

UVM ScholarWorks

Copepods As A Model System For Exploring The Impacts Of Climate Change On Marine Ectotherms

Item Type	dissertation;article
Authors	Ashlock, Lauren
Download date	2026-06-12 08:35:25
Link to Item	https://hdl.handle.net/20.500.14849/3557

COPEPODS AS A MODEL SYSTEM FOR EXPLORING THE IMPACTS OF CLIMATE CHANGE ON
MARINE ECTOTHERMS

A Dissertation Presented

by

Lauren W. Ashlock

to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements
For the Degree of Doctor of Philosophy
Specializing in Biology

October, 2022

Defense Date: August 31, 2022
Dissertation Examination Committee:

Melissa Pespeni, Ph.D., Advisor
Stephen Keller, Ph.D., Chairperson
Hans Dam, Ph.D.
Brent Lockwood, Ph.D.
Jason Stockwell, Ph.D.
Cynthia J. Forehand, Ph.D., Dean of the Graduate College

ABSTRACT

Marine ecosystems provide essential habitat to ecologically and economically impactful species and provide humans with a wealth of ecosystem services. With climate change, marine ecosystems are increasing in mean temperature and temperature variability. Marine ectotherms are vulnerable to this change and are important sentinels of warming, as their internal physiology is dependent on the external thermal environment.

Copepods are marine ectotherms that play a critical role in trophic transfer and nutrient cycling. Importantly, copepods are relatively short-lived, allowing them to track ocean change as it happens. Together, these qualities make copepods a reliable model for understanding the impacts of global change on marine ectotherms. In this dissertation, I assess the impact of climate change on copepods through three strategies, 1) Examining the vulnerability of the nutrient rich copepod *Neocalanus plumchrus* to ocean warming, 2) Investigating the roles of plasticity and adaptation in copepod response to climate change stressors, 3) Testing the importance of latitude and life history stage in determining copepod thermal tolerance.

My results reveal the nutrient rich copepod *Neocalanus plumchrus* and the numerically dominant copepod *Acartia tonsa* are potentially vulnerable to continued warming. Integrating ten years of plankton survey data with satellite temperature data, I find that thermal thresholds vary by copepod species and that the most nutrient rich species, *Neocalanus plumchrus*, has a relatively low upper thermal threshold for occurrence of 11.5°C. Additionally, results demonstrate that *N. plumchrus* abundance was reduced during the 2014-2016 marine heatwave. With experimental methods, I demonstrate that *Acartia tonsa* thermal tolerance is reduced after rapid salinity shock, indicating its susceptibility to simultaneous salinity and temperature change. Together these results indicate that copepods are vulnerable to continued climate change.

My research also elucidates important determinants of thermal tolerance in the copepod *Acartia tonsa*. Using experimental evolution, I find that development at ocean warming conditions imparts the same level of thermal tolerance as >50 generations of ocean warming. Additionally, through field collections and two generations of common garden conditions, I find that thermal tolerance varies with latitude, with copepods from Maine having lower thermal tolerances than copepods collected in New York and Florida. Importantly, we also find that juvenile *A. tonsa* are more thermally tolerant than larvae and adults. These results highlight the importance of plasticity in defining thermal tolerance, emphasizing the importance of the developmental environment for marine ectotherms. Additionally, results reveal that thermal tolerance is specific to life history stage and latitude of collection.

CITATIONS

Material from this dissertation has been published in the following form:

Ashlock L, García-Reyes M, Gentemann C, Batten S, Sydeman W.. Temperature and Patterns of Occurrence and Abundance of Key Copepod Taxa in the Northeast Pacific. *Frontiers in Marine Science*. 2021:1271.

DEDICATION

To my grandma Stella.

ACKNOWLEDGEMENTS

Thank you to my advisor, Dr. Melissa Pespeni, for believing in me and giving me a scientific home for the past six years. There is no better person with whom to share new scientific results or brainstorm new ideas. This dissertation would not have been possible without your guidance and support. Thank you to my committee chair, Dr. Stephen Keller, for your kindness and essential advice on statistics and experimental design. Thank you to Dr. Jason Stockwell for expanding my copepod knowledge and for grounding my experimental methods in ecology. Thank you to Dr. Brent Lockwood for giving me a second home in the Lockwood Lab and always being willing to nonjudgmentally listen to my new scientific ideas and questions. Thank you to Dr. Hans Dam for making my work with *Acartia tonsa* possible. From sourcing our original copepod populations, to hosting me in your lab so I could learn new copepod methods, and answering all of my many questions, I am so appreciative of your support.

Thank you to the past and present members of the Pespeni Lab for your friendship, support and collaboration. You made this process of independent research feel much less lonely and much more fun. Thank you also to all of the students I worked with that helped make this research possible: Emily Kaloudis, Emily Shore, Chelsea Darwin, Jessica Crooker, Anya Steinhart, Hannah Edmonds, Erika Petterson, and Maria Cayia.

Thank you to the Dam lab for teaching me all about copepods, and for your kindness and advice. Thank you to the members of the Lockwood Lab for kindly giving me advice about thermal physiology, and letting me use your water baths for my temperature assays. Thank you to my collaborators and co-authors at the Farallon Institute for making me feel

welcome in a new environment. Thank you especially to Dr. Marisol García-Reyes for mentoring me throughout my internship and beyond. Thank you to Dr. Lori Stevens and April Berteau for making my QuEST internship possible.

Thank you to past and present students and faculty in the biology department and QuEST program. Thank you especially to Dr. Lauren Ash, Dr. Emily Mikucki, and Dr. Lindsey Pett for their friendship and mentorship. I am also grateful to Dr. Brittany Verrico, Natalia Aristizábal, and Mariah Boudreau whose care and support made the completion of this degree possible.

Thank you to my friends and family, who fill my life with meaning and inspire me every day. I am endlessly grateful for your love and support. Each of you played an essential role in the completion of this dissertation. Thank you also to my sweet little family, Steven and Zeke. You make me grateful for each day.

TABLE OF CONTENTS

CITATIONS	II
DEDICATION	III
ACKNOWLEDGEMENTS.....	IV
INTRODUCTION.....	1
Literature Cited.....	5
CHAPTER I: TEMPERATURE AND PATTERNS OF OCCURRENCE AND ABUNDANCE OF KEY COPEPOD TAXA IN THE NORTHEAST PACIFIC	9
Abstract.....	9
Introduction.....	10
Methods.....	13
Results.....	18
Discussion.....	21
Literature cited.....	50
Tables and Figures.....	55
CHAPTER II: DEVELOPMENTAL TEMPERATURE MORE THAN LONG-TERM EVOLUTION DEFINES THERMAL TOLERANCE IN AN ESTUARINE COPEPOD.....	61
Abstract.....	61
Introduction.....	62
Methods.....	65
Results.....	69
Discussion	71
Figures and Tables.....	79
Literature Cited.....	82
CHAPTER III: LATITUDINAL AND LIFE STAGE DIFFERENCES IN HIGH TEMPERATURE AND LOW SALINITY TOLERANCE IN AN ESTUARINE COPEPOD.....	88
Abstract.....	88
Introduction.....	89
Materials & Methods.....	92
Results.....	95
Discussion	96
Tables & Figures	110
CONCLUDING REMARKS	115
COMPREHENSIVE BIBLIOGRAPHY	121

INTRODUCTION

The ocean serves as a habitat for marine organisms, protects coastal communities from storms, and provides humans with a critical food source (Palumbi et al., 2009, Sandifer and Sutton-Grier, 2014, Scavia et al., 2002). Additionally, the ocean buffers the impacts of anthropogenic climate change by absorbing and sequestering greenhouse gases and heat (Booth et al., 2017, Reid et al., 2010, Bates et al., 2012). The ocean is also threatened by climate change induced increases in mean sea surface temperatures, temperature variability, and marine heatwaves (Scheffers et al., 2016, Harley et al., 2006, Frölicher *et al.*, 2018, Oliver *et al.*, 2018). Changing environmental temperatures will directly impact the physiology of ectotherms (Huey and Kingsolver, 1989, Angilletta et al., 2010). Importantly, marine ectotherms have narrower thermal safety margins than terrestrial ectotherms, indicating they may be more vulnerable to climate change (Pinsky et al., 2019). Additionally, these thermal safety margins will narrow with continued warming (Gunderson and Stillman 2015). Advancement of research on the mechanisms of thermal tolerance in marine ectotherms may help mitigate the detrimental effects of anthropogenic climate change on marine ecosystems.

Thermal tolerance is a complex and plastic trait that changes across life stages, populations, and acclimation temperatures. The thermal tolerance breadth of marine ectotherms increases with latitude, while upper and lower thermal limits decline with latitude (Sunday et al., 2019). Importantly, the thermal limits of ectotherms show a positive relationship with extreme thermal temperatures (Sunday et al., 2019), indicating that the increasing prevalence of temperature extremes and marine heat waves under

global change may influence thermal tolerance. Response to these changes will depend in part on the life history strategies of marine organisms, which are complex and diverse with many species having a planktonic life stage (Marshall and Morgan 2011). Life history stages can have distinct morphologies, physiologies, and habitats (Tangwancharoen and Burton 2014, Marshall et al., 2016). This results in life history stage dependent thermal tolerance, where primarily early life stages are more vulnerable than later life stages (Pandori and Sorte 2019, but see Tangwancharoen and Burton 2014). In addition to life history stage differences in thermal tolerance, local adaptation to temperature in marine invertebrates is evident (Gleason and Burton 2013, Willett 2010, Kelly et al., 2012). Even within life stages and populations, acclimation temperature can impact thermal tolerance, with animals acclimated to higher temperatures having higher thermal tolerance limits (Sasaki and Dam 2019, Stillman and Somero 2000).

Marine ectotherms are not simply subject to environmental temperatures, but can adjust their thermal tolerance through plasticity and adaptation. Plasticity is the capacity for genotypes to express different phenotypes in response to environmental change (Fordyce et al., 2006, West-Eberhard 2003, Somero et al., 2017, Whitman et al., 2009). Importantly, plasticity can cause phenotypic change within a generation (Somero et al., 2017). Therefore, plasticity provides short-term resilience to changing climate conditions by buying populations time to adapt in response to change across generations (Snell-Rood et al., 2018, Diamond and Martin 2021). Another potential coping strategy for changing environmental conditions is adaptation, the process of genetic change in a population across generations in response to selection that improves population fitness in a given environment (Hartl, 2000, Hendry, 2016). *Drosophila subobscura* exhibits

genome-wide shifts in allele frequencies in response to a heat wave, indicating genetic adaptation to thermal conditions (Rodríguez-Trelles et al., 2013). Additionally, coccolithophores and copepods exhibit rapid adaptation in response to increased experimental temperatures (Schlüter et al., 2014, Dam et al., 2021, Sasaki and Dam 2021). Exploring the dynamics and limitations of plasticity and adaptation in response to climate change is critical for understanding ectotherm mechanisms of response to changing climate conditions.

Copepods are an excellent model system for understanding patterns of plasticity, adaptation, and thermal tolerance in marine ectotherms. Copepods are highly impactful aquatic organisms and are one of the most abundant metazoans on the planet (Humes, 1994, Turner 2004). They drive nutrient cycling in the ocean (Dam and Baumann 2017, Steinberg *et al.*, 2017, Richardson *et al.*, 2008, Frangoulis et al., 2010), connect primary producers and the microbial food web to upper-level consumers (Dam and Baumann 2017, Liu et al., 2017), and transport carbon to the benthos (Dam and Baumann 2017, Richardson *et al.*, 2008). Additionally, copepods have relatively short generation times, making them valuable sentinels of change in aquatic ecosystems (Hays et al., 2005, Liu et al., 2017, Richardson et al., 2008, Dam, 2013). Their short generation time also allows for tractable studies regarding evolutionary response to environmental change. Importantly, copepods are a critical food source for ecologically and economically important fishes (Steinberg et al., 2017, Richardson et al., 2008). Therefore, knowledge of their tolerance to climate change is relevant to food web stability and fisheries health.

Here, I combined experimental methods with environmental data science to reveal determinants of thermal tolerance and potential vulnerability of copepods to changing climate conditions. My work is subdivided into three chapters 1) Temperature and Patterns of Occurrence and Abundance of Key Copepod Taxa in the Northeast Pacific, 2) Developmental Temperature more than Long-Term Evolution Defines Thermal Tolerance in an Estuarine Copepod, and 3) Latitudinal and Life Stage Differences in High Temperature and Low Salinity Tolerance in an Estuarine Copepod. Each chapter fulfills the respective specific aims, 1) Identify vulnerability of an ecologically and economically impactful copepod to ocean warming and marine heatwaves, 2) Reveal the relative roles and limitations of plasticity and adaptation in the copepod response to ocean warming, 3) Elucidate the impact of collection location and life history stage on the thermal tolerance of copepods. The fulfillment of these aims contributes to the fields of marine ecology, evolutionary biology, and thermal physiology while also furthering knowledge about aquatic ectotherm resilience to global change. Assessments of thermal tolerance and adaptive capacity are important for determining future species persistence in the face of climate change (Nicotra et al., 2015, Bernhardt et al., 2013, Collin et al., 2021). Additionally, the literature calls for vulnerability assessments and biodiversity management efforts that incorporate evolutionary capacity and plasticity (Hoffmann and Sgrò 2011, Ofori et al., 2017). Importantly, ignoring the components of adaptive capacity can influence the results of species vulnerability assessments (Ofori et al., 2017). This motivates my research on the thermal limits, evolutionary capacity, and thermal tolerance plasticity of copepods.

Literature Cited

- Angilletta Jr, M. J., Cooper, B. S., Schuler, M. S., & Boyles, J. G. (2010). The evolution of thermal physiology in endotherms. *Frontiers in Bioscience-Elite*, 2: 861-881.
- Bates, N. R., Best, M. H. P., Neely, K., Garley, R., Dickson, A. G., & Johnson, R. J. (2012). Detecting anthropogenic carbon dioxide uptake and ocean acidification in the North Atlantic Ocean. *Biogeosciences*, 9: 2509-2522.
- Bernhardt, J. R., & Leslie, H. M. (2013). Resilience to climate change in coastal marine ecosystems. *Annual review of marine science*, 5: 371-392.
- Booth, D. J., Poloczanska, E., Donelson, J. M., Molinos, J. G., & Burrows, M. (2017). Biodiversity and climate change in the oceans. *Climate change impacts on fisheries and aquaculture: A global analysis*, 1: 63-89.
- Collin, R., Rebolledo, A. P., Smith, E., & Chan, K. Y. K. (2021). Thermal tolerance of early development predicts the realized thermal niche in marine ectotherms. *Functional Ecology*, 35: 1679-1692.
- Dam, H. G. (2013). Evolutionary adaptation of marine zooplankton to global change. *Annual Review of Marine Science*, 5: 349-370.
- Dam, H. G., & Baumann, H. (2017). Climate change, zooplankton and fisheries. *Climate change impacts on fisheries and aquaculture: A global analysis*, 2: 851-874.
- Dam, H. G., deMayo, J. A., Park, G., Norton, L., He, X., Finiguerra, M. B., ... & Pespeni, M. H. (2021). Rapid, but limited, zooplankton adaptation to simultaneous warming and acidification. *Nature Climate Change*, 11: 780-786.
- Diamond, S. E., & Martin, R. A. (2021). Buying time: Plasticity and population persistence. In *Phenotypic plasticity & evolution*, 185-209. CRC Press.
- Fordyce, J. A. (2006). The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *Journal of Experimental Biology*, 209: 2377-2383.
- Frölicher, T. L., Fischer, E. M., & Gruber, N. (2018). Marine heatwaves under global warming. *Nature*, 560: 360-364.
- Gleason, L. U., & Burton, R. S. (2013). Phenotypic evidence for local adaptation to heat stress in the marine snail *Chlorostoma* (formerly *Tegula*) *funnebralis*. *Journal of experimental marine biology and ecology*, 448: 360-366.
- Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*, 282: 20150401.

- Harley, C. D., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J., Thornber, C. S., ... & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology letters*, 9: 228-241.
- Hartl, D. L. (2020). *A primer of population genetics and genomics*. Oxford University Press.
- Hays, G. C., Richardson, A. J., & Robinson, C. (2005). Climate change and marine plankton. *Trends in ecology & evolution*, 20: 337-344.
- Hendry, A. P. (2016). *Eco-evolutionary dynamics*. Princeton university press.
- Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, 470: 479-485.
- Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends in ecology & evolution*, 4: 131-135.
- Humes, A. G. (1994). How many copepods?. In *Ecology and morphology of copepods*, 1-7. Springer, Dordrecht.
- Kelly, M. W., Sanford, E., & Grosberg, R. K. (2012). Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proceedings of the Royal Society B: Biological Sciences*, 279: 349-356.
- Liu, H., Zhang, X., Yang, Q., Zuo, T., & Quigg, A. (2017). Mesozooplankton dynamics in relation to environmental factors and juvenile fish in a subtropical estuary of the Gulf of Mexico. *Journal of Coastal Research*, 33: 1038-1050.
- Marshall, D. J., & Morgan, S. G. (2011). Ecological and evolutionary consequences of linked life-history stages in the sea. *Current Biology*, 21: R718-R725.
- Marshall, D. J., Burgess, S. C., & Connallon, T. (2016). Global change, life-history complexity and the potential for evolutionary rescue. *Evolutionary Applications*, 9: 1189-1201.
- Nicotra, A. B., Beever, E. A., Robertson, A. L., Hofmann, G. E., & O'Leary, J. (2015). Assessing the components of adaptive capacity to improve conservation and management efforts under global change. *Conservation Biology*, 29: 1268-1278.
- Ofori, B. Y., Stow, A. J., Baumgartner, J. B., & Beaumont, L. J. (2017). Influence of adaptive capacity on the outcome of climate change vulnerability assessment. *Scientific reports*, 7: 1-12.
- Oliver, E. C., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., ... & Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature communications*, 9: 1-12.

- Palumbi, S. R., Sandifer, P. A., Allan, J. D., Beck, M. W., Fautin, D. G., Fogarty, M. J., ... & Wall, D. H. (2009). Managing for ocean biodiversity to sustain marine ecosystem services. *Frontiers in Ecology and the Environment*, 7: 204-211.
- Pandori, L. L., & Sorte, C. J. (2019). The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. *Oikos*, 128: 621-629.
- Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., & Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature*, 569: 108-111.
- Reid, P. C., Fischer, A. C., Lewis-Brown, E., Meredith, M. P., Sparrow, M., Andersson, A. J., ... & Washington, R. (2009). Impacts of the oceans on climate change. *Advances in marine biology*, 56: 1-150.
- Richardson, A. J. (2008). In hot water: zooplankton and climate change. *ICES Journal of Marine Science*, 65: 279-295.
- Rodríguez-Trelles, F., Tarrío, R., & Santos, M. (2013). Genome-wide evolutionary response to a heat wave in *Drosophila*. *Biology Letters*, 9: 20130228.
- Sandifer, P. A., and Sutton-Grier, A. E. (2014). Connecting stressors, ocean ecosystem services, and human health. In *Natural Resources Forum* 38: 157-167.
- Sasaki, M. C., & Dam, H. G. (2019). Integrating patterns of thermal tolerance and phenotypic plasticity with population genetics to improve understanding of vulnerability to warming in a widespread copepod. *Global change biology*, 25: 4147-4164.
- Sasaki, M. C., & Dam, H. G. (2021). Negative relationship between thermal tolerance and plasticity in tolerance emerges during experimental evolution in a widespread marine invertebrate. *Evolutionary applications*, 14: 2114-2123.
- Scavia, D., Field, J. C., Boesch, D. F., Buddemeier, R. W., Burkett, V., Cayan, D. R., ... & Titus, J. G. (2002). Climate change impacts on US coastal and marine ecosystems. *Estuaries*, 25: 149-164.
- Scheffers, B. R., De Meester, L., Bridge, T. C., Hoffmann, A. A., Pandolfi, J. M., Corlett, R. T., ... & Watson, J. E. (2016). The broad footprint of climate change from genes to biomes to people. *Science*, 354: aaf7671.
- Schlüter, L., Lohbeck, K. T., Gutowska, M. A., Gröger, J. P., Riebesell, U., & Reusch, T. B. (2014). Adaptation of a globally important coccolithophore to ocean warming and acidification. *Nature Climate Change*, 4: 1024-1030.

- Snell-Rood, E. C., Kobiela, M. E., Sikkink, K. L., & Shephard, A. M. (2018). Mechanisms of plastic rescue in novel environments. *Annual review of ecology, evolution, and systematics*, 49: 331-354.
- Somero G.N., Lockwood B.L., and Tomanek L. (2017). Biochemical adaptation: response to environmental challenges, from life's origins to the Anthropocene. Sinauer Associates, Incorporated Publishers
- Steinberg, D. K., & Landry, M. R. (2017). Zooplankton and the ocean carbon cycle. *Annual review of marine science*, 9: 413-444.
- Stillman, J. H., & Somero, G. N. (2000). A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology*, 73: 200-208.
- Sunday, J., Bennett, J. M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A. L., ... & Morales-Castilla, I. (2019). Thermal tolerance patterns across latitude and elevation. *Philosophical Transactions of the Royal Society B*, 374: 20190036.
- Tangwancharoen, S., & Burton, R. S. (2014). Early life stages are not always the most sensitive: heat stress responses in the copepod *Tigriopus californicus*. *Marine Ecology Progress Series*, 517: 75-83.
- Turner, J. T. (2004). The importance of small planktonic copepods and their roles in pelagic marine food webs. *Zool. Stud*, 43: 255-266.
- West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.
- Whitman, D. W., and T. N. Ananthakrishnan. (2009). Phenotypic Plasticity of Insects: Mechanisms and Consequences. *Phenotypic Plasticity of Insects: Mechanisms and Consequences*.
- Willett, C. S. (2010). Potential fitness trade-offs for thermal tolerance in the intertidal copepod *Tigriopus californicus*. *Evolution: International Journal of Organic Evolution*, 64: 2521-2534.

**CHAPTER I: TEMPERATURE AND PATTERNS OF OCCURRENCE AND ABUNDANCE OF KEY
COPEPOD TAXA IN THE NORTHEAST PACIFIC**

Abstract

The Northeast Pacific is a highly heterogeneous and productive ecosystem, yet it is vulnerable to climate change and extreme events such as marine heat waves. Recent heat wave induced die-offs of fish, marine mammals, and seabirds in the Gulf of Alaska were associated with the loss of large, lipid-rich copepods, which are a vital food resource for forage fishes. The critical and temperature sensitive role of copepods in this ecosystem motivates our investigation into the impacts of temperature on copepod occurrence, abundance, and phenology. Here, we pair long term *in situ* copepod data from Continuous Plankton Recorder surveys with satellite temperature data to determine the influence of water temperature on three key copepod taxa: *Neocalanus plumchrus*, *Calanus pacificus*, and *Oithona spp.* Through the use of linear models and thermal threshold methods, we demonstrate that *N. plumchrus* is most vulnerable to warming and future marine heat waves in this region. Linear models demonstrate that *N. plumchrus* abundance is negatively related to temperature, and thermal threshold methods reveal that *N. plumchrus* has an upper thermal threshold of 11.5°C for occurrence, and 10.5°C for abundance. Additionally, examining *N. plumchrus* abundance before and during the 2014–2016 marine heat wave demonstrates reduced species abundance during past warming events. *Oithona spp.* and *C. pacificus* appear to be less vulnerable to warm temperatures. However, their presence will not be sufficient to supplement the loss of the larger-bodied and lipid-rich *N. plumchrus*. Our findings demonstrate the power of using

long-term *in situ* data to determine thermal tolerances, and suggest the need to further examine the potential resilience of *N. plumchrus* to climate change.

Keywords: copepod, thermal threshold, *in situ* data, Continuous Plankton Recorder, marine heat wave, phenology, climate change, Northeast Pacific

Introduction

The Northeast Pacific's highly productive and diverse ecosystems (Weingartner et al., 2002; Sousa et al., 2016) are marked by variation in temperature across regions, seasons, years, and long-term patterns of climate variability (Benson and Trites, 2002; Batten et al., 2006; Janout et al., 2010; Johnstone and Mantua, 2014; Hill et al., 2015). Recently, the Northeast Pacific (NEP) experienced increasingly frequent warm-water periods, including an intense marine heat wave (MHW) during 2014–2016 (Di Lorenzo and Mantua, 2016), and again in 2019 (Amaya et al., 2020). The biological effects of these MHWs were dramatic across trophic levels, with changes in plankton community composition and die-offs of fish, marine mammals, and seabirds (Cavole et al., 2016; Yang et al., 2018; Peña et al., 2019; Piatt et al., 2020). With the apparent increase in the magnitude and frequency of MHWs globally (Scheffers et al., 2016; Frölicher et al., 2018; Oliver et al., 2018), understanding the impact of these events is critical for predicting future ecosystem and fisheries health and productivity under changing conditions.

In the NEP, MHW induced die-offs of Coho salmon and Alaskan walleye pollock were attributed to the loss of large lipid-rich copepods in the diet of juvenile stages

(Cavole et al., 2016). Additionally, mortality seen in marine mammals and seabirds was likely caused by losses of forage fishes, which also rely on copepods for food (Cavole et al., 2016; Piatt et al., 2020). A challenge in studying the impact of MHW on copepods is the availability of long-term *in situ* data. While there are many laboratory studies investigating thermal responses of copepods (Kelly et al., 2012; Pereira et al., 2017; Sasaki and Dam, 2019), geographically comprehensive *in situ* datasets can be cost prohibitive. In the NEP, the Continuous Plankton Recorder survey (CPR; Batten et al., 2003a; Reid et al., 2003) has collected consistent *in situ* data each year since 2000, providing a unique opportunity to study the responses of multiple copepod taxa to a wide range of thermal conditions. In this study, we pair 16 years of CPR data with temperature data to examine the impact of temperature on patterns of occurrence, abundance, and phenology of copepods in the Gulf of Alaska (GoA). This study spans the extreme MHW of 2014–2016, allowing us to examine the impacts of this event on copepods in the region.

To represent taxa with different life histories and biogeographic affinities, we examined the abundance of three dominant taxa in the NEP: *Neocalanus plumchrus*, *Calanus pacificus*, and *Oithona spp.* *N. plumchrus* are large-bodied (3.0–4.5 mm) and lipid-rich, dominating the mesoplankton biomass in the spring and early summer (Batten et al., 2003b; Batten and Mackas, 2009; McKinstry and Campbell, 2018). *C. pacificus* are medium-sized (1.5–2.5 mm), and found throughout the NEP, and in mid to high latitudes (Coyle and Pinchuk, 2003, 2005; Lee et al., 2006; Liu and Hopcroft, 2007; Nuwer et al., 2008). *Oithona spp.* are smaller (~1 mm) and occur from tropical to polar environments (Hansen et al., 2004; Madsen et al., 2008; Dvoretzky and Dvoretzky, 2009). *C. pacificus*

and *Oithona spp.* contain modest lipid stores, yet are an important food resource for fish after *N. plumchrus* exit the surface waters in mid-summer to diapause at depth (Coyle and Pinchuk, 2003, 2005; Kobari et al., 2004; Hopcroft et al., 2005; Ward and Hirst, 2007). These taxa also have different thermal affinities, with *N. plumchrus* favoring generally cooler waters, *C. pacificus* favoring warm waters, and *Oithona spp.* being found at a wide range of temperatures (Kobari and Ikeda, 2001; Coyle and Pinchuk, 2003; Batten and Walne, 2011; Castellani et al., 2016). While *C. pacificus* and *Oithona spp.* may be resilient to changing ocean temperatures, *N. plumchrus* seasonal abundance is sensitive to warming. *N. plumchrus* biomass peaks appeared earlier in warmer years, and narrowed in width in 2000–2007, as compared to 1957–1965 (Mackas et al., 1998, 2007; Batten and Mackas, 2009). The mechanism for this shift is not well-known, though it is likely that warmer environmental temperatures play a role (Batten and Mackas, 2009).

Here, we investigate temperature associated changes in copepod occurrence and abundance by: (1) Examining the relationship between copepod occurrence and abundance and temperature in the NEP through the use of General Linear Models (GLM) and thermal threshold methods, (2) Identifying how copepod abundance varies in space by subdividing our study area, and characterizing regional thermal regimes, and (3) Demonstrating how copepod abundance varies in time, both across seasons and years in relation to temperature. We hypothesize that *Neocalanus plumchrus* occurrence and abundance will decrease with warming, *Calanus pacificus* occurrence and abundance will increase with warming, and *Oithona spp.* occurrence and abundance will show no significant relationship with temperature. Additionally, we hypothesize that warming will impact the phenology (timing of abundance) of *N. plumchrus*, resulting in earlier and

more narrow abundance peaks in warmer years. Results from this study have implications for the effects of continuous warming and MHWs on zooplankton, and the future health of this biologically diverse and vital marine ecosystem.

Methods

Data

We obtained copepod data from the North Pacific CPR Survey (Batten et al., 2003a; Reid et al., 2003). The CPR was towed behind commercial ships at about 7 m of depth, and filtered plankton from the water through a band of 270 μm mesh. The continuous mesh record was divided into discrete samples, each representing 18.5 km of each sampling voyage. Every fourth sample was microscopically processed to provide taxonomically-resolved abundance data for the zooplankton retained on the filtering mesh (CPR; Batten et al., 2003a; Reid et al., 2003).

The North Pacific CPR survey consists of transects that span the Gulf of Alaska (Figure 1). Sampling along these transects began in March 2000, and samples have been processed through 2016. The first transect is 2,200 km long, sampling from Juan de Fuca Strait to Cook Inlet, Alaska, monthly from approximately April to September. Prior to 2004 this transect began in California and ended at the entrance to Prince William Sound, Alaska. The second transect is 6,500 km long and samples from Juan de Fuca Strait to Asia, typically passing through the Aleutian Islands. This transect is sampled three times a year in the spring, summer, and fall. Additionally, we used data from a subregion where

two CPR transects overlap that were sampled at a higher temporal resolution (nine samplings over a six-month period) in the eastern Gulf of Alaska (48N to 55N and 145W to 130W). These data were analyzed by Batten and Mackas (2009), and were updated here to include data through 2018. The higher resolution samples from this subregion facilitated our analysis of seasonal shifts in *Neocalanus plumchrus* abundance with warming.

The plankton data were filtered to include only our three focal taxa: *Neocalanus plumchrus*, *Calanus pacificus*, and *Oithona spp.* Additionally, we filtered the data to include only samples from May through August, to capture warm months that may limit *N. plumchrus* occurrence and abundance. Counts for *Calanus pacificus* and *Oithona spp.* were of late stage copepodites [Copepodite stage 5 (CV)] and adult copepods. However, *Neocalanus plumchrus* counts were only of CV copepodites, as adults are found exclusively in deep waters and are not sampled by the CPR (Batten et al., 2003b; Batten and Mackas, 2009).

Sea surface temperature (SST) data for this analysis came from two sources: the NASA Multi-Scale Ultra High Resolution SST dataset (JPL MUR MEaSUREs Project, 2015), and the Met Office Hadley Centre SST reanalysis dataset (HadISST 1, Rayner et al., 2003). MUR-SST merges multiple satellite datasets to obtain daily global SST at 1km resolution starting in 2002. The MUR-SST data was co-located with each CPR sample and averaged over a region of 0.5° for the 15 days prior to the sampling date. We used the MUR-SST data from May through August for GLM and thermal threshold analyses. The HadISST dataset provides global SST at 1° spatial resolution and monthly temporal

resolution, with data available since the 1800's. We used the HadISST dataset for our phenology and annual SST analyses because it spans a longer time frame, and Spearman's correlation coefficient demonstrated that HadISST and MUR-SST are highly correlated ($r = 0.96$ at a 15-day resolution for MUR-SST, Supplementary Figure 1).

Bathymetric data to delimit regions were obtained from the ETOPO1 dataset, which has a 1 arc-minute resolution¹. This dataset was also co-located with each CPR sample to determine which region each sample belonged to. The entire study region was then subdivided into four areas: three regions over the continental slope out to 3000 m depth and one oceanic region deeper than 3000 m. The shelf regions were divided into western (between -175W and -155W), northern (between -155W and -140W) and eastern regions (east of -136W). The continental shelf and slope are characterized by strong currents, with the meandering and 'warm' Alaska Coastal Current in the east, to the faster and narrower Alaska Stream in the north, and leading to the fast and cold current through the Unimak Pass into the Bering Sea in the west. The oceanic area has less water movement, as it is home to the Alaska Gyre and mesoscale eddies that are separate from the Alaska Coastal Current (Crawford et al., 2007; Henson and Thomas, 2008).

¹ <https://www.ngdc.noaa.gov/mgg/global/>

Modeling Abundance

To explore the relationship of copepod abundance to temperature, we log-transformed counts [$\log_{10}(N + 1)$], and performed a GLM (assuming normal distributions). We examined the relationships between taxon abundance and co-located MUR-SST, date,

depth, and region. The Variance Inflation Factor was recorded for each model to identify the extent of multicollinearity in the data. While our emphasis is on the impact of temperature, date was included in the models because *Neocalanus plumchrus* are highly seasonal in their abundance. Additionally, region and depth were included due to the spatial heterogeneity of oceanographic characteristics in the study region. Regional definitions were the same as the ones used above (section “Data”). Water depth was defined as a binary variable, separating shelf/slope, and oceanic habitats as being less or greater than 3000 m of depth. The influence of two-way interaction terms was also explored in our models, when applicable.

To determine the impact of warm water years on *Neocalanus plumchrus* abundance, we used the marine heat wave of 2014–2016 as a natural experiment. This was done by categorizing years as marine heat wave years, or not, and running a one-way ANOVA to examine the effect of marine heat wave years on $\log_{10}(N + 1)$ counts of *N. plumchrus* abundance.

Thermal Thresholds

Thermal thresholds were calculated by looking at rapid and large changes in occurrence and abundance with the co-located MURSST for each taxon (Carstensen et al., 2012). The temperature range was divided into 1°C bins, and for copepod data falling in each bin we used two metrics: (i) percentage of occurrence relative to the total number of samples in the bin, and (ii) the 95th percentile of abundance. We used the 95th percentile for abundance because we were interested in how temperature limits

occurrence and abundance, and because it summarized the shape of the distribution of the abundance data (Supplementary Figures 2–4). A cut-off of 20% of the maximum value of the abundance metric for all temperatures was designated as an effective lower limit. When the percent occurrence or the 95th percentile of abundance for a given temperature-bin crossed and stayed beyond this cut-off, we classified this point as the thermal threshold for that taxon. Thresholds were calculated across all regions, and also for each region separately. We also examined differences in thermal thresholds by month and depth. Additionally, regional HadISST time series data were analyzed to investigate how often upper thermal thresholds were crossed. This was done to indicate how often taxa were outside of their thermal threshold, allowing for the inference of potential impacts of warming to future copepod occurrence and abundance in this region.

Phenology of *Neocalanus plumchrus* With Temperature

As noted above, *Neocalanus plumchrus* are highly seasonal in surface waters. Additionally, data prior to 2009 indicate that peaks in *N. plumchrus* abundance narrowed and shifted earlier in warmer years (Mackas et al., 1998, 2007; Batten and Mackas, 2009). To identify if these shifts in phenology continued through 2018, changes in the timing and width of the *N. plumchrus* abundance peak were assessed. This was done by using the high temporal resolution data set described in section “Data”. The higher temporal resolution allowed for a more accurate depiction of the shape and timing of the abundance peak. Cumulative integration was performed through each year of the abundance vs. date curve. Abundance peak was taken to be the midpoint of the season, when the cumulative curve reached the 50th percentile. The cohort width was the number

of days between the 25th and 75th percentile. These data were paired with annual mean HADISST and 1 year lagged annual mean HADISST to see the relationship between temperature and cohort width, and between temperature and the midpoint of the annual abundance peak (Batten and Mackas, 2009). This was done by running four separate linear models: one looking at the influence of annual SST on cohort width, one looking at the influence of lagged annual SST on cohort width, one looking at the influence of SST on abundance peak midpoint, and one looking at the influence of lagged annual SST on abundance peak midpoint. We examined annual 1 year lagged HadISST in addition to annual SST, because it was identified as the strongest determinant of cohort width and midpoint in Batten and Mackas (2009).

Results

Modeling Abundance

Neocalanus plumchrus abundance was best modeled by temperature, date, and water depth (Table 1). Increasing date and temperature reduced *N. plumchrus* abundance, and abundance was greater in oceanic habitats. In comparison, the best model for *Calanus pacificus* abundance included temperature and region as independent variables. *C. pacificus* abundance increased with increasing temperature, and the oceanic region had a higher abundance as compared to the shelf/slope regions in the east, north, and west. The best model for *Oithona spp.* abundance also included temperature and region as independent variables. Temperature was negatively related to *Oithona spp.* abundance, while the oceanic region had higher *Oithona spp.* abundance as compared to the eastern

and northern regions. All models had relatively low explanatory power, with the model for *N. plumchrus* explaining the largest amount of variation in abundance, at 15%. The low explanatory power in these models is likely due to the large sample size and high variability in the data (Table 1).

Results from our marine heat wave analysis demonstrated that *Neocalanus plumchrus* was less abundant during the 2014–2016 marine heat wave ($p = 0.0006$) (Supplementary Figure 5).

Thermal Thresholds

Neocalanus plumchrus was found at a range of temperatures from 3.68–12.99°C, with an upper-end abundance thermal threshold of 10.5°C (Figure 2), and an occurrence upper threshold of 11.5°C. *Calanus pacificus* was found at temperatures ranging from 4.25 to 15.56°C, with occurrence and abundance of *C. pacificus* showing a low-end thermal threshold of 7.5 and 8.5°C, respectively, (Figure 2). While *C. pacificus* abundance dipped below the 20% cut-off between 12 and 13°C, it did not have an upper thermal threshold (Figure 2) in this dataset. *Oithona spp.* was present at temperatures ranging from 3.68 to 15.66°C, with relatively low, but consistent occurrence across all temperatures (Figure 2). *Oithona spp.* abundance was variable, but overall lower for higher temperatures (Figure 2). While *Oithona spp.* abundance dipped below our 20% cut-off at 9.5°C and 12.5°C, it did not have an upper thermal threshold in this dataset. The profile of the 95th percentile of abundance with temperature was similar between

shelf/slope and oceanic habitats for each taxon (Supplementary Figure 6). The 95th percentile of abundance did vary for taxa among regions (Supplementary Figure 7).

Interestingly, in years where mean temperature was above the regional thermal threshold, *Neocalanus plumchrus* had reduced mean abundance (Figure 3). These results corroborate our Marine Heat Wave analysis, by showing reduced *N. plumchrus* abundance during the Marine Heat Wave of 2014–2016. Thermal thresholds were relatively stable across months for *N. plumchrus* and *Calanus pacificus* (Supplementary Figures 8–11). Monthly changes in thermal thresholds revealed a dip in *Oithona spp.* abundance in July and August (Supplementary Figures 10, 11).

Historical HadISST data demonstrated that temperatures in the eastern region rarely surpassed the 10.5°C *Neocalanus plumchrus* abundance threshold in May (Figure 4). However, temperatures in this region frequently exceeded the 10.5°C *N. plumchrus* abundance threshold and the 11.5°C *N. plumchrus* occurrence threshold in June. Temperatures in the eastern region were exclusively above both thresholds in July (Figure 4). In the northern, western, and oceanic regions temperatures never surpassed either thermal threshold in May. In June, the northern region rarely exceeded the 10.5°C abundance and 11.5°C occurrence thresholds, and the western and oceanic regions never exceeded these thresholds. Temperatures in the northern, and oceanic regions frequently surpassed both the 10.5°C abundance and 11.5°C occurrence threshold in July. However, temperatures in the western region rarely surpassed the 10.5°C abundance threshold in July.

Phenology of *Neocalanus plumchrus* With Warming

Our results supported those of Batten and Mackas (2009), with the midpoint of the *Neocalanus plumchrus* abundance peak appearing earlier in warmer years for both annual SST and 1 year lagged annual SST ($p = 0.019$ and $p = 0.006$, respectively) (Figure 5A). However, while Batten and Mackas (2009) saw a narrowing of cohort width, we did not find a significant relationship between annual mean SST or one-year lag mean HADISST and cohort width ($p = 0.45$ and $p = 0.24$, respectively) (Figure 5B). Interestingly our results show a more significant relationship between lagged annual mean SST than annual mean SST with no lag.

Discussion

Results from our GLM and thermal threshold analyses supported hypotheses that *Neocalanus plumchrus* occurrence/abundance had a negative relationship, and *Calanus pacificus* occurrence/abundance had a positive relationship with temperature. However, our results did not support our hypothesis that *Oithona spp.* occurrence/abundance had no relationship with temperature, with *Oithona spp.* abundance decreasing with increasing temperatures. Importantly, results from our marine heat wave analysis indicated that *N. plumchrus* had reduced abundance during the marine heat wave of 2014–2016.

Additionally, results from our *N. plumchrus* phenology analysis supported our hypothesis that warming affected the phenology of *N. plumchrus*. Overall, our results indicate that future warming may impact patterns of copepod occurrence/abundance, particularly for the subarctic-water favoring species *N. plumchrus*.

Modeling Occurrence and Abundance

Our GLM demonstrated that *Neocalanus plumchrus* abundance decreases with increasing temperature. Our analysis also showed a negative relationship between *N. plumchrus* abundance and date. This is in line with our hypothesis, as *N. plumchrus* has a strong seasonal expression in surface waters (Batten et al., 2003b; Batten and Mackas, 2009). We found that the *N. plumchrus* abundance increased with water depth. *N. plumchrus* is known to be a pelagic species that occupies areas off the shelf/slope (Mauchline, 1998). Therefore, it is not surprising that their occurrence increased with increasing water depth. Somewhat surprisingly, our results revealed that region did not explain any more of the variance in *N. plumchrus* abundance than water depth. Additionally, our marine heat wave and thermal threshold analyses demonstrated that *N. plumchrus* was less abundant in marine heat wave years (Figure 3 and Supplementary Figure 5). This aligns with our characterization of *N. plumchrus* as a cool water favoring species, and is supported by work done in the California Current demonstrating reductions in cool water favoring copepods during the 2014–2016 Marine Heatwave (Peterson et al., 2017). Together, this indicates that *N. plumchrus* and other cool water copepods may be vulnerable to increasing frequency and intensity of marine heat waves with warming.

While *Oithona spp.* abundance decreased with increasing temperature, *Calanus pacificus* abundance increased with increasing temperature. Neither *C. pacificus* or *Oithona spp.* had a significant relationship with date. However, region did add significant value to the models of *C. pacificus* and *Oithona spp.* abundance. Interestingly, *C.*

pacificus had the highest abundance in the oceanic region, indicating that *C. pacificus* and *Neocalanus plumchrus* are both oceanic species. *Oithona spp.* also had increased abundance in the oceanic region.

It is important to note that our models explained only up to 15% of the variance in taxon abundance. Therefore, this analysis serves as an initial exploration of potential drivers of abundance, rather than a method for prediction. The low explanatory power of our models is primarily due to the very large sampling region and substantial variability observed in the data. The variability in this dataset may be related to patchiness in the spatial distribution of plankton (Mackas et al., 1985). There are other potential drivers of spatial variability that we did not examine, such as current patterns, primary production, and predator-prey dynamics (Mackas et al., 1985). While these other variables may contribute to patterns of abundance, we focused here on SST due to its importance in our study region, its ability to be accurately measured, and the direct and indirect effects it has on copepod abundance.

Thermal Thresholds and Implications for Climate Change

In support of our regression analyses, the thermal threshold analysis revealed an upper thermal threshold of 10.5°C for *Neocalanus plumchrus* abundance, and an upper threshold of 11.5°C for *N. plumchrus* occurrence. Our analysis demonstrated that thermal thresholds limit abundance, with years where mean temperature exceeded regional *N. plumchrus* thermal thresholds coinciding with reduced *N. plumchrus* abundance (Figure 3). Additionally, we found that temperatures in the eastern, northern, and oceanic regions

regularly exceeded the occurrence and abundance thermal thresholds in months where *N. plumchrus* is known to occupy the surface waters (Figure 4). Together these results indicate that *N. plumchrus* may be vulnerable to continually warming water temperatures and future marine heatwaves. This potential vulnerability of *N. plumchrus* to warming is supported by other studies in the Northeast Pacific that have seen the loss of large lipid rich copepods with warming (Peterson et al., 2017).

Our threshold analysis revealed no upper thermal threshold for *Calanus pacificus*. This supports existing knowledge of *C. pacificus* as a warm water favoring species that has been shown to possess a positive relationship with temperature (Coyle and Pinchuk, 2003; Batten and Walne, 2011). Interestingly, there was a lower thermal threshold of occurrence at 7.5°C and abundance at 8.5°C. Regional climatology indicates that *C. pacificus* may appear earlier and in higher abundances in the western, northern, and oceanic regions with warming (Supplementary Figure 12). Our results align well with findings demonstrating increased abundances of *C. pacificus* in the Northeast Pacific in warm years associated with Marine Heat Waves (Peterson et al., 2017; Fisher et al., 2020). We did see a dip in abundance and occurrence for *C. pacificus* between temperatures 12–13°C. We hypothesize that this dip may correspond to the end of a first cohort and beginning of a second cohort in warmer shelf/slope regions in the north and the east (Supplementary Figure 3; Conover, 1988; Osgood and Frost, 1994).

Our thermal threshold analysis did not reveal any thresholds of occurrence or abundance for *Oithona spp.* This aligns well with the wide range of latitudes and temperatures that *Oithona spp.* is known to occupy (Ward and Hirst, 2007; Zamora-Terol

et al., 2014; Castellani et al., 2016). There was a dip in abundance and occurrence between 10 and 13°C. This break in abundance may reflect reduced abundance values seen in July and August (Supplementary Figures 10, 11). Alternatively, gaps in abundance and occurrence could correspond with differing species within the *Oithona* genus. Data from our study region demonstrates that species *O. similis* dominates the water column at >50% abundance (Pers.Comm. Moira Galbraith). However, *O. atlantica*, *O. setigera*, and *O. tenuis* are also found in our study region (Pers.Comm. Moira Galbraith). While warming temperatures may not limit *Calanus pacificus* and *Oithona spp.* occurrence or abundance, the vulnerability of *Neocalanus plumchrus* to warming could have dire consequences. *N. plumchrus* are a vital source of nutrient dense biomass that cannot be supplemented by smaller warm water species, such as *C. pacificus* or *Oithona spp.* (Mackas et al., 1998; Evanson et al., 2000; Batten et al., 2003b; Batten and Mackas, 2009; Cavole et al., 2016; McKinstry and Campbell, 2018). This is particularly concerning for the economically and culturally important fish, marine mammals, and seabirds that rely on *N. plumchrus* as a food source (Kawamura and Hirano, 1985; Mackas et al., 1998; McKinstry and Campbell, 2018).

Phenology of *Neocalanus plumchrus* With Warming

Our results demonstrated that the seasonal surface appearance of *Neocalanus plumchrus* was earlier with warmer temperatures. These results corroborate previous work demonstrating earlier shifts in the abundance peak of *N. plumchrus* (Batten and Mackas, 2009). Bertram et al. (2001) linked warm spring water temperatures, and the earlier appearance of copepods, with reduced reproductive success in Cassin's auklet.

With continued warming, we could see additional impacts of these seasonal shifts in abundance on upper trophic levels that rely on *N. plumchrus* as a primary food source. Unlike Batten and Mackas (2009), we did not see a statistically significant effect of temperature on cohort width in the longer time series examined here. However, with further temperature increases in this region there may be a stronger influence of temperature on cohort width in the future. Alternatively, there may be a limit to how narrow a cohort can be, or a limit to our power to detect changes in cohort width with our current sampling resolution.

Interestingly, we found 1 year lagged annual mean SST to be a stronger driver of abundance midpoint than annual mean SST. While same year mean SST influences the developmental time and time spent at the surface accumulating lipids for the current cohort, lagged annual mean SST reflects lipid stores and potential reproductive timing and success of adults that found the next generation. We hypothesize that temperatures experienced by the previous cohort have a stronger influence than temperatures experienced by earlier developmental stages of the same cohort due to the life history strategy of *Neocalanus plumchrus*. Subadult (copepodite stage V) and adult *N. plumchrus* spend the majority of time at depth, with only early to mid-copepodite stages occurring at the surface and experiencing the temperatures there (Batten and Mackas, 2009). At depth, animals are buffered from surface temperatures. Therefore, the temperatures and surface conditions experienced by copepodites of the previous annual cohort are likely to have a stronger impact on seasonal abundance timing of the next generation.

CONCLUSION

Here, we demonstrated that a critical species in the trophic and ecosystem dynamics of the Northeast Pacific, *Neocalanus plumchrus*, is vulnerable to continued warming of the oceans due to anthropogenic climate change. This is of particular concern in our region of study, because of the recent history of intense marine heat waves, and the trophic dominance and nutritional importance of this species. Our results indicate that with increasing temperatures, *N. plumchrus* biomass may decline and there may be changes in their phenology, which could impact fish, marine mammals, and seabirds that rely on *N. plumchrus* as a food resource.

Future research should be aimed at better understanding the thermal tolerance of this important species and the direct versus indirect effects of temperature. Acclimating *Neocalanus plumchrus* to a range of temperatures and assessing their upper thermal limits would provide valuable information regarding the isolated effect temperature has on survival and performance. In addition, it would be of great value to understand the capacity of *N. plumchrus* to adapt in response to warming in the Northeast Pacific. This would provide critical information regarding the potential of *N. plumchrus* to respond to and persist despite rapidly changing ocean conditions.

DATA AVAILABILITY STATEMENT

The data and code underlying this study have been made available on GitHub:

https://github.com/27lsevier-institute/ThermalPatterns_Copepods_NorthEastPacific.

AUTHOR CONTRIBUTIONS

LA wrote the manuscript text. LA, MG-R, and WS designed the experiment. LA and MG-R made the manuscript figures. WS assisted with statistical analyses. SB provided and advised on plankton data and helped write sections on phenology of *Neocalanus plumchrus* with temperature. MG-R and CG processed satellite temperature data. All authors contributed to the article and approved the submitted version.

FUNDING

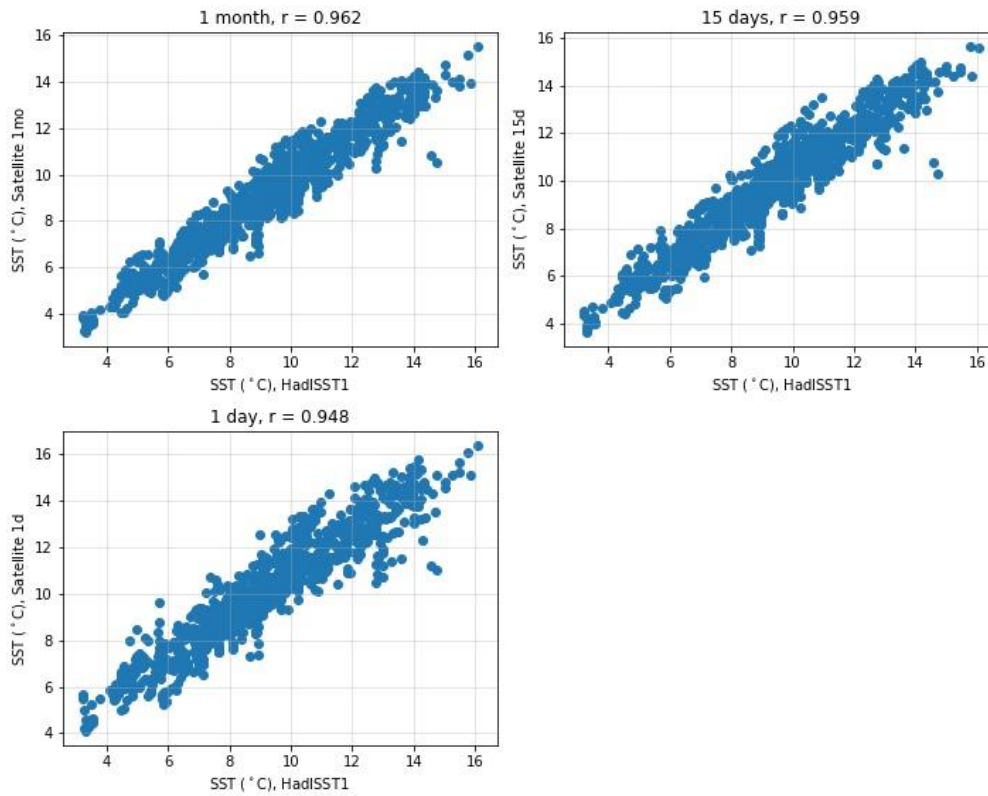
LA was funded by NSF-NRT QuEST program and the University of Vermont Biology Department (Grant # 1735316). MG-R, WS, and CG were funded by NASA's Life in the Moving Oceans Initiative (Award # 80NSSC17K0557). The North Pacific CPR survey is supported by a consortium that includes the North Pacific Research Board, Exxon Valdez Oil Spill Trustee Council, Fisheries and Oceans Canada, Marine Biological Association of the United Kingdom, and the North Pacific Marine Science Organization (PICES).

ACKNOWLEDGMENTS

We thank past and present members of the CPR program for processing the taxonomic data used in this study, as well as the many volunteer vessels and crews that towed CPR device. We thank Moira Galbraith for data on *Oithona spp.* We thank Zoe Burr and Brian Hoover for conversations that moved the progress of this manuscript forward. We also thank Brian Hoover and Caitlin Kroeger for helpful edits of the manuscript.

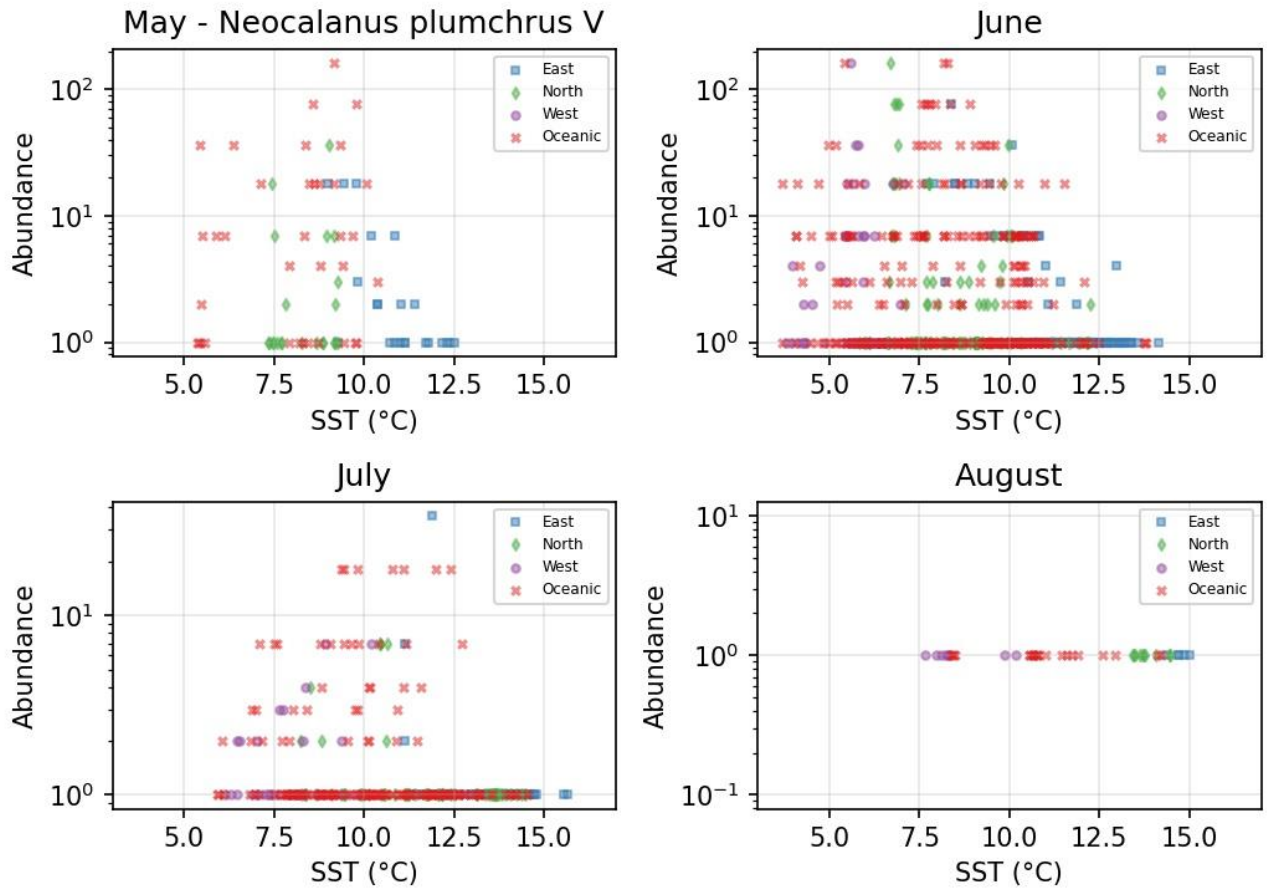
SUPPLEMENTARY MATERIAL

Supplementary Figure 1



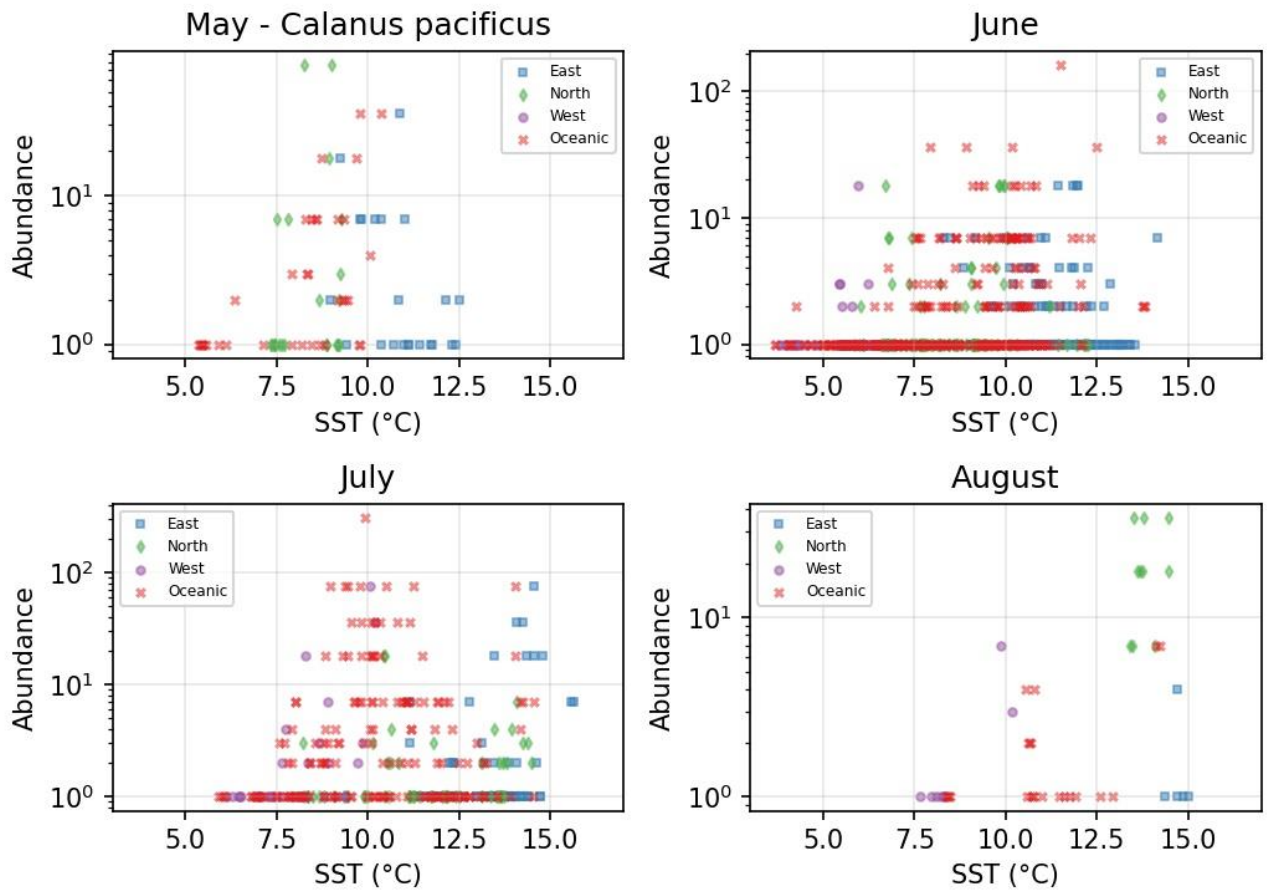
Supplementary Figure 1. Spearman correlations between HadISST and MURSST show that they represent SST similarly, and there is little difference between time scales. As expected, the one-month means are the best fit. However, it is more likely that this is due to the temporal resolution.

Supplementary Figure 2



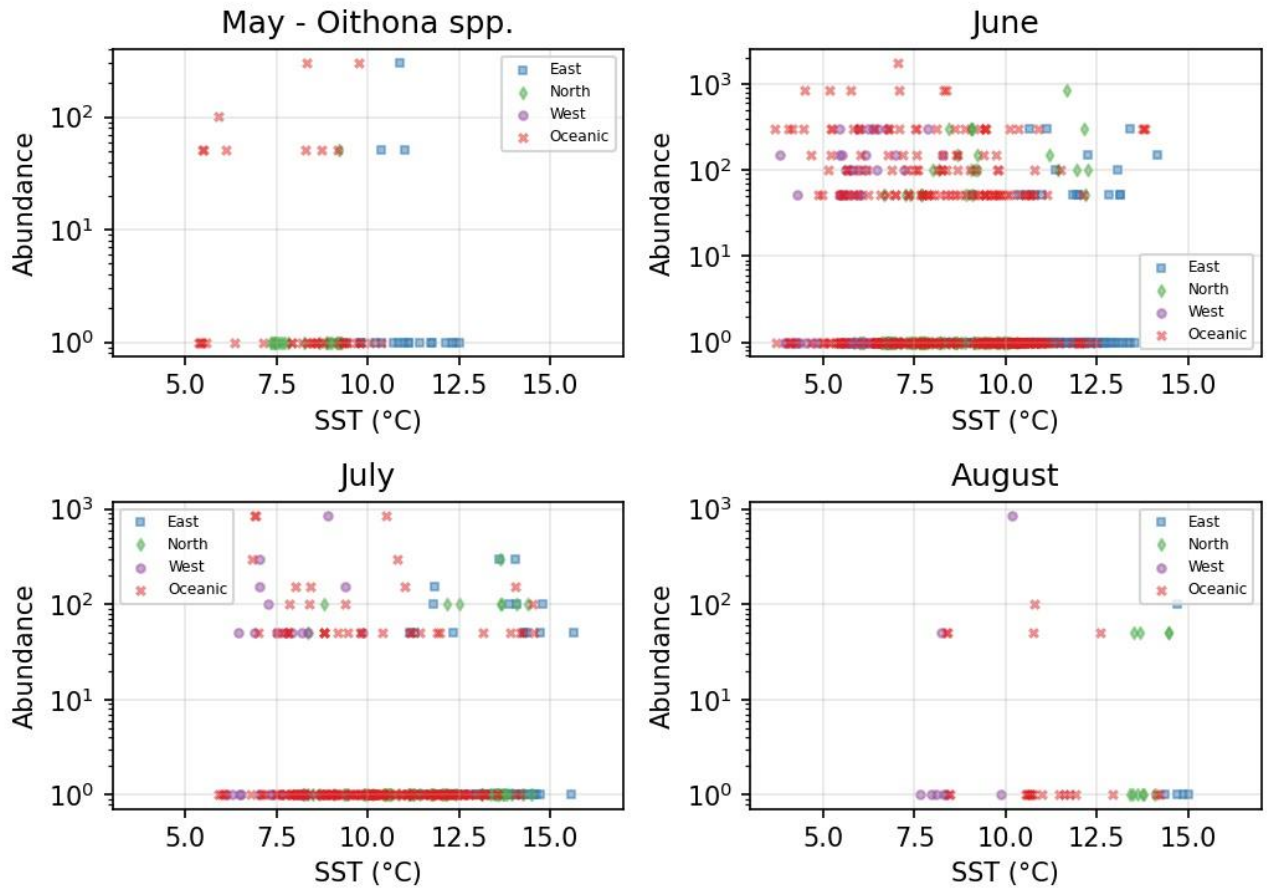
Supplementary Figure 2. Log abundance values by temperature for *N. plumchrus*. Colors and symbols denote subregion.

Supplementary Figure 3



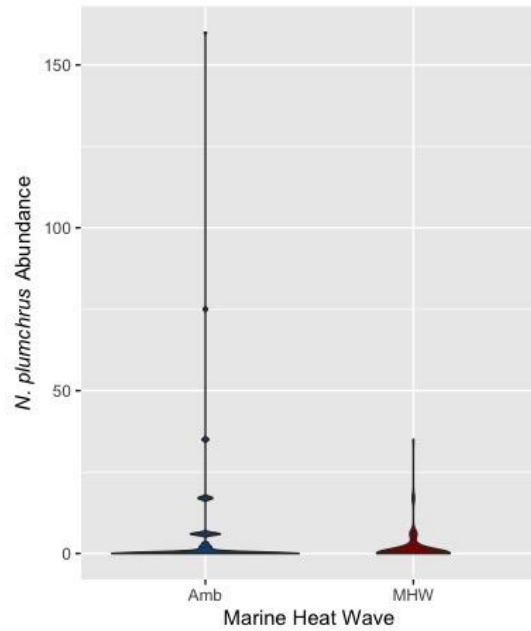
Supplementary Figure 3. Log abundance values by temperature for *C. pacificus*. Colors and symbols denote subregion.

Supplementary Figure 4



Supplementary Figure 4. Log abundance values by temperature for *Oithona* spp. Colors and symbols denote subregion.

Supplementary Figure 5



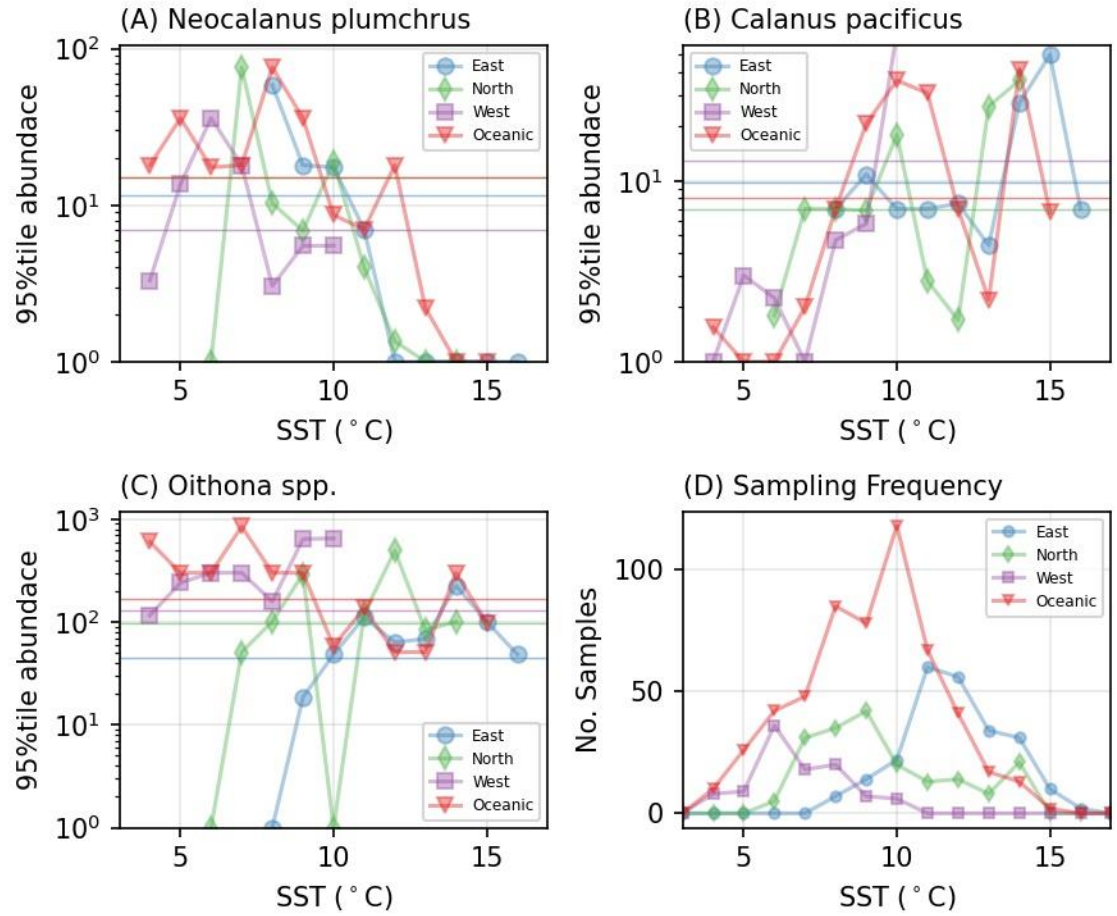
Supplementary Figure 5. *N. plumchrus* abundance by marine heat wave status. Amb denotes samples taken in non-marine heat wave years. MHW denotes samples taken during the 2014-2016 marine heatwave.

Supplementary Figure 6



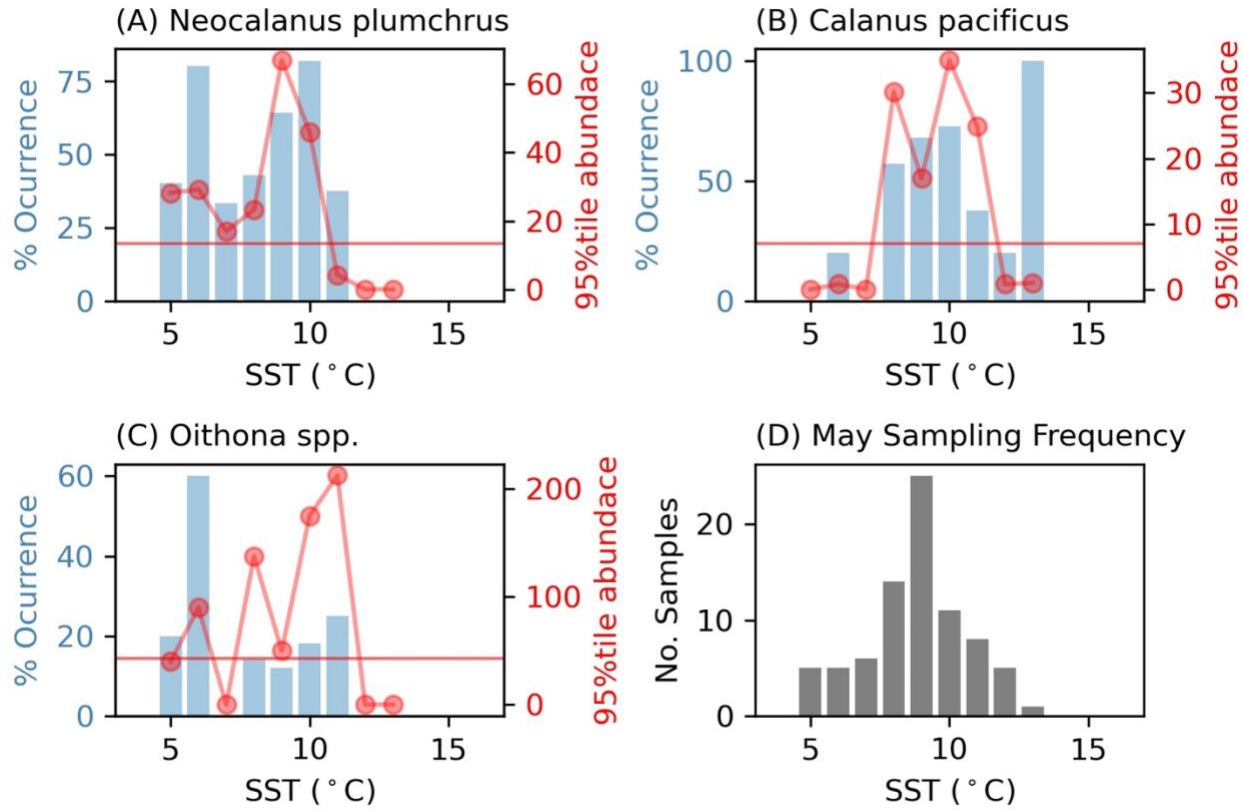
Supplementary Figure 6. Plots represent 95th percentile for abundance. Red points represent oceanic samples (>3000m) and blue points represent shelf/slope samples (<3000m). The horizontal lines represent 20% of the maximum 95th percentile of abundance. A. Plot of *N. plumchrus* abundance. B. Plot of *C. pacificus* abundance. C. Plot of *Oithona spp.* abundance. D. Histogram depicting the distribution of temperatures for samples collected from each habitat type.

Supplementary Figure 7



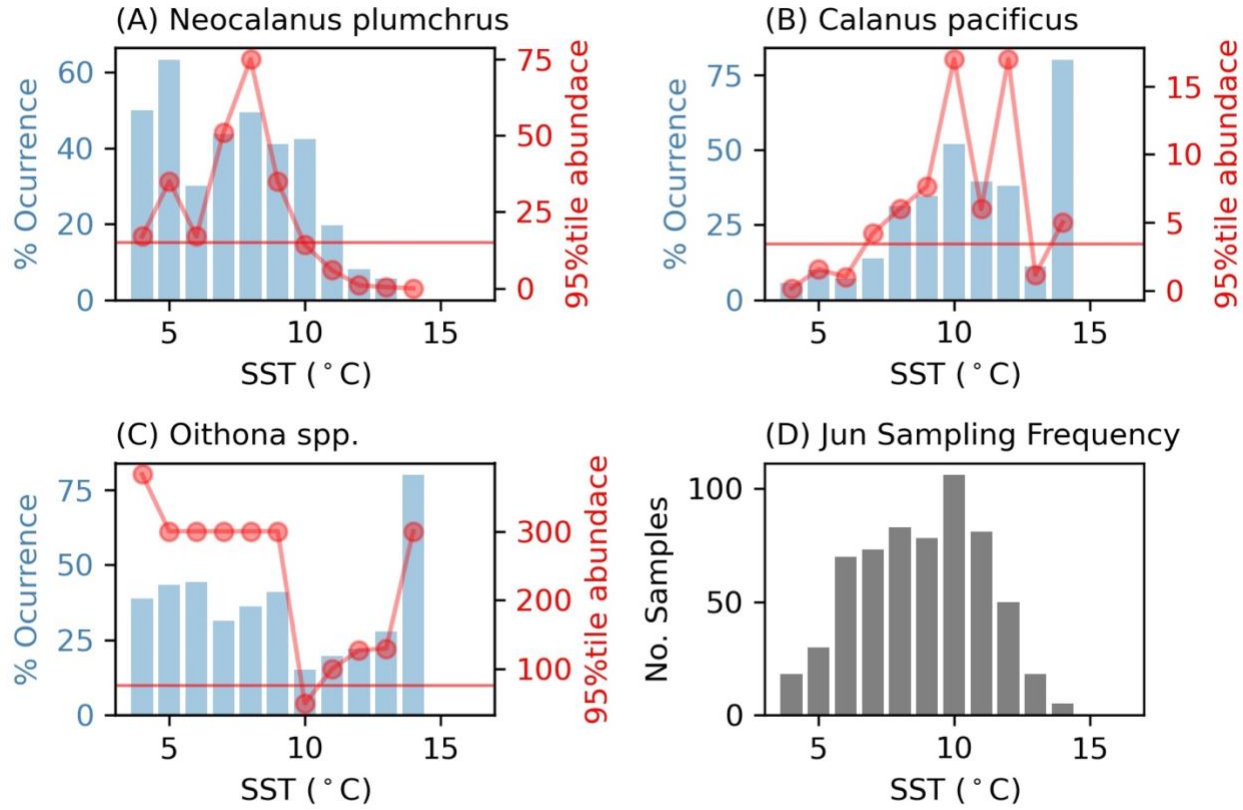
Supplementary Figure 7. Plots represent 95th percentile for abundance. Different colors and shapes represent different regions. The horizontal lines represent 20% of the maximum 95th percentile of abundance. A. Plot of *N. plumchrus* abundance. B. Plot of *C. pacificus* abundance. C. Plot of *Oithona spp.* abundance. D. Distribution of temperatures for samples collected from each region.

Supplementary Figure 8



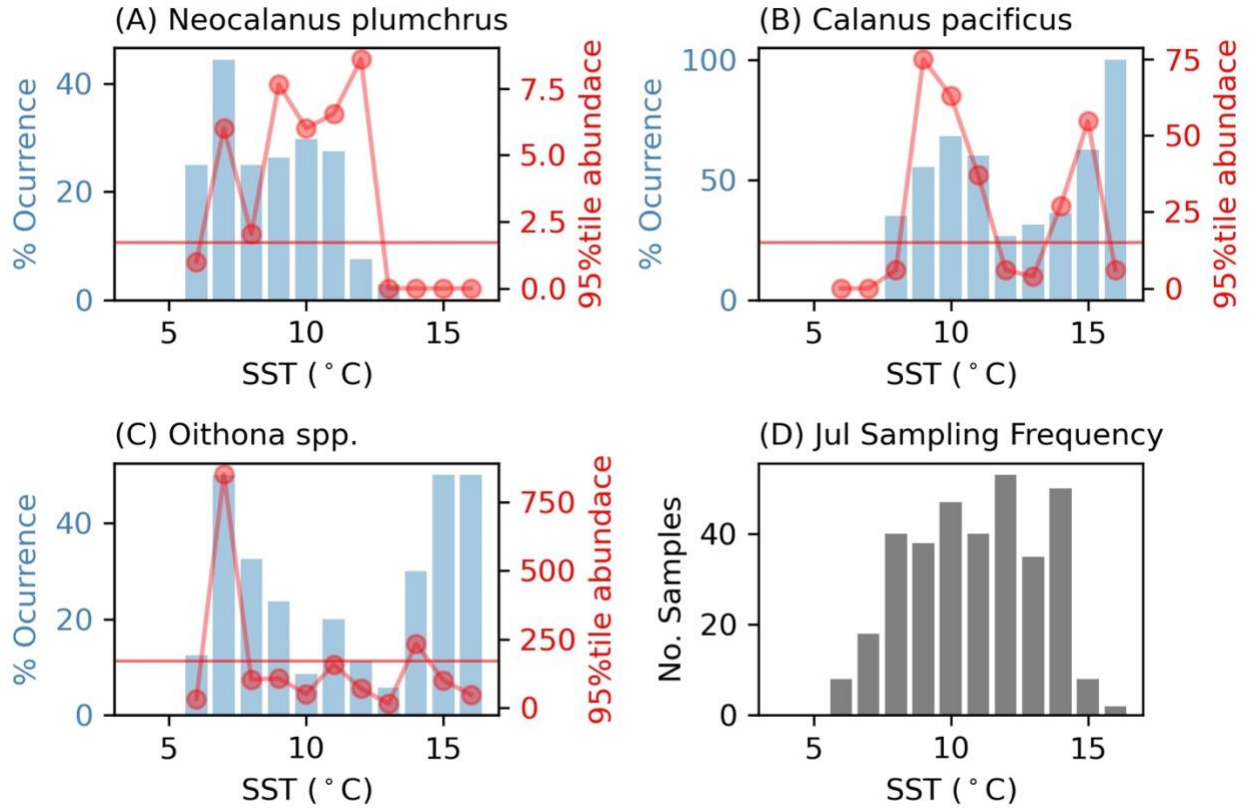
Supplementary Figure 8. Thermal threshold analysis for the month of May. Blue bars represent percent occurrence and red dots represent the 95th percentile of abundance. The horizontal line represents 20% of the maximum 95th percentile of occurrence. All data are divided by 1°C SST (based on MUR-SST) bins. A. Plot of *N. plumchrus* occurrence and abundance. B. Plot of *C. pacificus* occurrence and abundance. C. Plot of *Oithona spp.* occurrence and abundance. D. Histogram of sample temperatures.

Supplementary Figure 9



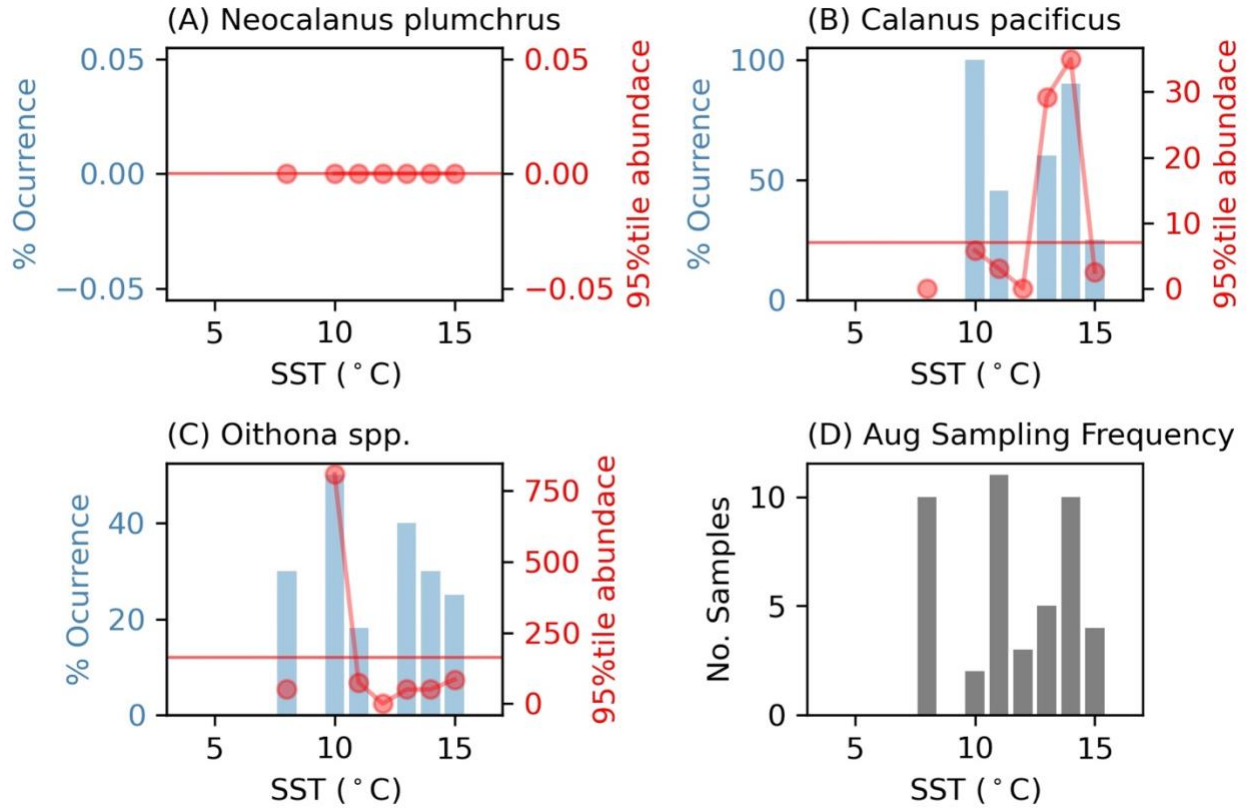
Supplementary Figure 9. Thermal threshold analysis for the month of June. Blue bars represent percent occurrence and red dots represent the 95th percentile of abundance. The horizontal line represents 20% of the maximum 95th percentile of occurrence. All data are divided by 1°C SST (based on MUR-SST) bins. A. Plot of *N. plumchrus* occurrence and abundance. B. Plot of *C. pacificus* occurrence and abundance. C. Plot of *Oithona spp.* occurrence and abundance. D. Histogram of sample temperatures.

Supplementary Figure 10



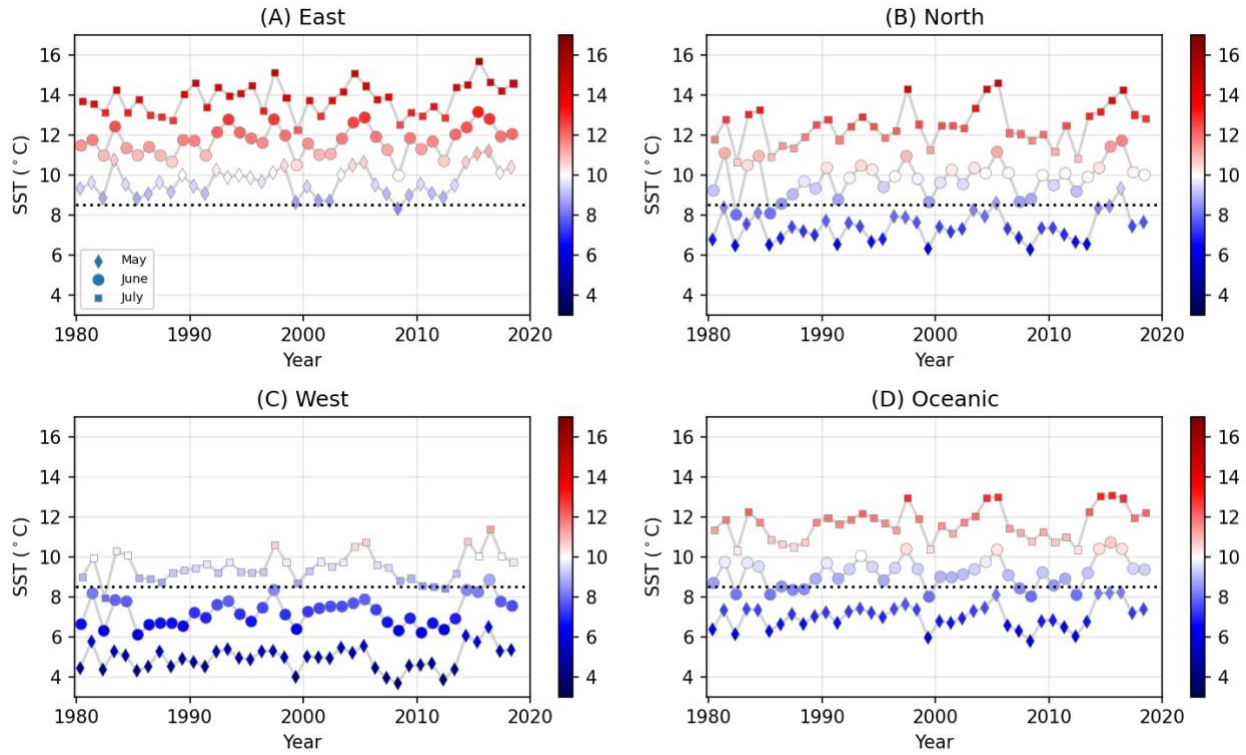
Supplementary Figure 10. Thermal threshold analysis for the month of July. Blue bars represent percent occurrence and red dots represent the 95th percentile of abundance. The horizontal line represents 20% of the maximum 95th percentile of occurrence. All data are divided by 1°C SST (based on MUR-SST) bins. A. Plot of *N. plumchrus* occurrence and abundance. B. Plot of *C. pacificus* occurrence and abundance. C. Plot of *Oithona spp.* occurrence and abundance. D. Histogram of sample temperatures.

Supplementary Figure 11



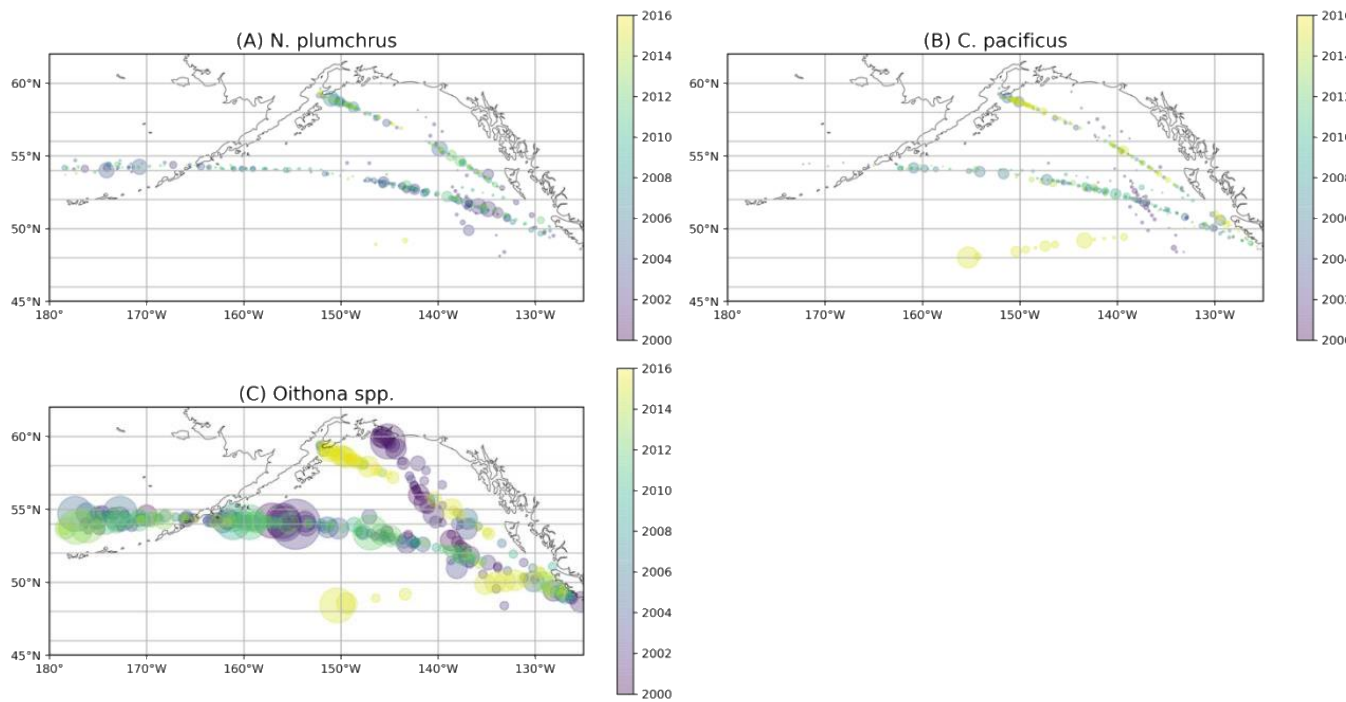
Supplementary Figure 11. Thermal threshold analysis for the month of August. Blue bars represent percent occurrence and red dots represent the 95th percentile of abundance. The horizontal line represents 20% of the maximum 95th percentile of occurrence. All data are divided by 1°C SST (based on MUR-SST) bins. A. *N. plumchrus* occurrence and abundance. B. *C. pacificus* occurrence and abundance. C. *Oithona spp.* occurrence and abundance. D. Histogram of sample temperatures.

Supplementary Figure 12



Supplementary Figure 12. Monthly mean HADISST per region per year. Color and y-axes indicate temperature. Black dotted line indicates the lower thermal threshold for *C. pacificus* occurrence and abundance (8.5°C). A. SST plots of eastern region. B. SST plots of northern region. C. SST plots of western region. D. SST plots of oceanic region.

Supplementary Figure 13



Supplementary Figure 13. Distribution plots of focal taxa. The size of points represents abundance and color of points represent year. A. *N. plumchrus* distribution plot. B. *C. pacificus* distribution plot. C. *Oithona* spp. distribution plot.

Supplementary Table 1

Supplementary Table 1. All models tested for *N. plumchrus* abundance. The highlighted row represents the selected model. Sample size is 1,069 observations.

Taxon	Model	DF	RSE	AIC	LRS	R2	Coefficients	VIF
<i>N. plumchrus</i>	Temp	1	0.45	1339.56	99.94	0.09	Est. SE t val. P (Int.) 0.75 0.05 15.09 <2e-16 Temp -0.05 5.20e-03 -10.23 <2e-16	Temp 1
	Date	1	0.44	1304.28	135.22	0.12	Est. SE t val. P (Int.) 2.10 0.15 13.65 <2e-16 Date -0.27 0.02 -11.99 <2e-16	Date 1
	Depth	1	0.47	1417.57	21.94	0.02	Est. SE t val. P (Int.) 0.33 0.02 16.21 <2e-16 Shelf -0.13 0.03 -4.70 2.9e-06	Shelf 1
	Region	3	0.47	1417.33	4.23	0.02	Est. SE t val. P (Int.) 0.32 0.02 16.20 <2e-16 East -0.18 0.04 -5.07 4.73e-07 North -0.09 0.04 -2.17 0.03 West -0.07 0.05 -1.45 0.15	East 1.12 North 1.11 West 1.07

Taxon	Model	DF	RSE	AIC	LRS	R²	Coefficients	VIF
<i>N. plumchrus</i>	Temp Date	2	0.44	1284.57	56.99	0.13	Est. SE t val. P (Int.) 1.90 0.16 12.01 < 2e-16 Date -0.20 0.03 -7.64 4.82e-14 Temp -0.03 0.01 -4.68 3.28e-06	Temp 1.42 Date 1.42
	Temp Date Depth	3	0.44	1272.9	13.67	0.15	Est. SE t val. P (Int.) 1.98 0.16 12.47 < 2e-16 Date -0.22 0.03 -8.08 1.7e-15 Temp -0.02 0.01 -3.61 3.23e-04 Shelf -0.10 0.03 -3.70 2.25e-04	Date 1.44 Temp 1.51 Shelf 1.07
	Temp Date Region	5	0.44	1274.2	2.7	0.15	Est. SE t val. P (Int.) 1.99 0.17 12.05 < 2e-16 Temp -0.02 0.01 -2.74 0.01 Date -0.22 0.03 -7.19 1.22e-12 East -0.12 0.04 -2.79 0.01 North -0.06 0.04 -1.51 0.13 West -0.14 0.05 -2.65 0.01	Temp 2.81 Date 1.83 East 1.74 North 1.14 West 1.31

Supplementary Table 2

Supplementary Table 2. All models tested for *C. pacificus* abundance. The highlighted row represents the selected model. Sample size is 1,069 observations.

Taxon	Model	DF	RSE	AIC	LRS	R2	Coefficients	VIF
<i>C. pacificus</i>	Temp	1	0.43	1233.61	28.02	0.02	Est. SE t val. P (Int.) 0.02 0.05 0.41 0.68 Temp 0.03 4.946e-03 5.32 1.24e-07	Temp 1
	Date	1	0.43	1246.77	14.86	0.01	Est. SE t val. P (Int.) -0.32 0.15 -2.11 0.04 Date 0.08 0.02 3.86 1.18e-04	Date 1
	Depth	1	0.43	1250.09	11.53	0.01	Est. SE t val. P (Int.) 0.31 0.02 16.29 < 2e-16 Shelf -0.09 0.03 -3.40 6.93e-04	Shelf 1
	Region	3	0.43	1246.13	7.96	0.02	Est. SE t val. P (Int.) 0.31 0.02 16.60 < 2e-16 East -0.09 0.03 -2.82 4.95e-03 North -0.04 0.04 -1.14 0.26 West -0.18 0.05 -3.95 8.39e-05	East 1.12 North 1.11 West 1.07

Taxon	Model	DF	RSE	AIC	LRS	R2	Coefficients	VIF
<i>C. pacificus</i>	Temp Date	2	0.43	1234.19	14.58	0.03	Est. SE t val. P (Int.) -0.16 0.15 -1.01. 0.31 Date 0.03 0.03 1.19 0.23 Temp 0.02 0.01 3.83 1.37e-04	Date 1.42 Temp 1.42
	Temp Depth	2	0.43	1212.76	39.34	0.04	Est. SE t val. P (Int.) 0.03 0.05 0.73 0.47 Temp 0.03 5.03e-03 6.32 3.81e-10 Shelf -0.13 0.03 -4.80 1.82e-06	Temp 1.05 Shelf 1.05
	Temp Region	4	0.42	1205.54	11.22	0.05	Est. SE t val. P (Int.) -0.04 0.06 -0.65 0.52 Temp 0.04 6.05e-03 6.58 7.51e-11 East -0.21 0.04 -5.68 1.77e-08 North -0.08 0.04 -2.09 0.04 West -0.08 0.05 -1.62 0.11	Temp 1.54 East 1.45 North 1.13 West 1.21

Taxon	Model	DF	RSE	AIC	LRS	R2	Coefficients	VIF
<i>C. pacificus</i>	Temp Date Depth	3	0.43	1214.37	12.81	0.04	Est. SE tval. P (Int.) -0.06 0.15 -0.37 0.71 Date 0.02 0.03 0.618 0.54 Temp 0.03 6.03e-03 4.93 9.55e-07 Shelf -0.13 0.03 -4.69 3.15e-06	Date 1.44 Temp 1.51 Shelf 1.07
	Temp Date Region	5	0.42	1207.02	11.35	0.05	Est. SE tval. P (Int.) 0.07 0.16 0.45 0.65 Temp 0.04 0.01 5.34. 1.11e-07 Date -0.02 0.03 -0.72 0.47 East -0.23 0.04 -5.47 5.56e-08 North -0.08 0.04 -2.15 0.03 West -0.07 0.05 -1.35 0.18	Temp 2.81 Date 1.83 East 1.74 North 1.14 West

Supplementary Table 3

Supplementary Table 3. All models tested for *Oithona spp.* abundance. The highlighted row represents the selected model. Sample size is 1,069 observations.

Taxon	Model	DF	RSE	AIC	LRS	R ²	Coefficients	VIF
<i>Oithona spp.</i>	Temp	1	0.9	2806.31	28.63	0.03	Est. SE t val. P (Int.) 1.05 0.10 10.62 < 2e-16 Temp -0.06 0.01 -5.38 9.05e-08	Temp 1
	Date	1	0.91	2827.52	7.42	0.01	Est. SE t val. P (Int.) 1.39 0.31 4.42 1.07e-05 Date -0.13 0.05 -2.73 6.52e-03	Date 1
	Depth	1	0.91	2824.38	10.56	0.01	Est. SE t val. P (Int.) 0.63 0.04 15.98 < 2e-16 Shelf -0.18 0.06 -3.25 1.17e-03	Shelf 1
	Region	3	0.9	2803.6	35.34	0.03	Est. SE t val. P (Int.) 0.62 0.04 16.20 < 2e-16 East -0.33 0.07 -4.66 3.52e-06 North -0.19 0.08 -2.48 0.01 West 0.21 0.10 2.21 0.03	East 1.12 North 1.11 West 1.07

Taxon	Model	DF	RSE	AIC	LRS	R2	Coefficients	VIF
<i>Oithona</i> <i>spp.</i>	Temp Date	2	0.9	2808.27	0.04	0.02	Est. SE tval. P (Int.) 0.99 0.32 3.05 2.35e-03 Date 0.01 0.05 0.20 0.84 Temp -0.06 0.01 -4.63 4.17e-06	Date 1.42 Temp 1.42
	Temp Date Depth	3	0.9	2805.78	0.03	0.02	Est. SE tval. P (Int.) 1.08 0.33 3.32 9.45e-04 Date -3.57e-03 0.05 -0.07 0.95 Temp -0.05 0.01 -3.94 8.59e-05 Shelf -0.12 0.06 -2.12 0.03	Date 1.44 Temp 1.51 Shelf 1.07
	Temp Date Region	5	0.89	2799.47	10.31	0.04	Est. SE tval. P (Int.) 1.36 0.34 4.03 5.95e-05 Temp -0.01 0.02 -0.79 0.43 Date -0.09 0.06 -1.50 0.13 East -0.29 0.09 -3.29 1.02e-03 North -0.17 0.08 -2.24 0.03 West 0.17 0.11 1.65 0.10	Temp 2.81 Date 1.83 East 1.74 North 1.14 West

Taxon	Model	DF	RSE	AIC	LRS	R2	Coefficients	VIF																														
<i>Oithona spp.</i>	Temp Region	4	0.89	2799.72	12.59	0.03	<table border="1"> <thead> <tr> <th></th> <th>Est.</th> <th>SE</th> <th>t val.</th> <th>P</th> </tr> </thead> <tbody> <tr> <td>(Int.)</td> <td>0.89</td> <td>0.12</td> <td>7.62</td> <td>5.48e-14</td> </tr> <tr> <td>Temp</td> <td>-0.03</td> <td>0.01</td> <td>-2.42</td> <td>0.02</td> </tr> <tr> <td>East</td> <td>-0.23</td> <td>0.08</td> <td>-2.94</td> <td>3.4e-03</td> </tr> <tr> <td>North</td> <td>-0.16</td> <td>0.08</td> <td>-2.11</td> <td>0.04</td> </tr> <tr> <td>West</td> <td>0.13</td> <td>0.10</td> <td>1.28</td> <td>0.20</td> </tr> </tbody> </table>		Est.	SE	t val.	P	(Int.)	0.89	0.12	7.62	5.48e-14	Temp	-0.03	0.01	-2.42	0.02	East	-0.23	0.08	-2.94	3.4e-03	North	-0.16	0.08	-2.11	0.04	West	0.13	0.10	1.28	0.20	Temp 1.54 East 1.45 North
	Est.	SE	t val.	P																																		
(Int.)	0.89	0.12	7.62	5.48e-14																																		
Temp	-0.03	0.01	-2.42	0.02																																		
East	-0.23	0.08	-2.94	3.4e-03																																		
North	-0.16	0.08	-2.11	0.04																																		
West	0.13	0.10	1.28	0.20																																		

Literature cited

- Amaya, D. J., Miller, A. J., Xie, S. P., & Kosaka, Y. (2020). Physical drivers of the summer 2019 North Pacific marine heatwave. *Nature communications*, 11: 1-9.
- Batten, S. D., Clark, R., Flinkman, J., Hays, G., John, E., John, A. W. G., ... & Walne, A. (2003a). CPR sampling: the technical background, materials and methods, consistency and comparability. *Progress in Oceanography*, 58: 193-215.
- Batten, S. D., Welch, D. W., & Jonas, T. (2003b). Latitudinal differences in the duration of development of *Neocalanus plumchrus* copepodites. *Fisheries Oceanography*, 12(3), 201-208.
- Batten, S. D., Hyrenbach, K. D., Sydeman, W. J., Morgan, K. H., Henry, M. F., Yen, P. P., & Welch, D. W. (2006). Characterising meso-marine ecosystems of the North Pacific. *Deep Sea Research Part II: Topical Studies in Oceanography*, 53: 270-290.
- Batten, S. D., & Mackas, D. L. (2009). Shortened duration of the annual *Neocalanus plumchrus* biomass peak in the Northeast Pacific. *Marine Ecology Progress Series*, 393: 189-198.
- Batten, S. D., & Walne, A. W. (2011). Variability in northwards extension of warm water copepods in the NE Pacific. *Journal of Plankton Research*, 33: 1643-1653.
- Benson, A. J., & Trites, A. W. (2002). Ecological effects of regime shifts in the Bering Sea and eastern North Pacific Ocean. *Fish and Fisheries*, 3: 95-113.
- Bertram, D. F., Mackas, D. L., & McKinnell, S. M. (2001). The seasonal cycle revisited: interannual variation and ecosystem consequences. *Progress in Oceanography*, 49: 283-307.
- Carstensen, J., Weydmann, A., Olszewska, A., & Kwaśniewski, S. (2012). Effects of environmental conditions on the biomass of *Calanus spp.* in the Nordic Seas. *Journal of Plankton Research*, 34: 951-966.
- Castellani, C., Licandro, P., Fileman, E., Di Capua, I., & Mazzocchi, M. G. (2016). *Oithona similis* likes it cool: evidence from two long-term time series. *Journal of Plankton Research*, 38(3), 703-717.
- Cavole, L. M., Demko, A. M., Diner, R. E., Giddings, A., Koester, I., Pagniello, C. M., ... & Franks, P. J. (2016). Biological impacts of the 2013–2015 warm-water anomaly in the Northeast Pacific: winners, losers, and the future. *Oceanography*, 29: 273-285.
- Conover, R. J. (1988). Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. *Hydrobiologia*, 167: 127-142.

- Coyle, K. O., & Pinchuk, A. I. (2003). Annual cycle of zooplankton abundance, biomass and production on the northern Gulf of Alaska shelf, October 1997 through October 2000. *Fisheries Oceanography*, 12: 327-338.
- Coyle, K. O., & Pinchuk, A. I. (2005). Seasonal cross-shelf distribution of major zooplankton taxa on the northern Gulf of Alaska shelf relative to water mass properties, species depth preferences and vertical migration behavior. *Deep Sea Research Part II: Topical Studies in Oceanography*, 52: 217-245.
- Crawford, W. R., Brickley, P. J., & Thomas, A. C. (2007). Mesoscale eddies dominate surface phytoplankton in northern Gulf of Alaska. *Progress in Oceanography*, 75: 287-303.
- Di Lorenzo, E., & Mantua, N. (2016). Multi-year persistence of the 2014/15 North Pacific marine heatwave. *Nature Climate Change*, 6: 1042-1047.
- Dvoretzky, V. G., & Dvoretzky, A. G. (2009). Life cycle of *Oithona similis* (Copepoda: Cyclopoida) in Kola Bay (Barents Sea). *Marine biology*, 156:1433-1446.
- Evanson, M., Bornhold, E. A., Goldblatt, R. H., Harrison, P. J., & Lewis, A. G. (2000). Temporal variation in body composition and lipid storage of the overwintering, subarctic copepod *Neocalanus plumchrus* in the Strait of Georgia, British Columbia (Canada). *Marine Ecology Progress Series*, 192: 239-247.
- Fisher, J., Kimmel, D., Ross, T., Batten, S., Bjorkstedt, E., Galbraith, M., ... & Perry, R. I. (2020). Copepod responses to, and recovery from, the recent marine heatwave in the Northeast Pacific. *PICES Sci. 2019: Notes Sci. Board Chair*, 28: 65.
- Frölicher, T. L., Fischer, E. M., & Gruber, N. (2018). Marine heatwaves under global warming. *Nature*, 560: 360-364.
- Hansen, F. C., Möllmann, C., Schütz, U., & Hinrichsen, H. H. (2004). Spatio-temporal distribution of *Oithona similis* in the Bornholm Basin (central Baltic Sea). *Journal of Plankton Research*, 26: 659-668.
- Henson, S. A., & Thomas, A. C. (2008). A census of oceanic anticyclonic eddies in the Gulf of Alaska. *Deep Sea Research Part I: Oceanographic Research Papers*, 55: 163-176.
- Hill, D. F., Bruhis, N., Calos, S. E., Arendt, A., & Beamer, J. (2015). Spatial and temporal variability of freshwater discharge into the Gulf of Alaska. *Journal of Geophysical Research: Oceans*, 120: 634-646.
- Janout, M. A., Weingartner, T. J., Royer, T. C., & Danielson, S. L. (2010). On the nature of winter cooling and the recent temperature shift on the northern Gulf of Alaska shelf. *Journal of Geophysical Research: Oceans*, 115: C5.

- Johnstone, J. A., & Mantua, N. J. (2014). Atmospheric controls on northeast Pacific temperature variability and change, 1900–2012. *Proceedings of the National Academy of Sciences*, 111: 14360-14365.
- JPL MUR MEaSURES Project (2015). GHRSSST Level 4 MUR Global Foundation Sea Surface Temperature Analysis (v4.1). Ver. 4.1. PO.DAAC, CA, USA. Available online at: <https://doi.org/10.5067/GHGMR-4FJ04> (accessed August, 2020)
- Kawamura, A., & Hirano, K. (1985). The spatial scale of surface swarms of *Calanus plumchrus* Marukawa observed from consecutive plankton net catches in the northwestern North Pacific. *Bulletin of marine science*, 37: 626-633.
- Kelly, M. W., Sanford, E., & Grosberg, R. K. (2012). Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proceedings of the Royal Society B: Biological Sciences*, 279: 349-356.
- Kobari, T., & Ikeda, T. (2001). Ontogenetic vertical migration and life cycle of *Neocalanus plumchrus* (Crustacea: Copepoda) in the Oyashio region, with notes on regional variations in body sizes. *Journal of Plankton Research*, 23: 287-302.
- Kobari, T., Nagaki, T., & Takahashi, K. (2004). Seasonal changes in abundance and development of *Calanus pacificus* (Crustacea: Copepoda) in the Oyashio–Kuroshio Mixed Region. *Marine Biology*, 144: 713-721.
- Lee, R. F., Hagen, W., & Kattner, G. (2006). Lipid storage in marine zooplankton. *Marine Ecology Progress Series*, 307: 273-306.
- Liu, H., & Hopcroft, R. R. (2007). A comparison of seasonal growth and development of the copepods *Calanus marshallae* and *C. pacificus* in the northern Gulf of Alaska. *Journal of Plankton Research*, 29: 569-581.
- Mackas, D. L., Denman, K. L., & Abbott, M. R. (1985). Plankton patchiness: biology in the physical vernacular. *Bulletin of Marine Science*, 37: 652-674.
- Mackas, D. L., Batten, S., & Trudel, M. (2007). Effects on zooplankton of a warmer ocean: recent evidence from the Northeast Pacific. *Progress in Oceanography*, 75: 223-252.
- Madsen, S. D., Nielsen, T. G., & Hansen, B. W. (2008). Annual population development and production by small copepods in Disko Bay, western Greenland. *Marine Biology*, 155: 63-77.
- Mauchline, J. (1998). *Adv. Mar. Biol.* 33: *The biology of calanoid copepods*.
- McKinstry, C. A., & Campbell, R. W. (2018). Seasonal variation of zooplankton abundance and community structure in Prince William Sound, Alaska, 2009–2016. *Deep Sea Research Part II: Topical Studies in Oceanography*, 147: 69-78.

- Nuwer, M. L., Frost, B. W., & Virginia Armbrust, E. (2008). Population structure of the planktonic copepod *Calanus pacificus* in the North Pacific Ocean. *Marine Biology*, 156: 107-115.
- Oliver, E. C., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., ... & Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature communications*, 9: 1-12.
- Osgood, K. E., & Frost, B. W. (1994). Comparative life histories of three species of planktonic calanoid copepods in Dabob Bay, Washington. *Marine Biology*, 118: 627-636.
- Peña, M. A., Nemcek, N., & Robert, M. (2019). Phytoplankton responses to the 2014–2016 warming anomaly in the northeast subarctic Pacific Ocean. *Limnology and Oceanography*, 64: 515-525.
- Pereira, R. J., Sasaki, M. C., & Burton, R. S. (2017). Adaptation to a latitudinal thermal gradient within a widespread copepod species: the contributions of genetic divergence and phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, 284: 20170236.
- Peterson, W. T., Fisher, J. L., Strub, P. T., Du, X., Risien, C., Peterson, J., & Shaw, C. T. (2017). The pelagic ecosystem in the Northern California Current off Oregon during the 2014–2016 warm anomalies within the context of the past 20 years. *Journal of Geophysical Research: Oceans*, 122: 7267-7290.
- Piatt, J. F., Parrish, J. K., Renner, H. M., Schoen, S. K., Jones, T. T., Arimitsu, M. L., ... & Sydeman, W. J. (2020). Extreme mortality and reproductive failure of common murrelets resulting from the northeast Pacific marine heatwave of 2014-2016. *PloS one*, 15: e0226087.
- Rayner, N. A. A., Parker, D. E., Horton, E. B., Folland, C. K., Alexander, L. V., Rowell, D. P., ... & Kaplan, A. (2003). Global analyses of sea surface temperature, sea ice, and night marine air temperature since the late nineteenth century. *Journal of Geophysical Research: Atmospheres*, 108: D14.
- Reid, P. C., Colebrook, J. M., Matthews, J. B. L., Aiken, J. C. P. R., & Team, C. P. R. (2003). The Continuous Plankton Recorder: concepts and history, from Plankton Indicator to undulating recorders. *Progress in Oceanography*, 58(2-4), 117-173.
- Sasaki, M. C., & Dam, H. G. (2019). Integrating patterns of thermal tolerance and phenotypic plasticity with population genetics to improve understanding of vulnerability to warming in a widespread copepod. *Global change biology*, 25: 4147-4164.

- Scheffers, B. R., De Meester, L., Bridge, T. C., Hoffmann, A. A., Pandolfi, J. M., Corlett, R. T., ... & Watson, J. E. (2016). The broad footprint of climate change from genes to biomes to people. *Science*, 354(6313), aaf7671.
- Sousa, L., Coyle, K. O., Barry, R. P., Weingartner, T. J., & Hopcroft, R. R. (2016). Climate-related variability in abundance of mesozooplankton in the northern Gulf of Alaska 1998–2009. *Deep Sea Research Part II: Topical Studies in Oceanography*, 132: 122-135.
- Ward, P., & Hirst, A. G. (2007). *Oithona similis* in a high latitude ecosystem: abundance, distribution and temperature limitation of fecundity rates in a sac spawning copepod. *Marine Biology*, 151: 1099-1110.
- Weingartner, T. J., Coyle, K., Finney, B., Hopcroft, R., Whitley, T., Brodeur, R., ... & Royer, T. (2002). The northeast Pacific GLOBEC program: coastal Gulf of Alaska. *Oceanography*, 15:2.
- Yang, B., Emerson, S. R., & Peña, M. A. (2018). The effect of the 2013–2016 high temperature anomaly in the subarctic Northeast Pacific (the “Blob”) on net community production. *Biogeosciences*, 15: 6747-6759.
- Zamora-Terol, S., Kjellerup, S., Swalethorp, R., Saiz, E., & Nielsen, T. G. (2014). Population dynamics and production of the small copepod *Oithona spp.* in a subarctic fjord of West Greenland. *Polar Biology*, 37: 953-965.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Ashlock, García-Reyes, Gentemann, Batten and Sydeman. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms

Tables and Figures

TABLE 1. Model selection for copepod abundance.

Taxon	Model	DF	RSE	AIC	LRS	R2	Coefficients		VIF					
<i>N. plumchrus</i>	Temp + Date + Depth	3	0.444	1272.90	13.67	0.15	Est.	SE	t val.	p	Date 1.44	Temp 1.51	Shell 1.07	
							(Int.)	1.98	0.16	12.47	<2e-16			
							Date	-0.22	0.03	-8.08	1.7e-15			
							Temp	-0.02	0.01	-3.61	3.23e-04			
							Shell.	-0.10	0.03	-3.70	2.25e-04			
							Est.	SE	t val.	p	Temp 1.54	East 1.45	North 1.13	West 1.21
							(Int.)	-0.04	0.06	-0.65	0.52			
							Temp	0.04	6.05e-03	6.58	7.51e-11			
							East	-0.21	0.04	-5.68	1.77e-08			
							North	-0.08	0.04	-2.09	0.04			
<i>Oithona</i> spp.	Temp + Region	4	0.89	2799.712	12.59	0.03	Est.	SE	t val.	p	Temp 1.54	East 1.45	North 1.13	West 1.21
							(Int.)	0.89	0.12	7.62	5.48e-14			
							Temp	-0.03	0.01	-2.42	0.02			
							East	-0.23	0.08	-2.94	3.4e-03			
North	-0.16	0.08	-2.11	0.04										
West	0.13	0.10	1.28	0.20										

Sample size for each model is 1,069 observations.

Figure legends

FIGURE 1. Map of the Gulf of Alaska and eastern Bering Sea with symbols representing locations of Continuous Plankton Recorder samples. Colors and symbols indicate region: eastern shelf/slope, northern shelf/slope, western shelf/slope, and oceanic. Shelf/slope regions include samples 3,000 m depth.

FIGURE 2. The blue bars represent taxon percent occurrence in samples. Red dots indicate the 95th percentile of taxon abundance. The horizontal line represents the thermal threshold based on the 95th percentile of abundance metric (20% of the maximum value of 95th percentile of abundance metric for the region). Percent occurrence and 95th percentile of abundance for each taxon are from the entire study region and span the entire seasonal period. All data are divided by 1°C SST (based on MUR-SST) bins. (A) Plot of *Neocalanus plumchrus* occurrence and abundance. (B) Plot of *Calanus pacificus* occurrence and abundance. (C) Plot of *Oithona* spp. occurrence and abundance. (D) Histogram of sample temperatures.

FIGURE 3. Annual mean *Neocalanus plumchrus* abundance plotted by region. Years where temperatures exceed regional thermal thresholds are marked in red.

FIGURE 4. Monthly SST above (red) and below (blue) the 10.5°C threshold of abundance for *Neocalanus plumchrus* for the months of May, June, and July for each region. The horizontal dashed lines indicate 11.5°C threshold for occurrence. (A) Temperature plots for the eastern region. (B) Temperature plots for the northern region. (C) Temperature plots for the western region. (D) Temperature plots for the oceanic region.

FIGURE 5. (A) Plot of cohort abundance midpoint against one-year lag mean HadISST. (B) Plot of cohort width against one-year lag mean HadISST.

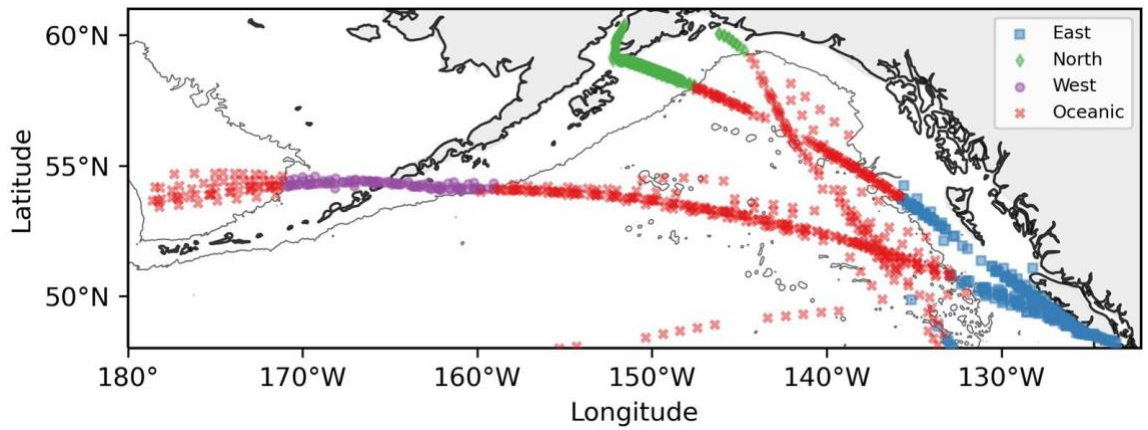


Figure 1

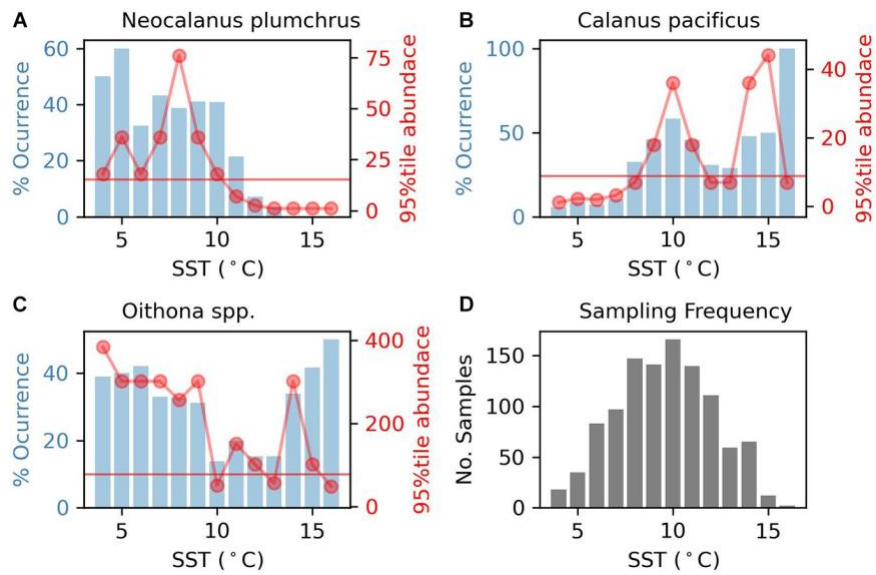


Figure 2

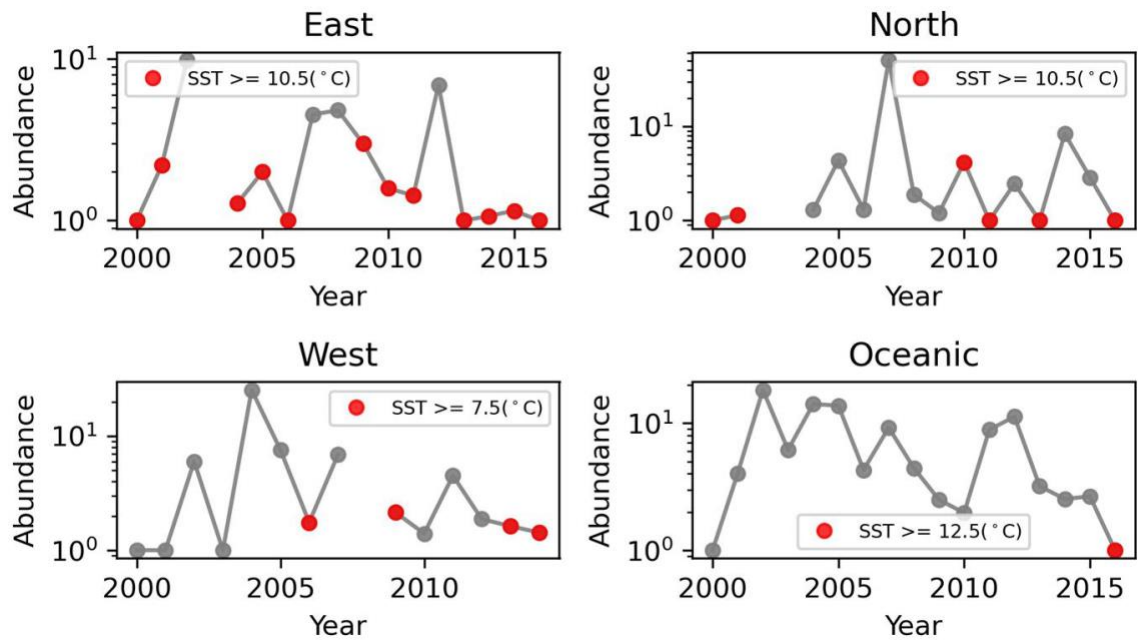


Figure 3

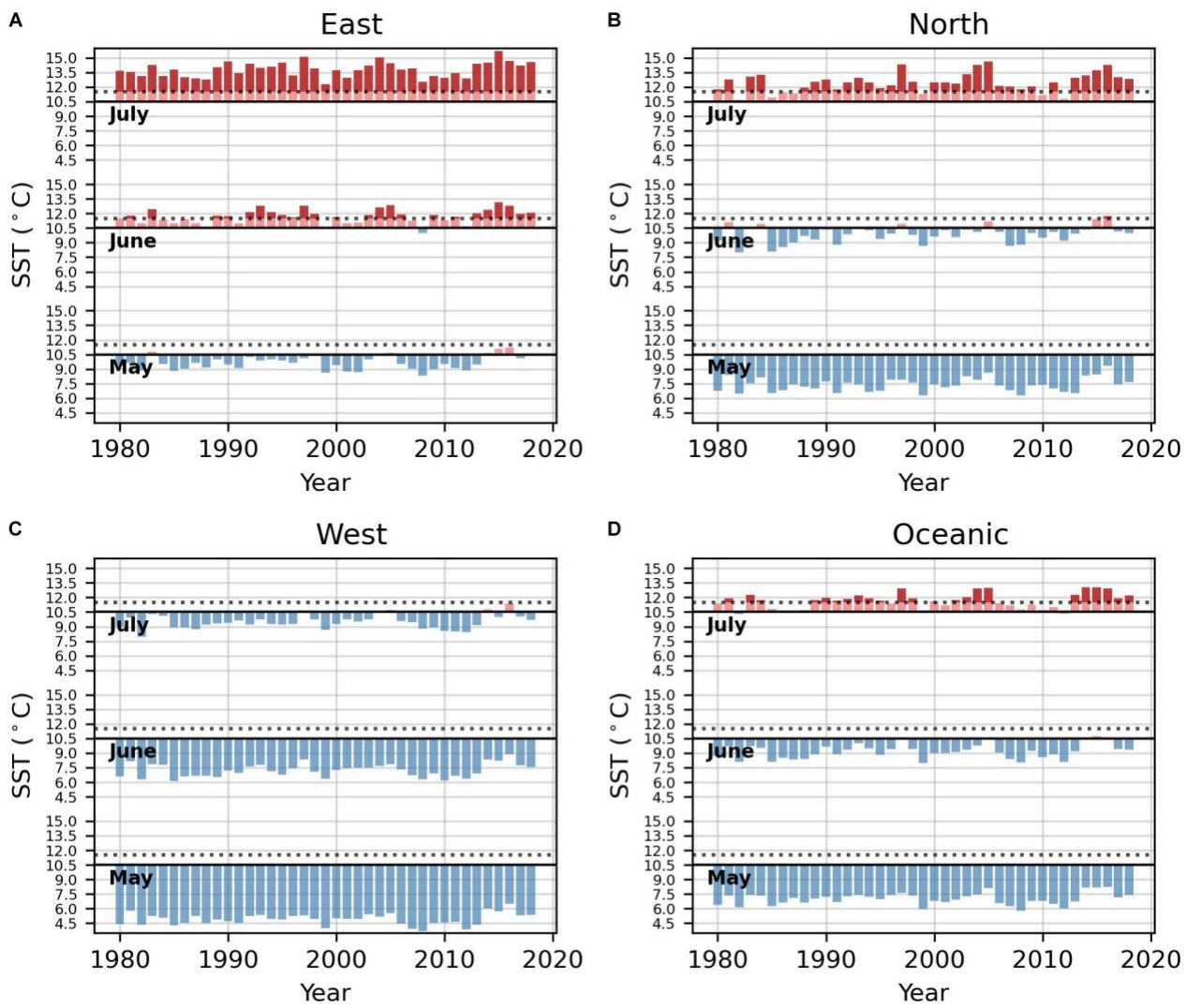


Figure 4

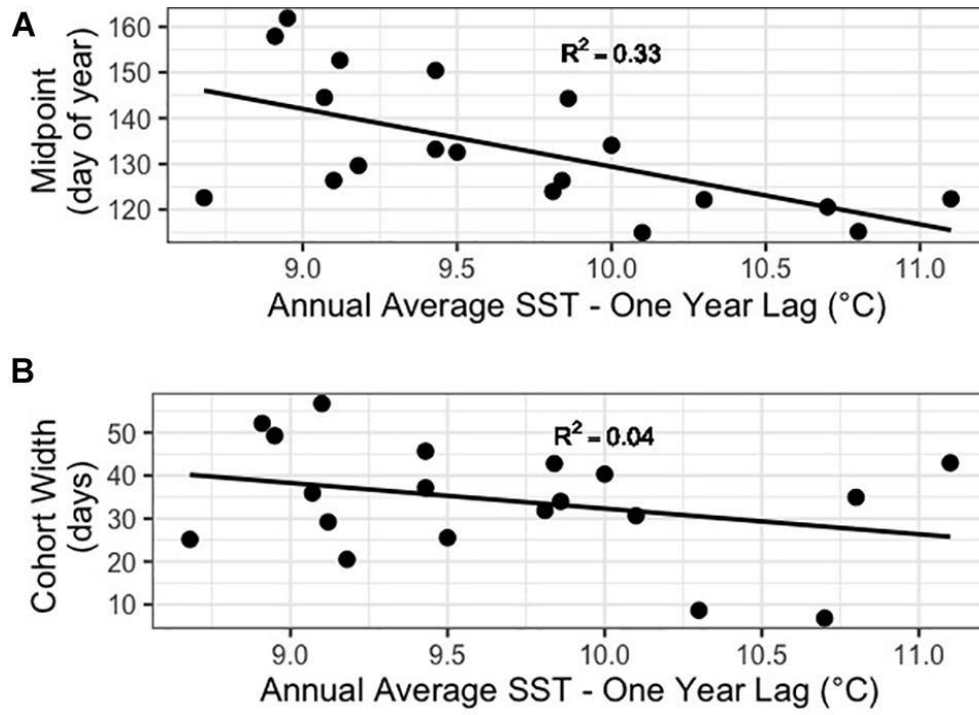


Figure 5

**CHAPTER II: DEVELOPMENTAL TEMPERATURE MORE THAN LONG-TERM EVOLUTION
DEFINES THERMAL TOLERANCE IN AN ESTUARINE COPEPOD**

Abstract

Estuarine organisms are experiencing increasingly variable temperature and salinity with climate change. Plasticity and adaptation are critical strategies for responding to environmental change, and understanding their role in response to climate change is essential for predicting species and ecosystem resilience. Here we characterized the response of the copepod, *Acartia tonsa*, to sequential temperature and salinity stress. Copepods originated from one of two >50 generation old temperature adapted lines: Ambient (AM, 18°C), and Ocean Warming (OW, 22°C). Copepods from these lines were subjected to one or three generations at the reciprocal temperature. Copepods from all treatments were then assessed for differences in temperature and salinity tolerance. Development, three generations, and >50 generations of warming increased thermal tolerance compared to Ambient conditions, with developmental OW resulting in equal thermal tolerance to three and >50 generations of OW. Strikingly, developmental OW and >50 generations of OW had no effect on low salinity tolerance relative to Ambient. By contrast, when environmental salinity was reduced first, copepods had lower thermal tolerances. These results highlight a critical role for plasticity in the copepod climate response and suggest that climate change may leave estuarine copepods vulnerable to increasingly variable temperature and salinity conditions.

Keywords: Plasticity, adaptation, thermal tolerance, salinity tolerance, climate change, copepod

Introduction

Mean water temperatures and variation in water temperature are expected to increase with climate change (Scheffers et al., 2016, Harley et al., 2006). Marine heat waves are also increasing in prevalence and duration, and the hydrological cycle is expected to intensify with increases in wet and dry extremes (Singh et al., 2013, Frölicher et al., 2018, Oliver et al. 2018). Organisms can use plasticity and adaptation to respond to these changes. Plasticity is the ability of genotypes to exhibit multiple phenotypes in response to environmental change (Fordyce et al., 2006, West-Eberhard 2003, Somero et al., 2017, Whitman et al., 2009). Adaptation is genetically-based phenotypic change driven by the process of selection that maximizes relative fitness in a given environment (Hartl, 2000, Hendry, 2016). Importantly, plasticity can result in new phenotypes within a generation, while adaptation acts across generations (Hartl, 2000, Somero et al., 2017). Understanding the relative roles of plasticity and adaptation in the response to a changing climate will reveal the potential tolerances and vulnerabilities of marine organisms.

Estuaries are excellent ecosystems for understanding population-level responses to a variety of stressors. Estuarine ecosystems are characterized by a temporally and spatially dynamic physical environment, with regular and stochastic variation in temperature and salinity (Najjar et al., 2000, Moyle et al., 2010). These complex ecosystems foster high productivity, and support large populations of fish, mammals, birds, and invertebrates (Harris et al., 2016, Moyle et al., 2010). Copepods are critical

members of estuarine ecosystems, connecting primary producers to higher trophic levels, contributing to biogeochemical cycling, and providing a vital food source to forage fishes (Richardson et al., 2008, Steinberg et al., 2017). Copepods are also excellent sentinels of environmental change, as their short generation times allow them to closely mirror changing environmental conditions (Dam 2013). Further, copepods are ideal models for lab experiments. They are easily cultured in a lab setting, and have short generation times, facilitating their study across generations.

Acartia tonsa is a globally distributed, numerically dominant, estuarine copepod (González 1974, Calliari et al., 2008, Johnson and Allen 2012), which makes a suitable model for global change studies. *Acartia tonsa* is a generalist with respect to temperature and salinity, and highly responsive and relatively robust to anthropogenic warming (Rice et al., 2015, Rice et al., 2016, Rahlff et al., 2017, Garzke et al., 2015). Both plasticity and adaptation play a critical role in the *A. tonsa* response to temperature variation. Plasticity affects the thermal tolerance of *A. tonsa*, with populations that develop at higher temperatures having higher thermal tolerances (Sasaki and Dam 2019). Additionally, *A. tonsa* subjected to 25 generations of experimental Ocean Warming show increased performance and fitness over time, indicating adaptation to Ocean Warming (Dam et al., 2021). While the influence of adaptation and plasticity on *A. tonsa* thermal tolerance is characterized, the relative importance and potential limitations of these two strategies remains unknown.

Despite thermal tolerance plasticity and adaptive capacity of *A. tonsa* to ocean warming, exposure to chronic ocean warming may leave *A. tonsa* vulnerable to additional stressors. With warming, ectotherms experience increases in metabolic rate and therefore have a higher energetic demand (Kelly et al., 2016, Sheridan and Bickford 2011, Garzke et al., 2015), which may come at the expense of energy available for the acute response to temperature or salinity change. Additionally, rapid response to temperature change may limit the capacity of *A. tonsa* to respond to additional stressors such as salinity, if traits lack modularity or if adaptation reduces standing genetic diversity for tolerance to other stressors (Ellegren and Galtier 2016, Jump and Penuelas 2005). *A. tonsa* populations exposed to warming demonstrate increases in thermal performance across generations which are correlated with decreases in thermal tolerance plasticity (Sasaki and Dam 2021). Therefore, increasingly dynamic estuaries may leave rapidly responsive *A. tonsa* populations vulnerable to unpredictable acute changes in temperature and salinity.

Here, we measured the impact of developmental, short-term, and long-term multigenerational exposure to Ocean Warming (OW) on acute temperature and salinity tolerance. This experimental design allowed us to examine plastic and genetic responses to temperature in *A. tonsa*. We predicted developmental exposure (one generation), short-term adaptation (three generations), and long-term adaptation (>50 generations) to OW would increase copepod tolerance to acute heat stress. Specifically, we anticipated that tolerance to acute heat stress would increase proportionally to the length of the temperature treatment. Additionally, we predicted that any experimental exposure to OW would reduce copepod tolerance to acute salinity stress.

Methods

2.1 Collection and Culture

Animals originated from Ambient (AM) and Ocean Warming (OW) lineages from a long-term multigenerational experiment (Dam et al., 2021). Adult *Acartia tonsa* were collected from the Long Island Sound (41.3°N, 72.0°W; Groton, CT, USA) in June 2016 using a 250 µm plankton net with a solid cod end. Animals were raised as stock cultures at the University of Connecticut, Avery Point campus (Dam et al. 2021) for three generations before splitting them into four replicates each at AM (18°C), and OW conditions (22°C). Replicates were seeded by eight stock cultures with 160 female, and 80 male adult copepods (Dam et al., 2021). Replicates were fed *ad libitum* with a mixture of the phytoplankters *Rhodomonas sp.*, *Tetraselmis sp.*, and *Thalassiosira weissflogii* (Dam et al., 2021). Replicates were maintained at a salinity ranging from 31-36ppt (Dam et al., 2021). Copepod and algal cultures were maintained on a 12:12 light dark cycle. Algal cultures were intentionally raised at AM temperature (18°C) to avoid potential changes in nutritional content if they were cultured at OW.

After >50 generations, animals from all eight replicates (two treatments, with four replicates per treatment) were transferred to the University of Vermont in October 2019. Organisms were transported at 18°C for five hours and returned to their respective temperature conditions upon arrival at UVM. Light and food conditions were maintained the same as at Avery Point. After an additional three generations in the lab, copepods from three replicates at AM and four replicates at OW were assessed for their Upper Lethal Temperature (ULT) (Figure 1).

2.2 Upper Lethal Temperature (ULT) Assay

To assess ULT, four adults from each replicate were placed in their own individual well of a 12-well plate with water at their treatment temperature, food replete conditions, and 30ppt salinity. Adults were isolated from the culture the day prior to the assay, and allowed to acclimate to the plate wells at their respective treatment temperatures overnight. The following day, plates were removed from incubators and plates from both treatments were equilibrated to room temperature. Plates were then sealed with parafilm and placed in a water bath where the temperature ramped from 22 to 34°C over 60 minutes (0.2°C/min), unmonitored. These temperatures were unmonitored because previous ULT assays in our lab demonstrated that they are sublethal to *Acartia tonsa* from the Long Island Sound (Supplemental Figure 1). Temperature was then ramped at a rate of 0.0125°C/ minute. Copepods were assessed for survival (no movement for 10 seconds after disturbing the water in the well) at every 0.5°C, until no surviving copepods remained.

2.3 Reciprocal Transplants

To assess the relative importance of plasticity and adaptation in defining thermal tolerance we created two sets of reciprocal transplants. Transplant cultures were created by setting all adults from each replicate aside to lay eggs for 48 hours. Total eggs produced by each replicate were separated in half by volume and split between the control and reciprocal condition. Three reciprocal transplant cultures were assessed for

ULT after development from egg to adult (AM_{DevOW}). The remaining three transplant cultures were assessed for ULT as adults after three generations at 22°C ($AM_{3GensOW}$). In comparing developmental warming (AM_{DevOW}), to three generations of warming ($AM_{3GensOW}$), to >50 generations of warming (from our OW line), we can determine which treatment condition had the largest impact on thermal tolerance.

To test for relaxed selection and potential loss of thermal tolerance, eggs from the warming line replicates were used to seed transplant cultures at 18°C. Of the animals that were moved to ambient conditions, three replicate populations stayed there for development from egg to adult (OW_{DevAM}), while the other three remained in ambient conditions for three generations ($OW_{3GensAM}$). We measured ULT of four adult animals from each condition.

2.4 Temperature and Salinity Relationship

To assess the effect of OW on salinity tolerance, animals from the AM, OW, and AM_{DevOW} lines were assessed for their Lower Lethal Salinity (LLS). The LLS assay consisted of four adults from each replicate being placed in a 12-well plate. Wells did not contain food, and had a starting salinity of 30ppt. Temperature was maintained the same as the respective treatment temperature conditions.

Adults were isolated from the culture the day prior to the assay, and allowed to acclimate to the plate wells overnight. Adults started at 30ppt, and then salinity was reduced stepwise by full water replacement every 30 minutes from 30ppt to 20ppt, 15ppt, 10ppt, 8ppt, 6ppt, 4ppt, 2ppt, and 0ppt. Survival was monitored at every step. Preliminary

experiments revealed limited mortality above 10ppt salinity (Supplemental Figure 2), therefore we focused on finer resolution increments at lower salinity levels.

To explore the relationship between temperature and salinity tolerance further, we reversed the order of events and measured the effect of low salinity acclimation on ULT. We took seven animals from each replicate of our AM line and acclimated them to three sublethal salinity conditions: 30ppt, 20ppt, and 15ppt. One third of adults ($n = 7$) started and remained at 30ppt, another set was moved stepwise from 30 ppt to 20ppt after 30 minutes ($n = 7$), and another set was moved from 20 ppt to 15ppt after an additional 30 minutes ($n = 7$). Animals were maintained at 18°C throughout the assay. After 12 hours of acclimation to the three salinity levels (with 100% survival), animals from all treatments were assessed for their ULT. ULT assays were performed at the respective acclimatized salinity.

To test how consistent the relationship between temperature and salinity tolerance was, we assessed animals from the experimental AM replicates as well as another more recent collection from Long Island Sound that had been acclimated to the lab (collected in July 2018, at Hammonasset Beach State Park, CT; 41.3°N, 72.5°W). For each population and salinity condition, we isolated seven adults, 42 adults total.

2.5 Data Analysis

We assessed the normality of the data using the Shapiro-Wilk test and tested for homogeneity of variance using the Levene test. Our results indicated that our data were not normally distributed and did not have equal variance across replicates therefore differences between ULT and LLS were assessed using non-parametric methods.

Additionally, we performed the Levene test to reveal potential differences in variance among treatments. We used the non-parametric Aligned Rank Transform ANOVA in cases with more than one independent variable (Wobbrock et al., 2011). The ART ANOVA, and multifactor contrast tests, were performed using the R package ARTool (Wobbrock et al., 2011, Elkin et al., 2021). We used the Kruskal Wallis test when there was only one independent variable. The Dunn test was used as a post-hoc test when applicable. All analyses were performed in R version 4.0.3 (R Core Team 2020).

Results

3.1 Upper Lethal Temperature Assays

Ocean warming (22°C), whether for one, three, or >50 generations resulted in increased thermal tolerance for copepods (Dunn test, $Z = -3.65, -3.78, 2.85, P_{adj} = 0.002, 0.002, \text{ and } 0.02$ respectively; Figure 2). Importantly, there was no additional thermal tolerance gained for AM_{3GensOW} or OW compared to AM_{DevOW} (Dunn test, $Z = -0.39, P_{adj} = 0.69$ (AM_{3GensOW}), $Z = -0.81, P_{adj} = 0.48$ (OW); Figure 2). In addition, AM_{DevOW} had greater variance in ULT compared to all other treatment groups (Levene Test, $P_{adj} = 0.001$ (AM), 0.009 (AM_{3GensOW}), and 0.004 (OW)), suggesting that elevated temperature during development reveals a diversity of ULT phenotypes that are lost over subsequent generations.

3.2 Reciprocal Transplants

To reveal any sustained benefits or costs of long-term adaptation to OW, animals from the OW line were transplanted into ambient conditions for one (OW_{DevAM}) and three

generations ($OW_{3GensAM}$). We found that after either OW_{DevAM} or $OW_{3GensAM}$, there was no significant loss of thermal tolerance (Figure 3, Dunn test, $Z= 1.17, 1.95, P_{adj}= 0.36$ and 0.10 , respectively). However, animals from both of these transplanted groups had intermediate thermal tolerances between the AM and OW line animals (Figure 3).

3.3 Temperature and Salinity Relationship

To test for an effect of long- and short-term ocean warming on lower lethal salinity, we exposed AM, AM_{DevOW} , and OW line animals to sequentially lower salinities. We found no effect of one or >50 generations of ocean warming on low salinity tolerance relative to AM (Kruskal Wallis, $X^2= 0.81, P=0.67$, Figure 4), suggesting no costs of elevated temperature on low salinity tolerance. To test the reverse, if a low salinity environment affects thermal tolerance, we sequentially lowered the salinity from 30ppt, to 20ppt, to 15ppt for AM animals as well as a fresh collection of copepods also from the Long Island Sound that had been acclimated to the lab for at least three generations. We found that one day at a lower salinity of 15ppt reduced mean Upper Lethal Temperature of AM animals by $0.6^{\circ}C$ (ART ANOVA, $F= 6.98, P = 0.002$; Figure 5). Comparing among the salinity treatments, ULT was higher for copepods that stayed at 30ppt than copepods that were moved to 15ppt (ART ANOVA contrasts $P < 0.004$). ULT was also higher for animals that were moved to 20ppt than animals that were moved to 15ppt (ART ANOVA contrasts $P < 0.01$). Interestingly, although the two populations were collected from sites only 50 km apart in the Long Island Sound, we saw an effect of population on ULT (ART ANOVA, $F= 48.94, P < 0.0001$), with the fresh collection

having higher thermal tolerance and broader range of tolerances (Levene test, $P_{adj} = 0.02$).

Discussion

Here, we examined the effect of short and long-term Ocean Warming on acute temperature and salinity tolerance in the estuarine copepod *A. tonsa*. As predicted, warming for one (AM_{DevOW}), three ($AM_{3GensOW}$), or >50 generations (OW) increased copepod tolerance to acute heat stress. However, thermal tolerance did not increase proportionally to the number of generations in elevated temperature conditions. Rather, copepods from Ambient conditions that developed at Ocean Warming (AM_{DevOW}), spent three generations ($AM_{3GensOW}$), or >50 generations at 22°C (OW) had the same mean thermal tolerance. Developmental OW also revealed phenotypic diversity in thermal tolerance not present in the AM line, this diversity was subsequently lost after three and >50 generations of OW. Surprisingly, our prediction that experimental warming would reduce copepod tolerance to acute salinity stress was not supported, with no duration of experimental warming affecting lower lethal salinity. However, we did see a relationship between salinity and temperature tolerance, with animals acclimatized to lower environmental salinities having lower thermal tolerances. These results indicate copepods may be vulnerable to increasingly dynamic temperature and salinity conditions in estuarine ecosystems. Additionally, our results indicate that tolerance to one stressor is impacted by the occurrence of additional stressors. As oceans are multifaceted

ecosystems with many concurrent changing variables, it is essential to consider how these environmental stressors interact to determine organism tolerance.

We found that AM_{DevOW} individuals had a higher thermal tolerance than AM animals. An additional three and >50 generations at 22°C did not further improve thermal tolerance. This differs from *A. tonsa* LD₅₀ after experimental evolution to warming, which continuously improved across 40 generations (Sasaki and Dam, 2021). Differences here may be due to differences in the thermal tolerance metric assessed. Sasaki and Dam, 2021, assessed LD₅₀, the temperature at which 50% the population dies. In this study we assessed Upper Lethal Temperature, the temperature at which each individual copepod dies. Upper Lethal Temperature represents a hard physiological limit, and is likely more constrained than LD₅₀ (Bennett et al., 2021).

Our results also indicate that adaptation does not impart any improvement in Upper Lethal Temperature, beyond plasticity. These results align well with existing research that demonstrates relatively high plasticity in the *A. tonsa* response to elevated temperature conditions (Rice et al., 2016, Rahlff et al., 2017, Garzke et al., 2015, Sasaki and Dam 2019). Our results also corroborate research in *Daphnia sp.*, demonstrating that acclimation temperature has a larger impact on thermal tolerance than local adaptation (Yampolsky et al., 2014). Additionally, plasticity plays a larger role than genetic variation in determining temperature tolerance in *Drosophila melanogaster* (Ayrinhac et al., 2004 and Hoffmann et al., 2005). Together these results indicate that plasticity plays a critical role in thermal tolerance, particularly in the context of changing climate conditions. *A. tonsa* exhibited reduced transcriptional plasticity after long-term adaptation

to concurrent warming and increased pCO₂, while adaptation to the same combination of stressors reduced thermal tolerance plasticity (deMayo, et al. 2021, Brennan et al., 2022a). Consequently, thermal tolerance plasticity in *A. tonsa* may be dampened in the face of multiple climate stressors. Additionally, in response to heat tolerance selection the tidepool copepod, *Tigriopus californicus* demonstrated reduced phenotypic and gene expression plasticity (Kelly et al., 2017). Therefore, exposure to temperature stress alone can reduce the thermal tolerance plasticity of marine copepods.

Interestingly, AM_{DevOW} revealed a wider range of Upper Lethal Temperature values than other treatment groups (Figure 2). This result supports theory and other empirical studies that suggest novel environments may disrupt organism homeostasis (Badyaev et al., 2005) and reveal trait variability that is otherwise hidden (Salinas et al., 2019, Badyaev et al., 2005). Importantly, AM_{DevOW} also exhibited the highest observed thermal tolerances in our study (ULT >38 °C) that were no longer observed after three and >50 generations at 22°C, suggesting a possible cost of adaptation to warming. Additionally, we see decreasing thermal tolerances in OW_{DevAM} and OW_{3GensAM} compared to the OW line. This indicates that maintaining high thermal tolerances is potentially costly under ambient conditions, further indicating a cost of adaptation to warming temperatures. Importantly, treatment temperatures were static. Therefore, copepods in the AM_{3GensOW} and >50 generations of warming (OW) treatments experienced no temperature variability in the generation prior to thermal tolerance assessment. This is distinct from the AM_{DevOW} that experienced a temperature change from 18 °C to 22 °C during early development. Differences in trait variability may be due to this difference in exposure to temperature

variability rather than costs of maintaining high thermal tolerance across generations. Future work in this system should vary the amplitude and predictability of treatment temperature (Bitter et al., 2021), to elucidate the influence of these factors on plasticity in thermal tolerance. Additionally, such experiments could test if variability that is lost beyond one generation at warming is due to constant temperature conditions in the lab, or if there are other costs to maintaining high thermal tolerances.

We also saw an intermediate thermal tolerance phenotype in OW_{DevAM} and $OW_{3GensAM}$. This loss of thermal tolerance under ambient conditions may indicate potential relaxed selection, where the removal of a selective force leads to trait loss (Lahti et al., 2009). Importantly, the developmental environment plays a critical role in defining thermal tolerance (Schaefer and Ryan 2006, Sasaki and Dam 2019, Ayirinhac et al., 2004, Hoffmann et al., 2005). Therefore, lower thermal tolerances may be due to acclimation to a lower developmental temperature. Despite this resultant loss of thermal tolerance, it is important to remember that thermal tolerance was gained within one generation at OW for the AM line animals. Therefore, even if extended periods of relaxed temperature selection occur, *A. tonsa* is likely capable of quickly regaining thermal tolerance during periods of warming.

Importantly, copepods in the Lower Lethal Salinity assay in the present study were assessed in the absence of food. Food was not used in this assay because we were focusing on relative differences in salinity tolerance between treatments. Copepods with higher resource availability perform better at suboptimal salinities (Rippingale and Hodgkin 1977 and Hammock 2016), although this is not a universal feature in copepods

(Van Someran Gréve et al., 2020). Therefore, it is possible that salinity tolerances may have been higher under food replete conditions.

Counter to our initial hypothesis, there was no effect of OW on hypo-salinity tolerance. Our results align well with empirical studies done in the intertidal copepod *Tigriopus californicus* that revealed selection for increased heat tolerance did not impact salinity tolerance (Kelly et al., 2016). In strong support of our results, Kelly et al., 2016 found that when the order of events was reversed, copepods from low salinity conditions had lower thermal tolerances. Kelly et al., 2016 hypothesized this may be due to competing energetic demands between osmoregulation and responding to increasing temperatures. These results together are particularly important, because *T. californicus* and *A. tonsa* are hardy species that experience regular salinity and temperature fluctuations. Our results indicate that even euryhaline species, like *A. tonsa*, are vulnerable to simultaneous salinity and temperature fluctuations. These conditions are environmentally relevant to southern populations of *A. tonsa*, as summer corresponds with the wet season in Southwest Florida estuaries. Therefore, copepods and other estuarine and nearshore animals are exposed to periods of extreme salinity fluctuations and warm temperatures at the same time (Heilmayer et al., 2008, Tolley et al., 2005). With climate change, we expect more precipitation extremes and marine heat waves (Singh et al., 2013, Frolicher et al., 2018, Oliver et al. 2018). Such conditions could have negative implications for *A. tonsa* and other copepods that are less tolerant of temperature and salinity fluctuations.

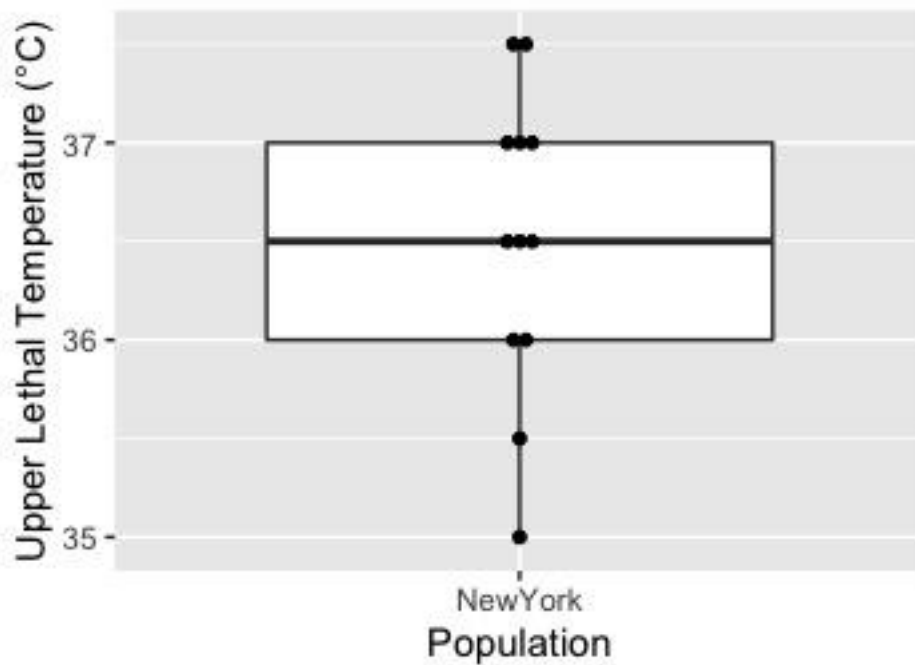
Despite being collected within 50 km of each other, AM copepods and copepods more recently collected from Hammonasset Beach (HB) had distinct mean thermal tolerances and variation in thermal tolerance. Differences in mean thermal tolerance among populations could be due to environmental differences between locations. Population level differences may also be due to different collection months, as *A. tonsa* has seasonal genetic differences in thermal tolerance (Sasaki and Dam, 2020). Additionally, *A. tonsa* is a cryptic species complex, with deeply diverging lineages (Figueroa et al., 2020). Therefore, these two populations may represent two distinct clades. Differences in variance among populations may indicate that extended culture in the lab impacts trait variability. The AM population spent ~50 more generations in the lab than the HB population. Therefore, reduced variability in the AM population compared to the HB population could be due to a longer history of static treatment temperatures in the AM animals. Further, AM animals maintained at the University of Connecticut Avery Point are shown to adapt to laboratory conditions (Sasaki and Dam 2021; Brennan et al., 2022b). Consequently, differences in trait variability may be due to lab adaptation reducing genetic variability in the AM line compared to the HB population.

We demonstrated the contribution of plasticity to the copepod climate response, indicating the importance of the developmental environment in determining copepod thermal tolerance. Additionally, we found that environmental salinity reductions of 15ppt resulted in decreased Upper Lethal Temperatures by 0.6°C in AM animals. These salinity reductions are well within the range already experienced by *A. tonsa* in the Long Island Sound. Therefore, our results indicate that *A. tonsa* in this region may be vulnerable to

continually increasing variation in temperature and salinity. Revealing the relationship between temperature and salinity tolerance in this species suggests that other less tolerant species may experience consequences of these two shifting variables as well. This motivates further exploration of the influence of temperature and salinity on survival and fitness in additional marine ectotherms.

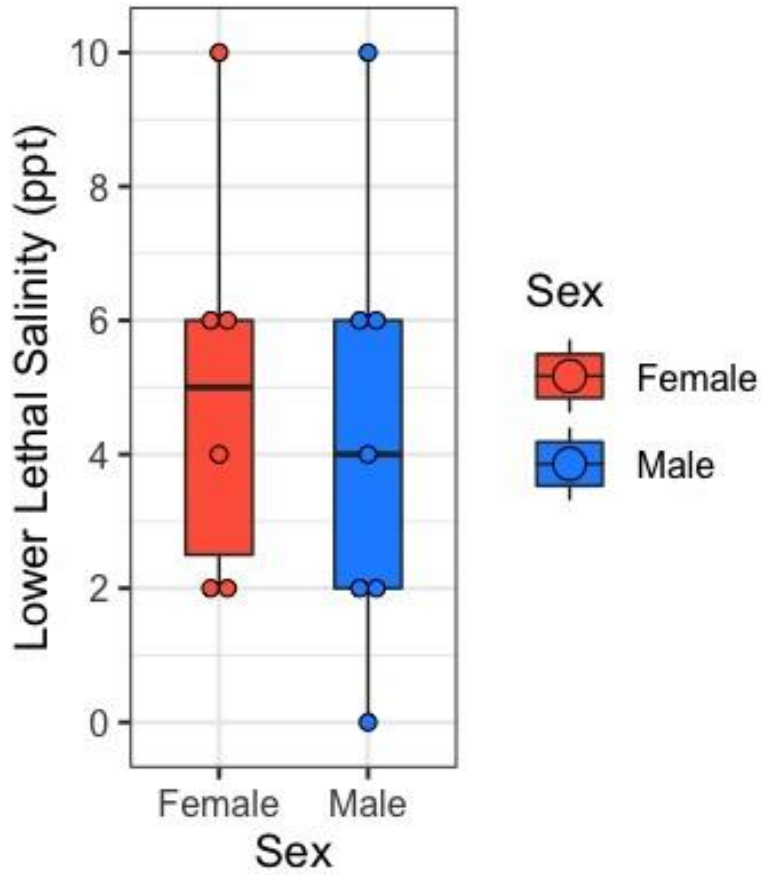
Supplemental Information

Supplemental Figure 1.



Supplemental Figure 1. Upper Lethal Temperature (ULT) for adult animals collected from Brookhaven, New York (n=20, N 40.95 °, W -73.03 °) after two generations in the lab at 18°C.

Supplemental Figure 2.



Supplemental Figure 2. Preliminary assessment of Lower Lethal Salinity (LLS) for animals from AM_{DevOW}.

Figures and Tables

Figure legends

Figure 1. Schematic of experimental design. Blue lines represent AM temperature conditions (18°C), red lines represent OW conditions (22°C). Plasticity vs. Adaptation bracket indicate the treatment groups necessary to compare the relative roles of plasticity and adaptation in the copepod climate response. The Relaxed selection bracket indicates the treatment groups necessary to identify a potential loss in thermal tolerance.

Figure 2. Upper Lethal Temperature (ULT) for ambient line animals moved into ocean warming conditions for one (AM_{DevOW}), three ($AM_{3GensOW}$), and >50 generations (OW). Dots represent individual copepods, each in their own well. Whiskers represent standard error, and bold horizontal bar represents the mean. Dots outside the box are outliers. Color denotes treatment temperature.

Figure 3. Upper Lethal Temperature for OW line animals moved into AM conditions. Dots represent individual copepods, each in their own well. Whiskers represent standard error. Bold horizontal line represents the mean. Dots are outliers. Color denotes treatment temperature. Note that >50Gens and Ambient groups are the same data represented in Fig. 2, presented separately for clarity of hypothesis testing.

Figure 4. Lower Lethal Salinity (LLS) after one (AM_{DevOW}) and >50-generations at warming (OW). Dots represent individual copepods, each in their own well. Whiskers represent standard error, and bold horizontal line represents the mean. Dots are outliers. Color denotes treatment temperature.

Figure 5. Upper Lethal Temperature after sequential lowering of salinity for AM animals (Esker Point) and a fresh collection from Long Island Sound (Hammonasset Beach). Dots represent individual copepods, each in their own well. Whiskers depict standard error. Bold horizontal line represents the mean. Dots are outliers. Colors denote population.

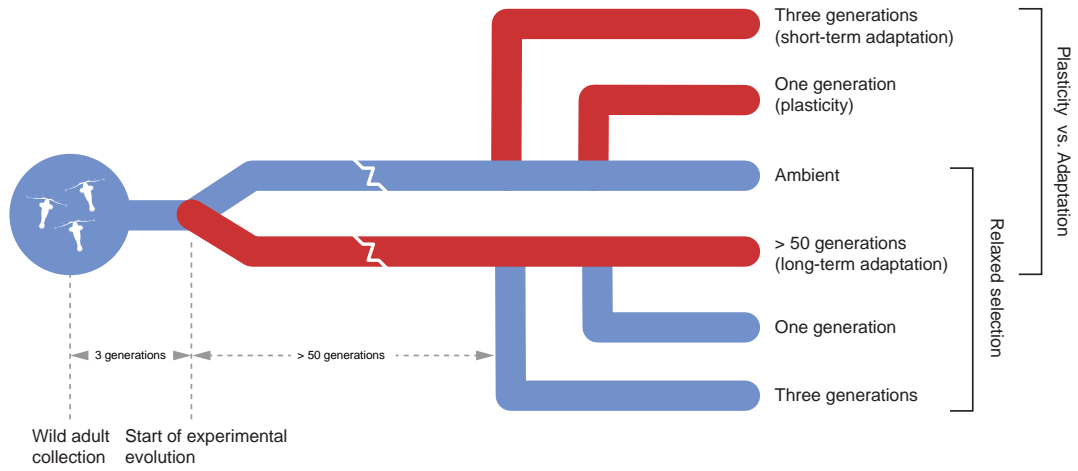


Figure 1

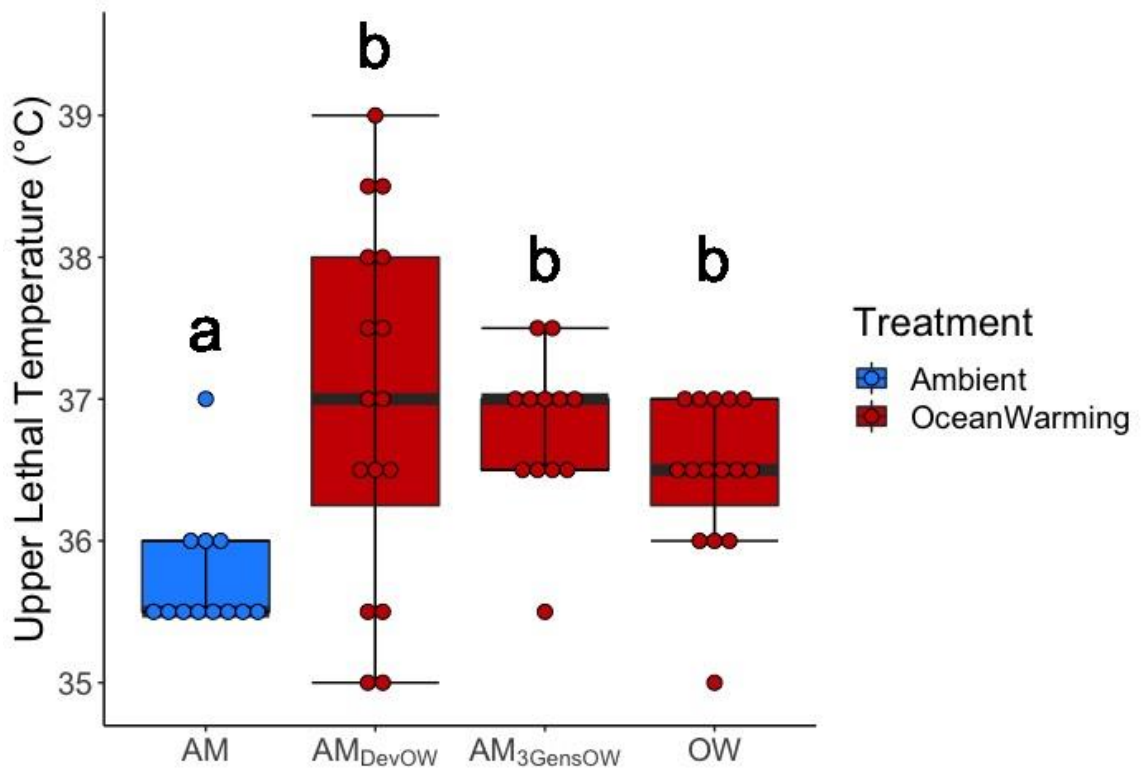


Figure 2

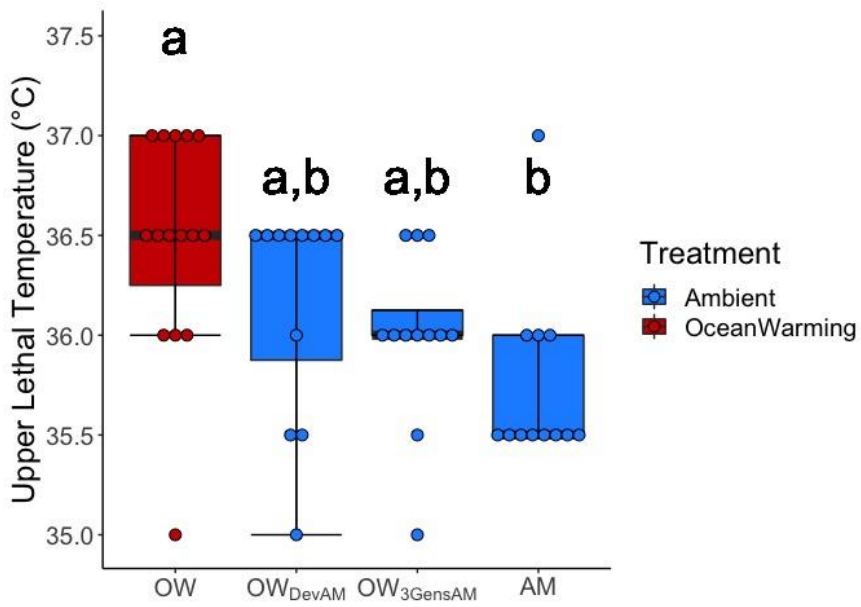


Figure 3

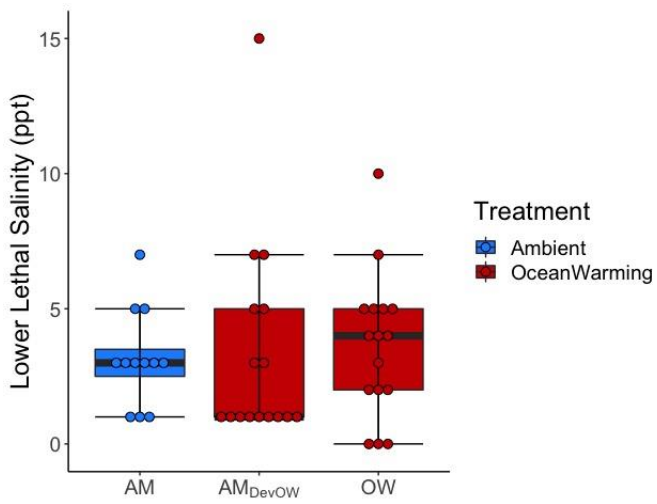


Figure 4

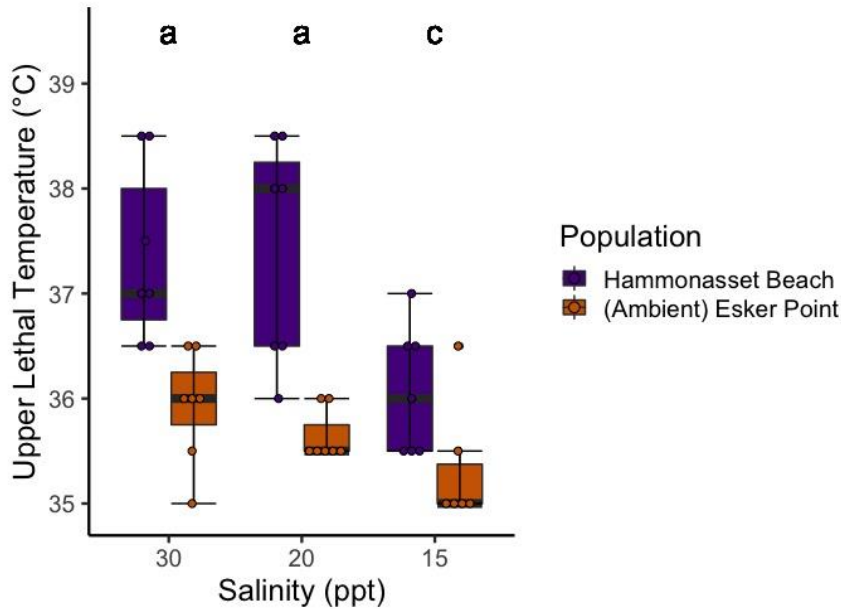


Figure 5

Literature Cited

- Ayrinhac, A., Debat, V., Gibert, P., Kister, A. G., Legout, H., Moreteau, B., ... & David, J. R. (2004). Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology*, 700-706.
- Badyaev, A. V. (2005). Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proceedings of the Royal Society B: Biological Sciences*, 272: 877-886.
- Bennett, J. M., Sunday, J., Calosi, P., Villalobos, F., Martínez, B., Molina-Venegas, R., ... & Olalla-Tárraga, M. Á. (2021). The evolution of critical thermal limits of life on Earth. *Nature communications*, 12, 1-9.
- Bitter, M. C., Wong, J. M., Dam, H. G., Donelan, S. C., Kenkel, C. D., Komoroske, L. M., ... & Lotterhos, K. E. (2021). Fluctuating selection and global change: a synthesis and review on disentangling the roles of climate amplitude, predictability and novelty. *Proceedings of the Royal Society B*, 288: 20210727.
- Brennan, R. S., DeMayo, J. A., Dam, H. G., Finiguerra, M. B., Baumann, H., & Pespeni, M. H. (2022a). Loss of transcriptional plasticity but sustained adaptive capacity after

- adaptation to global change conditions in a marine copepod. *Nature communications*, 13: 1-13.
- Brennan, R. S., deMayo, J. A., Dam, H. G., Finiguerra, M., Baumann, H., Buffalo, V., & Pespeni, M. H. (2022b). Experimental evolution reveals the synergistic genomic mechanisms of adaptation to ocean warming and acidification in a marine copepod. *Proceedings of the National Academy of Sciences*, 119: e2201521119.
- Calliari, D., Borg, M. C. A., Thor, P., Gorokhova, E., & Tiselius, P. (2008). Instantaneous salinity reductions affect the survival and feeding rates of the co-occurring copepods *Acartia tonsa* Dana and *A. clausi* Giesbrecht differently. *Journal of Experimental Marine Biology and Ecology*, 362: 18-25.
- Dam, H. G. (2013). Evolutionary adaptation of marine zooplankton to global change. *Annual Review of Marine Science*, 5: 349-370.
- Dam, H. G., deMayo, J. A., Park, G., Norton, L., He, X., Finiguerra, M. B., ... & Pespeni, M. H. (2021). Rapid, but limited, zooplankton adaptation to simultaneous warming and acidification. *Nature Climate Change*, 11: 780-786.
- deMayo, J. A., Girod, A., Sasaki, M. C., & Dam, H. G. (2021). Adaptation to simultaneous warming and acidification carries a thermal tolerance cost in a marine copepod. *Biology Letters*, 17: 20210071.
- Elkin, L. A., Kay, M., Higgins, J. J., & Wobbrock, J. O. (2021). An aligned rank transform procedure for multifactor contrast tests. In *The 34th Annual ACM Symposium on User Interface Software and Technology*. 754-768.
- Ellegren, H., & Galtier, N. (2016). Determinants of genetic diversity. *Nature Reviews Genetics*, 17: 422-433.
- Figuerola, N. J., Figuerola, D. F., & Hicks, D. (2020). Phylogeography of *Acartia tonsa* Dana, 1849 (Calanoida: Copepoda) and phylogenetic reconstruction of the genus *Acartia* Dana, 1846. *Marine Biodiversity*, 50: 1-20.
- Fordyce, J. A. (2006). The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *Journal of Experimental Biology*, 209: 2377-2383.
- Frölicher, T. L., Fischer, E. M., & Gruber, N. (2018). Marine heatwaves under global warming. *Nature*, 560: 360-364.
- Garzke, J., Ismar, S. M., & Sommer, U. (2015). Climate change affects low trophic level marine consumers: warming decreases copepod size and abundance. *Oecologia*, 177: 849-860.

- González, J. G. (1974). Critical thermal maxima and upper lethal temperatures for the calanoid copepods *Acartia tonsa* and *A. clausi*. *Marine Biology*, 27: 219-223.
- Hammock, B. G., Lesmeister, S., Flores, I., Bradburd, G. S., Hammock, F. H., & Teh, S. J. (2016). Low food availability narrows the tolerance of the copepod *Eurytemora affinis* to salinity, but not to temperature. *Estuaries and Coasts*, 39: 189-200.
- Harley, C. D., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J., Thornber, C. S., ... & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology letters*, 9: 228-241.
- Harris, P., Muelbert, J., Muniz, P., Yin, K., Ahmed, K., Folorunsho, R., ... & Rice, J. (2016). Estuaries and Deltas. In *United Nations World Ocean Assessment*. Cambridge: Cambridge University Press.
- Hartl, D. L. (2020). *A primer of population genetics and genomics*. Oxford University Press.
- 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: 179-185.
- Hendry, A. P. (2016). *Eco-evolutionary dynamics*. Princeton university press.
- Hoffmann, A. A., Shirriffs, J., & Scott, M. (2005). Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Functional Ecology*, 222-227.
- Johnson, W. S., & Allen, D. M. (2012). *Zooplankton of the Atlantic and Gulf coasts: a guide to their identification and ecology*. JHU Press.
- Jump, A. S., & Penuelas, J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology letters*, 8: 1010-1020.
- Kelly, M. W., DeBiase, M. B., Villela, V. A., Roberts, H. L., & Cecola, C. F. (2016). Adaptation to climate change: Trade-offs among responses to multiple stressors in an intertidal crustacean. *Evolutionary Applications*, 9: 1147-1155.
- Kelly, M. W., Pankey, M. S., DeBiase, M. B., & Plachetzki, D. C. (2017). Adaptation to heat stress reduces phenotypic and transcriptional plasticity in a marine copepod. *Functional Ecology*, 31: 398-406.
- Lahti, D. C., Johnson, N. A., Ajie, B. C., Otto, S. P., Hendry, A. P., Blumstein, D. T., ... & Foster, S. A. (2009). Relaxed selection in the wild. *Trends in ecology & evolution*, 24: 487-496.

- Moyle, P. B., Lund, J. R., Bennett, W. A., & Fleenor, W. E. (2010). Habitat variability and complexity in the upper San Francisco Estuary. *San Francisco Estuary and Watershed Science*, 8:3.
- Najjar, R. G., Walker, H. A., Anderson, P. J., Barron, E. J., Bord, R. J., Gibson, J. R., ... & Swanson, R. S. (2000). The potential impacts of climate change on the mid-Atlantic coastal region. *Climate Research*, 14: 219-233.
- Oliver, E. C., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., ... & Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature communications*, 9(1), 1-12.
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rahlff, J., Peters, J., Moyano, M., Pless, O., Claussen, C., & Peck, M. A. (2017). Short-term molecular and physiological responses to heat stress in neritic copepods *Acartia tonsa* and *Eurytemora affinis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 203: 348-358.
- Rice, E., Dam, H. G., & Stewart, G. (2015). Impact of climate change on estuarine zooplankton: surface water warming in Long Island Sound is associated with changes in copepod size and community structure. *Estuaries and coasts*, 38: 13-23.
- Rice, E., & Stewart, G. (2016). Decadal changes in zooplankton abundance and phenology of Long Island Sound reflect interacting changes in temperature and community composition. *Marine environmental research*, 120: 154-165.
- Richardson, A. J. (2008). In hot water: zooplankton and climate change. *ICES Journal of Marine Science*, 65: 279-295.
- Rippingale, R. J., & Hodgkin, E. P. (1977). Food availability and salinity tolerance in a brackish water copepod. *Marine and Freshwater Research*, 28: 1-7.
- Salinas, S., Irvine, S. E., Schertzing, C. L., Golden, S. Q., & Munch, S. B. (2019). Trait variation in extreme thermal environments under constant and fluctuating temperatures. *Philosophical Transactions of the Royal Society B*, 374: 20180177.
- Sasaki, M. C., & Dam, H. G. (2019). Integrating patterns of thermal tolerance and phenotypic plasticity with population genetics to improve understanding of vulnerability to warming in a widespread copepod. *Global change biology*, 25: 4147-4164.

- Sasaki, M. C., & Dam, H. G. (2020). Genetic differentiation underlies seasonal variation in thermal tolerance, body size, and plasticity in a short-lived copepod. *Ecology and evolution*, 10: 12200-12210.
- Sasaki, M. C., & Dam, H. G. (2021). Negative relationship between thermal tolerance and plasticity in tolerance emerges during experimental evolution in a widespread marine invertebrate. *Evolutionary applications*, 14: 2114-2123.
- Schaefer, J., & Ryan, A. (2006). Developmental plasticity in the thermal tolerance of zebrafish *Danio rerio*. *Journal of fish biology*, 69: 722-734.
- Scheffers, B. R., De Meester, L., Bridge, T. C., Hoffmann, A. A., Pandolfi, J. M., Corlett, R. T., ... & Watson, J. E. (2016). The broad footprint of climate change from genes to biomes to people. *Science*, 354: aaf7671.
- Sheridan, J. A., & Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nature climate change*, 1: 401-406.
- Singh, D., Tsiang, M., Rajaratnam, B., & Diffenbaugh, N. S. (2013). Precipitation extremes over the continental United States in a transient, high-resolution, ensemble climate model experiment. *Journal of Geophysical Research: Atmospheres*, 118: 7063-7086.
- Somero, G.N., Lockwood, B.L., and Tomanek L. (2017). Biochemical adaptation: response to environmental challenges, from life's origins to the Anthropocene. Sinauer Associates, Incorporated Publishers
- Steinberg, D. K., & Landry, M. R. (2017). Zooplankton and the ocean carbon cycle. *Annual review of marine science*, 9: 413-444.
- Tolley, S. G., Volety, A. K., & Savarese, M. (2005). Influence of salinity on the habitat use of oyster reefs in three southwest Florida estuaries. *Journal of Shellfish Research*, 24: 127-137.
- Van Someren Gréve, H., Jepsen, P. M., & Hansen, B. W. (2020). Does resource availability influence the vital rates of the tropical copepod *Apocyclops royi* (Lindberg, 1940) under changing salinities?. *Journal of Plankton Research*, 42: 467-478.
- West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.
- Whitman, D. W., & Ananthkrishnan, T. N. (2009). *Phenotypic plasticity of insects: mechanisms and consequences*. Science Publishers, Inc..
- Wobbrock, J. O., Findlater, L., Gergle, D., & Higgins, J. J. (2011). The aligned rank transform for nonparametric factorial analyses using only anova procedures.

In *Proceedings of the SIGCHI conference on human factors in computing systems*, 143-146.

Yampolsky, L. Y., Schaer, T. M., & Ebert, D. (2014). Adaptive phenotypic plasticity and local adaptation for temperature tolerance in freshwater zooplankton. *Proceedings of the Royal Society B: Biological Sciences*, 281: 20132744.

**CHAPTER III: LATITUDINAL AND LIFE STAGE DIFFERENCES IN HIGH TEMPERATURE AND
LOW SALINITY TOLERANCE IN AN ESTUARINE COPEPOD**

Abstract

Increases in mean temperature and temperature variability are manifestations of current climate change. Climate change is also characterized by changes in precipitation amount and variability, which impact salinity. In order to understand future ecosystem health, it is imperative that we examine tolerances of marine organisms to these changing conditions. The cosmopolitan estuarine copepod *Acartia tonsa* is an emerging model for understanding population persistence to changing temperature and salinity conditions. Importantly, *A. tonsa* have complex life histories and are broadly distributed, meaning the response to environmental change may be different across developmental stages and geographic space. Here, we aim to characterize the temperature and salinity tolerance of *A. tonsa* across life history stages and latitudes of collection. Results from this study will reveal the temperature and salinity tolerance of *A. tonsa*, and indicate potential patterns of stage and latitude specific tolerances. We found that temperature and salinity tolerance differ across life history stages, with the juvenile stage being the most thermally tolerant and least salinity tolerant, suggesting a potential trade-off between temperature and salinity tolerance. Interestingly, copepod larvae and adults had equivalent tolerances to temperature and salinity, contrasting with studies across a broad range of taxa that have found larval stages to be more sensitive to climate stressors than adults. Additionally, temperature and salinity tolerance differed with collection location. Temperature tolerance followed the expected relationship with latitude, Maine populations were the

least temperature tolerant and Florida populations were the most temperature tolerant. Interestingly, we found that Florida copepods were more tolerant of hyposalinity than Maine and Connecticut copepods. Taken together these results suggest that life history stage and latitude play important roles in determining the temperature and salinity tolerance of the copepod *A. tonsa*, and these factors should be considered in estimations of population dynamics and persistence with warming and increasing salinity variability.

Keywords: copepod, ontogeny, thermal tolerance, salinity tolerance

Introduction

As climate change persists, marine organisms are faced with increasing mean temperatures and an increase in the prevalence of marine heat waves (Frölicher *et al.*, 2018, Oliver *et al.*, 2018). Consequences of this warming include seasonal changes to plankton food web dynamics, mass mortality, and increasing prevalence of disease (Gibson *et al.*, 2011, Batten *et al.*, 2022, Oliver *et al.*, 2018). Extreme heat events are expected to be more frequent with time (Pandori and Sorte 2021, Oliver *et al.*, 2018, Frölicher *et al.*, 2018), which may result in further changes to marine community structure and ecosystem function. In addition to warming, the frequency of extreme precipitation events is increasing globally (Janssen *et al.*, 2014, Lehmann *et al.*, 2015). Precipitation changes are driven in part by anthropogenic climate change, are expected to intensify with continued warming (Lehmann *et al.*, 2015, Singh *et al.* 2013, Zhang *et al.*, 2007) and lead to rapidly shifting salinity conditions in marine and estuarine habitats.

Understanding the influence of increasing temperature and fluctuating salinity on marine organisms will provide critical knowledge about their persistence in a warming ocean.

Exposure to fluctuating temperature and salinity may impact organisms differently across their life history stages. Many marine organisms have complex life histories, with differences in physiology and ecology across each stage. Life history stages of marine ectotherms also often occupy different habitats, resulting in differential exposure to climatic stress across ontogeny (Makino and Ban 2000). Consequently, many marine organisms vary in stress tolerance throughout their life cycle (Pandori and Sorte, 2019, Tangwancharoen and Burton, 2014, Miller et al., 2013). A meta-analysis of 287 experiments, across Annelida, Arthropoda, Chordata, Cnidaria, Echinodermata, and Mollusca, revealed that earlier life stages were more negatively impacted by warming than older life stages (Pandori and Sorte 2019). However, there are examples in copepods, porcelain crabs, and kelp crabs where earlier life stages exhibit stronger tolerances than their older counterparts (Tangwancharoen and Burton, 2014, Miller et al., 2013, Storch et al., 2011). The most vulnerable stage will determine the vulnerability of the species to climate change. Therefore, it is critical to understand the stage-specific tolerance of marine organisms.

Copepods are a convenient system for examining the differences in stress tolerance across life stages. Copepods have complex life cycles with larvae, juveniles, and adults differing in habitat, swimming ability, feeding strategy, morphology, and physiology (Fulton, 1984, Meunier et al., 2016, Bradley et al., 2013, Mathews et al., 2018, Peterson 2001). Additionally, copepods play a critical role in food webs as a major connector of primary producers and the microbial food web to higher trophic levels (Calbet and Saiz

2005, Aberle et al., 2015, Dam and Baumann 2017, Liu et al., 2017). Many larval fish rely on copepods as a critical food source, tying copepods to fisheries health (Steinberg et al., 2017, Richardson et al., 2008). Copepods also make for excellent sentinels of change, as they are short-lived ectotherms that are highly responsive to environmental conditions (Liu et al., 2017, Richardson et al., 2008). *Acartia tonsa* is a coastal estuarine copepod that has 12 life history stages from egg to adult (Mauchline, 1998). *A. tonsa* is dominant in estuaries off the east coast of the United States, and is also found in many parts of the world (Gonzalez 1974, Johnson and Allen 2012). As an estuarine species, *A. tonsa* experiences temperature and salinity variability and extremes at a greater frequency than open ocean copepods. Therefore, *A. tonsa* is a tractable model for understanding the differences in temperature and salinity tolerance across ontogeny.

Here we examined the Upper Lethal Temperature (ULT) of the copepod *A. tonsa*, across larval, juvenile, and adult life stages for common garden reared populations originating from Maine, New York, and the gulf coast of Florida. Additionally, we investigated the attributes of environmental Sea Surface Temperature (SST) across different timescales most important for determining ULT. Further, we examined the Lower Lethal Salinity (LLS) of *A. tonsa* across stages for each population of origin. Drawing on previous results in the tidepool copepod, *Tigriopus californicus*, that show larvae and juveniles to be more temperature tolerant than adults, we predicted that larval and juvenile *A. tonsa* would be more tolerant of temperature and salinity stress than adults (Tangwancharoen and Burton, 2014). Additionally, we predicted that location-specific thermal tolerance would vary with source population with copepods from Maine populations less thermally tolerant than New York and Florida.

Materials & Methods

Collections and Laboratory Culture

We collected copepods from Hurricane Island, Maine (n = 222, Lat/Long = N 44.02°, W -68.54 °); Esker Point, Connecticut (n=1000, Lat/Long = N 41.32 °, W -72.00 °); Brookhaven, New York (n=20, Lat/Long = N 40.95 °, W -73.03 °); and Punta Gorda, Florida (n>1000, Lat/Long = N 26.48 °, W -82.01 °). Connecticut copepods were collected in June 2016, while Maine, New York, and Florida copepods were collected between July and September 2018. These collections took place across months where *A. tonsa* are present in the water column across all collection locations (Sasaki and Dam, 2019). Additionally, populations were all collected in summer months, minimizing seasonally dependent genetic differences in thermal tolerance (Sasaki and Dam, 2020)

Copepods were collected using a 200 µm mesh plankton net at 1-2 m depth. Adult *Acartia tonsa* were sorted from each plankton tow using a stereo microscope and maintained in a climate-controlled laboratory at 18°C and 30 ppt salinity using Instant Ocean® mixed with reverse-osmosis filtered water. *A. tonsa* were cultured using a 12:12 light:dark cycle and fed ad libitum on a mixed diet of the diatom *Thalassiosira weissflogii*, and the flagellates *Tetraselmis sp.* and *Rhodomonas lens*. Copepods from all locations were held under common temperature and salinity conditions (18°C and 30 ppt) for at least two full generations before assessing Upper Lethal Temperature (ULT).

Upper Lethal Temperature

We performed ULT assays on early nauplii (NII/NIII), early copepodites (CII/CIII), and adults (CVI). One day prior to the assay, 12-well plates were filled with 2 mL sea water at 30 ppt, with a mix of *T. weissflogii*, *Tetraselmis sp.*, and *R. lens* totaling 500 μg C/L (Feinberg and Dam 1998), and copepods were assigned to wells randomly, with one copepod in each well. Due to cultures being staggered with regard to life history stage and constraints on the number of plates that could be processed at a given time, the assessment of animals of different stages and different populations took place on different days. To ensure that environmental temperature and ramp speed were the same across days, we randomly placed an iButton temperature data logger (<https://www.maximintegrated.com/en/products/ibutton-one-wire/ibutton.html>, DS 1921G) in two wells, which recorded temperature every two minutes.

We assessed twelve copepods for each population at each life history stage. Each copepod was checked for survival prior to running the assay. We then added parafilm-sealed plates to a ThermoScientific PC200 water bath, where the temperature ramped from 20 to 30°C at a rate of 1°C every 15 minutes. Temperature ramp for copepodites from Maine and New York was 0.012°C/minute on average \pm 0.001°C/minute. All subsequent assessment of ULT was held at a consistent ramp of 0.012°C/minute. Individuals were checked for survival using a stereo microscope every 0.5°C until all individuals were scored as dead. Individuals were scored as dead if no movement was observed within 10 seconds following water disturbance in the well.

Environmental Temperature Data

To determine the influence of Sea Surface Temperature (SST) on ULT, we analyzed data from buoys near each of our four collection sites (Bar Harbor, ME Station ATGM1-8413320, New London, CT Station NLNC3-8461490, Fort Meyers, FL Station FMRF1-8725520). We examined the SST min, mean, max, and range for three different time frames: the decade prior to collection, the summer of collection, and 30 days prior to collection (Figure 1). For the decade prior and the summer of collection, we used data from June-September when copepods from all collection locations are present in the water column (Sasaki and Dam 2019). To determine which time frame and metric had the strongest relationship with ULT, we ran individual regressions for each SST metric. We then compared r^2 and AIC values across models to identify the strongest determinant of ULT.

Lower Lethal Salinity

Lower Lethal Salinity (LLS) assays were conducted on the Maine, Connecticut, and Florida populations. Copepods were held under the same temperature and salinity conditions for at least two full generations before assessing LLS. Stock solutions of 20, 15, 10, 8, 6, 4, 2, and 0 ppt were made by combining aerated tap water with Instant Ocean salt. Twelve copepods from each stage and population were picked from their cultures at 30 ppt and 18°C and moved into 12-well plates with water at the same conditions. Each well contained one copepod and 2 mL of water. Salinity was reduced stepwise every 30 minutes until each copepod died. Copepods were determined to be dead by disturbing well water and waiting 10 seconds without movement.

Data Analysis

The normality of the data was assessed using the Shapiro-Wilk test and homogeneity of variances were assessed with the Levene test. Results indicated that the Upper Lethal Temperature data were not normally distributed ($W = 0.96$, $p = 0.01$), but that there were equal variances across samples ($F = 0.87$, $p = 0.55$). Results for Lower Lethal Salinity indicated that the data were not normally distributed ($W = 0.87$, $p < 0.001$) and that variance was not homogeneous ($F = 2.25$, $p = 0.03$). Therefore, we compared Upper Lethal Temperature and Lower Lethal Salinity across life history stages and locations using the non-parametric Aligned Rank Transform (ART) ANOVA (Wobbrock et al., 2011). The ART ANOVA, and multifactor contrast tests, were performed using the R package ARTool (Wobbrock et al., 2011, Elkin et al., 2021).

Results

Both population ($F = 36.89$, $p < 0.0001$, $\eta_p^2 = 0.44$) and life history stage ($F = 26.20$, $p < 0.0001$, $\eta_p^2 = 0.36$) affected ULT (Figure 2). However, there was no population by stage interaction effect ($F = 1.7$, $p = 0.16$, $\eta_p^2 = 0.07$). Maine copepods were less thermally tolerant than New York copepods ($p < 0.0001$) and Florida copepods ($p < 0.0001$). Additionally, New York copepods were less thermally tolerant than Florida copepods ($p = 0.02$). When considering life history stage, copepodites were more thermally tolerant than adults ($p < 0.0001$) and nauplii ($p < 0.0001$), while adults and nauplii did not differ in ULT ($p = 0.17$). The mean, minimum, maximum, and range of temperatures measured across the decade prior to collection, the summer of collection, and 30 days prior to collection were highly correlated with each other (Figure 3). When modeling each

temperature variable separately, we found that the maximum temperature the summer of collection was the strongest predictor of ULT ($R^2 = 0.23$, $AIC = 410.91$) (Figure 4, Table 1).

Similarly, for low salinity tolerance, both population ($F = 4.03$, $p = 0.02$, $\eta_p^2 = 0.08$) and stage ($F = 17.37$, $p < 0.0001$, $\eta_p^2 = 0.27$) affected LLS (Figure 5). Florida copepods showed lower LLS than copepods from Connecticut ($p = 0.05$) and Maine ($p = 0.03$), while copepods from Maine and Connecticut showed no difference in LLS ($p = 0.98$). In terms of life history stage, copepodites were less tolerant of hyposalinity than adults ($p < 0.0001$) and nauplii ($p < 0.0001$). However, there was no difference in salinity tolerance between adults and nauplii ($p = 1.00$). In contrast to thermal tolerance, the effect of population on salinity tolerance depended on stage ($F = 7.56$, $p < 0.0001$, $\eta_p^2 = 0.24$), with Maine copepodites being less tolerant of low salinity than Florida copepodites ($p = 0.004$).

Discussion

We investigated the impact of life history stage and collection location on the Upper Lethal Temperature and Lower Lethal Salinity of the copepod *A. tonsa*. Results demonstrate a clear effect of life history stage on temperature and salinity tolerance. Juvenile copepods were more thermally tolerant than larval and adult copepods. Interestingly, the converse is true for salinity, with adults and larval stages being more salinity tolerant than juveniles. Additionally, predictions that location-specific thermal tolerance will be related to latitude were confirmed by our results. Our results indicate the

importance of considering life history stage and collection location when estimating the stress tolerance of marine ectotherms.

Together with previous studies, our results demonstrate that thermal tolerance across life history stages is specific to the species and method of assessment. We found that *A. tonsa* nauplii and adults have comparable ULTs. In contrast, *A. tonsa* nauplii and adults acclimated to the same treatment temperature (18°C) as our study have divergent tolerances, with nauplii less tolerant than adults (Sunar and Kir, 2021). Differences in results between these studies may be due to the faster ramp speed in Sunar and Kir, (2021), as differences in ramp speed can influence thermal tolerance metrics (Moyen et al., 2019, Allen et al., 2012, 2016). While we found that copepodites were the most thermally tolerant stage, Tangwancharoen and Burton 2014 observed that both nauplii and copepodites of *Tigriopus californicus* were more thermally tolerant than adults. Differences in stage-specific thermal tolerance may be due to ecological and/or physiological differences between species. *T. californicus* life history stages occupy the same tidepool habitat (Tangwancharoen and Burton 2014), while different life stages of *A. tonsa* can occupy different parts of the water column (Cuker and Watson, 2002, Fulton, 1984, Holliland et al., 2012). This may impact how stage influences temperature tolerance. There were also important differences in our methodology. In the present study, animals were gradually ramped over many hours to their acute temperature treatment, while in Tangwancharoen and Burton 2014, copepods were immediately immersed for one hour in their temperature treatments. It would be powerful for future studies to look at stage-specific thermal tolerance across multiple species of copepods

with consistent methodologies. This would provide important insight into the influence of ontogeny on temperature tolerance.

We found that copepodites were the least salinity tolerant stage. Stage-specific salinity tolerance is supported by previous studies demonstrating that crustaceans modulate their osmoregulation strategy and efficiency with ontogeny (Charmantier, 1998), and that copepods occupy waters with different salinities at different life history stages (Dur and Souissi 2018). We found that *Acartia tonsa* nauplii are equally tolerant of hyposalinity as adults, in contrast to salinity tolerance of the invasive shrimp, *Palaemon macrodactylus*, whose larvae tolerate a narrower range in salinity than adults (Vázquez et al., 2016). Our results also diverge from studies in *Cancer irroratus*, that found that the ability to osmoregulate and salinity tolerance were higher in adults than in earlier developmental stages (Charmantier and Charmantier-Daures 1991). Importantly, salinity tolerance in *C. irroratus* decreases in late larval stages and is lowest in post-larval megalopae (Charmantier and Charmantier-Daures 1991). This is consistent with our results demonstrating that salinity tolerance is lowest in early juvenile stages.

Additionally, our results align with research in *Acartia sp.*, finding copepodites to be less tolerant of dilution than adult females (Lance, 1964). Within the *Acartia tonsa* species complex, the F and S clades are delineated by environmental salinity (Chen and Hare, 2008, 2011, Plough et al., 2018). Additionally, salinity is important in defining plankton distribution more broadly (Dur and Suissi 2018). This demonstrates the importance of salinity in shaping biodiversity and species distributions and the need for more research on the stage-specific salinity tolerances of crustaceans.

Our results indicate a potential trade-off between temperature and salinity tolerance across all life history stages. In the tidepool copepod, *Tigriopus californicus*, selection studies demonstrated the absence of a genetic trade-off between high temperature and low salinity tolerance (Kelly et al., 2016). However, exposing *T. californicus* to simultaneous high temperatures and low salinities revealed a clear physiological trade-off, potentially due to competing energetic demands (Kelly et al., 2016). In previous studies, the same individual copepods experienced changes in temperature and salinity. However, in this study, temperature and salinity tolerance were measured in different individuals. Therefore, the trade-off we see is not due to competing energetic demands. Explorations of the trade-off between temperature and salinity tolerance are rare, motivating future studies examining the basis of this trade-off.

Results from our study indicate that upper thermal tolerance decreases with increasing latitude, consistent with previous observations of thermal performance in copepods and marine ectotherms more broadly (Tangwancharoen and Burton, 2014, Sunday et al., 2011, Sasaki and Dam 2019). By contrast, hyposalinity tolerance results show a non-continuous difference between source latitudes, with only the Florida population having a higher hyposalinity tolerance than Maine and Connecticut. *Tigriopus californicus* and *Acartia tonsa* are both found along salinity gradients with higher salinities in the south (Schoch et al., 2006, Gordon and Giulivi 2008). Conversely studies in the tidepool copepod *T. californicus* demonstrate an inverse relationship between latitude and hyposalinity tolerance, with northern populations more tolerant to hyposalinity conditions than southern populations (Leong et al., 2018). Results from Debiasse et al., 2018 provided evidence to support this trend. It would be informative for

a future study to deploy salinity loggers in each collection location, potentially revealing if local salinity variability drives differences in the latitudinal patterns of salinity tolerance in *A. tonsa*. Importantly, location-specific temperature and salinity tolerance may not be indicative of locally adapted populations of one species. Rather, animals collected from different locations may be more distantly related sister species (Figuroa et al., 2020). Location-specific tolerances in this case are even more critical, as local extirpation could mean the loss of a species or subspecies, rather than the loss of an interconnected population (Figuroa et al., 2020). Understanding of *A. tonsa* temperature and salinity tolerance would improve with a comprehensive examination of clade-specific temperature and salinity tolerance.

We found that maximum summer temperature explained the most variation in ULT and had the model with the lowest AIC. However, our results demonstrate that environmental temperature metrics are highly correlated with each other and explain a relatively low amount of the variability in ULT (Figure 3, Figure 4). This suggests that environmental temperature plays a limited role in defining ULT. Additionally, we do not see large differences in r^2 and AIC among models (Figure 4, Table 1). This suggests that across different timescales and metrics SST explains a similar amount of variance in ULT.

Our results suggest a trade-off between temperature and salinity tolerance in *A. tonsa*, indicating this species may be potentially vulnerable in the face of multiple varying stressors. This is particularly relevant for this species, as they are predominantly present in estuarine habitats. Additionally, we find that our Florida population had the highest temperature tolerance and the highest hyposalinity tolerance, indicating this may be our

most tolerant population as temperature and salinity variability continue to increase with climate change.

Acknowledgements

Funding: This work was funded by the National Science Foundation grants to M.H.P. (OCE 1559075; IOS 1943316). Thank you to Hans Dam and Matt Sasaki for the collection of the Florida population. Thank you to Phoebe Jekielek and the Hurricane Island Center for Science and Leadership for their help with the collection of the Maine population. Thank you to Emily Shore and Chelsea Darwin for help with copepod culture.

References

- Aberle, N., Malzahn, A. M., Lewandowska, A. M., & Sommer, U. (2015). Some like it hot: the protozooplankton-copepod link in a warming ocean. *Marine Ecology Progress Series*, 519: 103-113.
- Allen, J. L., Clusella-Trullas, S., & Chown, S. L. (2012). The effects of acclimation and rates of temperature change on critical thermal limits in *Tenebrio molitor* (Tenebrionidae) and *Cyrtobagous salviniae* (Curculionidae). *Journal of insect physiology*, 58: 669-678.

- Allen, J. L., Chown, S. L., Janion-Scheepers, C., & Clusella-Trullas, S. (2016). Interactions between rates of temperature change and acclimation affect latitudinal patterns of warming tolerance. *Conservation physiology*, 4: 1.
- Batten, S. D., Ostle, C., Hélaouët, P., & Walne, A. W. (2022). Responses of Gulf of Alaska plankton communities to a marine heat wave. *Deep Sea Research Part II: Topical Studies in Oceanography*, 195: 105002.
- Bradley, C. J., Strickler, J. R., Buskey, E. J., & Lenz, P. H. (2013). Swimming and escape behavior in two species of calanoid copepods from nauplius to adult. *Journal of Plankton Research*, 35: 49-65.
- Calbet, A., & Saiz, E. (2005). The ciliate-copepod link in marine ecosystems. *Aquatic Microbial Ecology*, 38(2), 157-167.
- Charmantier, G. (1998). Ontogeny of osmoregulation in crustaceans: a review. *Invertebrate Reproduction & Development*, 33: 177-190.
- Charmantier, G., & Charmantier-Daures, M. (1991). Ontogeny of osmoregulation and salinity tolerance in *Cancer irroratus*; elements of comparison with *C. borealis* (Crustacea, Decapoda). *The Biological Bulletin*, 180(1), 125-134.
- Chen, G., & Hare, M. P. (2008). Cryptic ecological diversification of a planktonic estuarine copepod, *Acartia tonsa*. *Molecular ecology*, 17: 1451-1468.

- Chen, G., & Hare, M. P. (2011). Cryptic diversity and comparative phylogeography of the estuarine copepod *Acartia tonsa* on the US Atlantic coast. *Molecular Ecology*, 20: 2425-2441.
- Cuker, B. E., & Watson, M. A. (2002). Diel vertical migration of zooplankton in contrasting habitats of the Chesapeake Bay. *Estuaries*, 25: 296-307.
- Dam, H. G., & Baumann, H. (2017). Climate change, zooplankton and fisheries. *Climate change impacts on fisheries and aquaculture: A global analysis*, 2: 851-874.
- DeBiasse, M. B., Kawji, Y., & Kelly, M. W. (2018). Phenotypic and transcriptomic responses to salinity stress across genetically and geographically divergent *Tigriopus californicus* populations. *Molecular ecology*, 27: 1621-1632.
- Dur, G., & Souissi, S. (2018). Ontogenetic optimal temperature and salinity envelopes of the copepod *Eurytemora affinis* in the Seine estuary (France). *Estuarine, Coastal and Shelf Science*, 200: 311-323.
- Elkin, L. A., Kay, M., Higgins, J. J., & Wobbrock, J. O. (2021). An aligned rank transform procedure for multifactor contrast tests. In *The 34th Annual ACM Symposium on User Interface Software and Technology*, 754-768.
- Feinberg, L. R., & Dam, H. G. (1998). Effects of diet on dimensions, density and sinking rates of fecal pellets of the copepod *Acartia tonsa*. *Marine Ecology Progress Series*, 175, 87-96.

- Figuerola, N. J., Figuerola, D. F., & Hicks, D. (2020). Phylogeography of *Acartia tonsa* Dana, 1849 (Calanoida: Copepoda) and phylogenetic reconstruction of the genus *Acartia* Dana, 1846. *Marine Biodiversity*, 50: 1-20.
- Frölicher, T. L., Fischer, E. M., & Gruber, N. (2018). Marine heatwaves under global warming. *Nature*, 560: 360-364.
- Fulton, R. S. (1984). Distribution and community structure of estuarine copepods. *Estuaries*, 7: 38-50.
- Gamfeldt, L., Wallén, J., Jonsson, P. R., Berntsson, K. M., & Havenhand, J. N. (2005). Increasing intraspecific diversity enhances settling success in a marine invertebrate. *Ecology*, 86: 3219-3224.
- Gibson, R., Atkinson, R., Gordon, J., Smith, I., & Hughes, D. (2011). Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. *Oceanogr Mar Biol Annu Rev*, 49: 1-42.
- González, J. G. (1974). Critical thermal maxima and upper lethal temperatures for the calanoid copepods *Acartia tonsa* and *A. clausi*. *Marine Biology*, 27(3), 219-223.
- Gordon, A. L., & Giulivi, C. F. (2008). Sea surface salinity trends: over fifty years within the subtropical North Atlantic. *Oceanography*, 21, 20-29.
- Holliland, P. B., Ahlbeck, I., Westlund, E., & Hansson, S. (2012). Ontogenetic and seasonal changes in diel vertical migration amplitude of the calanoid copepods

- Eurytemora affinis* and *Acartia* spp. in a coastal area of the northern Baltic proper. *Journal of Plankton Research*, 34: 298-307.
- Hughes, A. R., & Stachowicz, J. J. (2004). Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences*, 101: 8998-9002.
- Janssen, E., Wuebbles, D. J., Kunkel, K. E., Olsen, S. C., & Goodman, A. (2014). Observational-and model-based trends and projections of extreme precipitation over the contiguous United States. *Earth's Future*, 2: 99-113.
- Johnson, W. S., & Allen, D. M. (2012). *Zooplankton of the Atlantic and Gulf coasts: a guide to their identification and ecology*. JHU Press.
- Kelly, M. W., DeBiasse, M. B., Villela, V. A., Roberts, H. L., & Cecola, C. F. (2016). Adaptation to climate change: Trade-offs among responses to multiple stressors in an intertidal crustacean. *Evolutionary Applications*, 9: 1147-1155.
- Lance, J. (1964). The salinity tolerances of some estuarine planktonic crustaceans. *The Biological Bulletin*, 127: 108-118.
- Lehmann, J., Coumou, D., & Frieler, K. (2015). Increased record-breaking precipitation events under global warming. *Climatic Change*, 132: 501-515.
- Leong, W., Sun, P. Y., & Edmands, S. (2018). Latitudinal clines in temperature and salinity tolerance in tidepool copepods. *Journal of Heredity*, 109: 71-77.

- Liu, H., Zhang, X., Yang, Q., Zuo, T., & Quigg, A. (2017). Mesozooplankton dynamics in relation to environmental factors and juvenile fish in a subtropical estuary of the Gulf of Mexico. *Journal of Coastal Research*, 33: 1038-1050.
- Makino, W., & Ban, S. (2000). Response of life history traits to food conditions in a cyclopoid copepod from an oligotrophic environment. *Limnology and oceanography*, 45: 396-407.
- Mathews, L., Faithfull, C. L., Lenz, P. H., & Nelson, C. E. (2018). The effects of food stoichiometry and temperature on copepods are mediated by ontogeny. *Oecologia*, 188: 75-84.
- Mauchline, J. (1998). *Adv. Mar. Biol. 33: The biology of calanoid copepods*.
- Meunier, C. L., Boersma, M., Wiltshire, K. H., & Malzahn, A. M. (2016). Zooplankton eat what they need: copepod selective feeding and potential consequences for marine systems. *Oikos*, 125: 50-58.
- Miller, N. A., Paganini, A. W., & Stillman, J. H. (2013). Differential thermal tolerance and energetic trajectories during ontogeny in porcelain crabs, genus *Petrolisthes*. *Journal of Thermal Biology*, 38: 79-85.
- Moyen, N. E., Somero, G. N., & Denny, M. W. (2019). Impact of heating rate on cardiac thermal tolerance in the California mussel, *Mytilus californianus*. *Journal of Experimental Biology*, 222: jeb203166.

- Oliver, E. C., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., ... & Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature communications*, 9: 1-12.
- Pandori, L. L., & Sorte, C. J. (2019). The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. *Oikos*, 128: 621-629.
- Pandori, L. L., & Sorte, C. J. (2021). Spatial and temporal scales of exposure and sensitivity drive mortality risk patterns across life stages. *Ecosphere*, 12: e03552.
- Peterson, W. T. (2001). Patterns in stage duration and development among marine and freshwater calanoid and cyclopoid copepods: a review of rules, physiological constraints, and evolutionary significance. *Hydrobiologia*, 453: 91-105.
- Plough, L. V., Fitzgerald, C., Plummer, A., & Pierson, J. J. (2018). Reproductive isolation and morphological divergence between cryptic lineages of the copepod *Acartia tonsa* in Chesapeake Bay. *Marine Ecology Progress Series*, 597, 99-113.
- Reusch, T. B., Ehlers, A., Hämmerli, A., & Worm, B. (2005). Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences*, 102: 2826-2831.
- Richardson, A. J. (2008). In hot water: zooplankton and climate change. *ICES Journal of Marine Science*, 65: 279-295.
- Sasaki, M. C., & Dam, H. G. (2019). Integrating patterns of thermal tolerance and phenotypic plasticity with population genetics to improve understanding of

- vulnerability to warming in a widespread copepod. *Global change biology*, 25: 4147-4164.
- Sasaki, M. C., & Dam, H. G. (2020). Genetic differentiation underlies seasonal variation in thermal tolerance, body size, and plasticity in a short-lived copepod. *Ecology and evolution*, 10: 12200-12210.
- Schoch, G. C., Menge, B. A., Allison, G., Kavanaugh, M., Thompson, S. A., & A. Wood, S. (2006). Fifteen degrees of separation: Latitudinal gradients of rocky intertidal biota along the California Current. *Limnology and Oceanography*, 51: 2564-2585.
- Singh, D., Tsiang, M., Rajaratnam, B., & Diffenbaugh, N. S. (2013). Precipitation extremes over the continental United States in a transient, high-resolution, ensemble climate model experiment. *Journal of Geophysical Research: Atmospheres*, 118: 7063-7086.
- Steinberg, D. K., & Landry, M. R. (2017). Zooplankton and the ocean carbon cycle. *Annual review of marine science*, 9: 413-444.
- Storch, D., Fernández, M., Navarrete, S. A., & Pörtner, H. O. (2011). Thermal tolerance of larval stages of the Chilean kelp crab *Taliepus dentatus*. *Marine Ecology Progress Series*, 429: 157-167.
- Sunar, M. C., & Kır, M. (2021). Thermal tolerance of *Acartia tonsa*: In relation to acclimation temperature and life stage. *Journal of Thermal Biology*, 102: 103116.

- Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences*, 278: 1823-1830.
- Tangwancharoen, S., & Burton, R. S. (2014). Early life stages are not always the most sensitive: heat stress responses in the copepod *Tigriopus californicus*. *Marine Ecology Progress Series*, 517: 75-83.
- Vázquez, M. G., Bas, C. C., & Spivak, E. D. (2016). Ontogeny of salinity tolerance in the invasive shrimp *Palaemon macrodactylus* (Caridea: Palaemonidae). *Journal of Crustacean Biology*, 36: 214-219.
- Wobbrock, J. O., Findlater, L., Gergle, D., & Higgins, J. J. (2011, May). The aligned rank transform for nonparametric factorial analyses using only anova procedures. In *Proceedings of the SIGCHI conference on human factors in computing systems*, 143-146.
- Zhang, X., Zwiers, F. W., Hegerl, G. C., Lambert, F. H., Gillett, N. P., Solomon, S., ... & Nozawa, T. (2007). Detection of human influence on twentieth-century precipitation trends. *Nature*, 448: 461-465.

Tables & Figures

Figure legends

Figure 1. Buoy recorded Sea Surface Temperature near each collection site. Subplots represent data from three different time frames: A) decade prior to collection, B) summer of collection, C) and 30 days prior to collection. Color denotes collection location.

Figure 2. Upper Lethal Temperatures for individual nauplii, copepodites, and adults from three study populations. Color is by population. Whiskers represent standard error, and bold horizontal bar represents the median.

Figure 3. Correlation matrix of each SST metric and experimental Upper Lethal Temperature.

Figure 4. Scatter plots of each SST metric regressed against Upper Lethal Temperature values for each population. Collection refers to metrics for 30 days prior to collection, Decade is for temperature metrics the decade prior to collection, and Summer is for temperature metrics the summer prior to collection. The blue line represents the regression.

Figure 5. Lower Lethal Salinity for individual nauplii, copepodites, and adults from three study populations. Grey scale is by population. Whiskers represent standard error, and bold horizontal bar represents the median.

Table 1. Comparison of mixed models. Each model name represents the temperature metric assessed as the independent variable for that model. All models included Population and Stage as random effects.

Buoy Sea Surface Temperature Data at Different Timescales

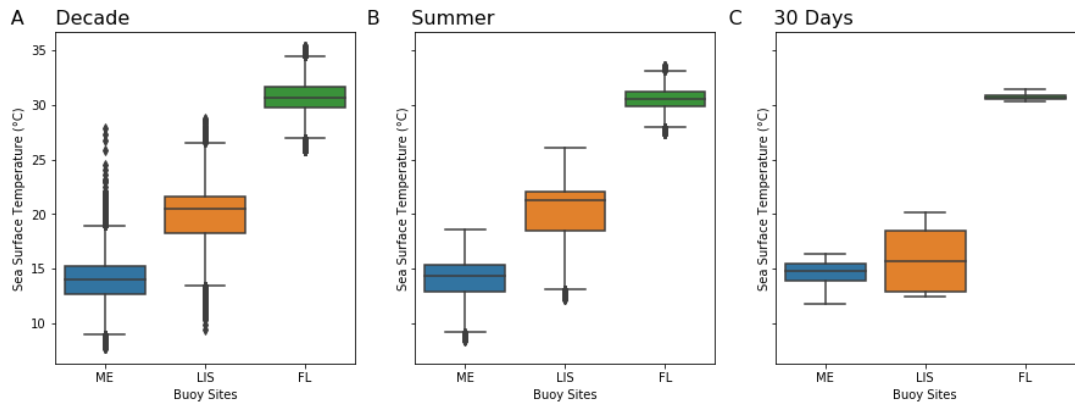


Figure 1.

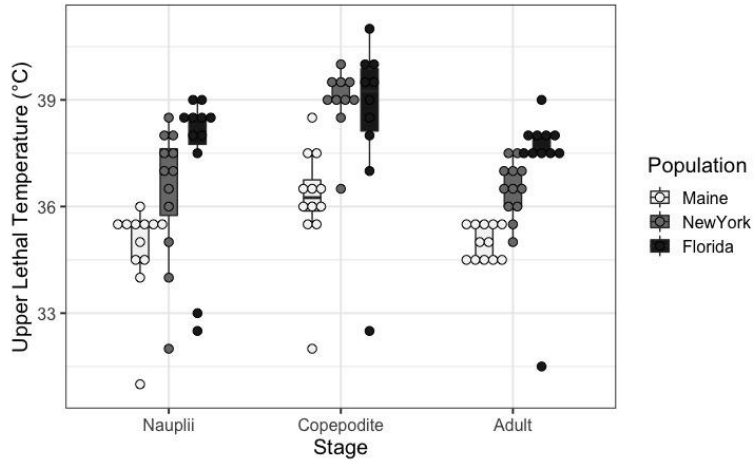


Figure 2.

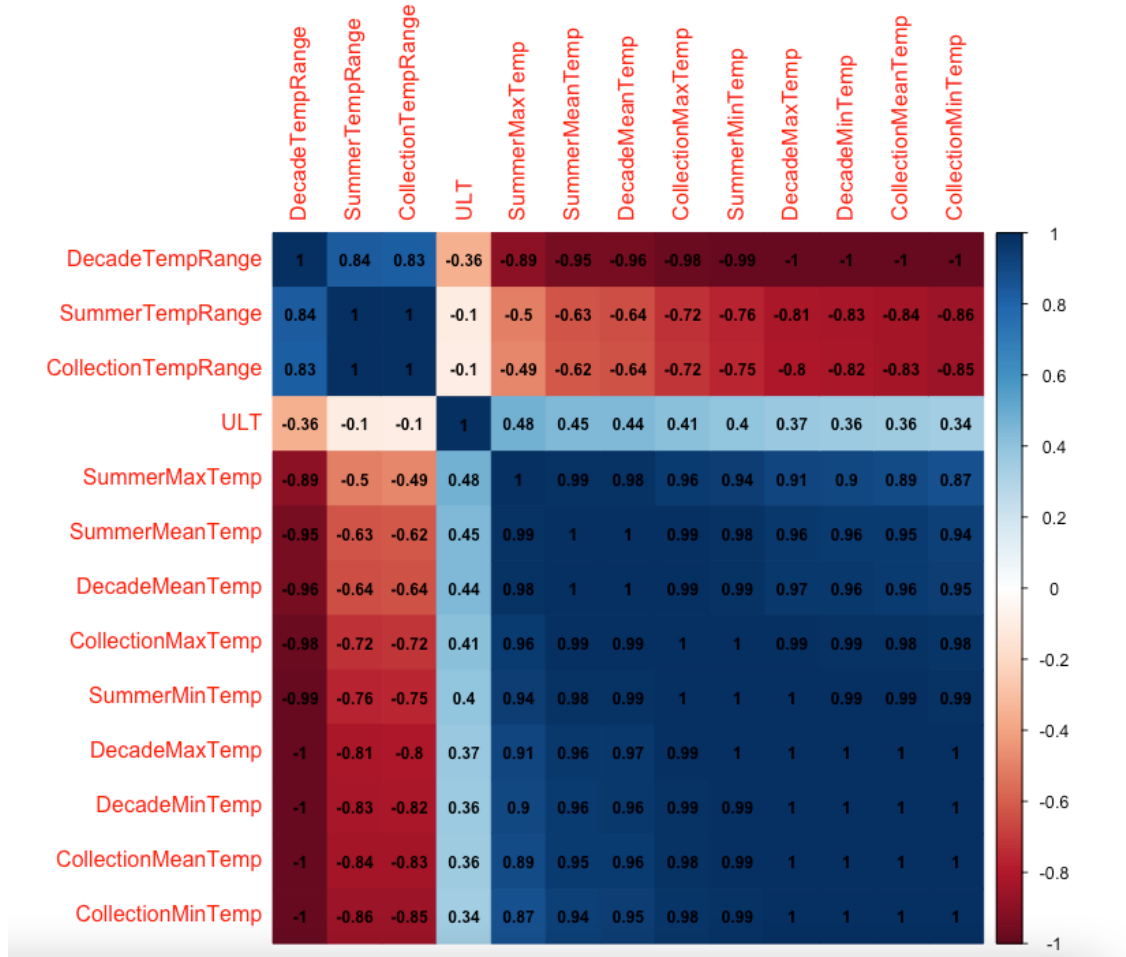


Figure 3.

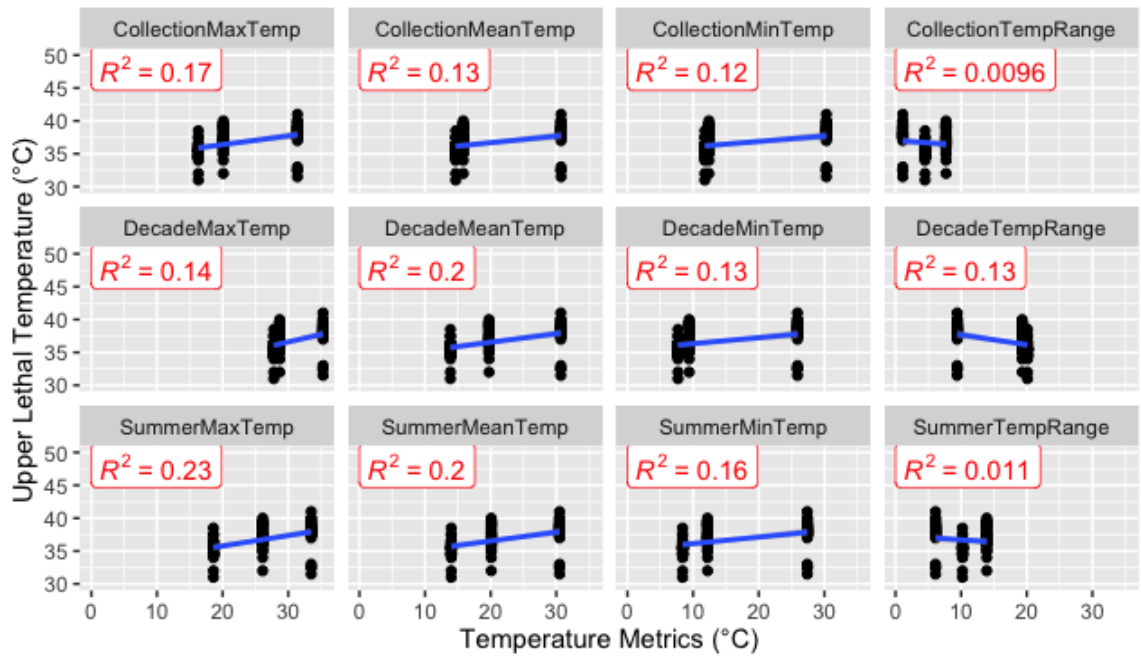


Figure 4.

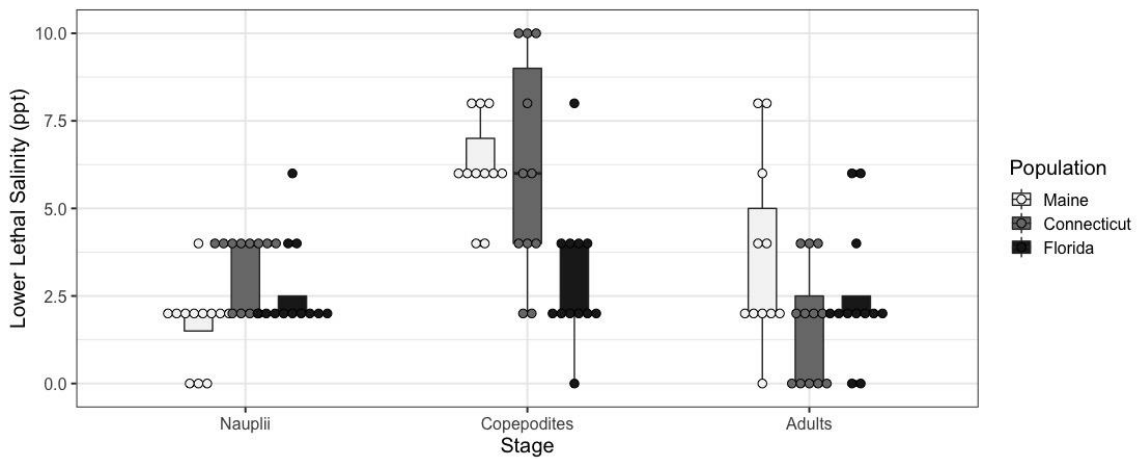


Figure 5.

Models	K	AICc	Delta_AICc	AICcWt	Cum.Wt	Res.LL
Summer_Max	5	410.91	0	0.14	0.14	-200.14
Decade_Max	5	411.18	0.27	0.12	0.27	-200.28
Collection_Range	5	411.48	0.57	0.11	0.37	-200.43
Summer_Rance	5	411.81	0.9	0.09	0.47	-200.59
Summer_Mean	5	411.85	0.94	0.09	0.55	-200.61
Decade_Mean	5	411.98	1.07	0.08	0.64	-200.68
Decade_Range	5	412.02	1.11	0.08	0.72	-200.7
Collection_Max	5	412.16	1.25	0.08	0.8	-200.77
Summer_Min	5	412.79	1.88	0.06	0.85	-201.08
Collection_Mean	5	412.84	1.93	0.05	0.91	-201.11
Decade_Min	5	413.03	2.12	0.05	0.96	-201.2
Collection_Min	5	413.23	2.32	0.04	1	-201.3

Table 1.

CONCLUDING REMARKS

In this work, I demonstrated that aquatic ectotherms are at once resilient and vulnerable to climate warming. Results from Chapter One indicate the ecologically and economically impactful marine copepod, *Neocalanus plumchrus*, is vulnerable to ocean warming and marine heatwaves (Ashlock et al., 2021). While other warm water species may supplement the loss of *N. plumchrus*, they contain lower nutrient content (Cavole et al., 2016). Importantly, loss of nutrient rich copepods is implicated in reduced recruitment and increased mortality in pollock and salmon fisheries (Cavole et al., 2016). Chapter Two demonstrates the importance of plasticity, with development conferring the same thermal tolerance benefits as > 50 generations of warming. Additionally, Chapter Two shows that *Acartia tonsa*, a copepod robust to temperature and salinity fluctuations is vulnerable in the face of increasingly variable temperature and salinity conditions with climate change. Chapter Three highlights the importance of collection location and life history stage in defining thermal tolerance. Results from this chapter also emphasize a need for standardized thermal tolerance methods across species of copepods, and marine ectotherms more broadly.

Results from this dissertation add to the fields of marine ecology and evolutionary biology. Work from Chapter One is novel in that it examines the temperature dependent abundance of *Neocalanus plumchrus* across unprecedented time and space (Ashlock et al., 2021, Sonia Batten, *Pers. Comm.*). Additionally, Chapter One establishes methods for identifying thermal thresholds of copepod abundance and occurrence, identifying

vulnerabilities of *N. plumchrus* to continued warming and marine heat waves. Chapter Two adds to the field of evolutionary biology in that it reveals the potential importance of plasticity in determining thermal tolerance. This highlights the importance of considering plasticity in species assessments of vulnerability to ocean warming (Hoffmann and Sgrò 2011, Ofori et al., 2017). Additionally, results from Chapter Two show a loss of the most thermally tolerant individuals after adaptation to ocean warming, suggesting that rapid adaptation to ocean warming may incur thermal tolerance costs. Chapter Three adds to the literature on the effects of life history stage and latitude on thermal tolerance of the dominant estuarine copepod *Acartia tonsa*. Importantly, juvenile stages of *A. tonsa* are more thermally tolerant and less salinity tolerant than adults and larvae. This indicates that copepodites will limit species salinity tolerance, while adults and nauplii will limit species temperature tolerance.

Reflecting back on this work, I recognize opportunities for improvement to scientific methods. In Chapter Two I found that plasticity has the same effect on thermal tolerance as >50 generations of Ocean Warming. This reveals the importance of plasticity and demonstrates the developmental environment is critical in determining thermal tolerance. In Chapter Two, copepods were not fed during salinity tolerance assays. While feeding was not critical in understanding the relative salinity tolerance between treatments, I am unable to rule out the potential effect of short-term starvation on copepod salinity tolerance. Chapter Three would be improved if I was able to identify the clades that each population represented. This would have allowed for a more specific understanding of thermal tolerance in this cryptic species complex. Additionally, Chapter Three would

have benefitted from the addition of transcriptomic data from the three different life history stages. This was originally planned to be included in this chapter, however, extracting sufficient levels of high-quality RNA from early life stages of copepods proved challenging. Importantly, the Upper Lethal Temperature assay used in Chapter Two and Chapter Three could be improved by reducing the time course of the assay and by being able to monitor copepod survival *in situ*, without removing them from the water bath. This is currently under development in the Pespeni Lab and will likely improve the efficiency of the assay and smooth out the profile of the temperature ramp.

Each chapter presented in this dissertation motivates prospective future studies. Chapter One would benefit from exploring *Neocalanus plumchrus* thermal tolerance plasticity. This would be relatively tractable to assess in late stage copepodites that are found in the surface waters of the Northeast Pacific (Batten et al., 2003). Additionally, the Northeast Pacific Continuous Plankton Recorder and the NASA Multi-Scale Ultra High-Resolution SST dataset (JPL MUR MEaSURES Project, 2015) are still running. This provides an opportunity to continue tracking changes in abundance with changing temperatures and potential future marine heatwaves. Future work for Chapter Two could use fluctuating temperature treatments instead of static temperature treatments. Fluctuating temperature treatments would more realistically mirror ocean conditions (Bernhardt et al., 2013). Additionally, temperature variability and predictability can impact thermal tolerance and plasticity (Reed et al., 2010, Lande, 2009, Bitter et al., 2021). Therefore, variable temperature would provide a more accurate picture of thermal tolerance and plasticity. Interestingly, in Chapter Two, I found that exposure to novel

environments reveals cryptic phenotypic variability in thermal tolerance. Replicating exposure to new developmental temperatures to see if this result is repeatable would reveal the influence of novel environments on trait variability. In Chapter Three, I identify a need for more studies that include multiple species with consistent methodologies when assessing thermal tolerance. Additionally, with the *Acartia tonsa* cryptic species complex (Figueroa et al., 2020), I could look at clade-level differences in thermal tolerance and adaptive capacity. This would identify potentially vulnerable and tolerant clades and indicate future clade persistence in the face of climate change.

The development of this dissertation expanded my biological and computational knowledge, while teaching me the value of collaboration with colleagues from different backgrounds. When I began my Ph.D., my experience was predominantly in marine field ecology and conservation. I pursued a degree in Dr. Melissa Pespeni's lab because I was inspired by her innovative research regarding the adaptive capacity of marine organisms to climate change. It was my hope that with my dissertation research, I could apply concepts of evolution to understand the potential persistence of marine organisms in response to global change stressors. In pursuit of this goal, I learned that knowledge of plasticity and evolutionary dynamics are essential for understanding the response of marine organisms to climate change. Additionally, the achievement of this goal is shared with my collaborators in the Dam lab at UCONN Avery Point, the Lockwood lab at the University of Vermont, and the Farallon Institute. Copepods are central to this dissertation and the Dam lab taught me everything I know about copepods with kindness, patience, and humor. Thermal tolerance is also a pillar of this work, using thermal

tolerance limits to understand copepod response to changing thermal conditions. The Lockwood Lab, in particular Dr. Brent Lockwood and Dr. Emily Mikucki, taught me about the influence of temperature on invertebrates from the molecular to the organismal level. My collaboration with the Farallon Institute taught me to work with scientists from all different backgrounds, ranging from ecology, to physical oceanography, to data science. Additionally, through this work I discovered the power of leveraging existing environmental and copepod data to ask scientific questions about the potential resilience of marine organisms to climate change. These collaborators, and my colleagues at the University of Vermont, inspire and motivate me to continue researching the impacts of climate change on marine organisms beyond the completion of this degree.

Literature Cited

- Ashlock, L., García-Reyes, M., Gentemann, C., Batten, S., & Sydeman, W. (2021). Temperature and Patterns of Occurrence and Abundance of Key Copepod Taxa in the Northeast Pacific. *Frontiers in Marine Science*, 1271.
- Batten, S. D., Welch, D. W., & Jonas, T. (2003). Latitudinal differences in the duration of development of *Neocalanus plumchrus* copepodites. *Fisheries Oceanography*, 12: 201-208.
- Bernhardt, J. R., & Leslie, H. M. (2013). Resilience to climate change in coastal marine ecosystems. *Annual review of marine science*, 5: 371-392.
- Bitter, M. C., Wong, J. M., Dam, H. G., Donelan, S. C., Kenkel, C. D., Komoroske, L. M., ... & Lotterhos, K. E. (2021). Fluctuating selection and global change: a synthesis and review on disentangling the roles of climate amplitude, predictability and novelty. *Proceedings of the Royal Society B*, 288: 20210727.
- Cavole, L. M., Demko, A. M., Diner, R. E., Giddings, A., Koester, I., Pagniello, C. M., ... & Franks, P. J. (2016). Biological impacts of the 2013–2015 warm-water anomaly in the Northeast Pacific: winners, losers, and the future. *Oceanography*, 29: 273-285.
- Figueroa, N. J., Figueroa, D. F., & Hicks, D. (2020). Phylogeography of *Acartia tonsa* Dana, 1849 (Calanoida: Copepoda) and phylogenetic reconstruction of the genus *Acartia* Dana, 1846. *Marine Biodiversity*, 50: 1-20.

- Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, 470: 479-485.
- JPL MUR MEaSURES Project (2015). GHRSSST Level 4 MUR Global Foundation Sea Surface Temperature Analysis (v4.1). Ver. 4.1. PO.DAAC, CA, USA. Available online at: <https://doi.org/10.5067/GHGMR-4FJ04> (accessed August, 2020)
- Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of evolutionary biology*, 22: 1435-1446.
- Ofori, B. Y., Stow, A. J., Baumgartner, J. B., & Beaumont, L. J. (2017). Influence of adaptive capacity on the outcome of climate change vulnerability assessment. *Scientific reports*, 7: 1-12.
- Reed, T. E., Waples, R. S., Schindler, D. E., Hard, J. J., & Kinnison, M. T. (2010). Phenotypic plasticity and population viability: the importance of environmental predictability. *Proceedings of the Royal Society B: Biological Sciences*, 277: 3391-3400.

COMPREHENSIVE BIBLIOGRAPHY

- Aberle, N., Malzahn, A. M., Lewandowska, A. M., & Sommer, U. (2015). Some like it hot: the protozooplankton-copepod link in a warming ocean. *Marine Ecology Progress Series*, 519: 103-113.
- Allen, J. L., Clusella-Trullas, S., & Chown, S. L. (2012). The effects of acclimation and rates of temperature change on critical thermal limits in *Tenebrio molitor* (Tenebrionidae) and *Cyrtobagous salviniae* (Curculionidae). *Journal of insect physiology*, 58: 669-678.
- Allen, J. L., Chown, S. L., Janion-Scheepers, C., & Clusella-Trullas, S. (2016). Interactions between rates of temperature change and acclimation affect latitudinal patterns of warming tolerance. *Conservation physiology*, 4: 1.
- Amaya, D. J., Miller, A. J., Xie, S. P., & Kosaka, Y. (2020). Physical drivers of the summer 2019 North Pacific marine heatwave. *Nature communications*, 11: 1-9.
- Angilletta Jr, M. J., Cooper, B. S., Schuler, M. S., & Boyles, J. G. (2010). The evolution of thermal physiology in endotherms. *Frontiers in Bioscience-Elite*, 2: 861-881.
- Ashlock, L., García-Reyes, M., Gentemann, C., Batten, S., & Sydeman, W. (2021). Temperature and Patterns of Occurrence and Abundance of Key Copepod Taxa in the Northeast Pacific. *Frontiers in Marine Science*, 1271.
- Ayrinhac, A., Debat, V., Gibert, P., Kister, A. G., Legout, H., Moreteau, B., ... & David, J. R. (2004). Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology*, 700-706.
- Badyaev, A. V. (2005). Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proceedings of the Royal Society B: Biological Sciences*, 272: 877-886.
- Bates, N. R., Best, M. H. P., Neely, K., Garley, R., Dickson, A. G., & Johnson, R. J. (2012). Detecting anthropogenic carbon dioxide uptake and ocean acidification in the North Atlantic Ocean. *Biogeosciences*, 9: 2509-2522.
- Batten, S. D., Clark, R., Flinkman, J., Hays, G., John, E., John, A. W. G., ... & Walne, A. (2003a). CPR sampling: the technical background, materials and methods, consistency and comparability. *Progress in Oceanography*, 58: 193-215.
- Batten, S. D., Welch, D. W., & Jonas, T. (2003b). Latitudinal differences in the duration of development of *Neocalanus plumchrus* copepodites. *Fisheries Oceanography*, 12(3), 201-208.

- Batten, S. D., Hyrenbach, K. D., Sydeman, W. J., Morgan, K. H., Henry, M. F., Yen, P. P., & Welch, D. W. (2006). Characterising meso-marine ecosystems of the North Pacific. *Deep Sea Research Part II: Topical Studies in Oceanography*, 53: 270-290.
- Batten, S. D., & Mackas, D. L. (2009). Shortened duration of the annual *Neocalanus plumchrus* biomass peak in the Northeast Pacific. *Marine Ecology Progress Series*, 393: 189-198.
- Batten, S. D., & Walne, A. W. (2011). Variability in northwards extension of warm water copepods in the NE Pacific. *Journal of Plankton Research*, 33: 1643-1653.
- Batten, S. D., Ostle, C., Hélaouët, P., & Walne, A. W. (2022). Responses of Gulf of Alaska plankton communities to a marine heat wave. *Deep Sea Research Part II: Topical Studies in Oceanography*, 195: 105002.
- Bennett, J. M., Sunday, J., Calosi, P., Villalobos, F., Martínez, B., Molina-Venegas, R., ... & Olalla-Tárraga, M. Á. (2021). The evolution of critical thermal limits of life on Earth. *Nature communications*, 12, 1-9.
- Benson, A. J., & Trites, A. W. (2002). Ecological effects of regime shifts in the Bering Sea and eastern North Pacific Ocean. *Fish and Fisheries*, 3: 95-113.
- Bernhardt, J. R., & Leslie, H. M. (2013). Resilience to climate change in coastal marine ecosystems. *Annual review of marine science*, 5: 371-392.
- Bertram, D. F., Mackas, D. L., & McKinnell, S. M. (2001). The seasonal cycle revisited: interannual variation and ecosystem consequences. *Progress in Oceanography*, 49: 283-307.
- Bitter, M. C., Wong, J. M., Dam, H. G., Donelan, S. C., Kenkel, C. D., Komoroske, L. M., ... & Lotterhos, K. E. (2021). Fluctuating selection and global change: a synthesis and review on disentangling the roles of climate amplitude, predictability and novelty. *Proceedings of the Royal Society B*, 288: 20210727.
- Booth, D. J., Poloczanska, E., Donelson, J. M., Molinos, J. G., & Burrows, M. (2017). Biodiversity and climate change in the oceans. *Climate change impacts on fisheries and aquaculture: A global analysis*, 1: 63-89.
- Bradley, C. J., Strickler, J. R., Buskey, E. J., & Lenz, P. H. (2013). Swimming and escape behavior in two species of calanoid copepods from nauplius to adult. *Journal of Plankton Research*, 35: 49-65.
- Brennan, R. S., DeMayo, J. A., Dam, H. G., Finiguerra, M. B., Baumann, H., & Pespeni, M. H. (2022a). Loss of transcriptional plasticity but sustained adaptive capacity after adaptation to global change conditions in a marine copepod. *Nature communications*, 13: 1-13.

- Brennan, R. S., deMayo, J. A., Dam, H. G., Finiguerra, M., Baumann, H., Buffalo, V., & Pespeni, M. H. (2022b). Experimental evolution reveals the synergistic genomic mechanisms of adaptation to ocean warming and acidification in a marine copepod. *Proceedings of the National Academy of Sciences*, 119: e2201521119.
- Calbet, A., & Saiz, E. (2005). The ciliate-copepod link in marine ecosystems. *Aquatic Microbial Ecology*, 38(2), 157-167.
- Calliari, D., Borg, M. C. A., Thor, P., Gorokhova, E., & Tiselius, P. (2008). Instantaneous salinity reductions affect the survival and feeding rates of the co-occurring copepods *Acartia tonsa* Dana and *A. clausi* Giesbrecht differently. *Journal of Experimental Marine Biology and Ecology*, 362: 18-25.
- Carstensen, J., Weydmann, A., Olszewska, A., & Kwaśniewski, S. (2012). Effects of environmental conditions on the biomass of *Calanus spp.* in the Nordic Seas. *Journal of Plankton Research*, 34: 951-966.
- Castellani, C., Licandro, P., Fileman, E., Di Capua, I., & Mazzocchi, M. G. (2016). *Oithona similis* likes it cool: evidence from two long-term time series. *Journal of Plankton Research*, 38(3), 703-717.
- Cavole, L. M., Demko, A. M., Diner, R. E., Giddings, A., Koester, I., Pagniello, C. M., ... & Franks, P. J. (2016). Biological impacts of the 2013–2015 warm-water anomaly in the Northeast Pacific: winners, losers, and the future. *Oceanography*, 29: 273-285.
- Charmantier, G. (1998). Ontogeny of osmoregulation in crustaceans: a review. *Invertebrate Reproduction & Development*, 33: 177-190.
- Charmantier, G., & Charmantier-Daures, M. (1991). Ontogeny of osmoregulation and salinity tolerance in *Cancer irroratus*; elements of comparison with *C. borealis* (Crustacea, Decapoda). *The Biological Bulletin*, 180(1), 125-134.
- Chen, G., & Hare, M. P. (2008). Cryptic ecological diversification of a planktonic estuarine copepod, *Acartia tonsa*. *Molecular ecology*, 17: 1451-1468.
- Chen, G., & Hare, M. P. (2011). Cryptic diversity and comparative phylogeography of the estuarine copepod *Acartia tonsa* on the US Atlantic coast. *Molecular Ecology*, 20: 2425-2441.
- Collin, R., Rebolledo, A. P., Smith, E., & Chan, K. Y. K. (2021). Thermal tolerance of early development predicts the realized thermal niche in marine ectotherms. *Functional Ecology*, 35: 1679-1692.
- Conover, R. J. (1988). Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. *Hydrobiologia*, 167: 127-142.

- Coyle, K. O., & Pinchuk, A. I. (2003). Annual cycle of zooplankton abundance, biomass and production on the northern Gulf of Alaska shelf, October 1997 through October 2000. *Fisheries Oceanography*, 12: 327-338.
- Coyle, K. O., & Pinchuk, A. I. (2005). Seasonal cross-shelf distribution of major zooplankton taxa on the northern Gulf of Alaska shelf relative to water mass properties, species depth preferences and vertical migration behavior. *Deep Sea Research Part II: Topical Studies in Oceanography*, 52: 217-245.
- Crawford, W. R., Brickley, P. J., & Thomas, A. C. (2007). Mesoscale eddies dominate surface phytoplankton in northern Gulf of Alaska. *Progress in Oceanography*, 75: 287-303.
- Cuker, B. E., & Watson, M. A. (2002). Diel vertical migration of zooplankton in contrasting habitats of the Chesapeake Bay. *Estuaries*, 25: 296-307.
- Dam, H. G. (2013). Evolutionary adaptation of marine zooplankton to global change. *Annual Review of Marine Science*, 5: 349-370.
- Dam, H. G., & Baumann, H. (2017). Climate change, zooplankton and fisheries. *Climate change impacts on fisheries and aquaculture: A global analysis*, 2: 851-874.
- Dam, H. G., deMayo, J. A., Park, G., Norton, L., He, X., Finiguerra, M. B., ... & Pespeni, M. H. (2021). Rapid, but limited, zooplankton adaptation to simultaneous warming and acidification. *Nature Climate Change*, 11: 780-786.
- DeBiase, M. B., Kawji, Y., & Kelly, M. W. (2018). Phenotypic and transcriptomic responses to salinity stress across genetically and geographically divergent *Tigriopus californicus* populations. *Molecular ecology*, 27: 1621-1632.
- deMayo, J. A., Girod, A., Sasaki, M. C., & Dam, H. G. (2021). Adaptation to simultaneous warming and acidification carries a thermal tolerance cost in a marine copepod. *Biology Letters*, 17: 20210071.
- Di Lorenzo, E., & Mantua, N. (2016). Multi-year persistence of the 2014/15 North Pacific marine heatwave. *Nature Climate Change*, 6: 1042-1047.
- Diamond, S. E., & Martin, R. A. (2021). Buying time: Plasticity and population persistence. In *Phenotypic plasticity & evolution*, 185-209. CRC Press.
- Dur, G., & Souissi, S. (2018). Ontogenetic optimal temperature and salinity envelopes of the copepod *Eurytemora affinis* in the Seine estuary (France). *Estuarine, Coastal and Shelf Science*, 200: 311-323.
- Dvoretzky, V. G., & Dvoretzky, A. G. (2009). Life cycle of *Oithona similis* (Copepoda: Cyclopoida) in Kola Bay (Barents Sea). *Marine biology*, 156:1433-1446.

- Elkin, L. A., Kay, M., Higgins, J. J., & Wobbrock, J. O. (2021). An aligned rank transform procedure for multifactor contrast tests. In The 34th Annual ACM Symposium on User Interface Software and Technology. 754-768.
- Ellegren, H., & Galtier, N. (2016). Determinants of genetic diversity. *Nature Reviews Genetics*, 17: 422-433.
- Evanson, M., Bornhold, E. A., Goldblatt, R. H., Harrison, P. J., & Lewis, A. G. (2000). Temporal variation in body composition and lipid storage of the overwintering, subarctic copepod *Neocalanus plumchrus* in the Strait of Georgia, British Columbia (Canada). *Marine Ecology Progress Series*, 192: 239-247.
- Feinberg, L. R., & Dam, H. G. (1998). Effects of diet on dimensions, density and sinking rates of fecal pellets of the copepod *Acartia tonsa*. *Marine Ecology Progress Series*, 175, 87-96.
- Figueroa, N. J., Figueroa, D. F., & Hicks, D. (2020). Phylogeography of *Acartia tonsa* Dana, 1849 (Calanoida: Copepoda) and phylogenetic reconstruction of the genus *Acartia* Dana, 1846. *Marine Biodiversity*, 50: 1-20.
- Fisher, J., Kimmel, D., Ross, T., Batten, S., Bjorkstedt, E., Galbraith, M., ... & Perry, R. I. (2020). Copepod responses to, and recovery from, the recent marine heatwave in the Northeast Pacific. *PICES Sci. 2019: Notes Sci. Board Chair*, 28: 65.
- Fordyce, J. A. (2006). The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *Journal of Experimental Biology*, 209: 2377-2383.
- Frölicher, T. L., Fischer, E. M., & Gruber, N. (2018). Marine heatwaves under global warming. *Nature*, 560: 360-364.
- Fulton, R. S. (1984). Distribution and community structure of estuarine copepods. *Estuaries*, 7: 38-50.
- Gamfeldt, L., Wallén, J., Jonsson, P. R., Berntsson, K. M., & Havenhand, J. N. (2005). Increasing intraspecific diversity enhances settling success in a marine invertebrate. *Ecology*, 86: 3219-3224.
- Garzke, J., Ismar, S. M., & Sommer, U. (2015). Climate change affects low trophic level marine consumers: warming decreases copepod size and abundance. *Oecologia*, 177: 849-860.
- Gibson, R., Atkinson, R., Gordon, J., Smith, I., & Hughes, D. (2011). Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. *Oceanogr Mar Biol Annu Rev*, 49: 1-42.

- Gleason, L. U., & Burton, R. S. (2013). Phenotypic evidence for local adaptation to heat stress in the marine snail *Chlorostoma* (formerly *Tegula*) *funnebralis*. *Journal of experimental marine biology and ecology*, 448: 360-366.
- González, J. G. (1974). Critical thermal maxima and upper lethal temperatures for the calanoid copepods *Acartia tonsa* and *A. clausi*. *Marine Biology*, 27(3), 219-223.
- Gordon, A. L., & Giulivi, C. F. (2008). Sea surface salinity trends: over fifty years within the subtropical North Atlantic. *Oceanography*, 21, 20-29.
- Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*, 282: 20150401.
- Hammock, B. G., Lesmeister, S., Flores, I., Bradburd, G. S., Hammock, F. H., & Teh, S. J. (2016). Low food availability narrows the tolerance of the copepod *Eurytemora affinis* to salinity, but not to temperature. *Estuaries and Coasts*, 39: 189-200.
- Hansen, F. C., Möllmann, C., Schütz, U., & Hinrichsen, H. H. (2004). Spatio-temporal distribution of *Oithona similis* in the Bornholm Basin (central Baltic Sea). *Journal of Plankton Research*, 26: 659-668.
- Harley, C. D., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J., Thornber, C. S., ... & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology letters*, 9: 228-241.
- Harris, P., Muelbert, J., Muniz, P., Yin, K., Ahmed, K., Folorunsho, R., ... & Rice, J. (2016). *Estuaries and Deltas*. In *United Nations World Ocean Assessment*. Cambridge: Cambridge University Press.
- Hartl, D. L. (2020). *A primer of population genetics and genomics*. Oxford University Press.
- Hays, G. C., Richardson, A. J., & Robinson, C. (2005). Climate change and marine plankton. *Trends in ecology & evolution*, 20: 337-344.
- 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: 179-185.
- Hendry, A. P. (2016). *Eco-evolutionary dynamics*. Princeton university press.
- Henson, S. A., & Thomas, A. C. (2008). A census of oceanic anticyclonic eddies in the Gulf of Alaska. *Deep Sea Research Part I: Oceanographic Research Papers*, 55: 163-176.

- Hill, D. F., Bruhis, N., Calos, S. E., Arendt, A., & Beamer, J. (2015). Spatial and temporal variability of freshwater discharge into the Gulf of Alaska. *Journal of Geophysical Research: Oceans*, 120: 634-646.
- Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, 470: 479-485.
- Hoffmann, A. A., Shirriffs, J., & Scott, M. (2005). Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Functional Ecology*, 222-227.
- Holliland, P. B., Ahlbeck, I., Westlund, E., & Hansson, S. (2012). Ontogenetic and seasonal changes in diel vertical migration amplitude of the calanoid copepods *Eurytemora affinis* and *Acartia spp.* in a coastal area of the northern Baltic proper. *Journal of Plankton Research*, 34: 298-307.
- Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends in ecology & evolution*, 4: 131-135.
- Hughes, A. R., & Stachowicz, J. J. (2004). Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences*, 101: 8998-9002.
- Humes, A. G. (1994). How many copepods?. In *Ecology and morphology of copepods*, 1-7. Springer, Dordrecht.
- Janout, M. A., Weingartner, T. J., Royer, T. C., & Danielson, S. L. (2010). On the nature of winter cooling and the recent temperature shift on the northern Gulf of Alaska shelf. *Journal of Geophysical Research: Oceans*, 115: C5.
- Janssen, E., Wuebbles, D. J., Kunkel, K. E., Olsen, S. C., & Goodman, A. (2014). Observational-and model-based trends and projections of extreme precipitation over the contiguous United States. *Earth's Future*, 2: 99-113.
- Johnson, W. S., & Allen, D. M. (2012). *Zooplankton of the Atlantic and Gulf coasts: a guide to their identification and ecology*. JHU Press.
- Johnstone, J. A., & Mantua, N. J. (2014). Atmospheric controls on northeast Pacific temperature variability and change, 1900–2012. *Proceedings of the National Academy of Sciences*, 111: 14360-14365.
- JPL MUR MEaSURES Project (2015). GHRSSST Level 4 MUR Global Foundation Sea Surface Temperature Analysis (v4.1). Ver. 4.1. PO.DAAC, CA, USA. Available online at: <https://doi.org/10.5067/GHGMR-4FJ04> (accessed August, 2020)
- Jump, A. S., & Penuelas, J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology letters*, 8: 1010-1020.

- Kawamura, A., & Hirano, K. (1985). The spatial scale of surface swarms of *Calanus plumchrus* Marukawa observed from consecutive plankton net catches in the northwestern North Pacific. *Bulletin of marine science*, 37: 626-633.
- Kelly, M. W., Sanford, E., & Grosberg, R. K. (2012). Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proceedings of the Royal Society B: Biological Sciences*, 279: 349-356.
- Kelly, M. W., DeBiasse, M. B., Villela, V. A., Roberts, H. L., & Cecola, C. F. (2016). Adaptation to climate change: Trade-offs among responses to multiple stressors in an intertidal crustacean. *Evolutionary Applications*, 9: 1147-1155.
- Kelly, M. W., Pankey, M. S., DeBiasse, M. B., & Plachetzki, D. C. (2017). Adaptation to heat stress reduces phenotypic and transcriptional plasticity in a marine copepod. *Functional Ecology*, 31: 398-406.
- Kobari, T., & Ikeda, T. (2001). Ontogenetic vertical migration and life cycle of *Neocalanus plumchrus* (Crustacea: Copepoda) in the Oyashio region, with notes on regional variations in body sizes. *Journal of Plankton Research*, 23: 287-302.
- Kobari, T., Nagaki, T., & Takahashi, K. (2004). Seasonal changes in abundance and development of *Calanus pacificus* (Crustacea: Copepoda) in the Oyashio–Kuroshio Mixed Region. *Marine Biology*, 144: 713-721.
- Lahti, D. C., Johnson, N. A., Ajie, B. C., Otto, S. P., Hendry, A. P., Blumstein, D. T., ... & Foster, S. A. (2009). Relaxed selection in the wild. *Trends in ecology & evolution*, 24: 487-496.
- Lance, J. (1964). The salinity tolerances of some estuarine planktonic crustaceans. *The Biological Bulletin*, 127: 108-118.
- Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of evolutionary biology*, 22: 1435-1446.
- Lee, R. F., Hagen, W., & Kattner, G. (2006). Lipid storage in marine zooplankton. *Marine Ecology Progress Series*, 307: 273-306.
- Lehmann, J., Coumou, D., & Frieler, K. (2015). Increased record-breaking precipitation events under global warming. *Climatic Change*, 132: 501-515.
- Leong, W., Sun, P. Y., & Edmands, S. (2018). Latitudinal clines in temperature and salinity tolerance in tidepool copepods. *Journal of Heredity*, 109: 71-77.
- Liu, H., & Hopcroft, R. R. (2007). A comparison of seasonal growth and development of the copepods *Calanus marshallae* and *C. pacificus* in the northern Gulf of Alaska. *Journal of Plankton Research*, 29: 569-581.

- Liu, H., Zhang, X., Yang, Q., Zuo, T., & Quigg, A. (2017). Mesozooplankton dynamics in relation to environmental factors and juvenile fish in a subtropical estuary of the Gulf of Mexico. *Journal of Coastal Research*, 33: 1038-1050.
- Mackas, D. L., Denman, K. L., & Abbott, M. R. (1985). Plankton patchiness: biology in the physical vernacular. *Bulletin of Marine Science*, 37: 652-674.
- Mackas, D. L., Batten, S., & Trudel, M. (2007). Effects on zooplankton of a warmer ocean: recent evidence from the Northeast Pacific. *Progress in Oceanography*, 75: 223-252.
- Madsen, S. D., Nielsen, T. G., & Hansen, B. W. (2008). Annual population development and production by small copepods in Disko Bay, western Greenland. *Marine Biology*, 155: 63-77.
- Makino, W., & Ban, S. (2000). Response of life history traits to food conditions in a cyclopoid copepod from an oligotrophic environment. *Limnology and oceanography*, 45: 396-407.
- Marshall, D. J., & Morgan, S. G. (2011). Ecological and evolutionary consequences of linked life-history stages in the sea. *Current Biology*, 21: R718-R725.
- Marshall, D. J., Burgess, S. C., & Connallon, T. (2016). Global change, life-history complexity and the potential for evolutionary rescue. *Evolutionary Applications*, 9: 1189-1201.
- Mathews, L., Faithfull, C. L., Lenz, P. H., & Nelson, C. E. (2018). The effects of food stoichiometry and temperature on copepods are mediated by ontogeny. *Oecologia*, 188: 75-84.
- Mauchline, J. (1998). *Adv. Mar. Biol.* 33: The biology of calanoid copepods.
- McKinstry, C. A., & Campbell, R. W. (2018). Seasonal variation of zooplankton abundance and community structure in Prince William Sound, Alaska, 2009–2016. *Deep Sea Research Part II: Topical Studies in Oceanography*, 147: 69-78.
- Meunier, C. L., Boersma, M., Wiltshire, K. H., & Malzahn, A. M. (2016). Zooplankton eat what they need: copepod selective feeding and potential consequences for marine systems. *Oikos*, 125: 50-58.
- Miller, N. A., Paganini, A. W., & Stillman, J. H. (2013). Differential thermal tolerance and energetic trajectories during ontogeny in porcelain crabs, genus *Petrolisthes*. *Journal of Thermal Biology*, 38: 79-85.
- Moyen, N. E., Somero, G. N., & Denny, M. W. (2019). Impact of heating rate on cardiac thermal tolerance in the California mussel, *Mytilus californianus*. *Journal of Experimental Biology*, 222: jeb203166.

- Moyle, P. B., Lund, J. R., Bennett, W. A., & Fleenor, W. E. (2010). Habitat variability and complexity in the upper San Francisco Estuary. *San Francisco Estuary and Watershed Science*, 8:3.
- Najjar, R. G., Walker, H. A., Anderson, P. J., Barron, E. J., Bord, R. J., Gibson, J. R., ... & Swanson, R. S. (2000). The potential impacts of climate change on the mid-Atlantic coastal region. *Climate Research*, 14: 219-233.
- Nicotra, A. B., Beever, E. A., Robertson, A. L., Hofmann, G. E., & O'Leary, J. (2015). Assessing the components of adaptive capacity to improve conservation and management efforts under global change. *Conservation Biology*, 29: 1268-1278.
- Nuwer, M. L., Frost, B. W., & Virginia Armbrust, E. (2008). Population structure of the planktonic copepod *Calanus pacificus* in the North Pacific Ocean. *Marine Biology*, 156: 107-115.
- Ofori, B. Y., Stow, A. J., Baumgartner, J. B., & Beaumont, L. J. (2017). Influence of adaptive capacity on the outcome of climate change vulnerability assessment. *Scientific reports*, 7: 1-12.
- Oliver, E. C., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., ... & Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature communications*, 9: 1-12.
- Osgood, K. E., & Frost, B. W. (1994). Comparative life histories of three species of planktonic calanoid copepods in Dabob Bay, Washington. *Marine Biology*, 118: 627-636.
- Palumbi, S. R., Sandifer, P. A., Allan, J. D., Beck, M. W., Fautin, D. G., Fogarty, M. J., ... & Wall, D. H. (2009). Managing for ocean biodiversity to sustain marine ecosystem services. *Frontiers in Ecology and the Environment*, 7: 204-211.
- Pandori, L. L., & Sorte, C. J. (2019). The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. *Oikos*, 128: 621-629.
- Pandori, L. L., & Sorte, C. J. (2021). Spatial and temporal scales of exposure and sensitivity drive mortality risk patterns across life stages. *Ecosphere*, 12: e03552.
- Peña, M. A., Nemcek, N., & Robert, M. (2019). Phytoplankton responses to the 2014–2016 warming anomaly in the northeast subarctic Pacific Ocean. *Limnology and Oceanography*, 64: 515-525.
- Pereira, R. J., Sasaki, M. C., & Burton, R. S. (2017). Adaptation to a latitudinal thermal gradient within a widespread copepod species: the contributions of genetic divergence and phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, 284: 20170236.

- Peterson, W. T. (2001). Patterns in stage duration and development among marine and freshwater calanoid and cyclopoid copepods: a review of rules, physiological constraints, and evolutionary significance. *Hydrobiologia*, 453: 91-105.
- Peterson, W. T., Fisher, J. L., Strub, P. T., Du, X., Risien, C., Peterson, J., & Shaw, C. T. (2017). The pelagic ecosystem in the Northern California Current off Oregon during the 2014–2016 warm anomalies within the context of the past 20 years. *Journal of Geophysical Research: Oceans*, 122: 7267-7290.
- Piatt, J. F., Parrish, J. K., Renner, H. M., Schoen, S. K., Jones, T. T., Arimitsu, M. L., ... & Sydeman, W. J. (2020). Extreme mortality and reproductive failure of common murrelets resulting from the northeast Pacific marine heatwave of 2014-2016. *PloS one*, 15: e0226087.
- Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., & Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature*, 569: 108-111.
- Plough, L. V., Fitzgerald, C., Plummer, A., & Pierson, J. J. (2018). Reproductive isolation and morphological divergence between cryptic lineages of the copepod *Acartia tonsa* in Chesapeake Bay. *Marine Ecology Progress Series*, 597, 99-113.
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rahlff, J., Peters, J., Moyano, M., Pless, O., Claussen, C., & Peck, M. A. (2017). Short-term molecular and physiological responses to heat stress in neritic copepods *Acartia tonsa* and *Eurytemora affinis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 203: 348-358.
- Rayner, N. A. A., Parker, D. E., Horton, E. B., Folland, C. K., Alexander, L. V., Rowell, D. P., ... & Kaplan, A. (2003). Global analyses of sea surface temperature, sea ice, and night marine air temperature since the late nineteenth century. *Journal of Geophysical Research: Atmospheres*, 108: D14.
- Reed, T. E., Waples, R. S., Schindler, D. E., Hard, J. J., & Kinnison, M. T. (2010). Phenotypic plasticity and population viability: the importance of environmental predictability. *Proceedings of the Royal Society B: Biological Sciences*, 277: 3391-3400.
- Reid, P. C., Colebrook, J. M., Matthews, J. B. L., Aiken, J. C. P. R., & Team, C. P. R. (2003). The Continuous Plankton Recorder: concepts and history, from Plankton Indicator to undulating recorders. *Progress in Oceanography*, 58: 117-173.

- Reid, P. C., Fischer, A. C., Lewis-Brown, E., Meredith, M. P., Sparrow, M., Andersson, A. J., ... & Washington, R. (2009). Impacts of the oceans on climate change. *Advances in marine biology*, 56: 1-150.
- Reusch, T. B., Ehlers, A., Hämmerli, A., & Worm, B. (2005). Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences*, 102: 2826-2831.
- Rice, E., Dam, H. G., & Stewart, G. (2015). Impact of climate change on estuarine zooplankton: surface water warming in Long Island Sound is associated with changes in copepod size and community structure. *Estuaries and coasts*, 38: 13-23.
- Rice, E., & Stewart, G. (2016). Decadal changes in zooplankton abundance and phenology of Long Island Sound reflect interacting changes in temperature and community composition. *Marine environmental research*, 120: 154-165.
- Richardson, A. J. (2008). In hot water: zooplankton and climate change. *ICES Journal of Marine Science*, 65: 279-295.
- Rippingale, R. J., & Hodgkin, E. P. (1977). Food availability and salinity tolerance in a brackish water copepod. *Marine and Freshwater Research*, 28: 1-7.
- Rodríguez-Trelles, F., Tarrío, R., & Santos, M. (2013). Genome-wide evolutionary response to a heat wave in *Drosophila*. *Biology Letters*, 9: 20130228.
- Salinas, S., Irvine, S. E., Schertzing, C. L., Golden, S. Q., & Munch, S. B. (2019). Trait variation in extreme thermal environments under constant and fluctuating temperatures. *Philosophical Transactions of the Royal Society B*, 374: 20180177.
- Sandifer, P. A., and Sutton-Grier, A. E. (2014). Connecting stressors, ocean ecosystem services, and human health. In *Natural Resources Forum* 38: 157-167.
- Sasaki, M. C., & Dam, H. G. (2019). Integrating patterns of thermal tolerance and phenotypic plasticity with population genetics to improve understanding of vulnerability to warming in a widespread copepod. *Global change biology*, 25: 4147-4164.
- Sasaki, M. C., & Dam, H. G. (2020). Genetic differentiation underlies seasonal variation in thermal tolerance, body size, and plasticity in a short-lived copepod. *Ecology and evolution*, 10: 12200-12210.
- Sasaki, M. C., & Dam, H. G. (2021). Negative relationship between thermal tolerance and plasticity in tolerance emerges during experimental evolution in a widespread marine invertebrate. *Evolutionary applications*, 14: 2114-2123.

- Scavia, D., Field, J. C., Boesch, D. F., Buddemeier, R. W., Burkett, V., Cayan, D. R., ... & Titus, J. G. (2002). Climate change impacts on US coastal and marine ecosystems. *Estuaries*, 25: 149-164.
- Schaefer, J., & Ryan, A. (2006). Developmental plasticity in the thermal tolerance of zebrafish *Danio rerio*. *Journal of fish biology*, 69: 722-734.
- Scheffers, B. R., De Meester, L., Bridge, T. C., Hoffmann, A. A., Pandolfi, J. M., Corlett, R. T., ... & Watson, J. E. (2016). The broad footprint of climate change from genes to biomes to people. *Science*, 354(6313), aaf7671.
- Schlüter, L., Lohbeck, K. T., Gutowska, M. A., Gröger, J. P., Riebesell, U., & Reusch, T. B. (2014). Adaptation of a globally important coccolithophore to ocean warming and acidification. *Nature Climate Change*, 4: 1024-1030.
- Schoch, G. C., Menge, B. A., Allison, G., Kavanaugh, M., Thompson, S. A., & A. Wood, S. (2006). Fifteen degrees of separation: Latitudinal gradients of rocky intertidal biota along the California Current. *Limnology and Oceanography*, 51: 2564-2585.
- Sheridan, J. A., & Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nature climate change*, 1: 401-406.
- Singh, D., Tsiang, M., Rajaratnam, B., & Diffenbaugh, N. S. (2013). Precipitation extremes over the continental United States in a transient, high-resolution, ensemble climate model experiment. *Journal of Geophysical Research: Atmospheres*, 118: 7063-7086.
- Snell-Rood, E. C., Kobiela, M. E., Sikkink, K. L., & Shephard, A. M. (2018). Mechanisms of plastic rescue in novel environments. *Annual review of ecology, evolution, and systematics*, 49: 331-354.
- Somero, G.N., Lockwood, B.L., and Tomanek L. (2017). *Biochemical adaptation: response to environmental challenges, from life's origins to the Anthropocene*. Sinauer Associates, Incorporated Publishers
- Sousa, L., Coyle, K. O., Barry, R. P., Weingartner, T. J., & Hopcroft, R. R. (2016). Climate-related variability in abundance of mesozooplankton in the northern Gulf of Alaska 1998–2009. *Deep Sea Research Part II: Topical Studies in Oceanography*, 132: 122-135.
- Steinberg, D. K., & Landry, M. R. (2017). Zooplankton and the ocean carbon cycle. *Annual review of marine science*, 9: 413-444.
- Stillman, J. H., & Somero, G. N. (2000). A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology*, 73: 200-208.

- Storch, D., Fernández, M., Navarrete, S. A., & Pörtner, H. O. (2011). Thermal tolerance of larval stages of the Chilean kelp crab *Taliepus dentatus*. *Marine Ecology Progress Series*, 429: 157-167.
- Sunar, M. C., & Kır, M. (2021). Thermal tolerance of *Acartia tonsa*: In relation to acclimation temperature and life stage. *Journal of Thermal Biology*, 102: 103116.
- Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences*, 278: 1823-1830.
- Sunday, J., Bennett, J. M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A. L., ... & Morales-Castilla, I. (2019). Thermal tolerance patterns across latitude and elevation. *Philosophical Transactions of the Royal Society B*, 374: 20190036.
- Tangwancharoen, S., & Burton, R. S. (2014). Early life stages are not always the most sensitive: heat stress responses in the copepod *Tigriopus californicus*. *Marine Ecology Progress Series*, 517: 75-83.
- Tolley, S. G., Volety, A. K., & Savarese, M. (2005). Influence of salinity on the habitat use of oyster reefs in three southwest Florida estuaries. *Journal of Shellfish Research*, 24: 127-137.
- Turner, J. T. (2004). The importance of small planktonic copepods and their roles in pelagic marine food webs. *Zool. Stud*, 43: 255-266.
- Van Someren Gréve, H., Jepsen, P. M., & Hansen, B. W. (2020). Does resource availability influence the vital rates of the tropical copepod *Apocyclops royi* (Lindberg, 1940) under changing salinities?. *Journal of Plankton Research*, 42: 467-478.
- Vázquez, M. G., Bas, C. C., & Spivak, E. D. (2016). Ontogeny of salinity tolerance in the invasive shrimp *Palaemon macrodactylus* (Caridea: Palaemonidae). *Journal of Crustacean Biology*, 36: 214-219.
- Ward, P., & Hirst, A. G. (2007). *Oithona similis* in a high latitude ecosystem: abundance, distribution and temperature limitation of fecundity rates in a sac spawning copepod. *Marine Biology*, 151: 1099-1110.
- Weingartner, T. J., Coyle, K., Finney, B., Hopcroft, R., Whitledge, T., Brodeur, R., ... & Royer, T. (2002). The northeast Pacific GLOBEC program: coastal Gulf of Alaska. *Oceanography*, 15:2.
- West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

- Whitman, D. W., & Ananthakrishnan, T. N. (2009). Phenotypic plasticity of insects: mechanisms and consequences. Science Publishers, Inc..
- Willett, C. S. (2010). Potential fitness trade-offs for thermal tolerance in the intertidal copepod *Tigriopus californicus*. *Evolution: International Journal of Organic Evolution*, 64: 2521-2534.
- Wobbrock, J. O., Findlater, L., Gergle, D., & Higgins, J. J. (2011, May). The aligned rank transform for nonparametric factorial analyses using only anova procedures. In Proceedings of the SIGCHI conference on human factors in computing systems, 143-146.
- Yampolsky, L. Y., Schaer, T. M., & Ebert, D. (2014). Adaptive phenotypic plasticity and local adaptation for temperature tolerance in freshwater zooplankton. *Proceedings of the Royal Society B: Biological Sciences*, 281: 20132744.
- Yang, B., Emerson, S. R., & Peña, M. A. (2018). The effect of the 2013–2016 high temperature anomaly in the subarctic Northeast Pacific (the “Blob”) on net community production. *Biogeosciences*, 15: 6747-6759.
- Zamora-Terol, S., Kjellerup, S., Swalethorp, R., Saiz, E., & Nielsen, T. G. (2014). Population dynamics and production of the small copepod *Oithona spp.* in a subarctic fjord of West Greenland. *Polar Biology*, 37: 953-965.
- Zhang, X., Zwiers, F. W., Hegerl, G. C., Lambert, F. H., Gillett, N. P., Solomon, S., ... & Nozawa, T. (2007). Detection of human influence on twentieth-century precipitation trends. *Nature*, 448: 461-465.