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STUDENT AWARDEE PAPER

In Hot Water: Current Thermal Threshold Methods Unlikely to Predict Invasive Species Shifts in NW Atlantic

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Synopsis As global temperatures continue to rise, accurate predicted species distribution models will be important for forecasting the movement of range-shifting species. These predictions rely on measurements of organismal thermal tolerance, which can be measured using classical threshold concepts such as Arrhenius break temperatures and critical thermal temperatures, or through ecologically relevant measurements such as the temperature at which reproduction and growth occur. Many species, including invasive species, exhibit thermal plasticity, so these thresholds may change based on ambient temperature, life stage, and measurement techniques. Here, we review thermal thresholds for 15 invertebrate species invasive to the Gulf of Maine. The high degree of variability within a species and between applied conceptual frameworks suggests that modeling the future distribution of these species in all ecosystems, but especially in the rapidly warming northwest Atlantic and Gulf of Maine, will be challenging. While each of these measurement techniques is valid, we suggest contextualization and integration of threshold measurements for accurate modeling.

Introduction

Anthropogenic climate change driven by greenhouse gas emissions has led to unprecedented rates of warming (IPCC 2022). Marine heat waves, which occur when temperatures reach the 90th percentile for 5 or more days (compared to a 30-year historical baseline period), are also increasing in frequency as climate change continues (Hobday et al. 2016; Oliver et al. 2018; Laufkötter et al. 2020). Warming temperatures are causing species to shift deeper in the water or poleward to find favorable thermal conditions (Perry et al. 2005; Sunday et al. 2012). Many marine organisms are ectothermic, whose temperature relies on external sources of body heat, and/or poikilothermic, whose temperature varies with environmental temperature. For these organisms, temperature changes affect their physiology by increasing their metabolic rate, typically at a ratio of 2–3 times base metabolic rate for every 10°C change (Q10; Cossins and Bowler 1987; Conant et al. 2011; Frederich and Lancaster 2024). At thermal extremes, this Q10 can deviate largely from the typical 2–3, and multiple different mechanisms or failures of mechanisms can limit an organism's ability to survive. In order to understand how climate change will affect ectothermic animals, accurate measurements of thermal tolerance and thermal thresholds are essential.

Several metrics are commonly used to measure thermal tolerance thresholds across the animal kingdom. These thresholds can be applied to species modeling by assessing the maximum and minimum temperatures for reproduction, growth, or survival based on oxygen transport, enzyme kinetics, and other markers of stress. One classical measurement is lethal dose 50 or LD50 (or LT50 for lethal temperature), which is derived from toxicology and is the temperature at which 50% of individuals die (see Nagabhushanam and Krishnamoorthy 1992). The Arrhenius break temperature (ABT) is a break from linearity and, while originally described for enzymatic reactions (Arrhenius 1889), is usually measured for heart rates at increasing or decreasing temperatures (Harrington and Hamlin 2019). The concept of the Oxygen and Capacity Limited Thermal Tolerance (OCLTT) hypothesis assumes the delivery of oxygen to peripheral tissues to be the

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factor determining scope for activity and, ultimately, survival (Pörtner et al. 2017). OCLTT describes two thresholds: the pejus temperature Tp, the temperature at which the animal's condition worsens, and the aerobic scope is limited (Frederich and Pörtner 2000), and the critical temperature Tc, the temperature at which the animal's oxygen demand exceeds the oxygen supply due to failing circulatory and/or ventilatory systems, and the subsequent buildup of anaerobic end-products (Pörtner et al. 2017). Critical thermal maxima and minima (CT_{max} and CT_{min}) measure the point at which the animal loses controlled motion (Cowles and Bogert 1944; Brett 1956; Kelty and Lee 2001; Jost et al. 2012). Lastly, the framework of Multiple Performances, Multiple Optima (MPMO) rejects the idea of one unifying parameter limiting survival during temperature extremes, but posits that organ systems, ion transport, and other processes within the animal fail at different temperatures for different species and avoids defining one general mechanism responsible for system failure at thermal thresholds (Städele et al. 2015).

Each of these frameworks outlines thermal thresholds but is typically focused on certain specific questions and is not necessarily ecologically the most relevant. For example, temperatures measured in CT_{max} are so high that they are rarely experienced in the field, except perhaps for intertidal organisms, which are exposed to the air (Stillman and Somero 2000). Thermal thresholds determined as the critical temperature Tc within the OCLTT framework for rock crabs, *Cancer irroratus*, are far above temperatures that these animals ever experience in nature (Frederich et al. 2009). If a threshold falls outside of water temperatures found in nature, it is not necessarily a helpful threshold for predicting species behavior or ability to survive. Outside of ecological relevance, measurements vary greatly due to acclimation temperature, measuring styles, differences in populations, and even misinterpretation of framework measurements (see, e.g., McGaw and Whitley 2012; Tepolt 2024). With different starting temperatures, acclimation temperatures, rates of temperature change, and population differences, there is no basis for statistical modeling (Forero et al. 2019).

Plasticity in thermal thresholds has been observed in many taxa at every life stage (see, e.g., Padilla and Savedo 2013). Geographic location can also influence thermal plasticity, which has been well documented for *Carcinus maenas*, whose CT_{max} values vary nearly 10°C based on acclimation temperature and location (Tepolt and Somero 2014). Some life stages have different energy requirements, so this plasticity may be limited for developing larvae and reproducing females, among others (deRivera et al. 2007).

In addition to characterizing thermal thresholds in the different frameworks outlined above, studies also focus on the temperature effects on factors such as larval development, survival, reproduction, or presence or absence of a species. These measurements help inform local abundance of organisms; however, predicting accurate species distribution requires a physiological understanding of organisms to be applied over a broader scale, measured in precise and replicable ways. Thus, without the mechanistic understanding of physiological limitations, the respective models may fall short. This is increasingly important as climate change and general warming move organisms towards the poles or deeper in the water column (Sunday et al. 2012). Furthermore, some studies use survival at the minimum and maximum regional temperature in the species range as temperature thresholds, which are likely underestimating the true limits of potential invaders (see, e.g., Willis et al. 2009).

Invasive species (defined here as organisms moved from one area to another by humans) are of special interest in context of climate-driven range shifts of species due to their negative impact on the recipient ecosystem and potentially even local economy. These invasive species will only have a chance at success if the temperatures in the recipient community fall within the thermal thresholds of the species. In general, many marine invasive species have a wide range of thermal tolerance and are able to live in many areas they are introduced to. Diet generalism, salinity tolerance, and high fecundity are also predictors of invasion success. For example, the European green crab (*C. maenas*) is native to Europe and northern Africa, but has established invasive populations nearly worldwide, on every continent except Antarctica (Carlton and Cohen 2003; Compton et al. 2010; Frederich and Lancaster 2024). Fortunately, *C. maenas* is well studied in regard to thermal tolerance, so we can predict their future range expansion (see Frederich and Lancaster 2024). Due to warming ocean temperatures and their extreme thermal tolerance (as low as −1.8°C), temperature is likely not the limiting factor for *C. maenas* spreading (Tepolt and Somero 2014).

The Gulf of Maine is a particularly well-suited test system for addressing the question of thermal tolerance in context of climate change importance due to the unprecedented rate of warming in this region (Pershing et al. 2021). This warming has temporarily, positively affected the lobster fishery, but warmer temperatures may facilitate poleward movement of invasive species from lower latitudes (Sorte et al. 2010; Duffy et al. 2017; Goode et al. 2019). Depending on the rate of species spread, which is influenced by larval duration and transport, lifecycle, and bathymetric barriers (among other

Table 1 List of invasive species, including their native and invasive ranges.

Species name	Phylum	Native range	Temperature in native range	Invasive range
Ascidiella aspersa	Chordata	Europe	Up to 26°C	Australia, Japan, New Zealand, North America, South America; possibly also India and South Africa
Botrylloides violaceus		Asia	−0.6–27.4°C	Australia, Europe, and North America
Botryllus schlosseri		Europe	−1–30°C	Asia, Australia, New Zealand, North America, and South America
Ciona intestinalis		Cryptogenic	0–27°C	Asia, Africa, Australia, Europe, New Zealand, North America, and South America
Didemnum vexillum		Asia	−2–24°C	Australia, Europe, New Zealand, and North America
Diplosoma listerianum		Europe	2.2–30°C	Asia, Australia, Europe, Madagascar, New Zealand, North America, and South America
Styela clava		Asia	−2–26.6°C	Australia, Europe, New Zealand, North America; possibly also in Africa
Caprella mutica	Arthropoda	Asia	−2–28°C	North America and Europe
Carcinus maenas		Europe	−1–35°C	Asia, Australia, North America, and South America
Hemigrapsus sanguineus		Asia	1.8–30°C	Europe, North America; possibly also Australia and India
Palaemon elegans		Europe, Africa	2–25°C	North America
Bugula neritina	Bryozoa	Europe	2.2–30°C	Africa, Asia, Australia, New Zealand, North America, South America; possibly also Antarctica; present on several islands including the Galapagos and Vanuatu
Membranipora membranacea		Europe	−1.8–27°C	Africa, Asia, Australia, New Zealand, and North America
Diadumene lineata	Cnidaria	Asia	0–27.5°C	Australia, Europe, New Zealand, North America, and South America
Ostrea edulis	Mollusca	Europe	5–25°C	Africa, Australia, New Zealand, and North America

Information compiled from the global invasive species database (GBIF Secretariat 2021) and the Smithsonian Marine Invasions Lab (Fofonoff et al. 2018).

factors), high-latitude ecosystems may face invasions sooner rather than later, especially as temperatures continue to climb. Due to the rate of warming in the Gulf of Maine, as well as the latitudinal gradient, this area is ideal for projecting how species distribution might change in other regions. While the rate of warming will affect species success, the extreme rate of warming in the Gulf of Maine serves as a "worst case scenario"; if organisms can survive this rate, they will likely be successful in other, less drastically changing areas. The implications of this study can be applied elsewhere in the world, especially for forecasting studies, which presently use field distribution to determine thermal tolerance (see, e.g., Holland et al. 2021). In this context, we provide suggestions for future physiological studies beyond the species here to increase their applicability to species modeling worldwide. By focusing on many taxa across phyla, we believe the findings here can be generalized to other species. Here, we do not run models, but shed light on the variation in threshold data, which has the potential to misinform models and predictions based on the threshold data selected by a modeler.

Here, we review the multiple reports on thermal tolerances of 15 ecologically important Gulf of Maine invasive species to highlight the importance of contextualizing thermal threshold measurements for predictive species distribution models (Table 1). These studies report thermal thresholds from field observations and laboratory experiments from all continents, including Africa (4), Antarctica (3), Asia (28), Australia (24), Europe (127), North America (146), South America (5), and Worldwide (21), with the rest being either unlisted or multi-continent. The species list for this review was chosen from the Marine Invaders Monitoring and Information Collaborative (MIMIC), a project based in New England hosted by the Massachusetts Office of Coastal Zone Management. While these species are not necessarily the most harmful, they are the most easily identifiable, allowing trained volunteers to make observations across the area. These observations began in

2008 and continue to be collected, creating a distribution of species over time. One species, *Ciona intestinalis*, is not listed on MIMIC surveys, but is considered cryptogenic in Maine (Hewitt et al. 2002) and thus was added to this analysis. The 15 species represented here span 5 phyla (urochordata, arthropoda, bryozoa, mollusca, and cnidaria) and consist of a variety of bauplans and metabolic strategies.

Many of the species examined here are key members of fouling communities. Fouling communities are one of the most common habitats for benthic invasive species in harbors and on boats. Assemblages of fouling communities compared in the Great Bay Estuary in New Hampshire have shown a 33% difference in community members since the late 1970s (Harris and Dijkstra 2007). Furthermore, increases in marine heatwave frequency will lead to more erratic temperature changes, which could alter invasive species communities (Sorte et al. 2010). This difference is likely due to newly introduced species and warming waters. Not all invasive species are transported through fouling species or ballast; other common methods of introduction are the pet trade and seafood industry (Rius et al. 2014). Due to the diversity of invertebrate phyla, observations and measurements are grouped by phylum rather than other functional characteristics to generalize species similarities to a broad audience. Other databases cover thermal thresholds for many species; one of the largest is GlobTherm, which only contains one thermal threshold for two of the species here (CT for *P. elegans* and *C. maenas*) (Bennett et al. 2019). Thus, this analysis expands greatly upon existing resources by adding more species and more thresholds.

Characterization of thermal thresholds

Using a variety of search queries (Supplementary Table 1), we collected records of thermal thresholds for the 15 species of interest. Although the search queries were based on the mechanistic frameworks, they also captured a variety of measurement techniques outside of the frameworks. Indeed, most of the studies included did not measure traditional thermal threshold metrics, but instead investigated factors such as survival, larval development, reproduction, or growth. Information recorded from each publication included the maximum or minimum temperature threshold and exactly what was measured. Some frameworks could be assigned to data if the framework was not explicitly listed in the publication. Furthermore, some publications used a different title for a framework that was previously established and was reassigned for the purpose of this review. For example, in 2014, Tepolt and Somero studied cardiac function of *C. maenas* and measured CT_{max}, whereas by our definition, this measurement might be ABT or MPMO (Tepolt and Somero 2014). Other publications did not specify a framework but did measure relevant values and were captured in the "general" search queries, so a descriptive term was chosen from those studies for what was being measured (i.e., development, survival, and reproduction).

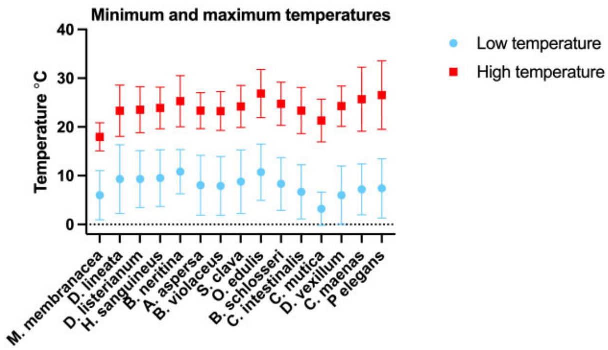

Fig. 1 Summary of nearly 450 measured thermal thresholds for all of the study species, represented by means and standard deviations of the high (square) and low (circle) temperature measurements. Species are organized from lowest maximum high temperature to highest maximum high temperature on the *x* axis.

This literature review compiled nearly 450 thermal threshold records for the species of interest. Most of the records did not align with any of the classical thermal physiology models, but instead focused on what temperatures the organisms were reproducing in the field, the temperatures at which they grew, and general records of survival in an area with specific temperatures. A summary of the average upper and lower measurements, and especially the broad range of the respective thresholds, can be found in Fig. 1.

Thresholds by phylum

Ascidian thermal thresholds

The largest group of organisms in our analysis are ascidians. *Ascidiella aspersa*, *B. violaceus*, *B. schlosseri*, *C. intestinalis*, *D. vexillum*, *D. listerianum*, and *S. clava*. *Ascidiella aspersa*, *C. intestinalis*, and *S. clava* are solitary tunicates, whereas the others live as thin-layer colonies. A breakdown of all measured thresholds can be found in Fig. 2. This group is fairly well studied, as *C. intestinalis* and others are used as model organisms. They are generally suspension feeders and are dominant members of fouling communities. In the Gulf of Maine, they have been introduced through equipment fouling and aquaculture (Lambert 2009; Carman et al. 2014). They frequently outcompete other species in fouling communities and grow over native bivalves such as mussels, oysters, and scallops, as well as eelgrass (Gittenberger 2007; Fletcher 2013; Long and Grosholz 2015).

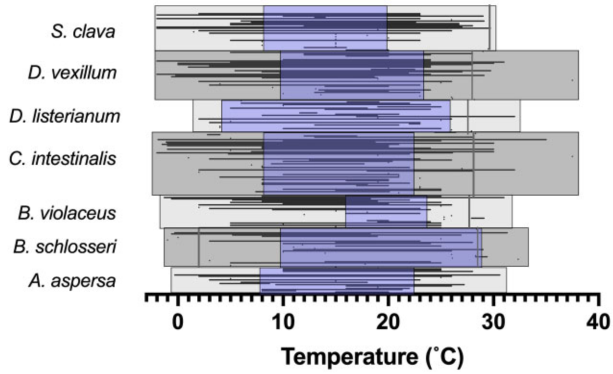

Fig. 2 Measured thermal thresholds (dots) and ranges between thresholds (lines) for the ascidian species included in this study. For *A. aspersa*, none of the traditional thermal threshold measurements were taken; *B. schlosseri* has lower and upper LD50 measurements (3–28.4 ± 2.14°C); *B. violaceus* has an upper LD50 measurement (26.72 ± 1.52°C); *C. intestinalis* has an ABT (21°C) and LD50 (27°C); *D. listerianum* has an LD50 (26.6 ± 1.40°C); *D. vexillum* only has an LD50 (26.77 ± 1.01°C); and lastly, *S. clava* has an LD50 of 29.5°C. Large gray boxes indicate maximum and minimum measured thermal tolerance; small gray boxes indicate a reproductive threshold.

Didemnum vexillum is particularly harmful to Gulf of Maine fisheries and ecosystems. At George's Bank, located in the center of the Gulf of Maine, over 200 km^2 have been colonized by *D. vexillum*, which is damaging nursery habitat for commercially valuable fish like Atlantic cod (*Gadus morhua*) (Valentine et al. 2007). Unfortunately, dredging, scraping, and trawling fragments ascidians, and many of those fragments can settle and start new colonies, so the issue is compounded by traditional fishing practices.

The invasive ascidians in Maine have wide temperature tolerances and are able to reproduce early in the year, allowing them to quickly dominate fouling communities in the spring. They inhabit a variety of ecosystems, from harbors and tide pools to large swaths of benthic areas (Dijkstra et al. 2007; Valentine et al. 2007; Sorte and Stachowicz 2011). Many species exhibit a lower thermal threshold that is below the temperatures required for reproduction. For example, *S. clava* requires a temperature >15°C to reproduce, so it is not feasible for populations to exist if the maximum temperature does not exceed 15°C, even though the species can survive down to −2°C (Davis et al. 2007; Davis and Davis 2008). Many species exhibit population-level variation, which leads to differences in thermal maxima and minima. For example, larvae of *B. schlosseri* have been reared at 10°C (which took nearly 66 days), while other studies did not have successful reproduction at 13°C (Sabbadin et al. 1955; Brunetti et al. 1974). Whether this difference is from laboratory versus field observations or interspecific variation is unknown.

For *C. intestinalis* and others, temperature may affect life cycle length. Individuals growing in cooler temperatures live longer (2–3 years), while individuals in warmer waters may reproduce several times per year or produce up to 4 generations in 1 year and live shorter lives (Berrill 1947; Dybern 1965; Yamaguchi 1975). *Botrylloides violaceus* in the Great Bay Estuary in New Hampshire now experience more than one reproductive cycle in a year, compared to the 1970s, where cooler temperatures and shorter heat extremes limited their reproductive cycles to 0.7 annual reproductive cycles (Dijkstra et al. 2011). Multiple generations per year could extend the impacted area and allow for increased genetic diversity, so understanding how temperature impacts the reproductive capacity of each species is important for modeling.

For each species studied in this group, there was a high amount of variability within measurements. Horizontal lines indicate the range between high and low thresholds measured from the same study, whereas small dots are from studies that only list an upper or lower threshold. Indeed, many studies only measured up to a certain temperature (usually 25–30°C) before ending an experiment prematurely, potentially labeling the maximum temperature measured as a thermal maximum. Studies using the frameworks above, such as LD50 and CTmax, which elicit the maximum survivable temperatures, show that the maximum temperatures for all ascidians studied here are >27°C, some falling well >27°C.

Bryozoan thermal thresholds

The invasive bryozoans *Membranipora membranacea* and *B. neritina* can be found in fouling communities, but also exist in ecosystems that are less directly influenced by humans. A summary of their thermal thresholds can be found in Fig. 3. *Membranipora membranacea* is frequently found as a biofouler in kelp forests, where it grows over the kelp and leads to decreased flexibility, which causes breakage and increased mortality (Dixon et al. 1981; Saunders and Metaxas 2007; Førde et al. 2016). Kelps are an excellent aquaculture food source that could be threatened by invasive bryozoans, and kelp forest composition in the Gulf of Maine is changing from tall canopies of brown kelps to short, dense, and red algae (Witman and Lamb 2018). This species composition may change habitat function, as kelp forests are usually considered nursery habitats due to their complex structure and wave-damping properties. Furthermore, kelp aquaculture is an emerging field in the Gulf of Maine in the winter, but warming

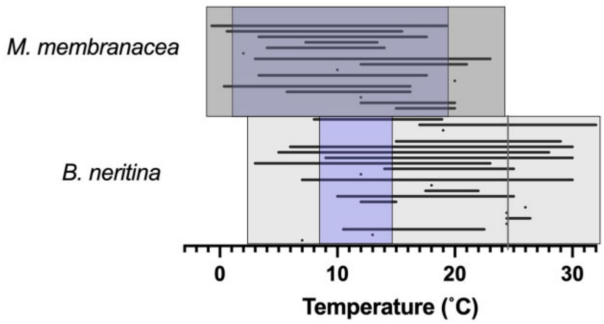

Fig. 3 Measured thermal thresholds (dots) and ranges between thresholds (lines) for the bryozoan species included in this study. *Bugula neritina* has a measured LD50 value (25.12 ± 0.89°C) and *M. membranacea* has no classical measured threshold values in the literature. Largest gray boxes indicate maximum and minimum measured thermal tolerance, smallest gray boxes indicate a reproductive threshold.

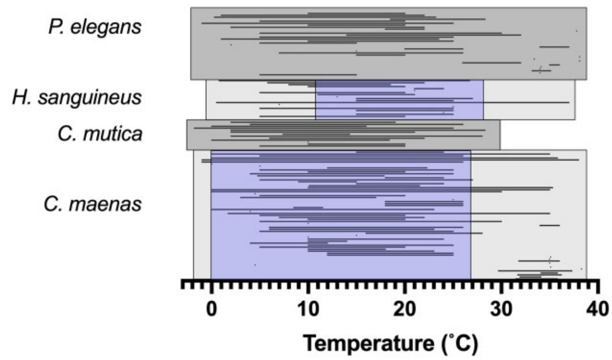

Fig. 4 Measured thermal thresholds (dots) and ranges between thresholds (lines) for the arthropod species included in this study. *Palaemon elegans* CTmax (34.08 ± 3.01°C). Large gray boxes indicate maximum and minimum measured thermal tolerance, small gray boxes indicate an estimated reproductive threshold, if one exists.

temperatures may increase biofouling risk at this time of year (Førde et al. 2016; Forbord et al. 2020).

These two species usually inhabit different parts of nearshore ecosystems. Whereas *B. neritina* is an upright bryozoan that attaches to hard structures nearshore, *M. membranacea* is found where kelps are found in subtidal regions. According to the measurements, *M. membranacea* is able to reproduce across most of its thermal range, whereas *B. neritina* has a narrower range of reproduction closer to the bottom of its thermal range. Importantly, *M. membranacea* reproduces below the minimum threshold measured by some populations, suggesting either population-level variation or vastly different measurement techniques.

Arthropod thermal thresholds

Invasive arthropods in the Gulf of Maine include *Carcinus maenas*, *Caprella mutica*, *Hemigrapsus sanguineus*, and *Palaemon elegans*. They arrived in New England through ballast water or rocks, aquaculture, and fouled equipment (McDermott 1998; Ashton et al. 2007; Edgell and Hollander 2011). *Carcinus maenas* is one of the most damaging invasive species in the Gulf of Maine, but in southern regions such as New Jersey, *H. sanguineus* has become the dominant invasive arthropod in the tide pools. These invasive species have documented impacts on nearshore ecosystems and prediction models must take thermal preferences into account for accuracy.

While arthropods have some of the widest thermal tolerances of the studied species, reproduction is a limiting factor at low temperatures, despite surviving at near-freezing temperatures (Fig. 4). One example of this is *C. mutica*, which survives down to 0°C in its native range, but in Scotland, juveniles were not present in the winter at some sampling sites despite the low temperature only reaching 7.4°C (Ashton et al. 2010). Some research suggests that marine species ranges conform to their thermal tolerance; if they can survive the temperatures in an area, they likely inhabit it (Sunday et al. 2012). However, other oceanographic factors, such as wave intensity, may limit the distribution of certain arthropods, despite temperatures well within their survivable ranges (Hampton and Griffiths 2007).

Carcinus maenas is a worldwide invader with high thermal tolerance. Despite the survival of these crabs at exceptionally high and low temperatures, different populations of crabs may struggle at middling temperatures on a physiological level. For example, *C. maenas* from Helgoland, Germany, had lower oxygen consumption rates at medium temperatures (12–21°C) compared to crabs from Cadiz, Spain (Laspoumaderes et al. 2022). However, even with the differences in oxygen consumption rates, the crabs continued to eat and grow at similar rates at increasing temperatures. As one of the better studied organisms here (66 measured temperature thresholds), population-level variation in thermal tolerance is well documented for *C. maenas*. Critical thermal thresholds range from water temperatures of 29.7–38.3°C based on haplotype and acclimation temperature.

Cnidarian thermal thresholds

The only cnidarian included in this study is *D. lineata*, who has thermal thresholds ranging from −0.6 to 30°C at the extremes (Fig. 5). Peak reproduction falls in the middle of this thermal range. Although they can survive low temperatures, they do not start growing or reproducing asexually until water temperatures reach >10°C.

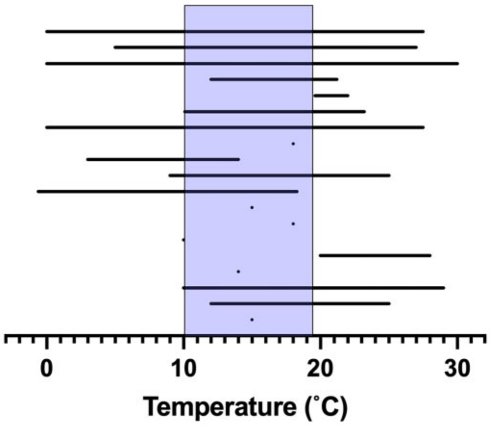

Fig. 5 Measured thermal thresholds (dots) and ranges between thresholds (lines) and ranges for *D. lineata*. Box indicates temperature range over which reproduction is possible.

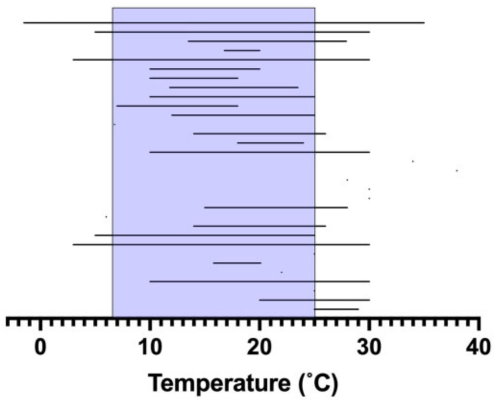

Fig. 6 Measured thermal thresholds (dots) and ranges between thresholds (lines) for *O. edulis* included LD50 measurements (34–38°C) and measurement of HSP70 expression, which begins at 25°C. Box indicates temperature range over which reproduction is possible.

Molluscan thermal thresholds

For the only invasive mollusc in this study, acclimation temperature seems to have an impact on minimum reproductive temperatures for *O. edulis* across its native range (Fig. 6). In Spain, oysters begin reproducing ∼12°C; however, in Norway, spawning onset does not occur until water temperatures reach 14°C (Bromley et al. 2016; Colsoul et al. 2021). For adult oysters reaching sexual maturity, temperature has an effect on sex ratios, where the first gametogenesis usually produces sperm, but sequential reproductions can switch between egg and sperm production and are affected by temperature (Zapata-Restrepo et al. 2019). Thus, while certain temperatures may not prove lethal to the oysters, raised temperatures may affect spawning viability for certain populations.

Which metric is most reliable and recommendations for the future?

Many of the species studied had well-defined thermal reproductive ranges for sexual reproduction and growth (Fig. 7). The widest reproductive range belongs to *C. maenas*, which reproduces year-round in the Gulf of Maine (Frederich and Lancaster 2024). The organism requiring the highest temperature for reproduction is *B. violaceus*, whereas *M. membranacea* and *C. maenas* have the lowest reproductive temperatures, just above freezing. Of importance, colony growth through budding and asexual reproduction was not included as reproduction for the purpose of this study, so all of the reproduction here is from sexual reproduction. In the growth-specific graph, *S. clava* has the narrowest range for growth and *O. edulis* has the widest. When comparing these ranges to the ranges in Fig. 1, which ranked organisms from lowest to highest upper mean thermal threshold, there is no similar pattern in reproductive or growth thresholds. In other words, understanding just the temperature range over which an organism reproduces or grows in the field does not indicate what their maximum and minimum survivable temperatures are.

One factor contributing to the broad range of reported thermal thresholds is certainly the difference between whole animal studies versus specific organ systems. Different levels of biological organization also affect thermal thresholds. Other reviews have shown the variation among taxonomic levels in thermal tolerances. Along with intra-individual variation, one study found varying thresholds among diverse insects ranging all the way down to the species level with the most variance occurring at the family level (Chown 2001). Studies have linked molecular biology to whole ecosystem functioning with regard to thermal stress across a wide range of taxa, including invertebrates and vertebrates (Pörtner 2012). Acclimation capacity of a whole organism is, in part, based on aerobic scope on a molecular level. Thermal physiology itself consists of layers, including pejus temperatures and critical temperatures, which have different levels of severity. Much of the variation in these data can be explained by looking at different levels of organization, but that does not simplify the decision for choosing the correct threshold for modeling. Here, by compiling and generalizing from diverse invasive taxa in the Gulf of Maine, we continue the narrative of the importance of studying different levels to fully understand thermal thresholds.

Frequent examples of genetic variation and local adaptation to acclimation temperatures cause different temperature thresholds in different regions. Some of the variation comes from acclimation temperature, the temperature at which the organism in question is ac-

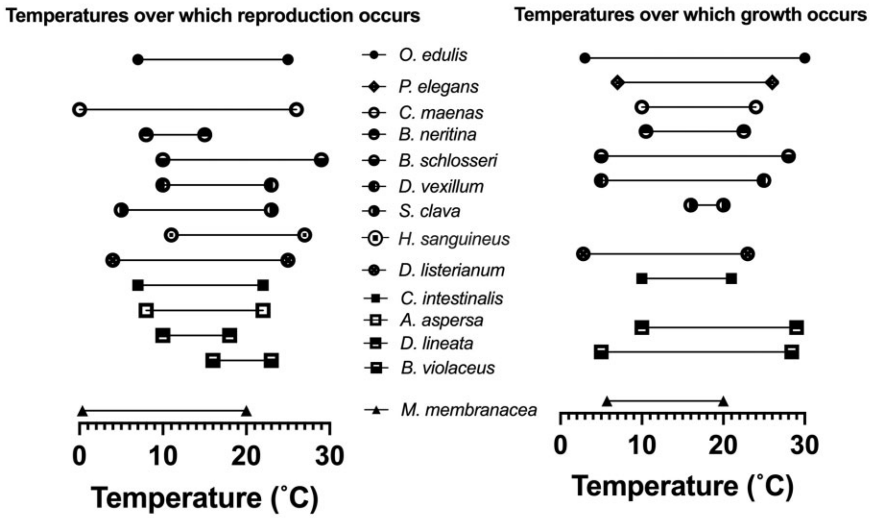

Fig. 7 A summary of the temperatures over which the organisms in this study reproduce and grow. The order of species in this figure is the same as Fig. 1, which ranks species from low to high mean high temperature. *Membranipora membranacea* has the lowest mean high thermal thresholds and *O. edulis* has the highest mean high thermal thresholds. Based on the width of reproductive and growth thermal thresholds, there is no trend between reproductive window and measured thermal thresholds. Thus, maximum, minimum, reproductive, and growth temperatures are all important in species survival in an area.

customed to in its environment. For example, an animal that lives in temperate and tropical regions has different acclimation temperatures along the gradient of its distribution. Even in different temperate regions, *P. elegans* experiences different osmoregulation capabilities at low temperatures, with Baltic Sea populations being better adapted to colder temperatures than populations near the UK (Janas and Spicer 2010).

One point for further study is air exposure, at which organisms may experience warmer temperatures than in the water. Many of these species survive in the intertidal zone, and several studies have looked at the impact of dry heat exposure on survival (see, e.g., Helmuth et al. 2010). One example for *S. clava* found that exposure at warmer temperatures (15–29°C) was more damaging than exposure at 10°C and that body size played a role in survival under these conditions (Hillock and Costello 2013). Asian shore crab *H. sanguineus* has a 4× higher metabolic rate out of the water at similar temperatures (Fletcher et al. 2022). Air temperatures in some regions may even reach lethal levels; in Australia, coastal temperatures reach >40°, which is the LT50 for *C. maenas* (Garside and Bishop 2014). Despite these temperatures, *C. maenas* is capable of evading unfavorable temperatures by moving into the shade, which tunicates are unable to achieve. The same is true for Asian shore crabs, which can change their distribution in tide pools in Long Island Sound to escape air temperatures reaching >40°C (Kraemer et al. 2007). These extreme temperatures are usually temporary, but the length of time can also affect species survival at high temperatures, which is used to prevent spread of biofoulers (Piola and Hopkins 2012). While thermal limits in air exposure are not shared in the figures above, for species distribution modeling of individuals living in the upper intertidal, these limits may be important in future work.

No matter what measurement method was used, all examples show high variance in thermal tolerance measurements within the same species. From a functional perspective, these data should only be considered useful in the region and season in which the measurement was taken. This lack of continuity has potentially alarming consequences for predicting species distribution modeling in worldwide, dynamic ecosystems. If a threshold were haphazardly chosen from one of the above studies, the conclusions may be misleading. Underestimation of potential spread could lead to invasive species outbreaks in unmonitored areas and overestimation could lead to wasted management resources.

It is important in future studies to use consistent measurement strategies with larval and juvenile forms as well. In Maine and worldwide, invasive species are impacting the livelihood of fishermen, decreasing harvestable food from the oceans, and disturbing natural habitats. With climate change predicted to increase sea surface temperatures, an understanding of thermal tolerance for all species will be important for management and mitigation at a variety of developmental stages. At the same temperature, 21°C, larvae of *H. sanguineus* have different oxygen consumption rates as they develop (Marsh et al. 2001). Under different temperatures, larvae of *C. maenas* and other species take longer to develop in colder conditions (deRivera et al. 2007). For organisms able to reproduce sexually and asexually through fragmentation or budding, winter temperatures may limit larval development but still allow settlement of fragmented adults (VKM 2023).

Of note, temperature is not the only factor that dictates species presence. For example, in Nova Scotia, it was found that neither temperature nor salinity were predictive in *C. intestinalis* distribution. Some species, such as *H. sanguineus*, may also rely on metamorphic cues from nearby adult populations, dictating metamorphosis outside of temperature cues (Anderson and Epifanio 2010). For *C. mutica* living in Scotland, some populations do not reproduce year-round despite mild temperatures, suggesting another factor limiting their reproductive success (Ashton et al. 2010). Other factors, such as heavy metal pollutants or pesticides, may influence settlement or development success for certain species in anthropogenically impacted areas (Rodrigues et al. 2015; Lange and Marshall 2017). These factors are important, as some invasions begin in harbors whose water quality is usually poor (Carlton 1996; Schiff et al. 2007). So, even for studies that include temperature, species distribution models should never negate visual surveys (Murphy et al. 2019).

Based on the present study, there is so much inconsistency in the data, and it is challenging to put species' thermal tolerance into perspective from an ecological and future projection standpoint. An integrative approach that contextualizes these thresholds will be important for the success of future models (Fig. 8). Thermal thresholds can be measured through—omics (e.g., Jane et al. 2024), population genetics, development, observation, and plasticity; each of these method measurements is valid in its own context. Understanding how growth and development are affected by temperature through in-field observations of gamete development, larval supply, settlement, and proliferation. Laboratory studies that pinpoint the maximum and minimum survivable temperatures for multiple populations, which may be outside of the bounds of ecological relevance but can inform a true upper limit to survival. Rius and colleagues do an excellent example of integrating different thresholds, showing field abundances and investigating larval development, metamorphosis, and settlement in the laboratory (Rius et al. 2014).

While physiological and metabolic signs of stress are important to understanding the mechanistic causes of organisms struggling at certain temperatures, these thresholds only provide limited information to predicting invasive range with climate change. Thus, focusing on the range in which an organism thrives enough to reproduce, grow, and remain healthy, as well as maximum and minimum survivable temperatures, reduces the minimum amount of critical information. Of course, traditional thermal thresholds do have importance on the cellular and molecular levels. Solely measuring survival in the field may gloss over physiological mechanisms and limitations that may hinder organism success. For example, at 20°C, both *H. sanguineus* and *C. maenas* survive easily in the field, but respirometry showed that *H. sanguineus* respiratory rate per gram is twofold higher at higher temperatures, suggesting a greater energetic cost to fill basic survival needs (Jungblut et al. 2018). Newer studies are focusing on transcriptomics and other large datasets to focus on changes in gene expression across life stages (Jane et al. 2024). In the American lobster, *Homarus americanus*, gene expression of DnaJ homologues and other proteins varied across the four pelagic larval stages with heat and UV exposure, suggesting a molecular response to stabilize proteins across ontology. By integrating across taxa and measurement techniques, a fuller understanding of thermal limits to invasion can be developed.

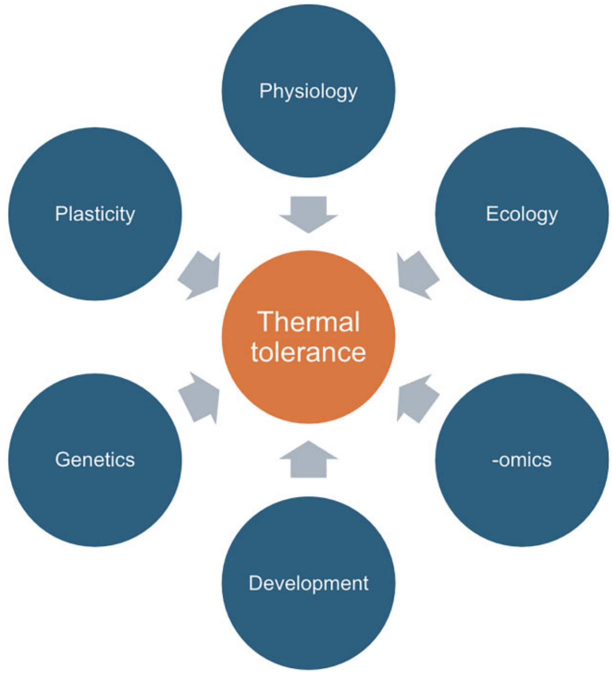

Fig. 8 Conceptual model outlining the contextualization required for modeling species distribution based solely on thermal thresholds. Measurements of thermal tolerances are not straightforward, integrating plasticity, physiology, ecology, omics, development, and genetics in a complex organism. Each of these metric factors into an organism's thermal tolerance, shifting thermal thresholds. As shown in this study, measurements of thermal thresholds without context cannot be equally applied to modeling due to variability.

The findings here apply beyond the Gulf of Maine. Invasive species (including some from the present study) threaten ecosystems in San Francisco Bay and have the opportunity to have economic impacts in the area as they disrupt fisheries (Cohen et al. 1995). There are at least 213 non-native marine and coastal species in China as of 2017, half of which are known to cause harm to ecosystems and economies (Xiong et al. 2017). In the East China Sea, researchers are using environmental DNA to understand invasion severity of *Sciaenops ocellatus*, among other invasive species (Wang et al. 2022). As the oceans continue to warm, studies show that prevalence of native species in fouling communities will decrease and invasive species will increase (Sorte et al. 2010).

Although oceans are generally warming, lower thermal thresholds should not be overlooked. Bugula neritina, *C. intestinalis*, and *M. membranacea* have been seen near Antarctica or are predicted to invade (Convey and Peck 2019; Avila et al. 2020). Though they have not established populations, invasions of Antarctica become more likely with climate change (Convey and Peck 2019; McCarthy et al. 2019; Holland et al. 2021). One area of particular concern is the Antarctic shelf, which is under threat of encroaching lithoid crabs and currently has no crushing predators (Smith et al. 2012; Aronson et al. 2015). These invasions are especially alarming when considering the current food web of the Antarctic Shelf, which currently has no durophagous, crushing predators, so the introduction of a crab such as *C. maenas* would be highly destructive. Without the evolutionary history of a shell-crushing predator, many organisms evolved to have soft bodies or thin shells. Their ability to survive at very low temperatures poses *C. maenas* as an excellent candidate to negatively impact the existing ecosystem (Frederich and Lancaster 2024). Due to anticipated climate changes and increased connectivity between continents, knowledge of thermal tolerance for invasive species is important to generate species distribution models, which could inform management strategies. These invasions are especially alarming when considering the current food web of the Antarctic Shelf, which currently has no durophagous, crushing predators, so the introduction of a crab such as *C. maenas* would be highly destructive (Aronson et al. 2015). Without the evolutionary history of a shell-crushing predator, many organisms evolved to have soft bodies or thin shells. Their ability to survive at very low temperatures poses C. maenas as an excellent candidate to negatively impact the existing ecosystem (Frederich and Lancaster 2024). This whole ecosystem will likely be threatened by crustaceans in the near future (Smith et al. 2012).

Here, we highlight inconsistencies in thermal performance measurements and uncertainties in thermal thresholds. A more integrative approach combining field and laboratory studies to capture the physiology as well as the ecology is required to forecast invasion probability. This study highlights the complexities of thermal thresholds and underscores the pivotal role of acclimation temperatures and consistent measurement techniques. All of the measured thresholds are valid in their own context, but we caution against arbitrarily selecting one threshold for species modeling. As our world warms and we continue influencing ecosystems, these models will become increasingly important, and threshold uncertainty will weaken their predictive power. By visualizing the existing data together, we highlight the importance for contextualizing thermal thresholds, ensuring we capture the nuanced intricacies of potential invasive species spread in a changing climate.

Author contributions

E.R.L.: Conceptualization, Methodology, Software, Validation, Investigation, Data Curation, Writing, Visualization.

D.C.B.: Methodology, Software, Validation, Resources, Review and editing.

M.F.: Conceptualization, Methodology, Validation, Investigation, Resources, Review and editing, Visualization.

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Supplementary data

Supplementary data available at ICB online.

Conflict of interest

The authors declare no conflicts of interest.

Data availability

All data analyzed in this study are available in the supplementary materials.

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