EFFECTS OF ASYNCHRONOUS STRESSORS ON THE EASTERN OYSTER, CRASSOSTREA VIRGINICA

A Thesis

by

ANIKA AGRAWAL

Submitted to the Graduate and Professional School
of Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Laura Jurgens
Committee Members,	Anna Armitage
	Lene Petersen
	Masami Fujiwara
Intercollegiate Faculty Chair,	Anja Schulze

August 2021

Major Subject: Marine Biology

ABSTRACT

With intensifying global change ecologists work to understand the effects of anthropogenic and environmental stressors on organisms. Importantly, prior stress can influence population response to later stress. This is often seen in population response to acute stress during extreme events, which may occur after exposure to other chronic environmental stressors. However, experiments on the effects of asynchrony in which stresses are imposed at different times on organisms are rare. I reviewed this small but growing body of literature and show how results in studies incorporating asynchrony often differ from those that examine the effects of multiple stressors imposed on organisms at the same time. In particular, I found that asynchrony rarely leads to additive effects, but rather elicited responses of cross-tolerance (mitigation of latter stress) or cross-susceptibility (exacerbation of the latter stress). Additionally, the majority of asynchronous stressor studies were conducted in North America and Europe and were rare in the rest of the world. I also conducted an experiment on Eastern oysters (*Crassostrea virginica*), for which there are few asynchronous stressor studies. In Galveston Bay, Texas, the hurricane season begins in May, following mild spring water temperatures, but continues through late summer months, when water temperatures can reach 32°C. I asked if oysters respond differently to hurricane-level low-salinity stress after periods of elevated late summer temperatures relative to more standard, early-season conditions. I exposed newly settled oyster spat to early-season (24°C) or late-season high temperatures (32°C) for one month followed by an acute low salinity (1 ppt) disturbance for 10 days (versus control). Oysters experienced the highest mortality

(46%) when high-temperature and low salinity stresses were imposed simultaneously with no prior conditioning. This was significantly different from oysters under asynchronous stressor treatments, which saw only 4% mortality. Additionally, prior thermal stress mitigated some of the negative effects of subsequent osmotic stress leading to significantly lower mortalities (4%) compared to treatments under acute salinity stress without prior heat exposure (16%). Results suggest that incorporating temporal dynamics, rather than simply crossing multiple stressors simultaneously, can have important consequences for our understanding of impacts from extreme events on early, important life stages of organisms.

DEDICATION

To my mom and dad. You paved the way.

ACKNOWLEDGEMENTS

I would like to first and foremost thank my advisor and committee chair, Dr. Laura Jurgens for her guidance and mentorship during my time at TAMUG. I would also like to thank my committee members, Dr. Anna Armitage, Dr. Lene Petersen, and Dr. Masami Fujiwara, for their support through the course of my master's thesis. Additionally, I could not have seen my experiment through to completion without the help of Katie St. Clair and the Sea Life Facility. Thank you also to Dr. Scott Rikard at the Auburn Island Shellfish Laboratory for shipping me oyster spat and providing me with husbandry knowledge.

A special thank you to my lab members Emily Hubbard and Melissa Ciesielski. Their support during my experiment was unparalleled. Additional thanks to Ume, Olivia, Candace, Janelle, Rayna, and Stephanie. Their help on various parts of my thesis from experimental execution to editing help made the process less overwhelming.

I would never have imagined going through a master's degree during a global pandemic. Thank you to the friends that kept me going through facetime calls and coworking with me. Thank you to my best friends Therese and Julia for being a place to land no matter what distance we have between us. Thank you to Erin, Andy, Sam, Stephanie, and Priya for being my role models and holding space for people like us to thrive in marine science.

Finally, the most gratitude to my family. A big thank you to Sneha. You've been my best friend since Day 1 and I cannot believe we are both going to start our PhDs at

the same time together this fall. Navigating the world of academia with you means so much to me. Thank you Lauren. You’re the light in my life and I’m so thankful for your love. I am so grateful for your constant support and for being patient with me while I work. And finally, thank you Mom and Dad, who have stayed awake with me while I’ve done lab work and have been there for me when everything has felt extremely hard. With their support, I knew I couldn’t give up. I am here today because of them and I am so grateful.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

Dr. Laura Jurgens supervised my thesis research with support from Dr. Anna Armitage, Dr. Lene Petersen, and Dr. Masami Fujiwara. Experimental design and set-up were organized with help from Dr. Jurgens, Dr. Armitage, and Dr. Petersen with assistance from Katie St. Clair.

Funding Sources

My master's thesis was supported by the Texas A&M University at Galveston 2-year competitive fellowship and a Marine Biology Department Mini Grant.

TABLE OF CONTENTS

LIST OF FIGURES

1. INTRODUCTION

Understanding interactive effects of environmental and anthropogenic stressors on populations is increasingly urgent for ecologists as global change intensifies (Breitburg et al. 1998; Darling and Côté 2008). Stressors rarely act in isolation, and multiple stressors can interact in surprising ways to influence populations (Folt et al. 1999; Sala et al. 2000; Crain et al. 2008). To date, most multi-stressor studies impose stresses simultaneously. Yet temporal variation in environmental stresses is nearly ubiquitous in nature (Easterling et al. 2000; Pincebourde et al. 2012; Gunderson et al. 2016). Although rarer, scientists are increasingly recognizing that studies incorporating asynchrony in the occurrence of multiple stressors (or single stressors imposed at different times) are important (Gunderson et al. 2016).

I approached this relatively novel form of stressor studies in two ways. In the first chapter, I provide insights from the small but growing body of asynchronous stressor literature on marine plants, invertebrates, and vertebrates. I identify trends within the asynchronous stressor literature and key research gaps. I also describe geographical trends in asynchronous stressor studies. In the second chapter, I delineate the effects of the timing of stressors on the Eastern oyster, *Crassostrea virginica*. Through an experimental study, I investigated whether a prior thermal stress mediates the effects of a subsequent salinity stress on oysters and show how recovery of oyster populations after an extreme event depends on the timing and order of stressors. Taken together, these two chapters provide both background on previous research and foundation for new research in the field of temporal dynamics in multiple stressor studies.

1.1. References

Breitburg DL, Baxter JW, Hatfield CA, et al (1998) Understanding Effects of Multiple Stressors: Ideas and Challenges. In: Pace ML, Groffman PM (eds) Successes, Limitations, and Frontiers in Ecosystem Science. Springer New York, New York, NY, pp 416–431 doi: 10.1007/978-1-4612-1724-4_17

Darling ES, Côté IM (2008) Quantifying the evidence for ecological synergies. Ecol Lett 11:1278–1286. doi: 10.1111/j.1461-0248.2008.01243.x

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecol Lett 11:1304–1315. doi: 10.1111/j.1461-0248.2008.01253.x

Easterling DR, Evans JL, Groisman PY, et al (2000) Observed Variability and Trends in Extreme Climate Events: A Brief Review. Bull Am Meteorol Soc 81:417–425. doi: 10.1175/1520-0477(2000)081<0417:OVATIE>2.3.CO;2

Folt CL, Chen CY, Moore MV, Burnaford J (1999) Synergism and antagonism among multiple stressors. Limnol Oceanogr 44:864–877. doi: 10.4319/lo.1999.44.3_part_2.0864

Gunderson AR, Stillman JH (2015) Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc R Soc B Biol Sci 282:20150401. doi: 10.1098/rspb.2015.0401

Sala OE, Chapin FS, Armesto JJ, et al (2000) Global biodiversity scenarios for the year 2100. Science 287:1770–1774. doi: 10.1126/science.287.5459.1770

Pincebourde S, Sanford E, Casas J, Helmuth B (2012) Temporal coincidence of environmental stress events modulates predation rates. Ecol Lett 15:680–688. doi: 10.1111/j.1461-0248.2012.01785.x

2. ECOLOGICAL EFFECTS OF ASYNCHRONOUS STRESSORS[1]

2.1. Introduction

Studying how asynchronous stressors impact individuals and populations provides new insights for conservation managers seeking to predict ecological responses to real-world variability in the timing of stressors. Recent studies indicate that population and community impacts of acute events, such as large rainfall events or heat waves, can depend on prior stressors and their timing. For example, with no time between stressors, oysters initially exposed to warm temperatures and subsequently to low salinity from rainfall events had higher mortality than when there were two or four weeks between stressors (Bible et al. 2017). At a community level, prior conditions affecting community structure can change responses to later events. For example, predator loss, which may occur from overfishing, decreased resistance to a subsequent hurricane-level salinity disturbance in tropical invertebrate communities (Jurgens et al. 2017).

Asynchrony in stressors can also exacerbate or mitigate effects on populations that may be masked by only studying multiple stressors simultaneously. For example, researchers observed that asynchronous sediment and nutrient disturbances on stream benthic assemblages led to a larger decrease in abundance and species diversity than when seen with simultaneous stressors (García Molinos and Donohue 2010). While they remain comparatively few, asynchronous stressor studies such as these are emerging as

---

[1] In review at *Oecologia*

an important body of research. They offer the potential for new insights into the realized impact of dynamic stresses to populations in a rapidly changing world.

Here I review the current state of empirical asynchronous stressor studies, focusing on aquatic systems globally. Marine and freshwater aquatic environments around the world experience a broad set of increasing global change stressors, including ocean acidification, high temperatures and marine heat waves, salinity changes, harmful algal blooms, eutrophication, sedimentation, increased frequency of major storms, sea level rise, habitat destruction, massive industrial fishing, and more (Doney et al. 2012; McCauley et al. 2015). These stresses span a wide variety of temporal scales from acute to chronic.

I examine whether the trends in the literature suggest predictable effects of asynchrony in stressors on populations and explore patterns in the abundance of asynchrony experiments relative to other multi-stressor work. I also investigated topical and geographical patterns in asynchrony studies to uncover regional trends and information gaps. This small, but informative body of literature offers exciting patterns in organismal response to stressors as well as opportunities for conservation managers and ecologists to understand how organisms respond to stressors in real-world, temporally dynamic situations.

2.2. Methods

Asynchronous stressor studies have added conditionality, and I define interactions between stressor effects on organisms slightly differently than is typical for synchronously imposed multi-stressor experiments, which tend to classify interactions as additive, synergistic, or antagonistic. Like synchronous studies, I use the term “additive” to describe situations in which the overall outcome is the sum of the two individual stressor effects, and there is no evidence of interactions between the stressors (Breitburg et al. 1998; Folt et al. 1999; Crain et al. 2008; Piggott et al. 2015). I use the term “cross-tolerance” to describe a situation where the first stressor increases tolerance to, or ameliorates, the effect of a second, subsequent stressor. One of the first instances of this term was in a medical context, where prior ethanol exposure increased later tolerance to heat and Adriamycin in mammalian cells (Li and Hahn 1978). When the opposite occurs, I term the increased susceptibility to the second stressor following exposure to the first “cross-susceptibility.” This term also had its origins in pharmacology in determining the toxicity of four drugs to mammalian cells (Nord et al. 1967). These terms are now frequently used in stressor literature.

I conducted literature searches to identify empirical studies of asynchronous stressors across a broad diversity of stressors in marine and aquatic systems. My first aim was to compare the number of asynchronous stressor papers against the number of single stressors and synchronous multiple stressor papers. I conducted literature searches on the Web of Science platform with the following criteria [“ecology” OR “marine”] AND [“temporal” OR “timing”] AND “multiple stressor*.” I used “marine” specifically as a search term to account for asynchronous stressor work in biological oceanography contexts. I then searched using the terms “asynchron*” AND “stressor*.” I conducted the same searches in Google Scholar using the above search criteria and also added the terms “cross-tolerance,” “timing of stressors,” and “experiment” to generate additional results. I removed irrelevant results, including papers focused predominantly on human health, paleobiology, geography, modelling, and management (where there was no empirical study described). I then categorized papers separately as asynchronous, versus those that examined single stressors and multiple stressors applied synchronously. For asynchronous stressor studies, I included papers examining different stressors imposed at different time points and also those studying a single stressor imposed at multiple time points. I focused on studies that exposed organisms to stressors within their own lifetime, or within the same generation. I did not include transgenerational experiments, as these incorporate additional complexities, including heritability and maternal effects. Finally, I removed literature reviews that did not include new experimental papers. To further fill out the repertoire of asynchronous stressor research, I did a one-step backwards search by examining all references in each publication.

To examine whether there were generalizable effects of stressor asynchrony, I classified experimental outcomes based on treatment effects compared to controls, for the endpoints studied. I used my definitions of additive effects, cross-tolerance, and cross-susceptibility as defined above to classify these outcomes.

In order to examine geographic trends in asynchrony research, I mapped studies based on location and focal stressors. I defined locations as either the field sites at which organisms were collected or the location of the laboratory where the studies were conducted in cases where the organisms were ordered from breeders or suppliers. Due to the absence of papers in many regions, I also conducted geographically focused literature searches, which did not reveal any additional papers in large regions such as Australia, Africa, Antarctica, the Arctic, Brazil, China, Europe, Japan, or the Mediterranean. These searches were [“marine” OR “ecology”] AND “multiple stressor*” AND [“Australia” OR “Africa” OR “Antarctica” OR “Arctic” OR “Brazil” OR “China” OR “Europe” OR “Japan” OR “Mediterranean”].

2.3. Trends in Asynchronous Stressor Literature

Of the 2275 search results from both Web of Science and Google Scholar that included the terms “temporal” or “timing”, only 2.5% of these discussed the effects of asynchronous stressors. This initial search yielded only 57 papers describing asynchronous stressor studies, of which 43 were empirical studies. Including the backwards search of literature cited in these papers yielded 81 total papers describing 90 asynchronous stressor experiments (Appendix A). An upward trend in the frequency of asynchronous studies over the last ten years nevertheless shows relatively few papers on this subject relative to simultaneous multiple stressor studies (Fig. 2.1).

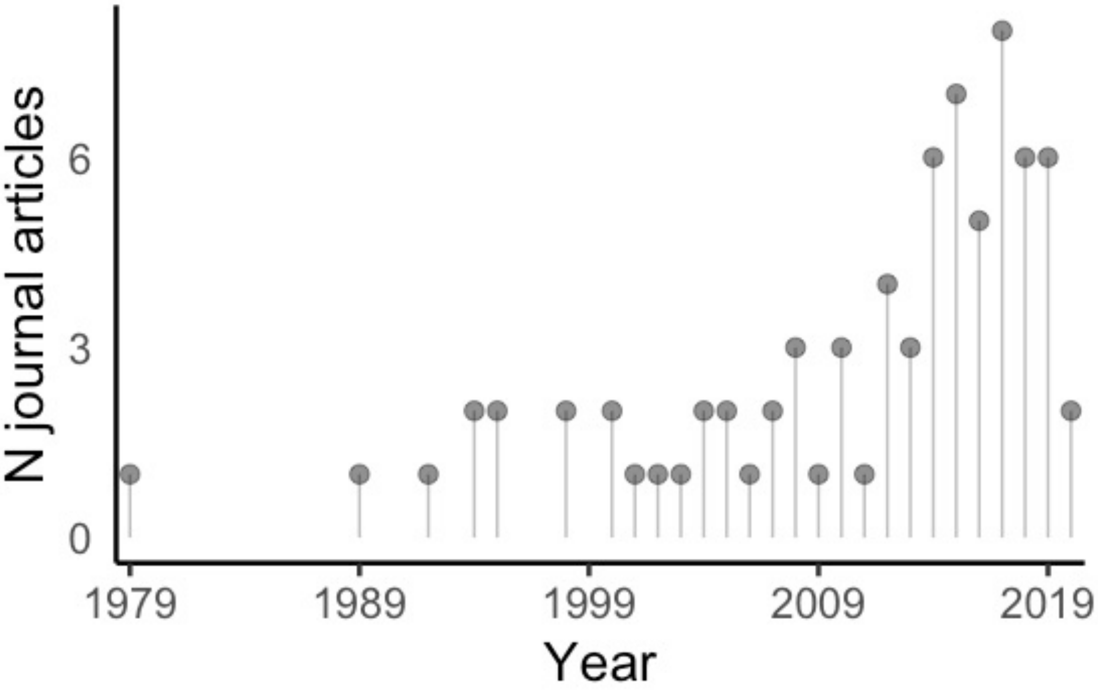

Figure 2.1 Trends in the number of peer-reviewed publications containing experiments on asynchronous multiple stressor effects on aquatic organisms from 1979-2020.

The 90 experiments I found spanned 12 stressors in varying combinations. Temperature was the most commonly studied stressor, followed by salinity, pollutants (including metals, pesticides and plastics), pH, biotic stressors (including viral and bacterial infections and predation), and hypoxia (Fig. 2.2). The least commonly studied stressors included changes in nutrition, physical disturbances, desiccation, ultraviolet radiation, ammonia, and sedimentation. Overall, experiments exhibited an almost even distribution of cross-tolerance (N = 40), in which the prior stress ameliorates the latter, and cross-susceptibility effects (N = 35), where the prior stress exacerbates the latter (Fig. 2.2). A small proportion of experiments yielded additive effects on organisms (N =

13), and two showed that interactions between stressors were not definitive as cross-tolerance, cross-susceptibility, or additive. I found additive effects in experiments studying physical disturbances or those in which there was no interaction between stressors. For example, in mussel bed communities, physical storm-like disturbances generally led to additive effects on presence of individual taxa and were mediated by other factors such as the presence of foundation species (Oliveira et al. 2014). Physical disturbance events tend to simply tack on to effects of prior stressors as they primarily lead to removal of organisms. In other cases, with enough time between stressors, organisms may fully recover, leading to uncoupling of effects. For example, in oysters, the seasonality of hypoxic, thermal, and osmotic stressors allowed oysters to recover from the effects of one stressor before exposure to a subsequent stressor (Cheng et al. 2015).

There were several experiments (N=24) conducted with the same stressor twice, which are commonly known as acclimation studies. Acclimation and acclimatization are well-known physiological responses to multiple exposures of the same stressor. It therefore is unsurprising that cross-tolerance in asynchronous stressor studies was commonly observed when organisms were exposed to the same stressor twice, including experiments with temperature (N = 10), salinity (N = 2), pollution (N = 1), pH (N = 1), and hypoxia (N = 2). These experiments form the largest body of asynchronous research to date and demonstrate the potential insights to be gleaned from studying asynchronous stressors more broadly.

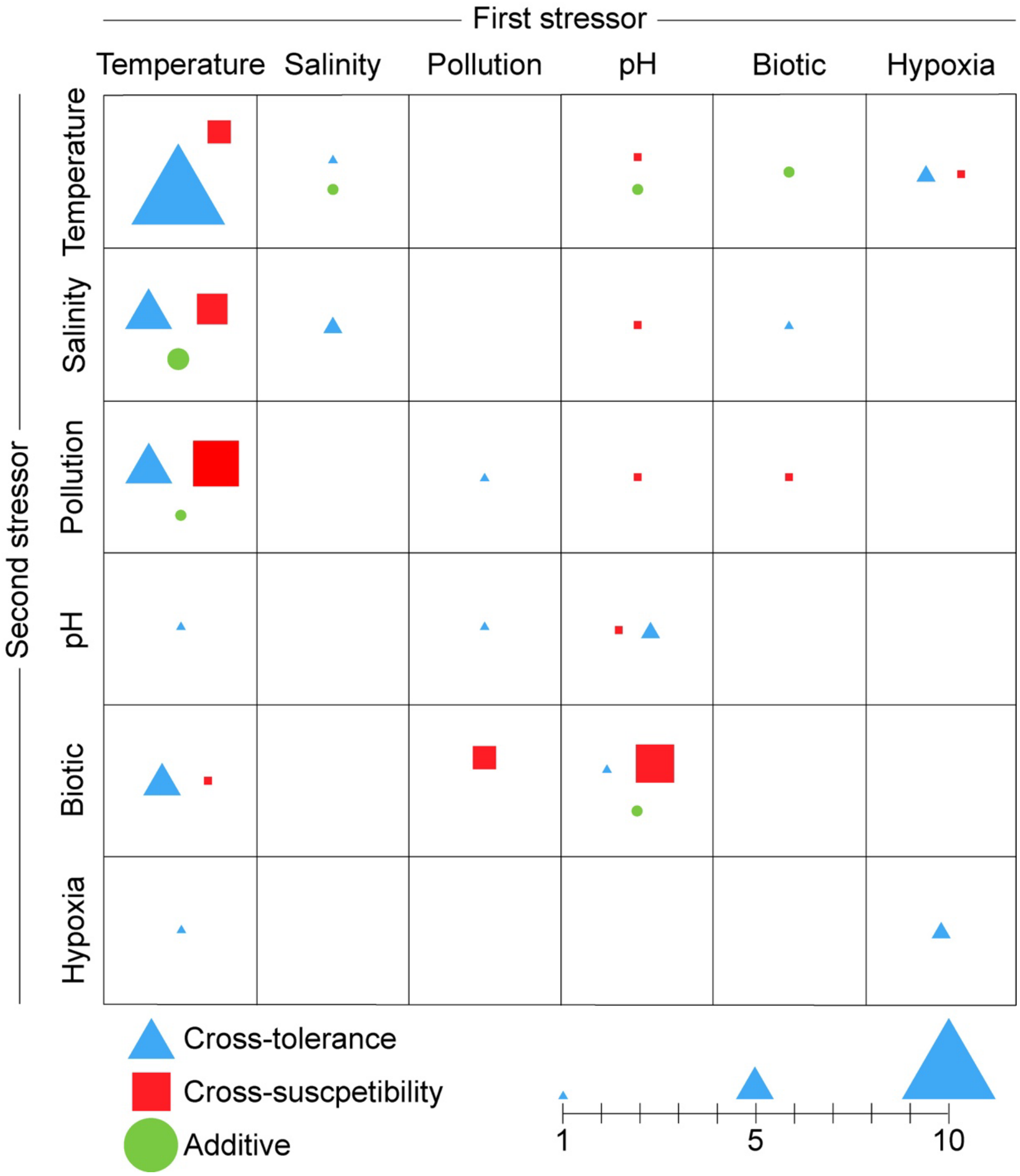

Figure 2.2 Trends in asynchronous stressor studies by stressor combination, showing outcomes on organisms. I included the six most common stressors studied. Symbol size scales with the number of studies on a specific stressor combination.

2.4. Insights and Surprises from Asynchronous Stressor Studies

Some of the most familiar and common asynchronous stress experiments — those of thermal acclimation — provide an example of the important insights for conservation that we can gain from considering stress timing. For example, thermal acclimation research has been critical to predicting potential population impacts and range shifts with climate warming (Calosi et al. 2008; Somero 2010; Gunderson and Stillman 2015). Thermal acclimation can expand the upper thermal range of organisms as they recruit the same metabolic pathways to cope with the subsequent thermal challenge. For example, work on goby fishes demonstrated that organisms exposed to temperatures above a normal functioning range recruit heat shock proteins (HSPs), which assist in protein re-folding during later, more extreme thermal stress exposure (Dietz and Somero 1992; Parsell 1993). Further research bears this out, including work showing oysters under an initial heat shock of 34°C induced HSPs, which then protected them against extreme heat shocks of up to 39°C (Brown et al. 2004). However, the production of HSPs itself does not confer tolerance, but rather the magnitude of the initial heat shock, frequency of exposure, and the timing between stress periods all play important roles. Importantly, HSP production ceases a few hours after the initial thermal shock and may not be stable for long periods. For example, newly synthesized HSPs were stable for only 12 hours following heat shock in salmon (DuBeau et al. 1998). Repeated exposure also does not guarantee acclimatization. Repeated exposure to sublethal temperatures in copepods actually reduced their thermal tolerance, leading to mortality (Siegle et al. 2018). Therefore, a variety of information is necessary to understand temporal dynamics in stress tolerance, even with single, repeated stressors.

Interestingly, HSPs appear to be a major mechanism of cross-tolerance even in situations where the latter stressor is not thermal. Changes in salinity, lowered pH, hypoxia or even exposure to pollutants such as pesticides, may all be mitigated by previously induced HSPs. For example, although a 10°C thermal shock had no effect, and a 15°C led to mortality, a 12°C thermal shock enabled tidepool sculpin to tolerate subsequent severe hypoxia and osmotic stress (Todgham et al. 2005). Similar responses have been seen in rainbow trout. Heat-shocked rainbow trout experienced lower plasma osmolarity and chloride levels during acute osmotic stress than their non-heat-shocked counterparts, showing some mild effects of prior thermal shock on subsequent salinity stress (Niu et al. 2008). HSPs may also influence pollutant responses. Blue mussels exposed to thermal stress recruited HSPs that increased tolerance against cadmium exposure (Tedengren et al. 2000). Therefore, where temperature is the first of two stressors imposed, organisms employ HSPs to respond, and these HSPs often protect against the second stressor. Other prior stressors, however, trigger different physiological responses, and may confer less resistance to later events.

As organisms use diverse mechanisms of stress response, the order of exposure may determine cross-tolerance or cross-susceptibility. For example, snails exposed to lowered pH followed by heavy metals experienced cross-susceptibility, but those from sites heavily polluted with metals had reduced sensitivity to acidified waters (Lefcort et al. 2015). This sort of change from cross-tolerance to cross-susceptibility is noted in other cases too. Aquatic beetles, for instance, experienced cross-tolerance when first exposed to salinity and then desiccation, but cross-susceptibility when the order of stressors was reversed (Pallarés et al. 2017). Some of this variability in outcomes can be attributed to certain prior stressors targeting organisms' immune responses.

When initial stressors compromise rather than boost immune response, they can undermine an organisms' capacity to respond to subsequent stressors. For example, calcifying organisms such as bivalves and gastropods may already be metabolically weakened under ocean acidification and experience immunosuppression. Oysters, energetically overwhelmed at lower pH, can undergo significant oxidative damage when exposed to pathogens such as *Vibrio* (Cao et al. 2018 a,b). Prior stressors can, therefore, physiologically, immunologically, and even behaviorally alter organisms, rendering them more susceptible to future stressors that specifically compromise these systems further.

Behavioral alterations in response to stressors may hamper organisms' predator responses. Larval topsmelt exposed to lowered pH or pollutants for short periods of time, for example, were unable to detect chemical cues of predators when later removed from prior stressors (Renick et al. 2015). In one study, fish eggs that were exposed to lowered pH, became attracted to predator species as settlement-stage larvae, thereby threatening their survival (Dixson et al. 2010). Similarly, fish under sublethal concentrations of pesticides for even four hours experienced behavioral changes and were more susceptible to subsequent predation risk seven days later in non-polluted water (Floyd et al. 2008). Imposing these stressors simultaneously would mask the ability to detect asynchronous multi-stressor interactions moderated by behavioral systems. However, the outcomes of some stressor combinations are not always as easily generalizable.

For some stressor combinations, there is a split between studies that find cross-tolerance versus cross-susceptibility, which appears to depend on the taxon in question. For example, sea mussels recruited HSPs in response to thermal stress, and experienced cross-tolerance to subsequent cadmium exposure, whereas other organisms such as rainbow trout and freshwater snails performed better under cadmium exposure when they were cold-acclimated (Roch and Maly 1979; Møller et al. 1994; Tedengren et al. 2000). Rainbow trout and freshwater snails, in these instances, experienced energy deficiencies when exposed to heat and then cadmium. We see a similar split between responses to initial thermal and later osmotic stresses. In all of the studies conducted on fish, HSPs produced during thermal stress alleviated the effects of osmotic stress, thereby leading to cross-tolerance (DuBeau et al. 1998; Todgham et al. 2005; Niu et al. 2008). Yet in most cases of phytoplankton or invertebrates, a prior thermal stress worsened the effects of a subsequent osmotic stress (Chen and Chen 2000; Stefanidou et al. 2019). However, sea cucumbers recruited HSPs to ameliorate the effects of osmotic stress (Dong et al. 2008). This organismal difference in response could be idiosyncratic or could be due to the specific experimental conditions needed to recruit HSPs to aid in thermal and osmotic pressures. Further, in some cases of cross-tolerance, the benefits of asynchrony appear to arise at the expense of other organismal functions. For example, oysters in acidified waters were better able to deal with pathogens, but at the cost of increased metabolism and oxidative stress, which could require higher rates of food consumption for survival and energy re-allocation (Cao et al. 2018a; Gurr et al. 2020).

Asynchronous stressor studies can also reveal consequences of stress variability on short, and even diurnal, time scales. Short-term stress asynchrony is commonly seen during tide cycles, when stressors fluctuate over the course of the day. Sea stars, for instance, may experience lasting impacts of thermal stress from daytime low tides. When this was followed by physiologically stressful high water temperatures at high tide, researchers observed reduced feeding performance (Pincebourde et al. 2012). These effects were only seen when study design incorporated realistic fluctuations in stressors, rather than conducting experiments with constant stressors (Pincebourde et al. 2012). Similarly, researchers have found that when diurnal temperature and pH fluctuations were varied asynchronously on porcelain crabs, there was a depression in metabolic activity (Paganini et al. 2014). There were, however, no negative effects of these stressors without diurnal asynchrony (Paganini et al. 2014). Considering the dynamic nature of most habitats, as well as the complexity of organismal physiology, asynchronous studies can offer novel information about stress responses.

It follows then, that the study of organismal stress must also take into consideration complex life histories, which can result in temporally variable stress exposure. Several aquatic species spend early life stages in one habitat, and later ones in a different habitat. Some species carry over effects from one life stage to another, leading to cross-susceptibility in later life stages. For example, if damselfly eggs are exposed to thermal stress, larval stages can have increased sensitivities to pesticides or future thermal stress

(Sniegula et al. 2017; Janssens et al. 2017). On the other hand, later, post-larval life stages had no stress memory from their earlier life stages and were unable to recruit mechanisms to defend against future thermal stressors (Sniegula et al. 2017; Janssens et al. 2017). While larval zebrafish exposed to higher temperatures better tolerated acute hypoxia, embryonic zebrafish exposed to higher temperatures did not carry over critical transcription factors to aid against hypoxia in the larval stage (Levesque et al. 2019). Therefore, studying asynchrony in this fashion provides a more complete story of the way an organism responds to stressors within its lifetime.

The vast differences seen across species and stressors in the studies I reviewed emphasize the need to study asynchronous stressors across a variety of systems, if we are to better predict how organisms will respond to continued anthropogenic change.

2.5. Geographical Distribution of Asynchronous Multiple Stressor Studies

Roughly 85% of asynchronous stressor studies were conducted in North America or Europe, with few other locations represented (Fig. 2.3). I note that I only considered English language journals in this review, which could have excluded studies published in non-English journals. That being said, I found no studies from Africa or Antarctica, and few from Central America, Asia, and Australia.

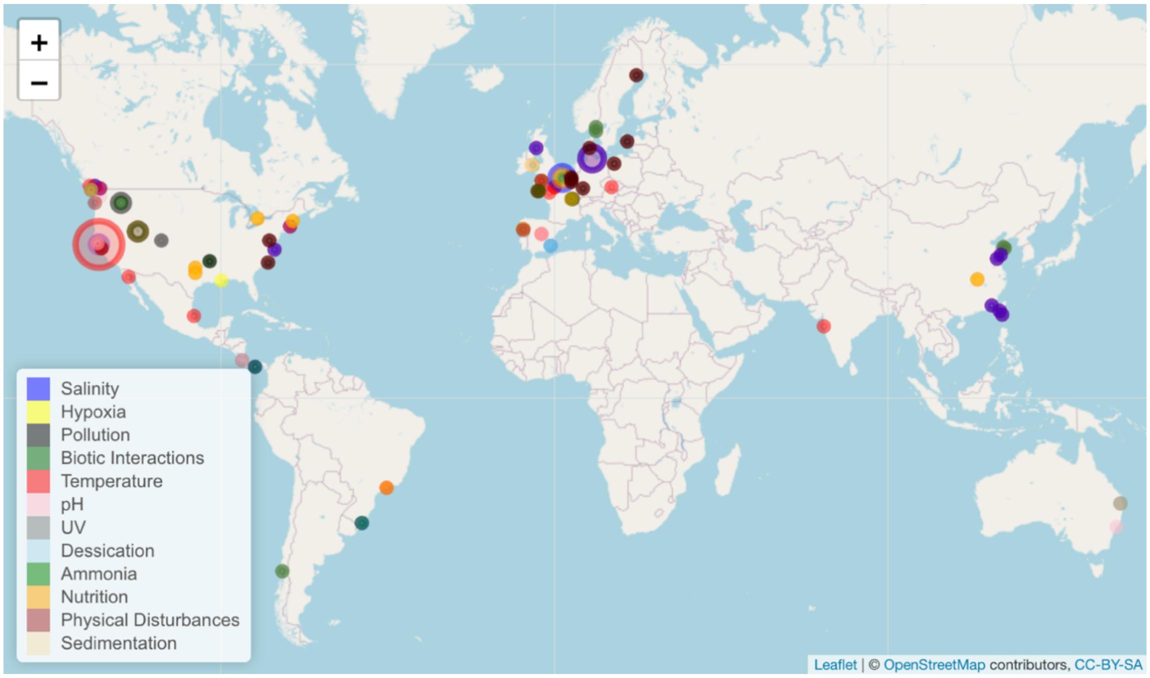

Figure 2.3 Global distribution of asynchronous multi-stressor studies classified by stressor, in aquatic systems. Circle size scales with the number of studies on a given stressor at the same location. Interactive map available on request.

This geographic bias, with the most number of papers from Europe and North America, is commonly seen in the fields of ecology and global change biology (Wilson et al. 2007; Pyšek et al. 2008; Martin et al. 2012; Archer et al. 2014). Reviews on terrestrial ecology and invasion ecology encompassing over 8000 and 2760 papers, respectively, found that studies were concentrated in Central America, North America, and Europe (Pyšek et al. 2008; Martin et al. 2012). Another review on pollinator ecology reported similar geographic bias, with only 4% of papers coming from the continent of Africa (Archer et al. 2014). Within the field of asynchronous multiple stressors, this geographic bias is likely due to the few research groups studying asynchronous multiple stressors.

Regional impacts of stressors may also explain regional trends in research as seen by a number of temperature-related studies in western Europe and salinity-related studies on the west coast of the United States. Several studies in western Europe specifically cite short-lived heat waves for their rationale in studying the effects of temperature on organisms in asynchronous stressor studies. Heat waves are acute stressor events, and are usually short-lived but can interact with prior or subsequent stressors on organisms in the region (Labaude et al. 2017; Péden et al. 2018). Following heat wave conditions, damselflies, for example, experienced greater mortality when exposed to agricultural pesticides (Dinh et al. 2016). These events increased the susceptibility of organisms to later stressors in most cases, explaining not only the extent of their detrimental effects, but also providing predictions on how organisms will fare under extreme events like heat waves.

Similarly, the frequency of severe rainfall events in the region explains the large number of studies on salinity stresses on the west coast of the United States. The San Francisco Bay receives about 40% of California's rainfall; but as climate change alters weather patterns, the intensity and frequency of low salinity events may increase (Miller et al. 2014; Bible et al. 2017; Chang et al. 2018). The majority of this rain and resulting decreases in salinity occur during winter months, asynchronous with other stressors such as summertime high temperatures. Similarly, in the Pacific Northwest of the U.S., runoff can be very sensitive to changes in precipitation and snowmelt (Elsner et al. 2010). A study that simulated lowered pH followed by lowered salinity from sudden runoff events found that this sequential nature of stressors led to cross-susceptibility and increased oxygen consumption in crab larvae as opposed to studies that exposed larvae to these stressors synchronously (Miller et al. 2014). Specific climatic events in different geographical regions, therefore, warrant studies that capture the seasonality of stressors on organisms if we are to accurately predict risk to populations.

There is, therefore, a need for future stressor studies on organisms to incorporate temporal dynamics of stressors as many regions experience stressors decoupled in time from one another. Doing so would allow for a better representation of how organisms respond to stressors in real-world scenarios. Overall, the 81 papers on asynchronous multiple stressors provide informative results on the interactions between stressors and the need for incorporating temporal variability of these stressors in experiments. However, due to the relative scarcity of these studies relative to other multiple stressor studies, we are not yet able to make broad-scale predictions on the effects of asynchronous multiple stressors on organisms. Additional asynchrony studies are especially important in poorly studied geographic regions and less-explored stressor combinations (Figs. 2.2 and 2.3) to amass critical information on how organisms will fare under current and future regimes of climate change.

2.6. Conclusion

To inform practical conservation strategies in light of global change, the most useful research works to understand mechanisms of stress impacts to populations while attempting to capture the complexities of real-world environmental variability. Laboratory studies allow us to tease apart those specific mechanisms by which organisms protect themselves against stressors, and therefore attempt to predict vulnerability. While all the dynamics and variability from the field can never be completely replicated in a controlled setting, it is of broad scientific interest to replicate field conditions, including temporal dynamics, where possible. Asynchrony in stressors is probably much more common than not for organisms in natural populations. As we see here, asynchrony can result in surprising outcomes impossible to predict from simultaneously imposed (e.g., simple factorial) stress experiments. Single and multiple stressor studies have certainly contributed much information to the fields of stressor physiology and conservation, but by adding temporal dynamics relevant to the ecosystems that organisms occupy, we arrive at more detailed and accurate mechanistic information coupled with novel real-world insights about organismal vulnerabilities.

Understanding the outcomes of stressor interactions on organisms can also enable conservation managers to better apply scarce resources. For example, managers may want to prioritize investments that mitigate situations where cross-susceptibility is more likely than cross-tolerance (Côté et al. 2016). Similar mechanistic details for synchronous multi-stressor situations have yielded such benefits before. For example, abating multiple, synergistic stresses in seagrass ecosystems provided benefits, but mitigating antagonistic stressors was detrimental to the ecosystem (Brown et al. 2013). Similarly, more detailed information from a growing body of asynchronous stressor studies will fill important gaps in understanding how to conserve species and populations facing an increasingly large array of anthropogenic changes occurring across a broad variety of time scales.

2.7. References

Archer CR, Pirk CWW, Carvalheiro LG, Nicolson SW (2014) Economic and ecological implications of geographic bias in pollinator ecology in the light of pollinator declines. Oikos 123:401–407. doi: 10.1111/j.1600-0706.2013.00949.x

Bible JM, Cheng BS, Chang AL, et al (2017) Timing of stressors alters interactive effects on a coastal foundation species. Ecology 98:2468–2478. doi: 10.1002/ecy.1943

Breitburg DL, Baxter JW, Hatfield CA, et al (1998) Understanding Effects of Multiple Stressors: Ideas and Challenges. In: Pace ML, Groffman PM (eds) Successes, Limitations, and Frontiers in Ecosystem Science. Springer New York, New York, NY, pp 416–431 doi: 10.1007/978-1-4612-1724-4_17

Brown CJ, Saunders MI, Possingham HP, Richardson AJ (2013) Managing for Interactions between Local and Global Stressors of Ecosystems. PLOS One 8:e65765. doi: 10.1371/journal.pone.0065765

Brown HM, Briden A, Stokell T, et al (2004) Thermotolerance and HSP70 profiles in adult and embryonic California native oysters, *Ostreola conchaphila* (Carpenter, 1857). J Shellfish Res 23:135–141

Calosi P, Bilton DT, Spicer JI (2008) Thermal tolerance, acclimatory capacity and vulnerability to global climate change. Biol Lett 4:99–102. doi: 10.1098/rsbl.2007.0408

Cao R, Liu Y, Wang Q, et al (2018a) Seawater acidification reduced the resistance of *Crassostrea gigas* to *Vibrio splendidus* challenge: An energy metabolism perspective. Front Physiol 9:880. doi: 10.3389/fphys.2018.00880

Cao R, Wang Q, Yang D, et al (2018b) CO2-induced ocean acidification impairs the immune function of the Pacific oyster against *Vibrio splendidus* challenge: An integrated study from a cellular and proteomic perspective. Sci Total Environ 625:1574–1583. doi: 10.1016/j.scitotenv.2018.01.056

Chang AL, Brown CW, Crooks JA, Ruiz GM (2018) Dry and wet periods drive rapid shifts in community assembly in an estuarine ecosystem. Glob Chang Biol 24:e627–e642. doi: 10.1111/gcb.13972

Chen JC, Chen WC (2000) Salinity tolerance of Haliotis diversicolor supertexta at different salinity and temperature levels. Aquaculture 181:191–203. doi: 10.1016/S0044-8486(99)00226-4

Cheng BS, Bible JM, Chang AL, et al (2015) Testing local and global stressor impacts on a coastal foundation species using an ecologically realistic framework. Glob Chang Biol 21:2488–2499. doi: 10.1111/gcb.12895

Côté IM, Darling ES, Brown CJ (2016) Interactions among ecosystem stressors and their importance in conservation. Proc R Soc B Biol Sci 283:20152592. doi: 10.1098/rspb.2015.2592

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecol Lett 11:1304–1315. doi: 10.1111/j.1461-0248.2008.01253.x

Darling ES, Côté IM (2008) Quantifying the evidence for ecological synergies. Ecol Lett 11:1278–1286. doi: 10.1111/j.1461-0248.2008.01243.x

Dietz TJ, Somero GN (1992) The threshold induction temperature of the 90-kDa heat shock protein is subject to acclimatization in eurythermal goby fishes (genus *Gillichthys*). Proc Natl Acad Sci U S A 89:3389–3393. doi: 10.1073/pnas.89.8.3389

Dinh KV, Janssens L, Stoks R (2016) Exposure to a heat wave under food limitation makes an agricultural insecticide lethal: a mechanistic laboratory experiment. Glob Chang Biol 22:3361–3372. doi: 10.1111/gcb.13415

Dixson DL, Munday PL, Jones GP (2010) Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. Ecol Lett 13:68–75. doi: 10.1111/j.1461-0248.2009.01400.x

Doney SC, Ruckelshaus M, Emmett Duffy J, et al (2012) Climate Change Impacts on Marine Ecosystems. Ann Rev Mar Sci 4:11–37. doi: 10.1146/annurev-marine-041911-111611

Dong Y, Dong S, Meng X (2008) Effects of thermal and osmotic stress on growth, osmoregulation and Hsp70 in sea cucumber (*Apostichopus japonicus* Selenka). Aquaculture 276:179–186. doi: 10.1016/j.aquaculture.2008.01.028

DuBeau SF, Pan F, Tremblay GC, Bradley TM (1998) Thermal shock of salmon in vivo induces the heat shock protein hsp 70 and confers protection against osmotic shock. Aquaculture 168:311–323. doi: 10.1016/S0044-8486(98)00358-5

Easterling DR, Evans JL, Groisman PY, et al (2000) Observed Variability and Trends in Extreme Climate Events: A Brief Review. Bull Am Meteorol Soc 81:417–425. doi: 10.1175/1520-0477(2000)081<0417:OVATIE>2.3.CO;2

Elsner MM, Cuo L, Voisin N, et al (2010) Implications of 21st century climate change for the hydrology of Washington State. Clim Change 102:225–260. doi: 10.1007/s10584-010-9855-0

Floyd EY, Geist JP, Werner I (2008) Acute, sublethal exposure to a pyrethroid insecticide alters behavior, growth, and predation risk in larvae of the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 27:1780–1787. doi: 10.1897/07-448.1

Folt CL, Chen CY, Moore MV, Burnaford J (1999) Synergism and antagonism among multiple stressors. Limnol Oceanogr 44:864–877. doi: 10.4319/lo.1999.44.3_part_2.0864

García Molinos J, Donohue I (2010) Interactions among temporal patterns determine the effects of multiple stressors. Ecol Appl 20:1794–1800. doi: 10.1890/10-0018.1

Gunderson AR, Armstrong EJ, Stillman JH (2016) Multiple Stressors in a Changing World: The Need for an Improved Perspective on Physiological Responses to the Dynamic Marine Environment. Ann Rev Mar Sci 8:357–378. doi: 10.1146/annurev-marine-122414-033953

Gunderson AR, Stillman JH (2015) Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc R Soc B Biol Sci 282:20150401. doi: 10.1098/rspb.2015.0401

Gurr SJ, Vadopalas B, Roberts SB, Putnam HM (2020) Metabolic recovery and compensatory shell growth of juvenile Pacific geoduck *Panopea generosa* following short-term exposure to acidified seawater. Conserv Physiol 8. doi: 10.1093/conphys/coaa024

Janssens L, Tüzün N, Stoks R (2017) Testing the time-scale dependence of delayed interactions: A heat wave during the egg stage shapes how a pesticide interacts with a successive heat wave in the larval stage. Environ Pollut 230:351–359. doi: 10.1016/j.envpol.2017.06.082

Jurgens LJ, Freestone AL, Ruiz GM, Torchin ME (2017) Prior predation alters community resistance to an extreme climate disturbance. Ecosphere 8:e01986. doi: 10.1002/ecs2.1986

Labaude S, Rigaud T, Cézilly F (2017) Additive effects of temperature and infection with an acanthocephalan parasite on the shredding activity of *Gammarus fossarum* (Crustacea: Amphipoda): the importance of aggregative behavior. Glob Chang Biol 23:1415–1424. doi: 10.1111/gcb.13490

Lefcort H, Cleary DA, Marble AM, et al (2015) Snails from heavy-metal polluted environments have reduced sensitivity to carbon dioxide-induced acidity. Springerplus 4:267. doi: 10.1186/s40064-015-1073-9

Levesque KD, Wright PA, Bernier NJ (2019) Cross talk without cross tolerance: effect of rearing temperature on the hypoxia response of embryonic zebrafish. Physiol Biochem Zool 92:349–364. doi: 10.1086/703178

Li GC, Hahn GM (1978) Ethanol-induced tolerance to heat and to adriamycin. Nature 274:699–701. doi: 10.1038/274699a0

Martin LJ, Blossey B, Ellis E (2012) Mapping where ecologists work: Biases in the global distribution of terrestrial ecological observations. Front Ecol Environ 10:195–201. doi: 10.1890/110154

McCauley DJ, Pinsky ML, Palumbi SR, et al (2015) Marine defaunation: Animal loss in the global ocean. Science 347:1255641–1255641. doi: 10.1126/science.1255641

Miller SH, Zarate S, Smith EH, et al (2014) Effect of elevated pCO2 on Metabolic Responses of Porcelain Crab (*Petrolisthes cinctipes*) Larvae Exposed to Subsequent Salinity Stress. PLOS One 9:. doi: 10.1371/journal.pone.0109167

Møller V, Forbes VE, Depledg MH (1994) Influence of acclimation and exposure temperature on the acute toxicity of cadmium to the freshwater snail *Potamopyrgus antipodarum* (hydrobiidae). Environ Toxicol Chem 13:1519–1524. doi: 10.1002/etc.5620130914

Niu CJ, Rummer JL, Brauner CJ, Schulte PM (2008) Heat shock protein (Hsp70) induced by a mild heat shock slightly moderates plasma osmolarity increases upon salinity transfer in rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol C Toxicol Pharmacol 148:437–444. doi: 10.1016/j.cbpc.2008.04.011

Nord NM, Watanabe F, Parker RH, Hoeprich PD (1967) Comparative Acute Toxicity of Four Drugs: A Study of Neomycin, Gentamicin, Kanamycin, and Dihydrostreptomycin. Arch Intern Med 119:493–502. doi: 10.1001/archinte.1967.00290230131004

Oliveira JP, Sousa-Pinto I, Weber GM, Bertocci I (2014) Interplay of experimental harvesting and climate-related disturbance on benthic assemblages of rocky seashores. Mar Ecol Prog Ser 495:131–142. doi: 10.3354/meps10574

Paganini AW, Miller NA, Stillman JH (2014) Temperature and acidification variability reduce physiological performance in the intertidal zone porcelain crab *Petrolisthes* *cinctipes*. J Exp Biol 217:3974–3980. doi: 10.1242/jeb.109801

Pallarés S, Botella-Cruz M, Arribas P, et al (2017) Aquatic insects in a multistress environment: Cross-tolerance to salinity and desiccation. J Exp Biol 220:1277–1286. doi: 10.1242/jeb.152108

Parsell D (1993) The Function of Heat-Shock Proteins in Stress Tolerance: Degradation and Reactivation of Damaged Proteins. Annu Rev Genet 27:437–496. doi: 10.1146/annurev.genet.27.1.437

Péden R, Rocher B, Chan P, et al (2018) Highly polluted life history and acute heat stress, a hazardous mix for blue mussels. Mar Pollut Bull 135:594–606. doi: 10.1016/j.marpolbul.2018.07.066

Piggott JJ, Townsend CR, Matthaei CD (2015) Reconceptualizing synergism and antagonism among multiple stressors. Ecol Evol 5:1538–1547. doi: 10.1002/ece3.1465

Pincebourde S, Sanford E, Casas J, Helmuth B (2012) Temporal coincidence of environmental stress events modulates predation rates. Ecol Lett 15:680–688. doi: 10.1111/j.1461-0248.2012.01785.x

Pyšek P, Richardson DM, Pergl J, et al (2008) Geographical and taxonomic biases in invasion ecology. Trends Ecol Evol 23:237–244. doi: 10.1016/j.tree.2008.02.002

Renick VC, Anderson TW, Morgan SG, Cherr GN (2015) Interactive effects of pesticide exposure and habitat structure on behavior and predation of a marine larval fish. Ecotoxicology 24:391–400. doi: 10.1007/s10646-014-1388-2

Roch M, Maly EJ (1979) Relationship of cadmium-induced hypocalcemia with mortality in rainbow trout (*Salmo gairdneri*) and the influence of temperature on toxicity. J Fish Res Board Canada 36:1297–1303. doi: 10.1139/f79-187

Sala OE, Chapin FS, Armesto JJ, et al (2000) Global biodiversity scenarios for the year 2100. Science 287:1770–1774. doi: 10.1126/science.287.5459.1770

Siegle MR, Taylor EB, O'Connor MI (2018) Prior heat accumulation reduces survival during subsequent experimental heat waves. J Exp Mar Bio Ecol 501:109–117. doi: 10.1016/j.jembe.2018.01.012

Sniegula S, Janssens L, Stoks R (2017) Integrating multiple stressors across life stages and latitudes: Combined and delayed effects of an egg heat wave and larval pesticide exposure in a damselfly. Aquat Toxicol 186:113–122. doi: 10.1016/j.aquatox.2017.02.029

Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine "winners" and "losers." J Exp Biol 213:912–920. doi: 10.1242/jeb.037473

Stefanidou N, Genitsaris S, Lopez-Bautista J, et al (2019) Response of a coastal Baltic Sea diatom-dominated phytoplankton community to experimental heat shock and changing salinity. Oecologia 191:461–474. doi: 10.1007/s00442-019-04502-0

Tedengren M, Olsson B, Reimer O, et al (2000) Heat pretreatment increases cadmium resistance and HSP 70 levels in Baltic Sea mussels. Aquat Toxicol 48:1–12. doi: 10.1016/S0166-445X(99)00030-2

Todgham AE, Schulte PM, Iwama GK (2005) Cross-tolerance in the tidepool sculpin: The role of heat shock proteins. Physiol Biochem Zool 78:133–144. doi:

10.1086/425205

Wilson JR, Procheş Ş, Braschler B, et al (2007) The (bio)diversity of science reflects the interests of society. Front Ecol Environ 5:409–414. doi: 10.1890/060077.1

3. EFFECTS OF ASYNCHRONOUS STRESSORS ON THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA)*

3.1. Introduction

Marine ecosystems are threatened by widespread environmental and anthropogenic stressors such as increasing temperatures, fluctuations in salinity, ocean acidification, and pollution (Doney et al. 2012; McCauley et al. 2015; Bland et al. 2018). As global change intensifies, ecologists have sought to understand the effects of these stressors on marine populations and ecosystems (Brietburg et al. 1998; Darling and Côté 2008). Previous single and multiple stressor studies have informed conservation planners how to prioritize resources towards more threatened ecosystems (Halpern et al. 2007, Côté et al. 2016). However, many of these studies did not fully explore the effects of stressors as they are typically experienced in the environment (Crain et al. 2008; Darling and Côté 2008; Gunderson et al. 2016). Notably, temporal variation —or asynchrony— in multiple stressors is largely understudied (Gunderson et al. 2016; Agrawal and Jurgens *in review*). Asynchronous multiple stressor experiments evaluate effects of multiple stressors imposed at different times on organisms.

Previous asynchronous stressor studies have shown that organisms can react to stressors in unexpected, and often different, ways than when stresses are imposed synchronously (Gunderson et al. 2016; Agrawal and Jurgens *in review*). While the outcomes of synchronous multiple stressor studies can be synergistic, antagonistic, or additive, these responses may not be seen in experiments where the timing of these stressors is considered (Crain et al. 2008; Gunderson et al. 2016; Agrawal and Jurgens *in review*). For example, stream benthic assemblages underwent greater decreases in species diversity following asynchronous stressors rather than synchronous stressors (Garcia Molinos and Donohue 2010). Similarly, prior exposure to stressors can alter organisms' physiology and immune responses, thereby altering their responses to subsequent stressors. For example, oysters under lower pH may undergo significant oxidative damage, which alters their immune responses when exposed to subsequent stressors such as pathogens (Cao et al. 2018a, b). Estuarine and coastal systems, experience a wide variety of asynchronous stressors. Estuarine and coastal regions commonly experience asynchronous stressors through seasonal variation in environmental and anthropogenic conditions (Lotze et al. 2006; Harley et al. 2006; Wetz and Yoskowitz 2013). Organisms within these ecosystems are often exposed to drastic changes in temperature and salinity. Yet even in these systems, asynchronous stress studies are comparatively rare.

Oysters occupy coastal and estuarine ecosystems and as a consequence are exposed to asynchronous stressors. The Eastern oyster, *Crassostrea virginica* is an important organism in the Gulf of Mexico and more specifically, Galveston Bay, both economically and ecologically due to ecosystem functions such as filtration capabilities and providing habitats for other marine organisms (Lenihan and Peterson 1998; Buzan et al. 2009; Hesterberg et al. 2020). Oysters have been lost to overfishing and habitat loss, among other anthropogenic stressors (Beck et al. 2011; Grabowski et al. 2012). There are many studies on the general effects of anthropogenic stressors on oysters; however, there are few that have varied stressors temporally. For instance, previous research on *Ostrea lurida,* the Olympia oyster, revealed that the timing of changes in temperature and salinity in the environment can significantly affect oyster mortality (Bible et al. 2017). These researchers conducted a synchronous and asynchronous stressor study on oysters where the former increased mortalities through synergistic effects, whereas the latter led to fewer mortalities than the synchronous study. Studying the effects of asynchronous multiple stressors on *C. virginica* would provide greater insight into the extent of survival of such an economically and ecologically important species in Galveston Bay.

Although *C. virginica* can tolerate a wide range of combinations of salinity and temperature due to the vast geographic range it occupies, certain stressful combinations of salinity and temperature may lead to increased mortality in populations (Heilmayer et al. 2008; La Peyre 2013; Lowe et al. 2017; Casas et al. 2018a; McCarty et al. 2020). For example, increased temperatures and lowered salinities negatively affected survival and growth rates across all life stages of *C. virginica* (Lowe et al. 2017). During the late summer months (July-August) in Galveston Bay, water temperature increases to upwards of 30°C, frequently reaching 32°C (Figure 3.1), which is physiologically stressful for oysters (Lowe et al. 2017). Oftentimes, floods associated with heavy rainfall and cyclonic depressions, such as tropical storms and hurricanes, coincide with these high temperature months. These events result in lowered salinities that can lead to oyster mortality since they are unable to tolerate prolonged periods of acute low salinity (Munroe et al. 2013). For example, during late August 2017, Hurricane Harvey made landfall in Texas and salinities in Galveston Bay dropped to 0 ppt within two days of its onset. Similarly, Tropical Storm Imelda lowered the salinity in the Bay in September 2019. The temperature in Galveston Bay also dropped as a consequence of the storms (Figure 3.1). Oysters, therefore, were asynchronously exposed to multiple stressors, that is, high temperatures followed by low salinities.

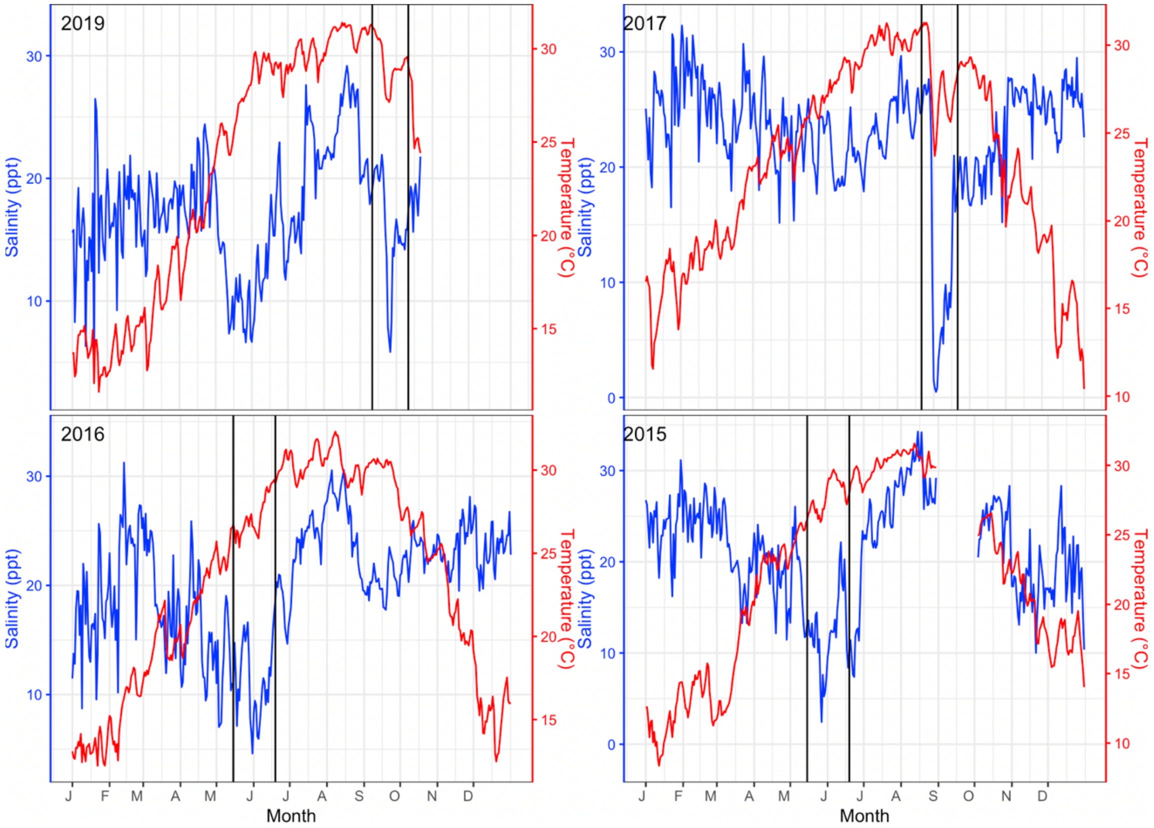

Figure 3.1 Daily mean salinity (blue) and temperature (red) at Pelican Island, Galveston Bay for four recent years in which flooding from storm events led to acute low-salinity events (bracketed with vertical black lines). Black arrows in the panels for 2017 and 2019 highlight simultaneous drops in salinity and temperature with heavy rainfall. Breaks in temperature and salinity lines indicate missing data. Data are publicly available from the Texas Water Development Board.

While hurricane season runs from June 1st through November 30th in the North Atlantic Ocean, large storm events can occur at any time of the year in any location and drive down the salinity in that area. For example, heavy rains during May 2015 and 2016 also lowered salinities. Prior to these events, the water temperature was not physiologically stressful for oysters as it was well below 30°C (Figure 3.1). There may be a difference in how oysters under these circumstances respond to osmotic stress based on their prior exposure to thermal stress.

Here, I tested the effects of asynchronous multiple stressors on newly settled *C. virginica* spat in an ecologically relevant asynchronous framework. I chose newly settled spat to gain a better understanding of the effects of asynchronous thermal and osmotic stressors during a crucial early life stage. Oysters spawn during the summer season and newly settled oysters have had a limited history of exposure to other stressors (Galstoff 1964). It is imperative to understand the effects of climate change on this early life stage as population growth depends on the survival of early life stages (e.g., Przeslawski et al. 2005; Pineda et al. 2012). Additionally, oyster spat are likely to be more susceptible to stressors than adult oysters because juveniles of marine species generally tend to be more sensitive to environmental changes than more mature life stages (Pineda et al. 2012; Mohammed 2013).

I broadly investigated how thermal stress, osmotic stress, and their interactions through time affected newly settled oyster spat. Asynchronous stressors could impact oyster populations through both lethal and sub-lethal effects on individuals. I, therefore, examined responses in terms of oyster mortality, growth, feeding, and metabolism among the different treatments. To examine sub-lethal effects on individuals, I asked three questions regarding growth: 1) whether the presence of a prior thermal stress led to greater growth, 2) if a salinity stress decreased growth, and 3) if a thermal stress during a salinity stress positively influenced growth. I also asked three specific questions regarding mortality effects: 1) whether a prior thermal stress mitigates the effects of a future salinity stress, 2) if decreases in temperature during a salinity stress reduce mortality, and 3) if asynchronous stressors lead to different mortality patterns than synchronous stressors. These questions address important gaps in our understanding about how stress timing on early life stages of an economically and ecologically important foundation species.

3.2. Methods

3.2.1. Experimental Design

3.2.1.1. Determining levels of stressors

I determined the levels of stressors for my experiment by considering the environmental data from Galveston Bay as well as the effects of stressors from prior stressor studies on oysters. I chose 32°C for the thermal stressor as Galveston Bay frequently experiences temperatures above 30°C, which is physiologically stressful for oysters (Figure 3.1). To determine the lethal salinity threshold for oyster spat (hereafter spat is referred to as oyster), I conducted a salinity trial to determine an extreme event threshold for salinity that would not result in 100% mortality. I subjected twenty oysters each to decreased salinities of 0, 1, 2, 3, 4, 5, 6 ppt with 24 ppt as a control. To establish the target salinity, I reduced the salinities from 24 ppt at roughly 0.5 ppt per hour. I conducted these experiments in 2-gallon tanks and covered them with lids to minimize the loss of water through evaporation and maintain a stable salinity between water changes, which I conducted every two days. I observed 25% mortality at 0.89 ppt and chose 1 ppt as the value for the acute salinity stressor, since excessive mortality in salinity treatments lower than 1 ppt could obscure other non-lethal physiological effects.

3.2.1.2. Pre-treatment conditions

I obtained oyster spat from Dauphin Island Hatchery, AL in May 2020. Newly settled oyster spat were roughly 2 weeks old and 2.5 mm in length. Oysters were screened for length at the hatchery prior to shipment. Upon arrival, I verified lengths by randomly measuring 5% of oysters with calipers.

I placed 300 oysters each in four 20-gallon tanks at hatchery conditions of 28°C and 24 ppt for three days to minimize additional stress following transport to Galveston. I preconditioned these tanks with denitrifying bacteria to prevent accumulation of nitrogenous compounds and supplied tanks with airlines and filters. Throughout the experiment, I fed oysters Shellfish Diet 1800®. To prevent shocking the oysters and introducing acute stressors prior to the start of the experiment, I began temperature changes three days after arrival, altering the temperature by 3°C every other day until I reached experimental conditions of 32°C (or control, 24°C).

3.2.1.3. Phase 1

To capture asynchrony in multiple stressors, my experiment had two phases. The timing of these stressors mimics a hurricane-level low-salinity event following hotter summer months (such as in 2017 and 2019; Figure 3.1) versus a low-salinity event in late spring or early summer (represented by the control temperature group in Phase 1). Phase 1 was a one-month thermal stress (Figure 3.2). While I exposed half of the oysters to thermal stress, the other half were kept at control temperatures.

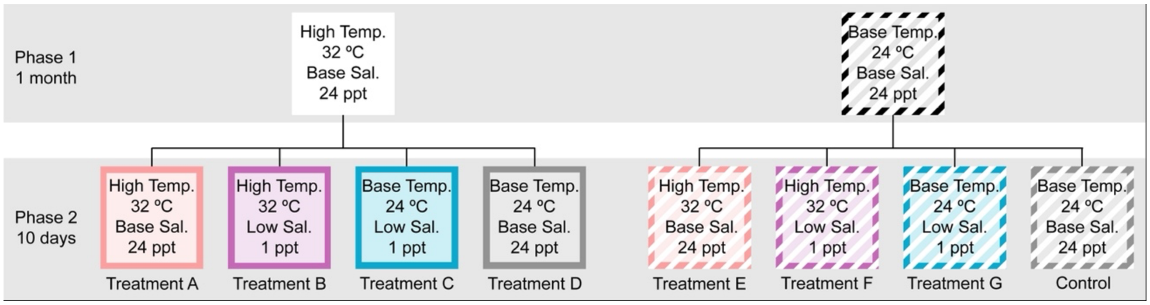

Figure 3.2 Experimental set-up (N = 15 oysters per tank, N = 6 tanks per treatment). Oysters were exposed to high (32 C) or control ("Base"; 24 C) temperatures for one month in Phase 1. In Phase 2, oysters were exposed to low (1 ppt) salinity for 10 days, mimicking a severe storm-driven flood event, or control (24 ppt). The combination of both low salinity and low temperature for 10 days after high temperature in Phase 1 (Treatment C), mimicks drops in temperature and salinity that can co-occur during a severe flood. Treatment F represents a more traditional, simultaneously conducted multi-stressor experiment without prior stress. Treatment A is the high temperature control, and the Control is the true control for the experiment (Treatments D and E were to aid interpretation).

3.2.1.4. Phase 2

This phase was a 10-day acute osmotic stress. Here, I use the term acute due to the abrupt, punctuated nature of the storm event. I used ten days for the acute stressor event, since this duration is representative of the duration of effects from a typical storm in the Bay. Lowered salinities from Hurricane Harvey lasted up to a month, but that storm was particularly anomalous. During the extreme low-salinity event, I also exposed a subset of oysters to lowered temperatures to capture the drops in temperature that occur during cyclonic depressions in the Bay.

During Phase 2, I exposed a subset of oysters to higher temperatures to create a fully crossed experiment (Figure 3.2). My fully crossed experiment of higher temperatures, acute salinities, and lowered temperatures that occur during large cyclonic events resulted in 7 different overall treatments and a control (Figure 3.2). Each treatment had N = 15 oysters per tank (up to N=17 if spat were growing on top of one another), and N = 6 tanks per treatment, yielding approximately N = 90 oysters per treatment.

Treatments A through D experienced thermal stress (32°C) during Phase 1, whereas Treatments E through G and the Control were at ambient temperatures (24°C, Figure 3.2). Treatment A represented the high temperature control as oysters were under thermal stress (32°C) and ambient salinity (24 ppt) during both Phase 1 and Phase 2. Treatments B and G, which were exposed to high (32°C) and control (24°C) temperatures in both Phases respectively, were exposed to low salinity (1 ppt) for ten days. Treatment C, which was exposed to high temperatures (32°C) during Phase 1, was exposed to both low salinity (1 ppt) and ambient temperature (24°C) for 10 days, mimicking drops in temperature and salinity during a severe storm-driven flood event. Treatment D, the final treatment at higher temperatures during Phase 1, was exposed to ambient salinity (24 ppt) and ambient temperatures (24°C) during Phase 2 and was included for a balanced design and to aid in interpretation of the data. Treatments E and F, which were both at ambient temperatures (24°C) during Phase 1, were exposed to increased temperatures (32°C) during Phase 2. I used Treatment E, which was at ambient salinity (24 ppt) during Phase 2 to determine the effects of thermal stress alone on oysters. Treatment F, on the other hand, was at a lowered salinity (1 ppt) during Phase 2, showing the effects of synchronous stressors on oysters. The Control treatment was at ambient temperature (24°C) and ambient salinity (24 ppt) during Phase 1 and Phase 2. I maintained 32°C treatments by placing tanks in water baths with heated water continuously circulating through a sump pump. I monitored temperature and salinity conditions daily using a YSI Pro 2030. I monitored ammonia, nitrates, and nitrites using an API Test Kit® and ensured none of these conditions led to stress or mortality through frequent water changes of 5 gallons per tank every other day.

On the last day of experimental conditions, at the end of Phase 2, I fasted oysters for 24 hours to avoid any differences in intestinal fullness between oysters prior to the respiration and clearance rate analyses (García-Esquivel et al. 2002; Bayne 2017). Thereafter, I conducted mortality, length, weight, clearance rate, and respiration rate analyses.

3.2.2. Endpoints

3.2.2.1. Mortality

I assessed mortality at four time points: before the start of Phase 1, at the end of Phase 1, at the end of Phase 2, and after one week of recovery at the end of the experiment. Oysters have adductor muscles, which keep the two valves of the oyster closed when alive. A failure in these muscles leads to valve gaping and, if irreversible after prodding, indicates mortality. I assessed mortality by manually prodding oysters that had their valves open. Oysters that exhibited prolonged adductor failure were pronounced dead.

3.2.2.2. Growth

I interpreted growth across treatments by measuring shell length of all individuals, as well as total weight (live tissue and shell mass) and final (dead) wet tissue mass for a subset of oysters per treatment. To measure length, I photographed all of the oysters in each tank, after Phase 1 and Phase 2, and measured the longest axis of the shell in each image using ImageJ® software. I weighed 48 oysters per treatment ($N = 8$ per tank; note that Treatment F had fewer oysters alive, and therefore, fewer than 8 oysters per tank). Following respiration rate analyses (see below), I dissected oysters, and extracted and weighed wet tissue mass for the same 48 oysters per treatment.

3.2.2.3. Clearance Rates

Clearance rates describe how much plankton an organism is able to consume from the water column within a given time period and are therefore useful measures of feeding performance. To determine differences in clearance rates between treatments, I selected five similarly sized oysters per tank (5-7 mm) in each treatment group for clearance rate analyses. I fed them 200 µL of Shellfish Diet 1800® and took aliquots of water every 20 minutes for an hour to determine algae concentration. I used a UV-Vis spectrophotometer to determine absorbance at 660 nm. I used absorbance as a proxy for algal concentration as recommended by Salerno et al. 2018. I calculated clearance rates as change in absorbance per time period. After clearance rate measurements, I brought the remaining oysters back to ambient temperature and salinity conditions and monitored for recovery or mortality.

3.2.2.4. Respiration Rates

To determine differences in respiration rates between treatments, I placed pre-massed individuals in either 500 µL or 1700 µL wells based on their size. These wells were part of a 24-well SensorDish that were attached to a micro-respirometer (Loligo Systems, Viborg, DK). Controls with no oysters made up 8 out of the 24 wells and confirmed that the background microbial oxygen uptake was negligible. I used Microresp™ (Loligo Sytems) to measure oxygen every five seconds for an hour. I calculated respiration rate (mg O_2 kg^{-1} hr^{-1}) using the following equation (Petersen and Gamperl 2010):

$$MO_2 = [\left(\left(C_i - C_f\right) x\ V\right) x\ 60](M\ x\ t^{-1})$$

where MO_2 is oxygen consumed, C_i is the initial oxygen concentration, C_f is the final oxygen concentration in the cell, V is the volume of the cell in liters (here, either 0.0005

L or 0.0017 L), M is the tissue weight of the oyster in kg, and t is the duration of the trial in minutes.

3.2.3. Statistical Analyses

To assess differences in mortality after the stressors, I used generalized linear mixed models with a binomial distribution and a logit link function. Treatment was the fixed factor with 8 levels, and Tank was the random factor. I used log likelihood and AICc to compare my models, and my analyses showed that Tank was not a significant factor. Thus, I was able to proceed with pooled oysters from each treatment to analyze differences in recovery mortality. I tested differences between treatments using pre-defined planned contrasts as defined by my three key questions (1: effects of prior thermal stress, 2: drops in temperature during salinity stress 3: asynchrony versus synchrony). I adjusted p-values to account for multiple comparisons using the "free" method within the multcomp package.

To assess differences in lengths, weights, clearance rates, and respiration rates, I used linear mixed models with Gaussian distributions. I used Treatment as the fixed factor and used log likelihood and AICc to examine the contribution of Tank as a random factor. I log-transformed lengths and weights to approximate normality. I tested differences between treatments using planned contrasts as defined by my three key questions for lengths and weights (1: effects of a prior thermal stress, 2: effects of a salinity stress, and 3) thermal stress during a salinity stress). I also conducted post-hoc tests for clearance rate and respiration rate models to determine what stressor combinations led to metabolism different from Control, and adjusted p-values to account for multiple comparisons using the "free" method within the multcomp package. I conducted all analyses in R (version 4.0.2) with the packages multcomp, lme4, ggplot2, ggpackage, and cowplot.

3.3. Results

3.3.1. Mortality

My experiments showed that specific combinations of stressors and their timing led to significant differences in mortality (Figure 3.3). There were no deaths observed in the Control tanks at any time point. Neither was there any mortality in any treatment after the acclimation period, or in elevated temperature treatments following Phase 1. Similarly, there were minimal effects of thermal stress from either Phase 1 or Phase 2 on oyster mortality, such as in treatments A, D, and E. There was no significant difference in mortality between treatments B and C ($p = 0.25$, result from planned comparison) at the end of Phase 2, showing that differences in temperature during an acute salinity stress did not confer differences in mortalities if oysters had a prior history of thermal stress. On the other hand, oysters with prior thermal stress experienced less mortality after a subsequent acute salinity stress since there was significantly greater mortality in treatment G (~16%; G: no stress in Phase 1, salinity stress in Phase 2, result from planned comparison) than in treatment C (~4%, C: thermal stress in Phase 1, salinity stress in Phase 2, $p = 0.038$, result from planned comparison). Furthermore, treatment C with asynchronous multiple stressors had significantly lower mortality than treatment F (no stress in Phase 1, both salinity and thermal stress in Phase 2, ~46%, p <0.001, result from planned comparison), where oysters were exposed to both stressors synchronously during Phase 2.

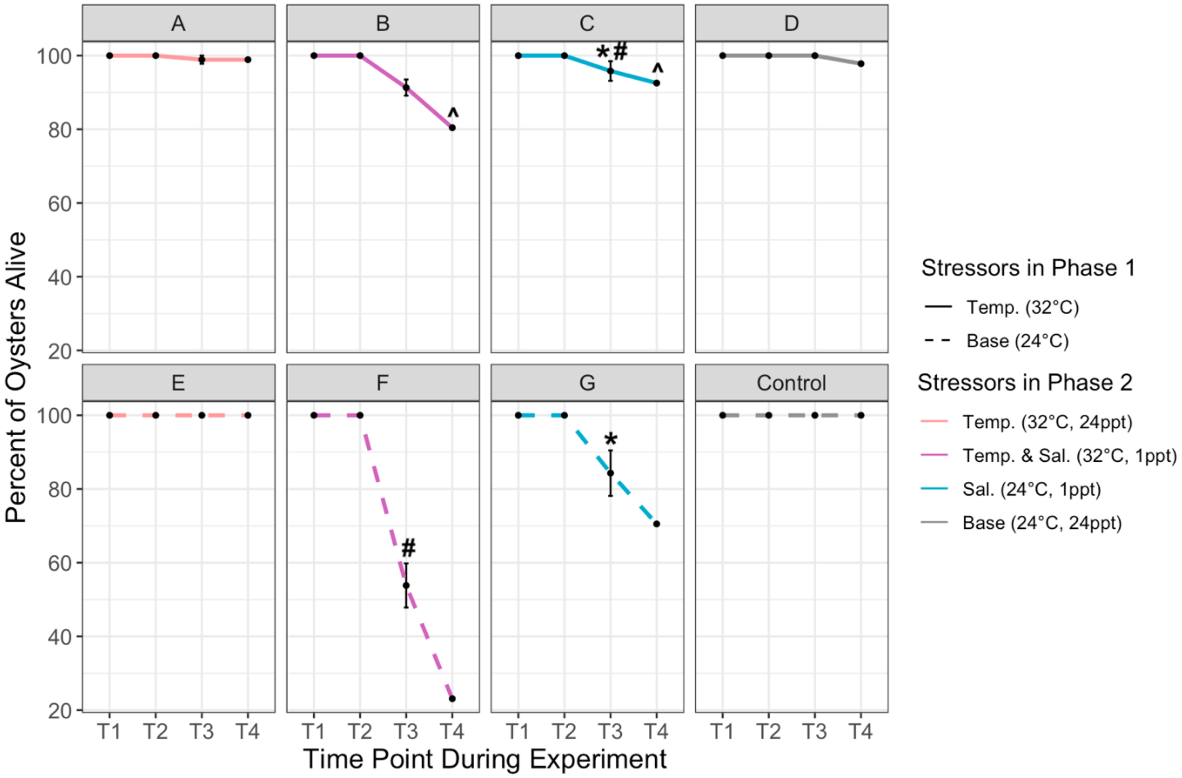

Figure 3.3 Mortality of oysters by treatment at four different time points during the experiment. T1 is prior to Phase 1, T2 is prior to Phase 2, T3 is at the end of Phase 2, and T4 is after one week of recovery. During recovery, we pooled oysters by treatment and there was no longer separation by tank. Error bars represent standard error. Symbols represent significant differences between treatments for specifically tested contrasts.

I found more pronounced differences between treatments one week after the end of the experiment at ambient temperature and salinity (Figure 3.3). Treatments under low salinity conditions in Phase 2 continued to accrue mortality. Treatment B had an increase in mortality from 9% to 20%, C from 5% to 8%, F from 46% to 77%, and G from 16% to 30% after the week-long recovery period. Importantly, there was a significant difference between post-recovery mortality between treatments B (thermal stress Phase 1, both salinity and thermal stress in Phase 2) and C (thermal stress in Phase 1, salinity stress only in Phase 2, $p = 0.019$, result from planned comparison). C had a significantly lower mortality than B, showing that while effects of thermal stress during an acute salinity stress were not immediately seen at the end of experimental conditions, there was a negative impact of synchronous stressors after a week of recovery (Figure 3.3).

3.3.2. Growth

In my experiment, oysters had higher growth rates and weight gains when exposed to higher temperatures in Phase 1 or Phase 2 under control salinity (Figures 3.4, 3.5, 3.6). After Phase 1, oysters under thermal stress were significantly longer than those in ambient temperatures ($p <0.001$, result from planned comparison). Oysters under thermal stress during Phase 1 grew to an average length of 6.25 mm, whereas those under ambient conditions only grew to an average length of 5 mm (Figure 3.4). After Phase 2, oysters exposed to acute salinity stress without prior thermal stress (Treatment G, no stress Phase 1, salinity stress Phase 2) were significantly smaller than oysters that had remained at ambient salinities (Treatment C, thermal stress Phase 1, salinity stress Phase 2, Figure 3.5, $p < 0.001$, result from planned comparison). There was also no difference in lengths between treatments F (no stress Phase 1, thermal and salinity stress

Phase 2) and G (no stress Phase 1, salinity stress Phase 2), showing that a thermal stress during an acute salinity stress event did not lead to additional growth. Oysters exposed to sequential thermal and salinity stressors were smaller in length than those lacking the subsequent salinity stress exposure, but this difference was not statistically significant. For the other aspect of growth, that is, body weight, oysters under salinity stress in Phase 2 regardless of Phase 1 conditions had significantly lower weights than oysters in ambient salinity conditions (Figure 3.6, $p < 0.01$, result from planned comparison).

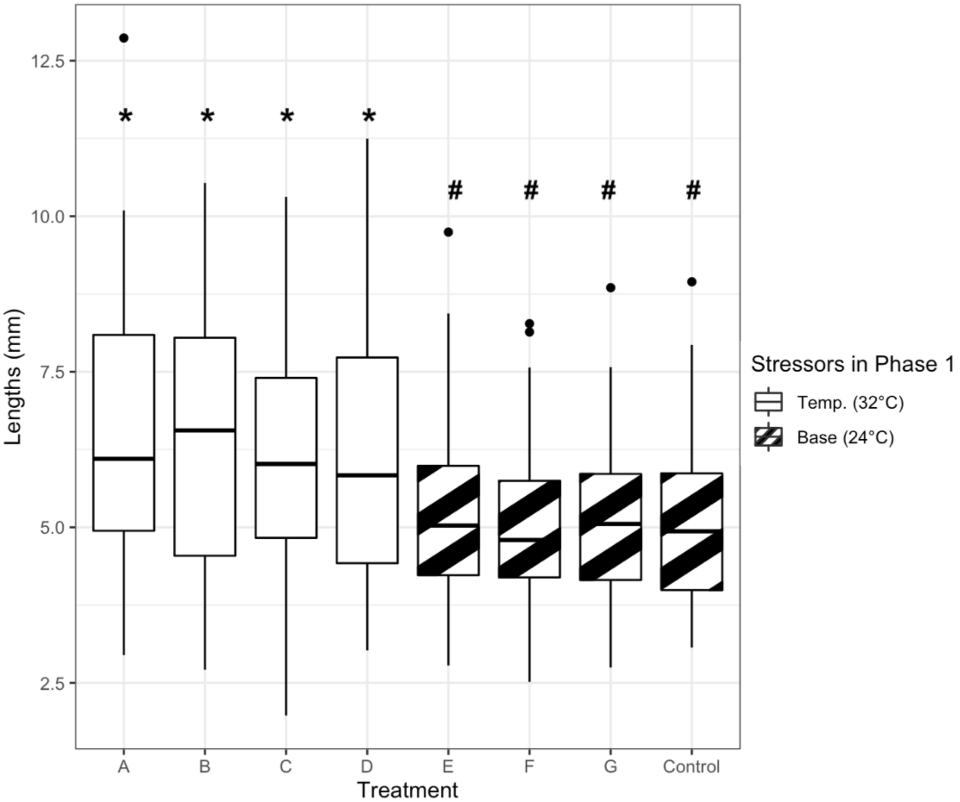

Figure 3.4 Length of oysters (Mean ± SE) following Phase 1 thermal stress. Striped bars indicate control treatments at 24 C in Phase 1. Treatments A, B, C, and D are significantly different from E, F, G, and control showing higher temperatures during Phase 1 led to higher growth rates as denoted by the different letters. Here, shared symbols denote no significant difference.

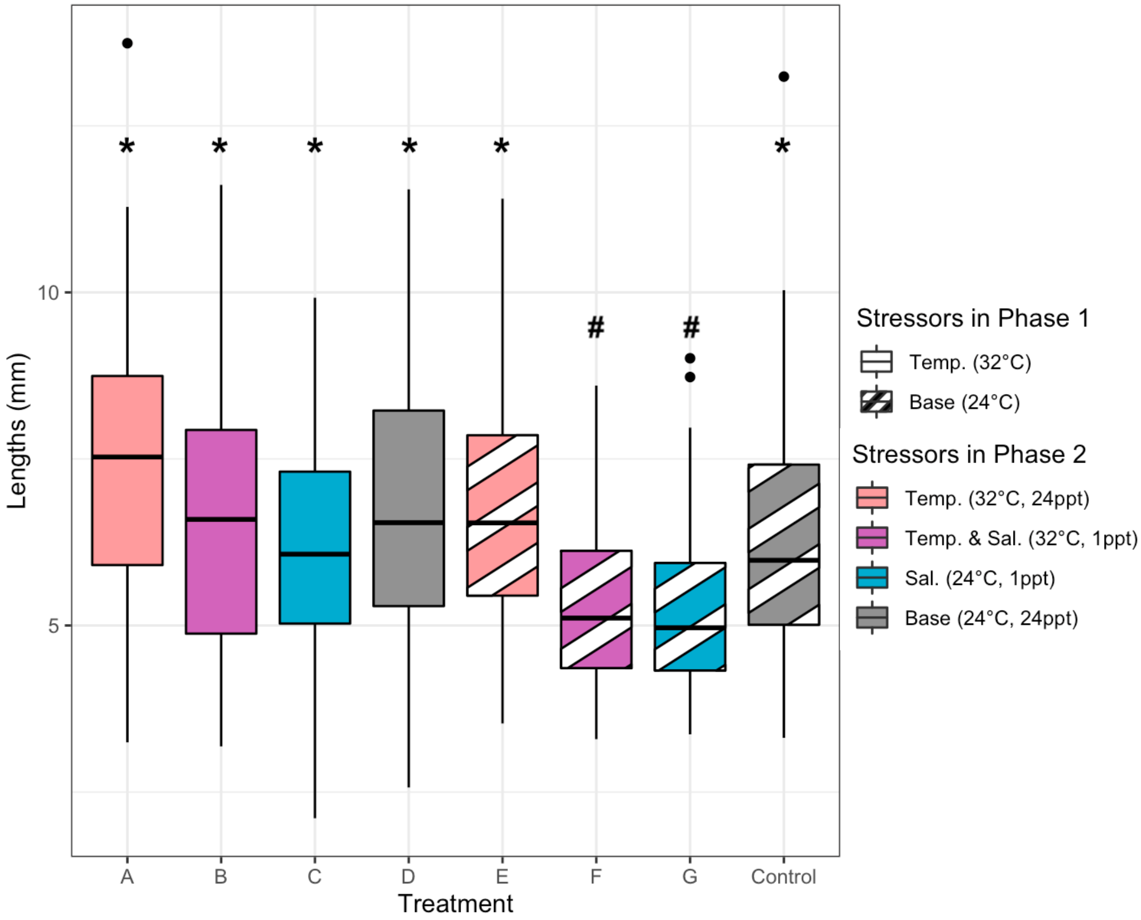

Figure 3.5 Length of oysters (Mean ± SE) at the end of Phase 2. Striped bars indicate the absence of a stressor in Phase 1. Colors indicate the type of stressor in Phase 2. Salinity stress in Phase 2 led to lower growth rates in oysters without a prior thermal stress. Here shared symbols indicate no significant difference. There was no significant difference between Treatments F and G, both of which had a salinity stress during Phase 2, but Treatment F also had a thermal stress.

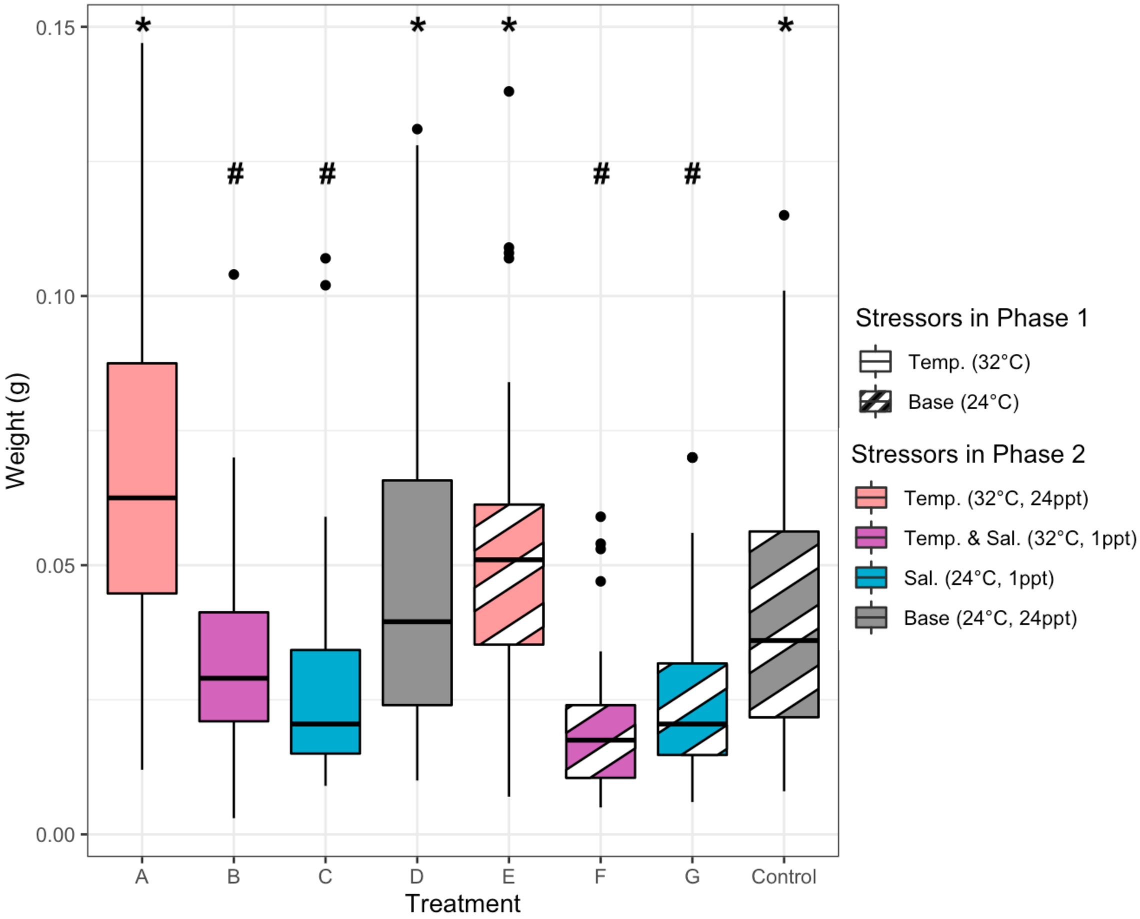

Figure 3.6 Whole body weights of oysters (Mean ± SE) at the end of Phase 2. Striped bars indicate the absence of a stressor in Phase 1. Colors indicate the type of stressor in Phase 2. Thermal stress during Phase 1 led to generally higher weights, whereas salinity stress in Phase 2 led to significantly large reductions in mass as denoted by the letters. Shared symbols here denote no significant difference.

3.3.3. Clearance Rates

Higher temperatures under control salinity during Phase 2 led to marginally higher clearance rates than Control in Treatment A, (Figure 3.7, p = 0.09, Tukey's post-hoc test) and Treatment E (p = 0.109, Tukey's post-hoc test). For instance, while the change in absorbance for Treatment A was twice as high than the change in absorbance for Control, it was not significantly higher. Oysters in lower salinities such as in treatments F and G did not have significantly different clearance rates from controls, indicating that oysters were feeding in all treatments regardless of prior temperature or subsequent salinity and temperature conditions ($p > 0.05$). A thermal stress during Phase 1 led to a generally higher clearance rate in treatments. Prior thermal stress led to a generally higher clearance rate in treatments with lower salinity, since Treatments B and C, with prior history of thermal stress had higher clearance rates than Treatments F and G.

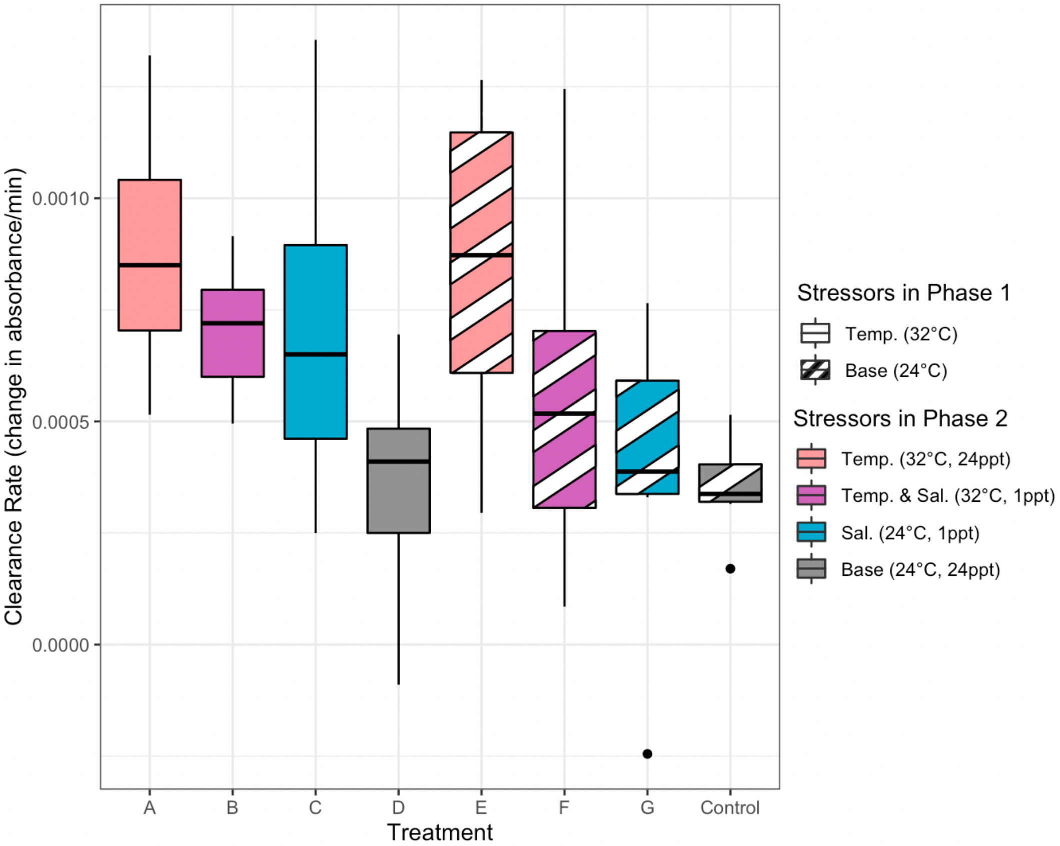

Figure 3.7 Clearance rates of oysters (Mean ± SE) at the end of Phase 2. Striped bars indicate the absence of a stressor in Phase 1. Colors indicate the type of stressor in Phase 2. Thermal stress in Phase 2 without salinity stress led to significantly higher clearance rates as denoted by the letters. There was no significant difference between treatments at a p-value < 0.05.

3.3.4. Respiration Rates

I found that Treatment G had the highest respiration rate, which was an order of magnitude higher than all other treatments, significantly greater than Control (Figure 3.8, $p < 0.05$, Tukey's post hoc test). There was no difference between respiration rates in Treatments B and C, suggesting that differences in temperature during an acute salinity stress did not lead to differences in respiration rate when oysters had a prior history of thermal stress ($p > 0.05$, Tukey's post hoc test). Further, higher temperatures without salinity stress led to generally the lowest respiration rates, as in Treatments A and E, and history of thermal stress also generally led to lower respiration rates as compared to Treatment G.

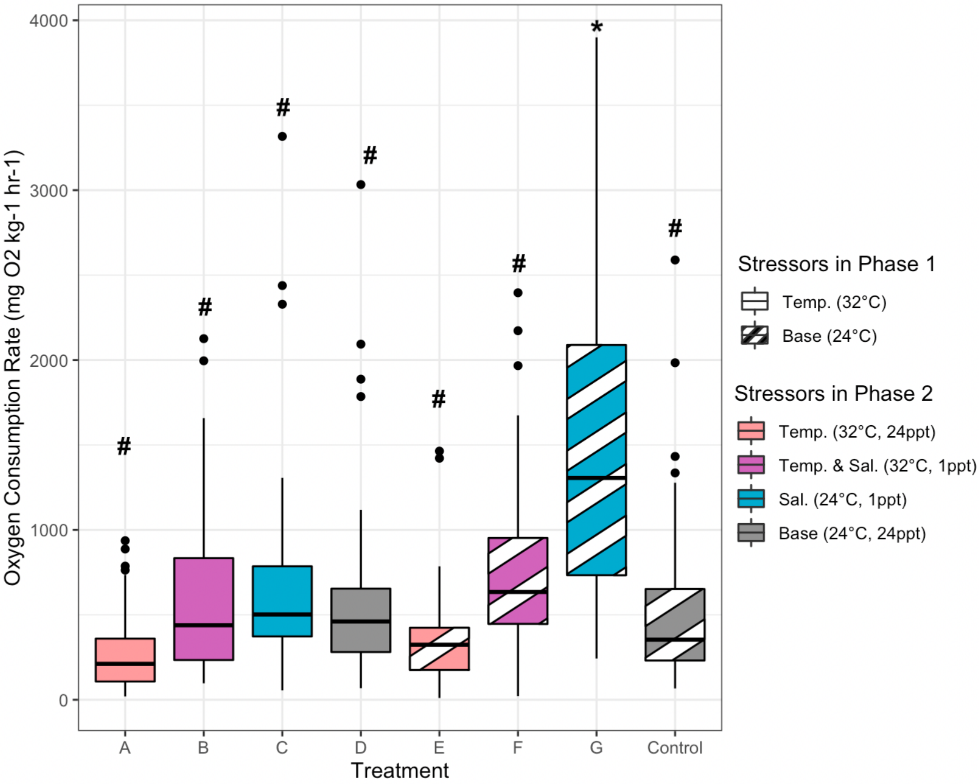

Figure 3.8 Respiration rates of oysters (Mean ± SE) at the end of Phase 2. Striped bars indicate the absence of a stressor in Phase 1. Colors indicate the type of stressor in Phase 2. Treatment G with no stress during Phase 1, and only salinity stress during Phase 2 had the significantly highest respiration rate. Shared symbols here denote no significant difference.

3.4. Discussion

My results demonstrate that the timing of environmental stressors affects oyster mortality. I found that oyster spat under asynchronous thermal and osmotic stressors experienced much lower mortality than oysters under synchronous thermal and salinity stress (Figure 3.3). This is consistent with prior research indicating that concurrent low salinities and increased temperatures can exacerbate mortality in adult oysters and spat (Heilmeyer et al. 2008; La Peyre 2013; Rybovich et al. 2016; Lowe et al. 2017; McCarty et al. 2020). These studies, however, focused on the outcomes of synchronous stressors on *C. virginica*. My results suggest there may be fewer mortalities in oyster populations that experience sequential or asynchronous thermal and osmotic stressors in the environment, at least for recently settled life stages. This has important implications for understanding how oyster populations will fare as temperatures continue to rise and storms become more frequent. Several studies worldwide have documented increased mass oyster mortality events following acute salinity stressors from large storm events. These have reported differing times to population recovery depending on the duration of the stressor as well as the water temperature during the event (Du et al. 2021).

After a week of recovery at ambient conditions, I saw continued mortalities in oysters exposed to the acute salinity stressor in Phase 2. Notably, I saw that a temperature decrease during the acute salinity stress during Phase 2 led to lower mortalities as compared to oysters under continued thermal stress and acute salinity stress. I saw this difference only after one week of recovery from experimental conditions, showing the importance of not only conducting environmentally relevant experiments from multiple facets, but also incorporating a recovery period to assess the full extent of multiple environmental stressors. Quantifying continuing mortality after the termination of an acute stressor event can inform the time to recovery of an oyster population, which is dependent on abundance and survivorship of early life stages (Munroe et al. 2013).

My results also show that in addition to the timing of stressors, the order of stressors influences oyster responses. Oysters under a prior chronic thermal stress experienced less mortality when exposed to an acute salinity stress versus those that only experienced an acute salinity stress (Figure 3.3). While initially founded in pharmacology, cross-tolerance (prior stress increases tolerance to a subsequent stressor; Li and Hahn 1973) is now commonly studied in physiology, especially in terms of thermal stress. When under thermal shock, organisms produce heat shock proteins (HSPs), which help in the refolding of proteins and allow a variety of organisms to tolerate thermal stress (Lindquist 1986; Parsell and Lindquist 1993). Though primarily produced in response to a thermal stress, HSPs may mitigate the negative effects associated with drops in salinity as well (DuBeau et al. 1998). For example, a 12°C thermal shock reduced the negative effects of subsequent osmotic stress in tidepool sculpin (Todgham et al. 2005). Similarly, heat stressed rainbow trout may modify plasma osmolarity through changes in their chloride levels, which may aid them in reducing a subsequent osmotic stress (Niu et al. 2008). Though I did not quantify HSPs in my experiment, there is evidence that *C. virginica,* like most other organisms, can produce HSPs under a thermal stress. For example, *C. virginica* produced HSPs under a wide range of temperatures including 32°C (Ivanina et al. 2009; Nash and Rehman 2019). Therefore, the production of HSPs in response to a chronic thermal stress could explain the reduced mortality in oyster treatments under a chronic thermal stress followed by an acute salinity stress. Although beyond the scope of this study, quantifying HSPs in oysters in future experiments could corroborate my hypotheses that HSPs produced during the initial thermal stress were responsible for cross-tolerance to a subsequent salinity stress.

Oyster growth was dependent on both temperature and salinity during both phases. The higher growth rates I observed under high temperature conditions and control salinities could be attributed to marginally higher clearance rates and lower than expected metabolic demands in oysters under thermal stress (Figures 3.7, 3.8). Adult *C. virginica* at higher temperatures tend to clear more water and, therefore, feed more (Lowe et al. 2017). Additionally, in the European flat oyster, *Ostrea edulis,* physiologically stressful conditions just outside the optimal thermal range led to increased clearance rates along with heart rates that were well below their peak activity (Eymann et al. 2002). As these heart rates were not drastically increased under a stressful, but not lethal, temperature range, their metabolic demand could be matched by higher filtration rates than controls. While my results only showed marginally higher filtration rates than controls, conducting longer filtration rate trials may further clarify differences in growth rates.

On the other hand, despite normal feeding rates during low salinity conditions in Phase 2, I found stunting in oysters, indicating that a lack of feeding did not lead to reduced growth. While previous studies have shown that oysters may close their valves under low salinity conditions, undergo anaerobic respiration, and stop feeding or clearing water leading to reduced weights and lengths (Michaelidis et al. 2015; La Peyre et al. 2013; Lowe et al. 2017; Casas et al. 2018b; Jones et al. 2019; McCarty et al. 2020), my experiments show no difference in clearance rates between low salinity treatments and controls. An acute salinity stress for ten days did not reduce feeding compared to controls (Figure 3.7). This could be due to the time length of the salinity stressor. For example, a previous study found that oysters under cyclical changes in salinity initially closed their valves quickly but opened them after 20 hours (Hand and Stickle 1977). This could have been the case in my experiment, where oysters opened their valves over the course of Phase 2 and began feeding. As there was no negative impact of the acute salinity stress on clearance rates during Phase 2, I found that differences in respiration rates could explain why oyster spat under osmotic stress were stunted.

Respiration rates have been used to explain the differences in energy budgets for many organisms including *C. virginica* (La Peyre et al. 2020). Higher oxygen consumption rates have been documented in oysters and in other invertebrates such as annelids and clams due to metabolism increases in response to stressful conditions (Matoo et al. 2013; Grimes et al. 2020). Under a salinity stress, oysters may be trying to osmoconform, that is altering their internal salinities to match that of the environment, which could lead to higher metabolic activity and thereby greater oxygen consumption (Soklova et al. 2012a). Energy may have been diverted from digestion towards osmoconforming, thereby stunting oyster spat in treatments under only a salinity stress during Phase 2 with no prior thermal stress. However, the same may not be true of treatments that had both salinity and thermal stress during Phase 2 with no prior history of stress. For example, Treatment F, which was under synchronous osmotic and thermal stressors in Phase 2, had a lower oxygen consumption rate than Treatment G, which was only exposed to an acute salinity stress in Phase 2 (Figure 3.8). The combination of stressors in Treatment F may have pushed oyster spat beyond their thermal optimal range, leading to a decrease in oxygen consumption (Soklova et al. 2012b). Despite not having as high of a stress level as Treatment F, Treatments with an acute salinity stress in Phase 2 that underwent a thermal stress in Phase 1 had similar rates of oxygen consumption to Treatment F. This could be attributed to the hypothesis that oxygen consumption may increase with increasing temperatures and decreasing salinities only to a certain point. For example, up to 20°C, decreases in salinities and increases in temperatures led to increased oxygen consumption. However, beyond that, combinations of the highest temperatures and lowest salinities did not lead to increased oxygen consumption (Shumway and Koehn 2002). Constructing thermal performance curves for *C. virginica* at this life stage would help to identify the balance between stress management and metabolic demand, which likely led to these results in oxygen consumption. Further, constructing dynamic energy budgets of oyster spat through clearance rate and respiration rate analyses, could inform stakeholders on how vulnerable the growth of a reef is during a given year.

3.5. Conclusion

Worsening climatic conditions in coastal and estuarine systems warrant studies on the organisms exposed to these everchanging environments (Knight and Davis 2009; Bindoff et al. 2019). Specifically, mass oyster kills are becoming more commonplace in these systems globally with the rise of large and frequent storms (Du et al. 2021). While several studies have investigated the effects of global warming and acute low salinity events, my study is among the first to describe the impact of the timing of these stressors on oyster spat. Importantly, I found that while *Crassostrea virginica* spat can tolerate a wide range of temperatures and salinities, as attested to in prior literature as well as in this study, the timing of these stressors can greatly alter the effects of these stressors. Further, characterizing stressor response at various life-stages will provide context to time to recovery of oyster populations following extreme events. For example, following two hurricanes in the 1980s within the Gulf of Mexico, oyster recovery hinged on the timing of the storm relative to oyster spawning (Livingston et al. 1999). My results suggest that oyster spat under a prior thermal stress, as could be seen during summer months, may fare better under an acute salinity stress, as seen during extreme rainfall events as compared to oysters that are exposed to an acute salinity stress after a cooler spring or autumn.

Studying the effects of stressors as they occur in the environment can provide greater understanding for how organisms respond to global change. As extreme events continue to become more frequent and more intense, they may follow pre-existing chronic stressors leading to organismal exposure to asynchronous multiple stressors. Assessing the consequences of these multiple, interacting stress dynamics can help inform predictions of population responses to global change (Smith 2011).

3.6. References

Agrawal, A., & Jurgens, J. L. In review. Ecological Effects of Asynchronous Stressors.

Bayne, B. L. (2017). Biology of Oysters. Elsevier.

Beck, M. W., Brumbaugh, R. D., Airoldi, L., Carranza, A., Coen, L. D., Crawford, C., … Guo, X. (2011). Oyster reefs at risk and recommendations for conservation, restoration, and management. *BioScience*, *61*(2). https://doi.org/10.1525/bio.2011.61.2.5

Bible, J. M., Cheng, B. S., Chang, A. L., Ferner, M. C., Wasson, K., Zabin, C. J., … Grosholz, E. D. (2017). Timing of stressors alters interactive effects on a coastal foundation species. *Ecology*, *98*(9), 2468–2478. https://doi.org/10.1002/ecy.1943

Bindoff, N. L., Cheung, W. W. L., Kairo, J. G., Arístegui, J., Guinder, V. A., Hallberg, R. … Williamson, P. (2019). Changing Ocean, Marine Ecosystems, and Dependent Communities. In H.-O Pörtner, D. C. Roberts, V. Masson-Delmotte, P. Zhai, M. Tignor, E. Poloczanska, et al. (Eds.) *IPCC Special Report on the Ocean and Cryosphere in a Changing Climate* (pp. 477 – 587). Intergovernmental Panel on Climate Change, Switzerland

Bland, L. M., Watermeyer, K. E., Keith, D. A., Nicholson, E., Regan, T. J., & Shannon, L. J. (2018). Assessing risks to marine ecosystems with indicators, ecosystem models and experts. *Biological Conservation*, *227*. https://doi.org/10.1016/j.biocon.2018.08.019

Breitburg, D. L., Baxter, J. W., Hatfield, C. A., Howarth, R. W., Jones, C. G., Lovett, G. M., & Wigand, C. (1998). Understanding Effects of Multiple Stressors: Ideas and Challenges. In *Successes, Limitations, and Frontiers in Ecosystem Science* (pp. 416–431). https://doi.org/10.1007/978-1-4612-1724-4_17

Buzan, D., Lee, W., Culbertson, J., Kuhn, N., & Robinson, L. (2009). Positive Relationship between Freshwater Inflow and Oyster Abundance in Galveston Bay, Texas. *Estuaries and Coasts*, *32*(1), 206–212. https://doi.org/10.1007/s12237-008-9078-z

Cao, R., Liu, Y., Wang, Q., Yang, D., Liu, H., Ran, W., … Zhao, J. (2018). Seawater acidification reduced the resistance of *Crassostrea gigas* to *Vibrio splendidus* challenge: An energy metabolism perspective. *Frontiers in Physiology*, *9*, 880. https://doi.org/10.3389/fphys.2018.00880

Cao, R., Wang, Q., Yang, D., Liu, Y., Ran, W., Qu, Y., … Zhao, J. (2018). CO2-induced ocean acidification impairs the immune function of the Pacific oyster against *Vibrio splendidus* challenge: An integrated study from a cellular and proteomic perspective. *Science of the Total Environment*, *625*, 1574–1583. https://doi.org/10.1016/j.scitotenv.2018.01.056

Casas, S. M., Filgueira, R., Lavaud, R., Comeau, L. A., La Peyre, M. K., & La Peyre, J. F. (2018). Combined effects of temperature and salinity on the physiology of two geographically-distant eastern oyster populations. *Journal of Experimental Marine Biology and Ecology*, *506*, 82–90. https://doi.org/10.1016/j.jembe.2018.06.001

Casas, S. M., Lavaud, R., La Peyre, M. K., Comeau, L. A., Filgueira, R., & La Peyre, J. F. (2018). Quantifying salinity and season effects on eastern oyster clearance and oxygen consumption rates. *Marine Biology*, *165*(5), 90. https://doi.org/10.1007/s00227-018-3351-x

Côté, I. M., Darling, E. S., & Brown, C. J. (2016). Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1824), 20152592. https://doi.org/10.1098/rspb.2015.2592

Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, *11*(12), 1304–1315. https://doi.org/10.1111/j.1461-0248.2008.01253.x

Darling, E. S., & Côté, I. M. (2008). Quantifying the evidence for ecological synergies. *Ecology Letters*, *11*(12), 1278–1286. https://doi.org/10.1111/j.1461-0248.2008.01243.x

Doney, S. C., Ruckelshaus, M., Emmett Duffy, J., Barry, J. P., Chan, F., English, C. A., … Talley, L. D. (2012). Climate Change Impacts on Marine Ecosystems. *Annual Review of Marine Science*, *4*(1), 11–37. https://doi.org/10.1146/annurev-marine-041911-111611

Du, J., Park, K., Jensen, C., Dellapenna, T., Zhang, W., & Shi, Y. (2021). Massive oyster kill in Galveston Bay caused by prolonged low-salinity exposure after Hurricane Harvey. *Science of the Total Environment, 774,* 145132. 10.1016/j.scitotenv.2021.145132

Eymann, C., Götze, S., Bock, C., Guderley, H., Knoll, A. H., Lannig, G., … Pörtner, H. O. (2020). Thermal performance of the European flat oyster, *Ostrea edulis* (Linnaeus, 1758)—explaining ecological findings under climate change. *Marine Biology*, *167*(2). https://doi.org/10.1007/s00227-019-3620-3

Galstoff, P. S. 1964. The American oyster, C. virginica. Fishery Bulletin of Fish Wildlife Service. 64:14–80. Washington, DC: US Government Printing Office.

García Molinos, J., & Donohue, I. (2010). Interactions among temporal patterns determine the effects of multiple stressors. *Ecological Applications*, *20*(7), 1794–1800. https://doi.org/10.1890/10-0018.1

Grabowski, J. H., Brumbaugh, R. D., Conrad, R. F., Keeler, A. G., Opaluch, J. J., Petersen, C. H., … Smyth, A. R. (2012). Economic Valuation of Ecosystem Services Provided by Oyster Reefs. *BioScience*, *62*(10), 900–909. https://doi.org/10.1525/bio.2012.62.10.10

Gunderson, A. R., Armstrong, E. J., & Stillman, J. H. (2016). Multiple Stressors in a Changing World: The Need for an Improved Perspective on Physiological Responses to the Dynamic Marine Environment. *Annual Review of Marine Science*, *8*(1), 357–378. https://doi.org/10.1146/annurev-marine-122414-033953

Halpern, B. S., Selkoe, K. A., Micheli, F., & Kappel, C. V. (2007). Evaluating and Ranking the Vulnerability of Global Marine Ecosystems to Anthropogenic Threats. *Conservation Biology*, *21*(5), 1301–1315. https://doi.org/10.1111/j.1523-1739.2007.00752.x

Harley, C. D. G., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J. B., Thornber, C. S., … Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, *9*(2), 228–241. https://doi.org/10.1111/j.1461-0248.2005.00871.x

Heilmayer, O., Digialleonardo, J., Qian, L., & Roesijadi, G. (2008). Stress tolerance of a subtropical Crassostrea virginica population to the combined effects of temperature and salinity. *Estuarine, Coastal and Shelf Science*, *79*(1), 179–185. https://doi.org/10.1016/J.ECSS.2008.03.022

Hesterberg, S. G., Herbert, G. S., Pluckhahn, T. J., Harke, R. M., Al-Qattan, N. M., Trevor Duke, C., … Sampson, C. P. (2020). Prehistoric baseline reveals substantial decline of oyster reef condition in a Gulf of Mexico conservation priority area. *Biology Letters*, *16*(2). https://doi.org/10.1098/rsbl.2019.0865

Ivanina, A. V., Taylor, C., & Sokolova, I. M. (2009). Effects of elevated temperature and cadmium exposure on stress protein response in eastern oysters *Crassostrea virginica* (Gmelin). *Aquatic Toxicology*, *91*(3), 245-254. https://doi.org/10.1016/j.aquatox.2008.11.016

Knight, D. B., & Davis, R. E. (2009). Contribution of tropical cyclones to extreme rainfall events in the southeastern United States. *Journal of Geophysical Research Atmospheres*, *114*(23). https://doi.org/10.1029/2009JD012511

La Peyre, M. K., Bernasconi, S. K., Lavaud, R., Casas, S. M., & La Peyre, J. F. (2020). Eastern oyster clearance and respiration rates in response to acute and chronic exposure to suspended sediment loads. *Journal of Sea Research*, *157*, 101831. https://doi.org/10.1016/j.seares.2019.101831

La Peyre, M. K., Eberline, B. S., Soniat, T. M., & La Peyre, J. F. (2013). Differences in extreme low salinity timing and duration differentially affect eastern oyster (*Crassostrea virginica*) size class growth and mortality in Breton Sound, LA. *Estuarine, Coastal and Shelf Science*, *135*, 146–157. https://doi.org/10.1016/j.ecss.2013.10.001

Lenihan, H. S., & Peterson, C. H. (1998). How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. *Ecological Applications*, *8*(1), 128-140. https://doi.org/10.1890/1051-0761(1998)008[0128:HHDTFD]2.0.CO;2

Livingston, R. J., Howell, R. L. IV., Niu, X., Lewis, G. F. III., & Woodsum, G. C. (1999). Recovery of Oyster Reefs (*Crassostrea virginica)* in a Gulf Estuary following disturbance by two hurricanes. *Bulletin of Marine Science, 64*(3), 465-483.

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., … Jackson, J. B. C. (2006). Depletion degradation, and recovery potential of estuaries and coastal seas. *Science*, *312*(5781), 1806-1809. https://doi.org/10.1126/science.1128035

Lowe, M. R., Sehlinger, T., Soniat, T. M., & Peyre, M. K. La. (2017). Interactive Effects of Water Temperature and Salinity on Growth and Mortality of Eastern Oysters, *Crassostrea virginica*: A Meta-Analysis Using 40 Years of Monitoring Data . *Journal of Shellfish Research*, *36*(3), 683–697. https://doi.org/10.2983/035.036.0318

Matoo, O. B., Ivanina, A. V., Ullstad, C., Beniash, E., & Sokolova, I. I. (2013). Interactive effects of elevated temperature and CO2 levels on metabolism and oxidative stress in two common marine bivalves (*Crassostrea virginica* and *Mercenaria mercenaria*). *Comparative Biochemistry and Physiology - A Molecular* *and Integrative Physiology*, *164*(4), 545-553. https://doi.org/10.1016/j.cbpa.2012.12.025

McCarty, A. J., McFarland, K., Small, J., Allen, S. K., & Plough, L. V. (2020). Heritability of acute low salinity survival in the Eastern oyster (*Crassostrea virginica*). *Aquaculture*, *529*, 735649. https://doi.org/10.1016/j.aquaculture.2020.735649

McCauley, D. J., Pinsky, M. L., Palumbi, S. R., Estes, J. A., Joyce, F. H., & Warner, R. R. (2015). Marine defaunation: Animal loss in the global ocean. *Science*, *347*(6219), 1255641–1255641. https://doi.org/10.1126/science.1255641

Michaelidis, B., Haas, D., & Grieshaber, M. K. (2005). Extracellular and intracellular acid-base status with regard to the energy metabolism in the oyster *Crassostrea gigas* during exposure to air. *Physiological and Biochemical Zoology*, *78*(3). https://doi.org/10.1086/430223

Mohammed, A. (2013). Why are early life stages of aquatic organisms more sensitive to toxicants than adults? New Insights into Toxicity and Drug Testing, InTech. https://doi.org/10.5772/55187

Munroe, D., Tabatabai, A., Burt, I., Bushek, D., Powell, E. N., & Wilkin, J. (2013). Oyster mortality in Delaware Bay: Impacts and recovery from Hurricane Irene and Tropical Storm Lee. *Estuarine, Coastal and Shelf Science*, *135*, 209-219. https://doi.org/10.1016/j.ecss.2013.10.011

Nash, S., & Rahman, M. S. (2019). Short-term heat stress impairs testicular functions in the American oyster, *Crassostrea virginica*: Molecular mechanisms and induction of oxidative stress and apoptosis in spermatogenic cells. *Molecular Reproduction and Development*, *86*(10), 1444-1458. https://doi.org/10.1002/mrd.23268

Pineda, M. C., McQuaid, C. D., Turon, X., López-Legentil, S., Ordóñez, V., & Rius, M. (2012). Tough Adults, Frail Babies: An Analysis of Stress Sensitivity across Early Life-History Stages of Widely Introduced Marine Invertebrates. *PLoS ONE*, *7*(10). https://doi.org/10.1371/journal.pone.0046672

Przeslawski, R., Davis, A. R., & Benkendorff, K. (2005). Synergistic effects associated with climate change and the development of rocky shore molluscs. *Global Change Biology*, *11*(3), 515-522. https://doi.org/10.1111/j.1365-2486.2005.00918.x

Rybovich, M., Peyre, M. K. La, Hall, S. G., & Peyre, J. F. La. (2016). Increased Temperatures Combined with Lowered Salinities Differentially Impact Oyster Size Class Growth and Mortality. *Journal of Shellfish Research*, *35*(1), 101–113. https://doi.org/10.2983/035.035.0112

Salerno, J., Gillis, P. L., Bennett, C. J., Sibley, P. K. & Prosser, R. S. (2018). Investigation of clearance rate as an endpoint in toxicity testing with freshwater mussels (Unionidae). *Ecotoxicology and Environmental Safety, 163*, 165-171. https://doi.org/10.1016/j.ecoenv.2018.07.054

Shumway, S., & Koehn, R. (1982). Oxygen consumption in the American oyster *Crassostrea virginica. Marine Ecology Progress Series, 9,* 59-68. 10.3354/meps009059

Smith, M.D. (2011). The ecological role of climate extremes: understanding and future prospects. *Journal of Ecology, 99*(3), 651-655. https://doi.org/10.1111/j.1365-2745.2011.01833.x

Sokolova, I. M., Frederich, M., Bagwe, R., Lannig, G., & Sukhotin, A. A. (2012). Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Marine Environmental Research*, *79,* 1-15. https://doi.org/10.1016/j.marenvres.2012.04.003

Sokolova, I. M., Sukhotin, A. A. & Lannig, G. (2012). Stress effects on metabolism and energy budgets in oysters. In D. Abele, J.P. Vazquez-Medina, and T. Zenteno-Savın. (Eds.), Oxidative Stress in Aquatic Ecosystems (pp. 263-280) Blackwell Publishing Ltd. https://doi.org/10013/epic.38895

Wetz, M. S., & Yoskowitz, D. W. (2013). An “extreme” future for estuaries? Effects of extreme climatic events on estuarine water quality and ecology. *Marine Pollution Bulletin, 69*(1-2), 7-18. https://doi.org/10.1016/j.marpolbul.2013.01.020

APPENDIX A

81 Asynchronous stressor articles resulting from the literature search, including articles not cited in the main text. ‘*’ indicates that the article is included in the references in the main text.

Amaral V, Cabral NH, Bishop MJ (2014) Prior exposure influences the behavioural avoidance by an intertidal gastropod, Bembicium auratum, of acidified waters. Estuar Coast Mar Sci 136:82–90. doi: 10.1016/j.ecss.2013.11.019

Amundrud SL, Srivastava DS (2019) Disentangling how climate change can affect an aquatic food web by combining multiple experiment approaches. Glob Chang Biol 25:3528-3538. doi: 10.1111/gcb.14717

Asplund ME, Baden SP, Russ S, et al (2014) Ocean acidification and host-pathogen interactions: blue mussels, *Mytilus edulis*, encountering *Vibrio tubiashii*. Environ Microbiol 16:1029-1039. doi: 10.1111/1462-2920.12307

Bertocci I, Godino JAD, Freitas C, et al (2016) Compounded perturbations in coastal areas: contrasting responses to nutrient enrichment and the regime of storm-related disturbance depends on life-history traits. Funct Ecol 31:1122-1134. doi: 10.1111/1365-2435.12815

*Bible JM, Cheng BS, Chang AL, et al (2017) Timing of stressors alters interactive effects on a coastal foundation species. Ecology 98:2468–2478. doi: 10.1002/ecy.1943

Bible JM, Evans TG, Sanford E (2020) Differences in induced thermotolerance among populations of Olympia oysters. Comp Biochem and Physiol A 239:110563.

doi: 10.1016/j.cbpa.2019.110563

Blake RE, Duffy JE (2010) Grazer diversity affects resistance to multiple stressors in an experimental seagrass ecosystem. Oikos 119:1625-1635. doi: 10.1111/j.1600-0706.2010.18419.x

Bond J, Bradley BP (1995) Heat-shock reduces the toxicity of malathion in *Daphnia magna*. Mar Environ Res 39:209-212. doi: 10.1016/0141-1136(94)00013-F

*Brown HM, Briden A, Stokell T, Griffin FJ, Cherr GN (2004) Thermotolerance and HSP70 profiles in adult and embryonic California native oysters, *Ostreola conchaphila* (Carpenter, 1857). J Shellfish Res 23:135–141.

Burleson ML, Silva PE (2011) Cross Tolerance to Environmental Stressors: Effects of Hypoxic Acclimation on Cardiovascular Responses of Channel Catfish (*Ictalurus punctatus*) to a Thermal Challenge. J Therm Biol 36:250-254. doi: 10.1016/j.jtherbio.2011.03.009

*Cao R, Liu Y, Wang Q, et al (2018a) Seawater acidification reduced the resistance of *Crassostrea gigas* to *Vibrio splendidus* challenge: An energy metabolism perspective. Front Physiol 9:880. doi: 10.3389/fphys.2018.00880

*Cao R, Wang Q, Yang D, et al (2018b) CO2-induced ocean acidification impairs the immune function of the Pacific oyster against *Vibrio splendidus* challenge: An integrated study from a cellular and proteomic perspective. Sci Total Environ 625:1574–1583. doi: 10.1016/j.scitotenv.2018.01.056

Cardinaud M, Offret C, Huchette S, Moraga D, Paillard C (2014) The impacts of handling and air exposure on immune parameters, gene expression, and susceptibility to vibriosis of European abalone *Haliotis tuberculata*. Fish Shellfish Immun 36:1-8. doi: 10.1016/j.fsi.2013.09.034

Castillo N, Saavedra LM, Vargas CA, Gallardo-Escárate C, Détrée C (2017) Ocean acidification and pathogen exposure modulate the immune response of the edible mussle *Mytilus chilensis*. Fish Shellfish Immun 70:149-155. doi: 10.1016/j.fsi.2017.08.047

*Chen JC, Chen WC (2000) Salinity tolerance of Haliotis diversicolor supertexta at different salinity and temperature levels. Aquaculture 181:191–203. doi: 10.1016/S0044-8486(99)00226-4

*Cheng BS, Bible JM, Chang AL, et al (2015) Testing local and global stressor impacts on a coastal foundation species using an ecologically realistic framework. Glob Chang Biol 21:2488–2499. doi: 10.1111/gcb.12895

Cherkasov AS, Biswas PK, Ridings DM, Ringwood AH, Sokolova IM (2006) Effects of acclimation temperature and cadmium exposure on cellular energy budgets in the marine mollusk *Crassostrea virginica*: Linking cellular and mitochondrial responses. J Exp Biol 209:1274–1284. doi: 10.1242/jeb.02093

Damgaard RM, Davenport J (1994) Salinity tolerance, salinity preference and temperature tolerance in the high-shore harpacticoid copepod Tigriopus brevicornis. Mar Biol 118:443–449. doi: 10.1007/BF00350301

de Orte MR, Clowez S, Calderia K (2019) Reached of bleached and symbiotic sea anemones to plastic microfiber exposure. Environ Pollut 249:512-517. doi: 10.1016/j.envpol.2019.02.100

*Dietz TJ, Somero GN (1992) The threshold induction temperature of the 90-kDa heat shock protein is subject to acclimatization in eurythermal goby fishes (genus *Gillichthys*). Proc Natl Acad Sci U S A 89:3389–3393. doi: 10.1073/pnas.89.8.3389

*Dinh KV, Janssens L, Stoks R (2016) Exposure to a heat wave under food limitation makes an agricultural insecticide lethal: a mechanistic laboratory experiment. Glob Chang Biol 22:3361–3372. doi: 10.1111/gcb.13415

*Dixson DL, Munday PL, Jones GP (2010) Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. Ecol Lett 13:68–75. doi: 10.1111/j.1461-0248.2009.01400.x

*Dong Y, Dong S, Meng X (2008) Effects of thermal and osmotic stress on growth, osmoregulation and Hsp70 in sea cucumber (*Apostichopus japonicus* Selenka). Aquaculture 276:179–186. doi: 10.1016/j.aquaculture.2008.01.028

Dong Y, Zhang S (2016) Ecological relevance of energy metabolism: transcriptional responses in energy sensing and expenditure to thermal and osmotic stresses in an intertidal limpet. Funct Ecol 30:1539-1548. doi: 10.1111/1365-2435.12625

Dorts J, Kestemont P, Thézenas M, Raes M, Silvestre F (2014) Effects of cadmium exposure on the gill proteome of *Cottus gobio*: Modulatory effects of prior thermal acclimation. Aquat Toxicol 154: 87-96. doi: 10.1016/j.aquatox.2014.04.030

*DuBeau SF, Pan F, Tremblay GC, Bradley TM (1998) Thermal shock of salmon in vivo induces the heat shock protein hsp 70 and confers protection against osmotic shock. Aquaculture 168:311-323. doi: 10.1016/S0044-8486(98)00358-5

Ellis RP, Widdicombe S, Parry H, Hutchinson TH, Spicer JI (2015) Pathogenic challenge revelas immune trade-off in mussels exposed to reduced seawater pH and increased temperature. J Exp Mar Biol Ecol 462:83-89. doi: 10.1016/j.jembe.2014.10.015

*Floyd EY, Geist JP, Werner I (2008) Acute, sublethal exposure to a pyrethroid insecticide alters behavior, growth, and predation risk in larvae of the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 27:1780–1787. doi: 10.1897/07-448.1

Freuchet F, Tremblay R, Flores AAV (2015) Interacting environmental stressors modulate reproductive output and larval performance in a tropical intertidal barnacle. Mar Ecol Prog Ser 532:161-175. doi: 10.3354/meps11377

*García Molinos J, Donohue I (2010) Interactions among temporal patterns determine the effects of multiple stressors. Ecol Appl 20:1794–1800. doi: 10.1890/10-0018.1

García Molinos, J, Donohue I (2011) Temporal variability within disturbance events regulates their effects on natural communities. Oecologia 166:795–806. doi: 10.1007/s00442-011-1923-2

*Gurr SJ, Vadopalas B, Roberts SB, Putnam HM (2020) Metabolic recovery and compensatory shell growth of juvenile Pacific geoduck *Panopea generosa* following short-term exposure to acidified seawater. Conserv Physiol 8. doi: 10.1093/conphys/coaa024

Hernroth B, Baden S, Tassidis H, et al (2016) Impact of ocean acification on antimicrobial activity in gills of the blue mussel (*Mytilus edulis*). Fish Shellfish

Immun 55:452-459. doi:10.1016/j.fsi.2016.04.007

Hettinger A, Sanford E, Hill TM, et al (2012) Persistent carry-over effects of planktonic exposure to ocean acidifcation in the Olympia oyster. Ecology 93: 2758-2768. doi: 10.1890/12-0567.1

Hu M, Li Q, Li L (2010) Effect of salinity and temperatuer on salinity tolerance of the sea cucumber *Apostichopus japonicus*. Fish Sci 76:267-273. doi: 10.1007/s12562-010-0214x

*Janssens L, Tüzün N, Stoks R (2017) Testing the time-scale dependence of delayed interactions: A heat wave during the egg stage shapes how a pesticide interacts with a successive heat wave in the larval stage. Environ Pollut 230:351–359. doi: 10.1016/j.envpol.2017.06.082

Jian CY, Cheng SY, Chen JC (2003) Temperature and salinity tolerances of yellowfin sea bream, *Acanthopagrus latus*, at different salinity and temperature levels. Aquac Res 34:175–185. doi: 10.1046/j.1365-2109.2003.00800.x

*Jurgens LJ, Freestone AL, Ruiz GM, Torchin ME (2017) Prior predation alters community resistance to an extreme climate disturbance. Ecosphere 8: e01986. doi: 10.1002/ecs2.1986

Kashian DR, Zuellig RE, Mitchell KA, Clements WH (2007) The cost of tolerance: Sensitivity of stream benthic communities to UV-B and metals. Ecol Appl 17: 365-375. doi: 10.1890/06-0396

Labaude S, Cézilly F, Tercier X, Rigaud T (2015) Influence of host nutritional condition on post-infection traits in the association between the manipulative acanthocephalan

Pomphorhynchus laevis and the amphipod *Gammarus pulex*. Parasites Vectors 8:403. doi: 10.1186/s13071-015-1017-9

*Labaude S, Rigaud T, Cézilly F (2017) Additive effects of temperature and infection with an acanthocephalan parasite on the shredding activity of *Gammarus fossarum* (Crustacea: Amphipoda): the importance of aggregative behavior. Glob Chang Biol 23:1415-1424. doi: 10.1111/gcb.13490

Lefcort H, Wehner EA, Cocco PL (2013) Pre-exposure to heavy metal pollution and the odor of predation decrease the ability of snails to avoid stressors. Arch Environ Contam Toxicol 64:273–280. doi: 10.1007/s00244-012-9821-0

*Lefcort H, Cleary DA, Marble AM, et al (2015) Snails from heavy-metal polluted environments have reduced sensitivity to carbon dioxide-induced acidity. Springerplus 4:267. doi: 10.1186/s40064-015-1073-9

*Levesque KD, Wright PA, Bernier NJ (2019) Cross talk without cross tolerance: effect of rearing temperature on the hypoxia response of embryonic zebrafish. Physiol Biochem Zool 92:349–364. doi: 10.1086/703178

Long Y, Yan J, Song G, et al (2015) Transcriptional events co-regulated by hypoxia and cold stresses in Zebrafish larvae. BMC Genomics 16:385. doi: 10.1186/s12864-015-1560-y

Manush SM, Pal AK, Chatterjee N, Das T, Mukherjee SC (2004) Thermal tolerance and oxygen consumption of *Macrobrachium rosenbergii* acclimated to three temperatures. J Therm Biol 29:15–19. doi: 10.1016/j.jtherbio.2003.11.005

Martín M, Hernández C, Bodega G, I, et al (1998) Heat-shock proteins expression in fish central nervous system and its possible relation with water acidosis resistance. Neurosci Res 31:97–106. doi: 10.1016/S0168-0102(98)00028-5

McBryan TL, Healy TM, Haakons KL, Schulte PM (2016) Warm acclimation improves hypoxia tolerance in Fundulus heteroclitus. J Exp Biol 219:474–484. doi: 10.1242/jeb.133413

Měráková E, Gvoždík L (2009) Thermal acclimation of swimming performance in newt larvae: The influence of diel temperature fluctuations during embryogenesis. Funct Ecol 23:989–995. doi: 10.1111/j.1365-2435.2009.01588.x

Metzger DCH, Healy TM, Schulte PM (2016) Conserved effects of salinity acclimation on thermal tolerance and *hsp70* expression in divergent populations of threespine stickleback (*Gasterosteus aculeatus*). J Comp Phys B 186:879-889. doi: 10.1007/s00360-016-0998-9

*Miller SH, Zarate S, Smith EH, et al (2014) Effect of elevated pCO2 on Metabolic Responses of Porcelain Crab (*Petrolisthes cinctipes*) Larvae Exposed to Subsequent Salinity Stress. PLOS ONE 9. doi: 10.1371/journal.pone.0109167

*Møller V, Forbes VE, Depledg MH (1994) Influence of acclimation and exposure temperature on the acute toxicity of cadmium to the freshwater snail *Potamopyrgus antipodarum* (hydrobiidae). Environ Toxicol Chem 13:1519–1524. doi: 10.1002/etc.5620130914

Müller WEG, Koziol C, Dapper J, et al (1995) Combinatory effects of temperature stress and nonionic organic pollutants on stress protein (hsp70) gene expression in the freshwater sponge *Ephydatia fluviatilis*. Environ Toxicol Chem 14:1203-1208. doi:

10.1002/etc.5620140712

*Niu CJ, Rummer JL, Brauner CJ, Schulte PM (2008) Heat shock protein (Hsp70) induced by a mild heat shock slightly moderates plasma osmolarity increases upon salinity transfer in rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol C 148:437–444. doi: 10.1016/j.cbpc.2008.04.011

*Oliveira JP, Sousa-Pinto I, Weber GM, Bertocci I (2014) Interplay of experimental harvesting and climate-related disturbance on benthic assemblages of rocky seashores. Mar Ecol Prog Ser 495:131–142. doi: 10.3354/meps10574

*Paganini AW, Miller NA, Stillman JH (2014) Temperature and acidification variability reduce physiological performance in the intertidal zone porcelain crab *Petrolisthes cinctipes*. J Exp Biol 217:3974–3980. doi: 10.1242/jeb.109801

Paillard C, Allam B, Oubella R (2004) Effect of temperature on defense parameters in Manila clam *Ruditapes philippinarum*. Dis Aquat Organ 59:249-262. doi: 10.3354/dao059249

*Pallarés S, Botella-Cruz M, Arribas P, Millán A, Velasco J (2017) Aquatic insects in a multistress environment: cross-tolernace to salinity and dessication. J Exp Biol 220:1277-1286. doi: 10.1242/jeb.152108

*Péden R, Rocher B, Chan P, et al (2018) Highly polluted life history and acute heat stress, a hazardous mix for blue mussels. Mar Pollut Bull 135:594-606. doi: 10.1016/j.marpolbul.2018.07.066

*Pincebourde S, Sanford E, Casas J, Helmuth B (2012) Temporal coincidence of environmental stress events modulates predation rates. Ecol Lett 15:680–688.

doi: 10.1111/j.1461-0248.2012.01785.x

Ravaux J, Léger N, Rabet N, et al (2012) Adaptation to thermally variable environments: capacity for acclimation of thermal limit and heat shock response in the shrimp *Palaemonetes varians*. J Comp Phys B 182:899-907. doi: 10.1007/s00360-012-0666-7

Re AD, Diaz F, Sierra E, Rodríguez J, Perez E (2005) Effect of salinity and temperature on thermal tolerance of brown shrimp *Farfantepenaeus aztecus* (Ives) (Crustacea, Penaeidae). J Therm Biol 30:618–622. doi: 10.1016/j.jtherbio.2005.09.004

Rees BB, Sudradjat FA, Love JW (2001) Acclimation to hypoxia increases survival time of zebrafish, Danio rerio, during lethal hypoxia. J Exp Zool 289:266–272. doi: 10.1002/1097-010X(20010401/30)289:4<266::AID-JEZ7>3.0.CO;2-5

*Renick VC, Anderson TW, Morgan SG, Cherr GN (2015) Interactive effects of pesticide exposure and habitat structure on behavior and predation of a marine larval fish. Ecotoxicology 24:391–400. doi: 10.1007/s10646-014-1388-2

*Roch M, Maly EJ (1979) Relationship of cadmium-induced hypocalcemia with mortality in rainbow trout (*Salmo gairdneri*) and the influence of temperature on toxicity. J Fish Res Board Can 36:1297–1303. doi: 10.1139/f79-187

Ruesink JL, Fitzpatrick JP, Dumbauld BR, et al (2012) Life history and morphological shifts in an intertidal seagrass following multiple disturbances. J Exp Mar Biol Ecol 424-425:25-31. doi: 10.1016/j.jembe.2012.05.002

Rutledge CJ, Beitinger TL (1989) The effects of dissolved oxygen and aquatic surface respiration on the critical thermal maxima of three intermittent-stream fishes.

Environ Biol Fishes 24:137–143. doi: 10.1007/BF00001283

Sampaio LA, Wasielesky W, Campos Miranda-Filho K (2002) Effect of salinity on acute toxicity of ammonia and nitrite to juvenile *Mugil platanus*. Bull Environ Contam Toxicol 68:668–674. doi: 10.1007/s001280306

*Siegle MR, Taylor EB, O'Connor MI (2018) Prior heat accumulation reduces survival during subsequent experimental heat waves. J Exp Mar Biol Ecol 501:109–117. doi: 10.1016/j.jembe.2018.01.012

Sinha AK, AbdElgawad H, Zinta G, et al (2015a) Nutritional Status as the Key Modulator of Antioxidant Responses Induced by High Environmental Ammonia and Salinity Stress in European Sea Bass (*Dicentrarchus labrax*). PLOS One 10. doi: 10.1371/journal.pone.0135091

Sinha AK, Rasoloniriana R, Dasan AF, et al (2015b) Interactive effect of high environmental ammonia and nutritional status on ecophysiological performance of European sea bass (*Dicentrarchus labrax*) acclimated to reduced seawater salinities. Aquat Toxicol 160:39–56. doi: 10.1016/j.aquatox.2015.01.005

Sinha AK, Dasan F, Rasoloniriana R, et al (2015c) Hypo-osmotic stress-induced physiological and ion-osmoregulatory responses in European sea bass (*Dicentrarchus labrax*) are modulated differentially by nutritional status. Comp Biochem Physiol A 181:87-99. doi: 10.1016/j.cbpa.2014.11.024

*Sniegula S, Janssens L, Stoks R (2017) Integrating multiple stressors across life stages and latitudes: Combined and delayed effects of an egg heat wave and larval pesticide exposure in a damselfly. Aquat Toxicol 186:113–122.

doi: 10.1016/j.aquatox.2017.02.029

Stefanidou N, Genitsaris S, Lopez-Bautista J, Sommer U, Moustaka-Gouni M (2018a) Unicellular Eukaryotic Communnity Response to Temperature and Salinity Variation in Mesocosm Experiments. Front Microbiol 9:2444. doi: 10.3389/fmicb.2018.02444

Stefanidou N, Genitsaris S, Lopez-Bautista J, Sommer U, Moustaka-Gouni M (2018b) Effects of heat shock and salinity changes on coastal Mediterranean phytoplankton in a mesocosm experiment. Mar Biol 165:154. doi: 10.1007/s00227-018-3415-y

*Stefanidou N, Genitsaris S, Lopez-Bautista J, Sommer U, Moustaka-Gouni M (2019) Response of a coastal Baltic Sea diatom-dominated phytoplankton community to experiment heat shock and changing salinity. Oecologia 191:461-474. doi: 10.1007/s00442-019-04502-0

Sung YY, Van Damme EJM, Sorgeloos P, Bossier P (2007) Non-lethal heat shock protects gnotobiotic *Artemia franciscana* larvae against virulent Vibrios. Fish Shellfish Immun 22:318-326. doi: 10.1016/j.fsi.2006.05.008

Sung YY, Pineda C, MacRae TH, Sorgeloos P, Bossier P (2008) Exposure of gnotobiotic *Artemia franciscana* larvae to abiotic stress promotes heat shock protein 70 synthesis and enhances resistance to pathogenic *Vibrio campbellii*. Cell Stress Chaperones 13:59-66. doi: 0.1007/s12192-008-0011-y

*Tedengren M, Olsson B, Reimer O, Brown DC, Bradley BP (2000) Heat pretreatment increases cadmium resistance and HSP 70 levels in Baltic Sea mussels. Aquat Toxicol 48:1–12. doi: 10.1016/S0166-445X(99)00030-2

*Todgham AE, Schulte PM, Iwama GK (2005) Cross-tolerance in the tidepool sculpin: The role of heat shock proteins. Physiol Biochem Zool 78:133–144. doi: 10.1086/425205

Vergauwen L, Knapen D, Hagenaars A, Blust R (2013) Hypothermal and hyperthermal acclimation differentially modulate cadmium accumulation and toxicity in the zebrafish. Chemosphere 91:521–529. doi: 10.1016/j.chemosphere.2012.12.028