassland ecosystem, and found that vulnerability to climate change increased by trophic position (primary producers < herbivores < predators), which contradict our results. However, my results are consistent with the work of Thackeray et al. (2016) that showed that secondary consumers were the most tolerant level in response to warming and precipitation. Disparities among these studies are likely due to dissimilar ecosystems, taxa, biological responses or stressors.

Why trophic levels generally showed different tolerance to climate changes cannot be determined at this stage. It is possible that the differential tolerance to climate may be caused by functional groups in different trophic levels responding differently to climate. Another possible explanation could be body size, as the size of an organism is not only closely related to its resource acquisition strategy, mobility, and sensory range (Andersen et al. 2016), but also to many physiological processes, including reproduction, metabolism, growth, and survival (Peters 1993, Brown et al. 2004). Larger-sized organisms with higher metabolic rates, higher levels of activity, and higher capacity to adjust growth may cope better with environmental stress than more inactive, sessile groups, with lower metabolic rates and activity as well as capacity to regulate physiology (Pörtner 2005, Widdicombe and Spicer 2008, Melzner et al. 2009b).

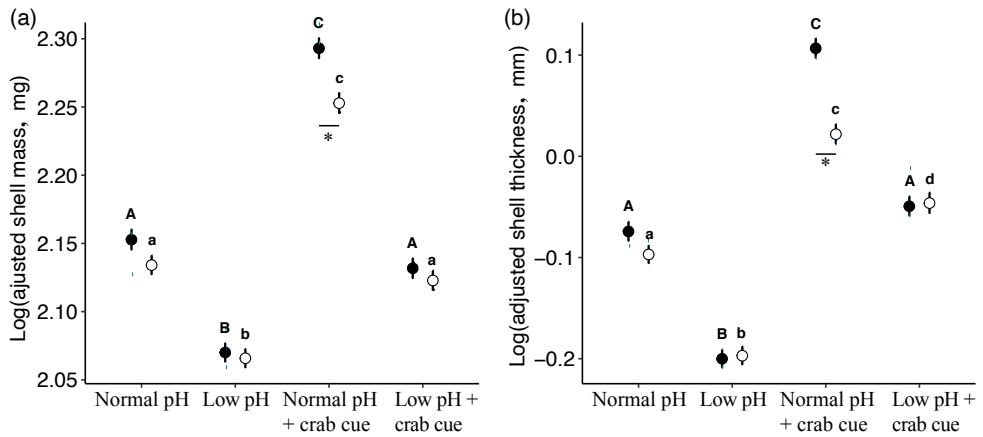


**Figure 5.** Orchard plots showing mean effect size, confidence interval (CIs, bold line) and individual effect size with precision (inverse variance) for ocean acidification (a) and warming (b) effects on marine trophic levels. Mean effect size and 95% confidence interval were estimated from multi-level meta-analytic models included trophic level as the moderator.  $k$  denotes the number of experiments of each trophic level. 95% confidence interval not overlapping with zero indicating significant effect, denoted here by an asterisk (\*).

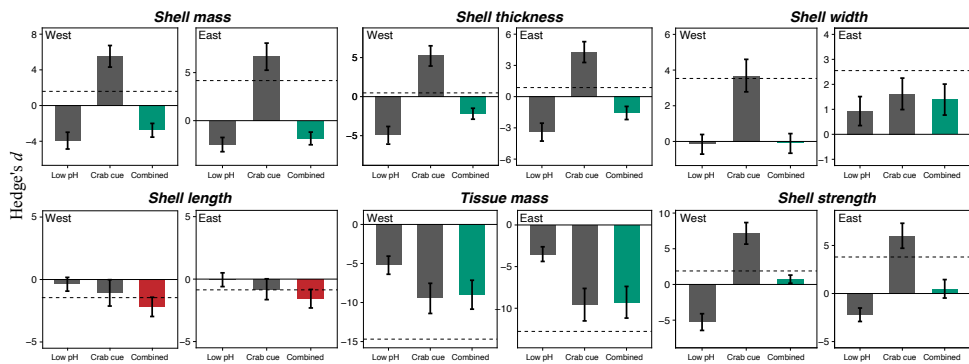
# Individual and interactive effect of ocean acidification and predation

**Paper III** demonstrated that various morphological characteristics of the mussels *Mytilus edulis* were affected by exposure to elevated  $p\text{CO}_2$  (low pH), crab cues as well as their combinations. High  $p\text{CO}_2$  significantly reduced shell mass and thickness, independently of where the mussel population originated from (Fig. 6), while crab cues significantly increased shell mass and thickness. West coast crab-experienced mussels developed significantly heavier and thicker shells than east crab-naïve mussels when exposed to crab cues (Fig. 6). However, and most interestingly, is that the predator-induced shell differences between west coast coevolved mussels and the east coast naïve mussels disappeared when the mussels were simultaneously exposed to low pH levels. The reason could be that ocean acidification either mask available cue (Draper and Weissburg 2019) or reduce available building material by lowering the rate of calcium carbonate disposition and dissolution (Fitzer et al. 2015b). As demonstrated, stressors normally do not act independently, but rather interact and produce combined effects on organisms. The interaction between low pH and crab cue was classified as antagonistic for all traits, except for shell length was classified as additive (Fig. 7). Between the west coast coevolved mussels and the east coast naïve mussels there was no population difference in the interaction type for all measured traits (Fig. 7).

The reduction in shell mass as well as the thickness of the shell can be explained by the fact that high  $p\text{CO}_2$  levels reduce calcification and induce shell dissolution (Doney et al. 2009, Fitzer et al. 2015b). Usually, shell thickness is positively correlated to mussel's ability to withstand crushing force (Leonard et al. 1999, Trussell and Nicklin 2002), and therefore, a thinner shell can explain, in part, the reduction in shell strength of mussels. However, a weaker shell under the high  $p\text{CO}_2$  level may also be explained by the composition and structure of calcium carbonate layers, which can be compromised under ocean acidification, leading to a brittle shell, that is less stiff and flexible (Fitzer et al. 2015a, 2015b, Byrne and Fitzer 2019). It was clear that west coast mussels developed stronger shells compared to the east coast mussels, suggesting genetically inherited differences between the populations, probably from strong natural selection and coevolution from crab predation. In addition, when I examined the combined effects of multiple stressors, which can be different from the sum of individual stressor effects, the results demonstrated antagonistic cumulative effects between ocean acidification and predation risk for all responses among the mussels except for shell length, which showed additive effects (Fig. 7). West and east coast mussels responded to the combined effect of the two stressors consistently with the same interaction type (Fig. 7).



**Figure 6.** (a) Adjusted mean log shell mass ( $\pm$  SE) at a covariate mean shell length of 16.7 (mm), and (b) adjusted mean log shell thickness at a covariate mean shell length of 16.7 (mm) for west (solid symbols) and east (open symbols) coast mussels in treatments of different pH (normal versus low) and predation risk (crab presence versus absence). Upper- and lower-case superscripts denote significant differences among treatments of west and east mussels, respectively. Asterisk (\*) denotes significant differences between two the populations in that treatment.



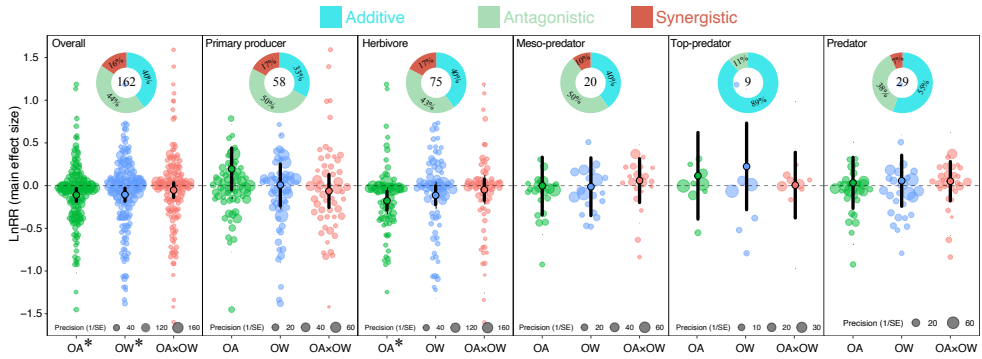
**Figure 7.** Interaction types between the low pH and crab cue treatments on different traits. Interaction types were classified by comparing the predicted additive expectation (dashed line) to observed combined effects. Additive effects were classified when the error bar (95% CI) overlapped with the dashed line. Green and red bars of combined effects represent antagonistic and additive interactions, respectively. No synergistic interaction was detected.

## Individual and interactive effect of ocean acidification and warming

While **Paper II** demonstrated that marine trophic levels varied in response to the individual effects of ocean acidification and warming, these two climatic stressors often interacted dependently on organisms. **Paper IV** included fully-factorial experimental data, and revealed that marine trophic levels also respond to combined stressors differently ( $Q_M = 22.28, p = 0.0345$ ). The results in **paper IV** support the findings of the previous meta-analysis (**paper II**), although only one-third of the number of species and studies were included in the analyses. The pattern of combined effects across trophic levels was similar to my previous results with the mean effect size increasing from, herbivore, meso-predators and top-predators, suggesting again that higher trophic levels are possibly more robust against ocean climate change effects. Although my results supported previously published findings that herbivores are the most sensitive trophic level, primary producers demonstrated a somewhat surprising result in response to combined effects with a negative effect size, where high levels of CO<sub>2</sub> and warming perhaps functioning as a resource for photosynthesizing organisms, and acting antagonistically.

Furthermore, the frequencies of interaction types (see donut charts in Fig. 8) differed significantly among trophic levels ( $\chi^2 = 14.24, p = 0.027, df = 6, n = 162$ ). The additive and the antagonistic effects prevailed across all trophic levels but their proportion changed with increasing rank of trophic levels. Synergistic effects, on the other hand, were the interaction type that occurred least, with less than 20% for all trophic levels. As predators showed less synergistic interactive effects between ocean acidification and warming compared to primary producers and herbivores, this may confirm that higher trophic levels are more tolerant to climate change-related stressors, both individually as well as combined (Fig. 8).

Concerning individual stressors, primary producers were positively affected by ocean acidification and this effect approached significance (Fig. 8). Meso-predators and top-predators were negligibly impacted by ocean acidification, with a mean effect size close to zero or above, and when meso-predators and top-predators were merged, we did not detect any greater change. Nevertheless, herbivores were the only trophic level significantly negatively affected by ocean acidification (Fig. 8). Regarding ocean warming, we detected similar patterns compared to the ocean acidification effects across trophic levels (Fig. 8). Ocean warming had positive effects on predators (i.e., meso-predators, top-predators, as well as merged). Although not significant, ocean warming demonstrated a negative effect among herbivores (Fig. 8). Overall, the individual effects of ocean acidification and ocean warming presented similar patterns on marine trophic levels – with the exception of primary producers, I consistently found that the negative effects from ocean acidification and warming decreased with increasing rank of marine trophic levels.



**Figure 8.** Orchard plots showing means of main effect sizes, confidence intervals (CIs, bold line) and individual effect sizes with precision (inverse variance) for ocean acidification (green), ocean warming (blue) and their combined effects (red) on marine trophic levels. Mean effect size and 95% confidence interval were estimated from multi-level meta-analytic models using the trophic level and stressor as moderators. 95% confidence interval not overlapping with zero indicate a significant effect denoted by an asterisk (\*). The additional panel Predator was formed by merging the Meso-predator and Top-predator groups. Donut charts indicate the frequencies (%) of additive, antagonistic and synergistic interaction types. Numbers inside donut charts indicate number of observations.

# Conclusions and future perspectives

In conclusion, I have demonstrated that marine trophic levels respond differently to both biotic (i.e. predation) and abiotic (i.e. climatic) stress. Higher trophic levels were often more tolerant in response to climatic stressors, while lower trophic levels showed greater phenotypic plasticity when exposed to predation cues. In **paper I**, I found that the meso-predatory snail *Nucella lapillus* was less sensitive to low pH than grazing snails *Littorina fabalis* and *Littorina saxatilis*, while grazers exhibited greater phenotypic plasticity in shell mass and thickness in response to crab cues. For **paper II**, I demonstrated that marine trophic levels respond differently to single stressors of ocean acidification and warming. Herbivores at the lower trophic level were the most sensitive level to climate change. Although primary producers are at the base of food webs, the elevated CO<sub>2</sub> acting as resources for them may offset the negative effects, making this level together with top-predators less sensitive compare herbivores. Results of **paper III** highlighted that despite the fact that of predation and low pH could have strong single effects on blue mussels, the combined effects can be antagonistic. Similarly, **paper IV** found that the effects of the combination of ocean acidification and warming differ from their individual effects and vary among trophic levels. Higher trophic levels (i.e., predators) showed stronger tolerance than lower trophic levels in response to ocean acidification and warming, as well as to their combined effects with a lower proportion of synergistic effects than for other trophic levels.



# References

- Andersen, K. H., T. Berge, R. J. Gonçalves, M. Hartvig, J. Heuschele, S. Hylander, N. S. Jacobsen, C. Lindemann, E. A. Martens, A. B. Neuheimer, K. Olsson, A. Palacz, A. E. F. Prowe, J. Sainmont, S. J. Traving, A. W. Visser, N. Wadhwa, and T. Kjørboe. 2016. Characteristic sizes of life in the oceans, from bacteria to whales. *Annual Review of Marine Science* **8**:217–241.
- Barnosky, A. D., N. Matzke, S. Tomiya, G. O. U. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L. McGuire, E. L. Lindsey, K. C. Maguire, B. Mersey, and E. A. Ferrer. 2011. Has the Earth's sixth mass extinction already arrived? *Nature* **471**:51–57.
- Bellard, C., C. Bertelsmeier, P. Leadley, W. Thuiller, and F. Courchamp. 2012. Impacts of climate change on the future of biodiversity: Biodiversity and climate change. *Ecology Letters* **15**:365–377.
- Bergmann, N., G. Winters, G. Rauch, C. Eizaguirre, J. Gu, P. Nelle, B. Fricke, and T. B. H. Reusch. 2010. Population-specificity of heat stress gene induction in northern and southern eelgrass *Zostera marina* populations under simulated global warming: *Zostera* gene expression under heat stress. *Molecular Ecology* **19**:2870–2883.
- Best, R. J., M. N. Stone, and J. J. Stachowicz. 2015. Predicting consequences of climate change for ecosystem functioning: variation across trophic levels, species and individuals. *Diversity and Distributions* **21**:1364–1374.
- Blumstein, D. T., J. C. Daniel, and B. P. Springett. 2004. A test of the multi-predator hypothesis: rapid loss of antipredator behavior after 130 years of isolation. *Ethology* **110**:919–934.
- Bonamour, S., L.-M. Chevin, A. Charmantier, and C. Teplitsky. 2019. Phenotypic plasticity in response to climate change: the importance of cue variation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **374**:20180178.
- Bopp, L., L. Resplandy, J. C. Orr, S. C. Doney, J. P. Dunne, M. Gehlen, P. Halloran, C. Heinze, T. Ilyina, R. Séférian, J. Tjiputra, and M. Vichi. 2013. Multiple stressors of ocean ecosystems in the 21st century: projections with CMIP5 models. *Biogeosciences* **10**:6225–6245.
- Bourdeau, P. E. 2009. Prioritized phenotypic responses to combined predators in a marine snail. *Ecology* **90**:1659–1669.
- Bourdeau, P. E. 2011. Constitutive and inducible defensive traits in co-occurring marine snails distributed across a vertical rocky intertidal gradient. *Functional Ecology* **25**:177–185.

- Bradshaw, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. Pages 115–155 *Advances in Genetics*. Elsevier.
- Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. *Ecology* **85**:1771–1789.
- Butchart, S. H. M., M. Walpole, B. Collen, A. van Strien, J. P. W. Scharlemann, R. E. A. Almond, J. E. M. Baillie, B. Bomhard, C. Brown, J. Bruno, K. E. Carpenter, G. M. Carr, J. Chanson, A. M. Chenery, J. Csirke, N. C. Davidson, F. Dentener, M. Foster, A. Galli, J. N. Galloway, P. Genovesi, R. D. Gregory, M. Hockings, V. Kapos, J.-F. Lamarque, F. Leverington, J. Loh, M. A. McGeoch, L. McRae, A. Minasyan, M. H. Morcillo, T. E. E. Oldfield, D. Pauly, S. Quader, C. Revenga, J. R. Sauer, B. Skolnik, D. Spear, D. Stanwell-Smith, S. N. Stuart, A. Symes, M. Tierney, T. D. Tyrrell, J.-C. Vié, and R. Watson. 2010. Global biodiversity: Indicators of recent declines. *Science* **328**:1164–1168.
- Byrne, M., and S. Fitzer. 2019. The impact of environmental acidification on the microstructure and mechanical integrity of marine invertebrate skeletons. *Conservation Physiology* **7**:coz062.
- Chevin, L.-M., R. Lande, and G. M. Mace. 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biology* **8**:e1000357.
- Connell, S. D., Z. A. Doubleday, S. B. Hamlyn, N. R. Foster, C. D. G. Harley, B. Helmuth, B. P. Kelaher, I. Nagelkerken, G. Sarà, and B. D. Russell. 2017. How ocean acidification can benefit calcifiers. *Current Biology* **27**:R95–R96.
- Convey, P., R. Bindschadler, G. di Prisco, E. Fahrbach, J. Gutt, D. A. Hodgson, P. A. Mayewski, C. P. Summerhayes, J. Turner, and the ACCE Consortium. 2009. Antarctic climate change and the environment. *Antarctic Science* **21**:541–563.
- Crain, C. M., K. Kroeker, and B. S. Halpern. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters* **11**:1304–1315.
- Császár, N. B. M., P. J. Ralph, R. Frankham, R. Berkelmans, and M. J. H. van Oppen. 2010. Estimating the potential for adaptation of corals to climate warming. *PLoS ONE* **5**:e9751.
- Darling, E. S., and I. M. Côté. 2008. Quantifying the evidence for ecological synergies. *Ecology Letters* **11**:1278–1286.
- Dieleman, W. I. J., S. Vicca, F. A. Dijkstra, F. Hagedorn, M. J. Hovenden, K. S. Larsen, J. A. Morgan, A. Volder, C. Beier, J. S. Dukes, J. King, S. Leuzinger, S. Linder, Y. Luo, R. Oren, P. De Angelis, D. Tingey, M. R. Hoosbeek, and I. A. Janssens. 2012. Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO<sub>2</sub> and temperature. *Global Change Biology* **18**:2681–2693.
- Donelson, J. M., J. M. Sunday, W. F. Figueira, J. D. Gaitán-Espitia, A. J. Hobday, C. R. Johnson, J. M. Leis, S. D. Ling, D. Marshall, J. M. Pandolfi, G. Pecl, G. G. Rodgers, D. J. Booth, and P. L. Munday. 2019. Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change.

- Doney, S. C., V. J. Fabry, R. A. Feely, and J. A. Kleypas. 2009. Ocean acidification: The other CO<sub>2</sub> problem. *Annual Review of Marine Science* **1**:169–192.
- Doney, S. C., M. Ruckelshaus, J. Emmett Duffy, J. P. Barry, F. Chan, C. A. English, H. M. Galindo, J. M. Grebmeier, A. B. Hollowed, N. Knowlton, J. Polovina, N. N. Rabalais, W. J. Sydeman, and L. D. Talley. 2012. Climate change impacts on marine ecosystems. *Annual Review of Marine Science* **4**:11–37.
- Draper, A. M., and M. J. Weissburg. 2019. Impacts of global warming and elevated CO<sub>2</sub> on sensory behavior in predator-prey interactions: a review and synthesis. *Frontiers in Ecology and Evolution* **7**:72.
- Duquette, A., J. B. McClintock, C. D. Amsler, A. Pérez-Huerta, M. Milazzo, and J. M. Hall-Spencer. 2017. Effects of ocean acidification on the shells of four Mediterranean gastropod species near a CO<sub>2</sub> seep. *Marine Pollution Bulletin* **124**:917–928.
- Edwards, M., and A. J. Richardson. 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature* **430**:881–884.
- Fitzer, S. C., L. Vittert, A. Bowman, N. A. Kamenos, V. R. Phoenix, and M. Cusack. 2015a. Ocean acidification and temperature increase impact mussel shell shape and thickness: problematic for protection? *Ecology and Evolution* **5**:4875–4884.
- Fitzer, S. C., W. Zhu, K. E. Tanner, V. R. Phoenix, N. A. Kamenos, and M. Cusack. 2015b. Ocean acidification alters the material properties of *Mytilus edulis* shells. *Journal of The Royal Society Interface* **12**:20141227.
- Folt, C. L., C. Y. Chen, M. V. Moore, and J. Burnaford. 1999. Synergism and antagonism among multiple stressors. *Limnology and Oceanography* **44**:864–877.
- Fox, R. J., J. M. Donelson, C. Schunter, T. Ravasi, and J. D. Gaitán-Espitia. 2019. Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences* **374**:20180174.
- Gattuso, J.-P., and R. W. Buddemeier. 2000. Calcification and CO<sub>2</sub>. *Nature* **407**:311–313.
- Ghalambor, C. K., J. K. McKAY, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* **21**:394–407.
- Gurevitch, J., J. Koricheva, S. Nakagawa, and G. Stewart. 2018. Meta-analysis and the science of research synthesis. *Nature* **555**:175–182.
- Gurevitch, J., J. A. Morrison, and L. V. Hedges. 2000. The interaction between competition and predation: a meta-analysis of field experiments. *The American Naturalist* **155**:435–453.
- Hairston, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. *The American Naturalist* **94**:421–425.

- Halpern, B. S., M. Frazier, J. Potapenko, K. S. Casey, K. Koenig, C. Longo, J. S. Lowndes, R. C. Rockwood, E. R. Selig, K. A. Selkoe, and S. Walbridge. 2015. Spatial and temporal changes in cumulative human impacts on the world's ocean. *Nature Communications* **6**:7615.
- Hansen, J., M. Sato, R. Ruedy, K. Lo, D. W. Lea, and M. Medina-Elizade. 2006. Global temperature change. *Proceedings of the National Academy of Sciences* **103**:14288–14293.
- Harley, C. D. G., K. M. Anderson, K. W. Demes, J. P. Jorve, R. L. Kordas, T. A. Coyle, and M. H. Graham. 2012. Effects of climate change on global seaweed communities. *Journal of Phycology* **48**:1064–1078.
- Harley, C. D. G., A. Randall Hughes, K. M. Hultgren, B. G. Miner, C. J. B. Sorte, C. S. Thornber, L. F. Rodriguez, L. Tomanek, and S. L. Williams. 2006. The impacts of climate change in coastal marine systems: Climate change in coastal marine systems. *Ecology Letters* **9**:228–241.
- Harvey, B. P., D. Gwynn-Jones, and P. J. Moore. 2013. Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecology and Evolution* **3**:1016–1030.
- Hawkins, L. A., A. E. Magurran, and J. D. Armstrong. 2008. Ontogenetic learning of predator recognition in hatchery-reared Atlantic salmon, *Salmo salar*. *Animal Behaviour* **75**:1663–1671.
- Hochachka, P. W., and G. N. Somero. 2002. *Biochemical adaptation: mechanism and process in physiological evolution*. Oxford University Press, New York.
- Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. *Nature* **470**:479–485.
- Hollander, J., and P. E. Bourdeau. 2016. Evidence of weaker phenotypic plasticity by prey to novel cues from non-native predators. *Ecology and Evolution* **6**:5358–5365.
- Hollander, J., M. L. Collyer, D. C. Adams, and K. Johannesson. 2006. Phenotypic plasticity in two marine snails: constraints superseding life history. *Journal of Evolutionary Biology* **19**:1861–1872.
- Hudson, C. M., G. P. Brown, and R. Shine. 2017. Evolutionary shifts in anti-predator responses of invasive cane toads (*Rhinella marina*). *Behavioral Ecology and Sociobiology* **71**:1–9.
- IPCC, editor. 2014. *Climate change 2014: the physical science basis: contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge ; New York.
- Jackson, M. C., S. Pawar, and G. Woodward. 2021. The temporal dynamics of multiple stressor effects: from individuals to ecosystems. *Trends in Ecology & Evolution* **36**:402–410.
- Jellison, B. M., A. T. Ninokawa, T. M. Hill, E. Sanford, and B. Gaylord. 2016. Ocean acidification alters the response of intertidal snails to a key sea star predator. *Proceedings of the Royal Society B: Biological Sciences* **283**:20160890.

- Jevrejeva, S., J. C. Moore, A. Grinsted, and P. L. Woodworth. 2008. Recent global sea level acceleration started over 200 years ago? *Geophysical Research Letters* **35**:8.
- Jin, P., S. Overmans, C. M. Duarte, and S. Agustí. 2019. Increasing temperature within thermal limits compensates negative ultraviolet-B radiation effects in terrestrial and aquatic organisms. *Global Ecology and Biogeography* **28**:1695–1711.
- Kelly, M. W., and G. E. Hofmann. 2013. Adaptation and the physiology of ocean acidification. *Functional Ecology* **27**:980–990.
- Koricheva, J., J. Gurevitch, and K. Mengersen, editors. 2013. *Handbook of meta-analysis in ecology and evolution*. Princeton University Press.
- Kroeker, K. J., R. L. Kordas, R. N. Crim, and G. G. Singh. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms: Biological responses to ocean acidification. *Ecology Letters* **13**:1419–1434.
- Leonard, G. H., M. D. Bertness, and P. O. Yund. 1999. Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. *Ecology* **80**:1–14.
- Leung, J. Y. S., S. D. Connell, I. Nagelkerken, and B. D. Russell. 2017. Impacts of near-future ocean acidification and warming on the shell mechanical and geochemical properties of gastropods from intertidal to subtidal zones. *Environmental Science & Technology* **51**:12097–12103.
- Levitus, S., J. I. Antonov, T. P. Boyer, R. A. Locarnini, H. E. Garcia, and A. V. Mishonov. 2009. Global ocean heat content 1955-2008 in light of recently revealed instrumentation problems: Global ocean heat content. *Geophysical Research Letters* **36**:7.
- Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. *Ecology* **23**:399–417.
- Llopiz, J., R. Cowen, M. Hauff, R. Ji, P. Munday, B. Muhling, M. Peck, D. Richardson, S. Sogard, and S. Sponaugle. 2014. Early life history and fisheries oceanography: new questions in a changing world. *Oceanography* **27**:26–41.
- Manríquez, P. H., M. E. Jara, C. P. González, M. E. Seguel, P. Domenici, S.-A. Watson, C. Anguita, C. Duarte, and K. Brokordt. 2021. The combined effects of climate change stressors and predatory cues on a mussel species. *Science of The Total Environment* **776**:145916.
- Marshall, D. J., and C. D. McQuaid. 2011. Warming reduces metabolic rate in marine snails: adaptation to fluctuating high temperatures challenges the metabolic theory of ecology. *Proceedings of the Royal Society B: Biological Sciences* **278**:281–288.
- Melzner, F., S. Göbel, M. Langenbuch, M. A. Gutowska, H.-O. Pörtner, and M. Lucassen. 2009a. Swimming performance in Atlantic Cod (*Gadus morhua*) following long-term (4–12 months) acclimation to elevated seawater PCO<sub>2</sub>. *Aquatic Toxicology* **92**:30–37.
- Melzner, F., M. A. Gutowska, M. Langenbuch, S. Dupont, M. Lucassen, M. C. Thorndyke, M. Bleich, and H.-O. Pörtner. 2009b. Physiological basis for high CO<sub>2</sub> tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences* **6**:2313–2331.

- Menge, B. A., and A. M. Olson. 1990. Role of scale and environmental factors in regulation of community structure. *Trends in Ecology & Evolution* **5**:52–57.
- Menge, B. A., and J. P. Sutherland. 1976. Species diversity gradients: synthesis of the roles of predation, competition, and temporal heterogeneity. *The American Naturalist* **110**:351–369.
- Menge, B. A., and J. P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *The American Naturalist* **130**:730–757.
- Miles, H., S. Widdicombe, J. I. Spicer, and J. Hall-Spencer. 2007. Effects of anthropogenic seawater acidification on acid–base balance in the sea urchin *Psammechinus miliaris*. *Marine Pollution Bulletin* **54**:89–96.
- Morris, W. F., R. A. Huffbauer, A. A. Agrawal, J. D. Bever, V. A. Borowicz, G. S. Gilbert, J. L. Maron, C. E. Mitchell, I. M. Parker, A. G. Power, M. E. Torchin, and D. P. Vázquez. 2007. Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. *Ecology* **88**:1021–1029.
- Munday, P. L., R. R. Warner, K. Monro, J. M. Pandolfi, and D. J. Marshall. 2013. Predicting evolutionary responses to climate change in the sea. *Ecology Letters* **16**:1488–1500.
- Nagelkerken, I., and S. D. Connell. 2015. Global alteration of ocean ecosystem functioning due to increasing human CO<sub>2</sub> emissions. *Proceedings of the National Academy of Sciences* **112**:13272–13277.
- Noble, D. W. A., M. Lagisz, R. E. O’dea, and S. Nakagawa. 2017. Nonindependence and sensitivity analyses in ecological and evolutionary meta-analyses. *Molecular Ecology* **26**:2410–2425.
- Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L.) **28**:155–182.
- Parmesan, C., N. Ryrholm, C. Stefanescu, J. K. Hill, C. D. Thomas, H. Descimon, B. Huntley, L. Kaila, J. Kullberg, T. Tammaru, W. J. Tennent, J. A. Thomas, and M. Warren. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **399**:579–583.
- Peck, L. 2002. Ecophysiology of Antarctic marine ectotherms: limits to life. *Polar Biology* **25**:31–40.
- Pecl, G. T., M. B. Araújo, J. D. Bell, J. Blanchard, T. C. Bonebrake, I.-C. Chen, T. D. Clark, R. K. Colwell, F. Danielsen, B. Evengård, L. Falconi, S. Ferrier, S. Frusher, R. A. Garcia, R. B. Griffiths, A. J. Hobday, C. Janion-Scheepers, M. A. Jarzyna, S. Jennings, J. Lenoir, H. I. Linnertved, V. Y. Martin, P. C. McCormack, J. McDonald, N. J. Mitchell, T. Mustonen, J. M. Pandolfi, N. Pettorelli, E. Popova, S. A. Robinson, B. R. Scheffers, J. D. Shaw, C. J. B. Sorte, J. M. Strugnelli, J. M. Sunday, M.-N. Tuanmu, A. Vergés, C. Villanueva, T. Wernberg, E. Wapstra, and S. E. Williams. 2017. Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science* **355**:eaai9214.

- Perry, A. L., P. J. Low, J. R. Ellis, and J. D. Reynolds. 2005. Climate change and distribution shifts in marine fishes. *Science* **308**:1912–1915.
- Petchey, O. L., P. T. McPhearson, T. M. Casey, and P. J. Morin. 1999. Environmental warming alters food-web structure and ecosystem function. *Nature* **402**:69–72.
- Peters, R. H. 1993. *The ecological implications of body size*. Cambridge University Press.
- Petit, J. R., J. Jouzel, D. Raynaud, N. I. Barkov, J.-M. Barnola, I. Basile, M. Bender, J. Chappellaz, M. Davis, G. Delaygue, M. Delmotte, V. M. Kotlyakov, M. Legrand, V. Y. Lipenkov, C. Lorius, L. PÉpin, C. Ritz, E. Saltzman, and M. Stievenard. 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* **399**:429–436.
- Piggott, J. J., C. R. Townsend, and C. D. Matthaei. 2015. Reconceptualizing synergism and antagonism among multiple stressors. *Ecology and Evolution* **5**:1538–1547.
- Pigliucci, M. 2001. *Phenotypic plasticity: beyond nature and nurture*. Johns Hopkins University Press, Baltimore.
- Poloczanska, E. S., C. J. Brown, W. J. Sydeman, W. Kiessling, D. S. Schoeman, P. J. Moore, K. Brander, J. F. Bruno, L. B. Buckley, M. T. Burrows, C. M. Duarte, B. S. Halpern, J. Holding, C. V. Kappel, M. I. O'Connor, J. M. Pandolfi, C. Parmesan, F. Schwing, S. A. Thompson, and A. J. Richardson. 2013. Global imprint of climate change on marine life. *Nature Climate Change* **3**:919–925.
- Poore, A. G. B., A. H. Campbell, R. A. Coleman, G. J. Edgar, V. Jormalainen, P. L. Reynolds, E. E. Sotka, J. J. Stachowicz, R. B. Taylor, M. A. Vanderklift, and J. Emmett Duffy. 2012. Global patterns in the impact of marine herbivores on benthic primary producers. *Ecology Letters* **15**:912–922.
- Pörtner, H. O. 2005. Synergistic effects of temperature extremes, hypoxia, and increases in CO<sub>2</sub> on marine animals: From Earth history to global change. *Journal of Geophysical Research* **110**:C9.
- Pörtner, H. O. 2006. Climate-dependent evolution of Antarctic ectotherms: An integrative analysis. *Deep Sea Research Part II: Topical Studies in Oceanography* **53**:1071–1104.
- Pörtner, H. O., and R. Knust. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**:95–97.
- Pörtner, H. O., L. Peck, and G. Somero. 2007. Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. *Philosophical Transactions of the Royal Society B: Biological Sciences* **362**:2233–2258.
- Przeslawski, R., M. Byrne, and C. Mellin. 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Global Change Biology* **21**:2122–2140.
- Reusch, T. B. H. 2014. Climate change in the oceans: evolutionary versus phenotypically plastic responses of marine animals and plants. *Evolutionary Applications* **7**:104–122.
- Riebesell, U., and P. D. Tortell. 2011. *Effects of ocean acidification on pelagic organisms and ecosystems*. Page Ocean Acidification. Oxford University Press.

- Rosenblatt, A. E., and O. J. Schmitz. 2014. Interactive effects of multiple climate change variables on trophic interactions: a meta-analysis. *Climate Change Responses* **1**:8.
- Sala, O. E., F. Stuart Chapin, Iii, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Global biodiversity scenarios for the year 2100. *Science* **287**:1770–1774.
- Schlichting, C., and M. Pigliucci. 1998. *Phenotypic evolution: a reaction norm perspective*. Sinauer, Sunderland, Mass.
- Somero, G. N. 2005. Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Frontiers in Zoology* **2**:1–9.
- Sørensen, J. G., T. N. Kristensen, and V. Loeschcke. 2003. The evolutionary and ecological role of heat shock proteins: Heat shock proteins. *Ecology Letters* **6**:1025–1037.
- Strain, E. M. A., R. J. Thomson, F. Micheli, F. P. Mancuso, and L. Airoidi. 2014. Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. *Global Change Biology* **20**:3300–3312.
- Sunday, J. M., P. Calosi, S. Dupont, P. L. Munday, J. H. Stillman, and T. B. H. Reusch. 2014. Evolution in an acidifying ocean. *Trends in Ecology & Evolution* **29**:117–125.
- Thackeray, S. J., P. A. Henrys, D. Hemming, J. R. Bell, M. S. Botham, S. Burthe, P. Helaouet, D. G. Johns, I. D. Jones, D. I. Leech, E. B. Mackay, D. Massimino, S. Atkinson, P. J. Bacon, T. M. Brereton, L. Carvalho, T. H. Clutton-Brock, C. Duck, M. Edwards, J. M. Elliott, S. J. G. Hall, R. Harrington, J. W. Pearce-Higgins, T. T. Høye, L. E. B. Kruuk, J. M. Pemberton, T. H. Sparks, P. M. Thompson, I. White, I. J. Winfield, and S. Wanless. 2016. Phenological sensitivity to climate across taxa and trophic levels. *Nature* **535**:241–245.
- Thomas, C. D., E. J. Bodsworth, R. J. Wilson, A. D. Simmons, Z. G. Davies, M. Musche, and L. Conradt. 2001. Ecological and evolutionary processes at expanding range margins. *Nature* **411**:577–581.
- Thompson, R. M., M. Hemberg, B. M. Starzomski, and J. B. Shurin. 2007. Trophic levels and trophic tangles: the prevalence of omnivory in real food webs. *Ecology* **88**:612–617.
- Trussell, G. C., and M. O. Nicklin. 2002. Cue sensitivity, inducible defense, and trade-off in a marine snail. *Ecology* **83**:1635–1647.
- Vermeij, G. J., and J. D. Currey. 1980. Geographical variation in the strength of Thaidid snail shells **158**:383–389.
- Viechtbauer, W. 2010. Conducting meta-analyses in *R* with the **metafor** Package. *Journal of Statistical Software* **36**:1–48.



- Voigt, W., J. Perner, A. J. Davis, T. Eggers, J. Schumacher, R. Bährmann, B. Fabian, W. Heinrich, G. Köhler, D. Lichter, R. Marstaller, and F. W. Sander. 2003. Trophic levels are differentially sensitive to climate. *Ecology* **84**:2444–2453.
- Waddington, C. H. 1942. Canalization of development and the inheritance of acquired characters. *Nature* **150**:563–565.
- Walpole, M., R. E. A. Almond, C. Besançon, S. H. M. Butchart, D. Campbell-Lendrum, G. M. Carr, B. Collen, L. Collette, N. C. Davidson, E. Dulloo, A. M. Fazel, J. N. Galloway, M. Gill, T. Govers, M. Hockings, D. J. Leaman, D. H. W. Morgan, C. Revenga, C. J. Rickwood, F. Schutyser, S. Simons, A. J. Stattersfield, T. D. Tyrrell, J.-C. Vié, and M. Zimsky. 2009. Tracking progress toward the 2010 biodiversity target and beyond. *Science* **325**:1503–1504.
- Walsh, J. E., and W. L. Chapman. 2001. 20th-century sea-ice variations from observational data. *Annals of Glaciology* **33**:444–448.
- Walther, K., F. J. Sartoris, C. Bock, and H. O. Pörtner. 2009. Impact of anthropogenic ocean acidification on thermal tolerance of the spider crab *Hyas araneus*. *Biogeosciences* **6**:2207–2215.
- West, R., M. Letnic, D. T. Blumstein, and K. E. Moseby. 2018. Predator exposure improves anti-predator responses in a threatened mammal. *Journal of Applied Ecology* **55**:147–156.
- Widdicombe, S., and J. I. Spicer. 2008. Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? *Journal of Experimental Marine Biology and Ecology* **366**:187–197.
- Williams, S. E., L. P. Shoo, J. L. Isaac, A. A. Hoffmann, and G. Langham. 2008. Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biology* **6**:e325.
- Wong, M. C., J. d'Entremont, and M. A. Barbeau. 2012. An approach for quantifying effects of multiple predators that forage on different time scales. *Journal of Experimental Marine Biology and Ecology* **420**:100–109.
- Yang, Y., H. Hillebrand, M. Lagisz, I. Cleasby, and S. Nakagawa. 2022. Low statistical power and overestimated anthropogenic impacts, exacerbated by publication bias, dominate field studies in global change biology. *Global Change Biology* **28**:969–989.

# Acknowledgements

It's definitely a special adventure for me to do my PhD in Sweden and now the journey is about to be finished. There is no doubt that it has been a great period of my life with so many wonderful memories and some tough time. I could not make it happen without the help, support and encouragement from so many of you. Here I would like to express my thanks to you all.

**Johan**, I am so happy and lucky that I have you as my supervisor! You have been such a good supervisor, encouraging me to express my opinions and giving me freedom to try my own "research plan". Thanks for your patience in the communication, highly valuable supervision on the projects. Many thanks for you not only being a research supervisor but also being a life "supervisor" to me. Let's remember these official thanks wording and then move to my warm memories. I will never forget the midnight around 11.30 when I just arrived the land of Sweden. You drove two hours from home to pick me up at the airport and then safely sent me to my apartment. I will never forget the "Well done" from you after my unit talk. I will never forget the reminder for buying a suitable pair of hiking shoes before the Bahamas trip. Thank you for the traditional (for me Special) Swedish Eastern herring lunch, and the best dinner I have ever had on your 50<sup>th</sup> birthday. I cannot forget the day you drove 6 hours to the sea for animal collection, and then you did all the work because I had sea-sick and could only huddled myself up on the boat. Not sure if this is a proper Acknowledgements, but let it be as it is my Acknowledgement. Thank you, Johan,!!

**Christer**, thank you for your insightful and constructive suggestions on the manuscript and for all the nice discussions during the examiner meetings. Moreover, your encouragement and positive feedback really mean a lot to me. Thank you!

**Paul**, even if we have not met in person, I can't overstate how important you were for the completion of this thesis. Thank you so much for all your hard work. Thank you for the meetings at nonworking time for you.

**Anders**, thank you for always being available when I needed advice and always being so kindly. I am so grateful to have you as my co-supervisor.

**Lars-Anders**, thank you for your kindly support and help as my examiner during the past five years. Thank you for the napkin when the tear welled up my eyes because of your care.

张欢师姐，谢谢你的积极回应，才有了我的这5年瑞典之旅，有了Johan作为我的导师的可能。虽然我们没能相处很久，但是谢谢你的盆栽，它们现在还在我家里茁壮成长呢！

咏翠师姐，多谢你陪伴了我大半程的博士生活，没事的时候去你的办公室唠唠嗑算是我很开心的一件事情。

**Emma**, thank you for the clothes for my son and thank you for the hug that really meant a lot at that moment.

**Gustaf**, thanks for the Christmas dinner and many great chats, but also thank you for your help to different things like reclaim tax income, day care application and so on.

**Marcus**, I am so glad to have you as my companion, you really helped me a lot.

**Raphael**, thank you for your countless times of help ranging from borrowing traffic tickets, buying food in the Asian shop to introducing me software for drawing poster and showing me the grey mailbox for first copy of thesis.

**Marie**, thank you for many many fun chats and “Hi Nan!”.

**Anders N**, thank you for the help regarding to the statistic problems.

**Emma K**, thank you for the always timely greetings. Many times, Johan said to me “I heard from Emma, ...”, thanks for all these direct and indirect suggestions and help.

Thank you, all **Aquatic Ecology people**, for creating such a friendly and nice working environment.

谢谢我的娟姐，一直陪在我身边，默默的为这个家付出。谢谢胡毅涵带给我许许多多的欢乐时光。谢谢陈妈妈帮我们照顾胡毅涵，照顾这个家。

最后，想对妈妈说声对不起，在你最困难的时刻，没有能够陪伴在你的身边。明明很痛苦，但我每次问你的时候，你都说“没事，不难受的”。妈，对不起！希望你能够等到我回去。

谢谢爸对妈妈的照顾，能让我完成这个博士再回去。

谢谢香逢在妈妈生病的时候帮我照顾妈妈。





## List of papers

---

- I. Hu, N., Brönmark, C., Bourdeau, P.E. and Hollander, J., 2022. Marine gastropods at higher trophic level show stronger tolerance to ocean acidification. *Oikos*, 2022 (9): e08890.
- II. Hu, N., Bourdeau., P.E., Harlos, C., Liu, Y. and Hollander, J., 2022. Meta-analysis reveals variance in tolerance to climate change across marine trophic levels. *Science of the Total Environment*, 827: 154244
- III. Hu, N., Hollander, J., Brönmark, C. and Persson, A., 2022. Ocean acidification and predation risk, in isolation and in combination, show strong effects on marine mussels. Manuscript.
- IV. Hu, N., Bourdeau., P.E. and Hollander, J., 2022. Marine trophic levels vary in magnitude of responses and interaction types to individual and combined effects of ocean acidification and warming. Manuscript