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Shell Shocked: A Peak into the Thermal Tolerance and Shell Utilization of *Clibanarius erythropus*

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Mestrado em Biologia Marinha

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Declaração de autoria de trabalho

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Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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Abstract

Thermal tolerance of species has become a topic of increasing interest due to climate change and global warming. This type of data is important for identifying vulnerable species and establishing management strategies. In this study, the thermal tolerance and shell utilization were investigated for the Mediterranean intertidal hermit crab, *Clibanarius erythropus* in the fast warming southeastern Mediterranean Sea. The thermal tolerance was determined using two methods 1) the critical thermal maximum (CTMax) and 2) physiological performance, with the change in respiration rates used as a performance parameter. Additionally, CTMax was tested for three other intertidal species: the crab *Eriphia verrucosa,* topshell snail *Phorcus turbinatus*, and snail *Pisania striata* in order to make a comparison between the hermit crab and other members of its habitat. It was hypothesized that *C. erythropus* will have a higher thermal tolerance than the other species in their habitat, and that the size of the hermit crab will correlate with the size of the occupied shell. As expected, the CTMax of *C. erythropus* (38.8 °C) was found to be higher than the other species. This was followed by *E. verrucosa* at 37.93 °C, *P. striata* at 37.48 °C, and *P. turbinatus* which had a considerably lower value at 34.19 °C. Additionally, *C. erythropus* was the only species that was found to have a positive thermal safety margin (TSM). The weight of the occupied shell was found to have a significant correlation with the size of the hermit crab. These findings indicate that this species of hermit crab may be better equipped to handle continuous regional warming and intensification of extreme events than the other tested native species. Future work should focus also on other proxies for thermal performance such as degree of mobility and juvenile thermal tolerance.

Resumo

As alterações climáticas têm sido alvo de grande preocupação no seio da comunidade científica global. Alguns cientistas argumentam que as condições ambientais em algumas comunidades estão a mudar antes que tenhamos tempo para estabelecer uma compreensão firme da linha de base. Apesar dos inúmeros estudos destinados a melhorar a nossa compreensão do planeta, existe um consenso de que são necessários mais esforços. Esta informação é imprescindível para obter uma avaliação abrangente do nosso estado atual e para nos equiparmos com as ferramentas necessárias para prever a nossa trajetória futura. As alterações climáticas são um desafio dinâmico e multifacetado que exige investigação contínua, colaboração internacional e

esforços concertados para mitigar os seus impactos. O desenvolvimento de uma sólida compreensão do estado atual do ambiente e a utilização de ferramentas preditivas são componentes essenciais para abordar esta questão crítica e moldar um futuro mais sustentável.

A temperatura é um dos fatores bióticos mais influentes nos ecossistemas. Pode modificar profundamente a atividade, o crescimento e a reprodução de um indivíduo, afetando em última análise a distribuição global, a fenologia e a estrutura da cadeia alimentar de uma espécie. Existem muitos habitats que hospedam organismos que vivem já em condições onde um ligeiro aumento de temperatura afetaria negativamente a sua performance. A "tolerância térmica", os "limites térmicos" e a "margem de segurança térmica" servem como medidas que permitem aos cientistas discernir quais as espécies que são mais susceptíveis aos desafios colocados pelas alterações climáticas. A margem de segurança térmica é uma medida que explica o grau de vulnerabilidade que uma espécie pode enfrentar e dependerá, em última análise, dos seus limites térmicos, resposta de aclimatação e potencial de adaptação genética. No entanto, estes dados ainda são em grande parte desconhecidos para a maioria das espécies.

Entre os habitats suscetíveis de serem afetados mais cedo por um aumento da temperatura encontra-se a zona entre-marés, situada nas margens entre os reinos terrestre e marinho. As espécies que habitam esta zona experimentam mudanças fortes, e por vezes extremas, nas condições ambientais. Por conseguinte, os organismos intertidais podem estar mais bem adaptados e ser mais resilientes às alterações ambientais que se prevê que ocorram devido às alterações climáticas. No entanto, é importante notar que estes organismos podem já estar a viver em temperaturas iguais ou mesmo acima do seu ótimo térmico. Mudanças na distribuição global e eventos de mortalidade em massa têm sido documentados entre espécies entremarés que exemplificam a pressão térmica que alguns desses organismos estão sofrendo.

Este estudo centra-se no caranguejo eremita intertidal mediterrânico, *Clibanarius erythropus* (Latreille, 1818) como um esforço para obter informações sobre a ecologia e tolerância térmica de uma espécie pouco estudada ao longo da costa israelita. Um dos objetivos foi explorar a relação entre o tamanho do caranguejo e o tamanho da concha de uma população de *C. erythropus* intertidais medindo o comprimento e o peso de uma amostra de caranguejos em referência ao comprimento e peso de suas conchas ocupadas. O comprimento foi medido com aproximação de 0,01 cm e o peso com aproximação de 0,001 g. Outro objetivo foi testar a tolerância térmica desta espécie através de várias experiências. Um experimento foi projetado para investigar o desempenho térmico, medindo a mudança na taxa metabólica de *C.*

erythropus quando submetido a uma gama de temperaturas da água. Este foi testado a curto prazo (6,5 dias) e a longo prazo (13,5 dias) através da simulação de uma gama de condições de temperatura num mesocosmo ao ar livre no Instituto de Investigação Oceanográfica e Limnológica de Israel (IOLR). Um experimento final utilizou o método dinâmico para avaliar e comparar o CTMax de *C. erythropus* com três outras espécies (*Eriphia verrucosa, Phorcus turbinatus* e *Pisania striata*) que podem ser encontradas dentro da zona entremarés israelense. Supõe-se que o tamanho dos caranguejos afetará o tamanho das conchas que eles escolherem habitar. Supõe-se também que estes caranguejos eremitas terão uma maior tolerância térmica e estarão mais bem equipados para suportar o aumento das temperaturas oceânicas do que outras espécies dentro do mesmo habitat.

Como esperado, o CTMax de C. erythropus (38,8 °C) foi superior ao das demais espécies. Isto foi seguido por E. verrucosa a 37,93 °C e depois P. striata a 37,48 °C, que apresentaram valores de CTMax semelhantes, enquanto P. turbinatus teve o valor mais baixo com 34,19 °C. Além disso, C. erythropus foi a única espécie que apresentou margem de segurança térmica (TSM) positiva, quando apresentada com temperaturas máximas de habitat de 38 °C. Descobriu-se que o peso da concha ocupada tinha uma correlação significativa com o tamanho do caranguejo eremita, mas o comprimento da concha não tinha uma correlação significativa. Embora exista uma correlação significativa entre o peso da concha e o tamanho do respectivo caranguejo as regressões lineares não são bem ajustadas o que pode indicar que existem outros factores que influenciam a selecção das conchas. Estas descobertas indicam que esta espécie de caranguejo eremita pode estar mais bem equipada para lidar com o aquecimento regional contínuo e a intensificação de eventos extremos, do que outras espécies com as quais partilha o habitat. No entanto, existem também outros factores (por exemplo, grau de mobilidade e tolerância térmica juvenil) que também podem influenciar o seu sucesso. Embora o aumento da temperatura da superfície do mar seja um tema crescente de discussão, o mínimo térmico crítico também deve ser explorado para estas espécies, em estudos futuros, uma vez que as temperaturas das poças de maré isoladas podem cair consideravelmente durante as noites frias, causando stress pelo frio e até mortalidade. Além disso, mais pesquisas devem ser feitas para testar a tolerância térmica de indivíduos que variam em estágios de desenvolvimento e zonas de maré (por exemplo, intertidal vs. subtidal), uma vez que os valores CTMax deste estudo podem não refletir uma análise abrangente de cada espécie devido ao teste exclusivo de indivíduos adultos entremarés.

Keywords:

Climate change; thermal tolerance; *Clibanarius erythropus;* rocky intertidal

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accomplishments are merely a reflection of those who helped us along the way, and for this collective, I am eternally grateful.

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List of abbreviations, acronyms, and symbols

CO_2- carbon dioxide

CTMax- Critical Thermal Maximum

EMED- eastern Mediterranean

IPCC- International Panel on Climate Change

LB- Levantine basin

MHT- maximum habitat temperature

MO_2- mean oxygen consumption rate

pCO_2- partial pressure of carbon dioxide

pH- potential of hydrogen

SMR- standard metabolic rate

SST- sea surface temperature

TSM- thermal safety margin

UK- United Kingdom

Chapter 1- General Introduction

1.1 Climate change in the world and Mediterranean

The most recent International Panel on Climate Change (2023) report warns that "every region in the world is projected to face further increases in climate hazards". It is predicted that shifts in the average levels of pCO_2, temperature, pH, and precipitation will occur (Zamir *et al.*, 2018). Increased warming of the world's oceans seems to be an unavoidable outcome in the near term (2021-2040) since cumulative CO_2 emissions are predicted to increase in nearly all considered scenarios (IPCC, 2023). Ocean warming is one major driver of change to marine life as temperature affects all biological and chemical processes (Madeira *et al.*, 2012; Rilov *et al.*, 2022). Under these changing conditions, it is important to identify which ecosystems and species are more vulnerable (Duarte *et al.*, 2011; Vinagre *et al.*, 2016). Marine ectotherms are especially susceptible to the threat of temperature changes since they are unable to regulate their body temperature physiologically (van der Walt *et al.*, 2021). Anthropogenic induced anomalies in temperature patterns are already apparent in many marine ecosystems around the world, resulting in distributional, physiological, and behavioral shifts for marine ectotherms (van der Walt *et al.*, 2021) or, in extreme cases, death (Coll *et al.*, 2010; Yeruham *et al.*, 2015; Garrabou *et al.*, 2022; Patterson & Early, 2022).

The Mediterranean Sea has been regarded as one of the leading hotspots for climate change (Rilov *et al.*, 2022) and several mass mortality events have already been recorded for marine biota in the region (Coll *et al.*, 2010; Rilov *et al.*, 2020; Garrabou *et al.*, 2022), primarily due to its exceptionally high ocean warming rates (Amsalem and Rilov, 2021). Accordingly, Yeruham et al. (2019) refers to the Mediterranean as "one of the most heavily compromised marine systems on earth". The eastern Mediterranean (EMED) swiftly responds to atmospheric variability and is inherently extreme since it hosts the hottest, saltiest, and least productive conditions (Coll *et al.*, 2010). Pastor et al. (2020) found that EMED was the sub-basin which displayed the highest warming trend between their study period of the years 1982-2019 (Figure 1.1). The southeastern basin of the Mediterranean, also known as the Levantine basin (LB), has already seen an increase of 2-3°C in sea surface temperature (SST) over the past three decades (Yeruham *et al.*, 2019; Amsalem and Rilov, 2021). The LB is considered the southeastern (trailing) edge of distribution for most Mediterranean and Atlanto-Mediterranean species

(Rilov *et al.*, 2019; Rilov *et al.*, 2020). As a result, this basin can serve as a suitable natural laboratory since it may be considered a relevant model of climate change hotspots in the world's oceans (Coll *et al.*, 2010; Amsalem and Rilov, 2021; Garrabou *et al.*, 2022) and could thus provide insights into ecosystem resilience (Rilov *et al.*, 2022).

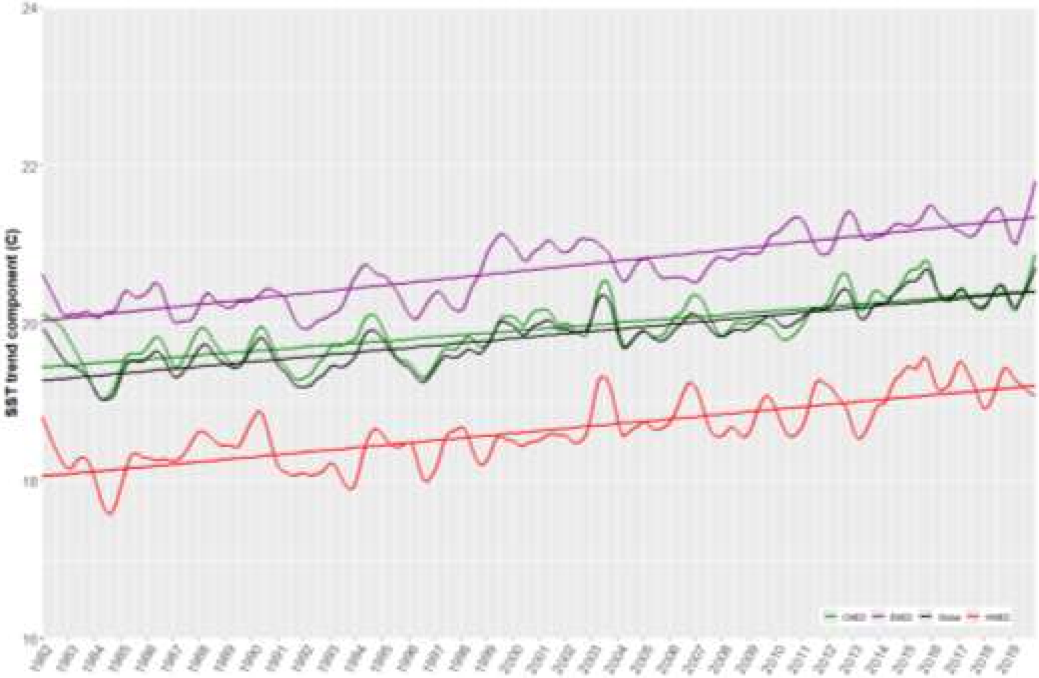

Figure 1.1- Deseasonalized SST trend component and linear regression (at 99% confidence level) for 1982-2019 for the global Mediterranean basin (black), WMED (red), CMED (green), and EMED (magenta). Adapted from Pastor et al., 2020.

1.2 Climate change impacts on intertidal ecosystems

Climate change is a global issue that will impact every habitat, but the intertidal zone is a marine habitat that will likely be the first to experience changes (Vinagre *et al.*, 2016). The intertidal zone exists in the space between terrestrial and marine environments, meaning that organisms who are part of this community are prone to experiencing strong and fast thermal fluctuations in both water and air temperatures because of the changing tides and wave action (Benvenuto & Gherartdi, 2001; Madeira *et al.*, 2012; Vinagre *et al.*, 2016; Amsalem and Rilov, 2021; Rilov *et al.*, 2022). As a result, these organisms seem to have evolved the ability to cope with thermal shock (Vinagre *et al.*, 2015; Benvenuto & Gherartdi, 2001). However, long-term monitoring reveals that climate change has already caused abnormal fluctuations in ecological patterns within rocky intertidal systems, indicating that some intertidal species may struggle to adapt to shifts in their environment's thermal range (Livore *et al.*, 2021). Moreover, the potential escalation of the occurrence in extreme intertidal desiccation events, driven by climate-induced alterations in temperature and wind patterns, may pose an additional risk to this ecosystem (Zamir *et al.*, 2018).

1.3 Hermit crab ecology

Hermit crabs are one group that has achieved remarkable success in the intertidal zone by adapting their biology and behavior to the characteristics of this habitat (Warburg & Shuchman, 1984; Benvenuto & Gherartdi, 2001). Many hermit crab species are omnivorous detritivores and nutrient availability is suggested to rarely be a limiting factor, even among closely related sympatric species (Hazlett, 1981). These decapod crustaceans, belonging to the Anomura clade, are typically characterized by their asymmetrically coiled, soft abdomen (pleon) which they protect using a hallow object (Tsang *et al.*, 2011). While various objects can be used, most hermit crab species prefer to occupy vacant gastropod shells (Botelho & Costa, 2000; Williams and McDermott, 2004; Caruso & Chemello, 2009), meaning that they are dependent upon gastropod mortality in their habitat (Hazlett, 1981; Benvenuto & Gherartdi, 2001; Tricarico *et al.*, 2009). This type of shelter use provides both mobility and protection which is likely why a large number of these crabs can be found in nearly all marine environments as well as tropical terrestrial coastlines (Hazlett, 1981). Crabs can acquire and transfer between shells (Figure 1.2) through various modalities (e.g. shell fighting or bargaining), but most often they find new shells by following odor cues to gastropod predation sites (Tricarico *et al.*, 2009). Additionally, hermit crabs are considered ecosystem engineers because their utilization of gastropod shells serves as a substrate for a diverse assembly of invertebrates, thus impacting the abundance and distribution of these associated species (Williams and McDermott, 2004).

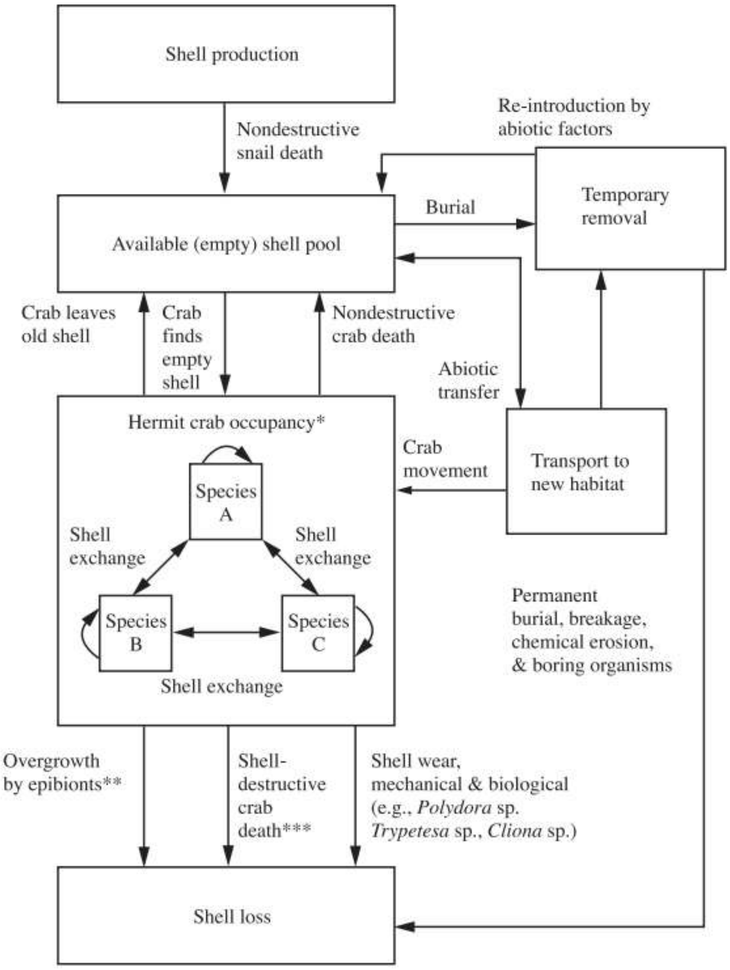

Figure 1.2- Flowchart showing shell movement in hermit crab populations along with the impacts of symbionts noted. Adapted from Williams and McDermott, 2004.

1.4 *Clibanarius erythropus*

Figure 1.3- Image of *C. erythropus* morphology, showing secondary loss of the abdominal exoskeleton and bilateral symmetry. Adapted from Dressler et al. 2021.

This study focuses on the Mediterranean intertidal hermit crab, *Clibanarius erythropus* (Latreille, 1818) (Figure 1.3). This hermit crab is known to inhabit intertidal to shallow waters (Tricarico *et al.*, 2009) within the Mediterranean Sea, Black Sea, and Atlantic coast, from Brittany to the Azores (Botelho & Costa, 2000; Benvenuto & Gherartdi, 2001). However, in 2016, the species was also found to have expanded up into the southwest UK (Patterson & Early, 2022). *C. erythropus* can be found in the high intertidal, meaning that at low tide these individuals can be exposed to severe conditions (Hazlett, 1981). Such exposure is hypothesized to have an effect on their tolerance to drastic temperature changes and influence seasonal migrations to more favorable habitats (Warburg & Shuchman, 1984).

Although this species is widely spread, highly abundant, and easily accessible, there is a considerable lack of information on the biology and ecology of this particular species of hermit crab (Gherartdi, 1990; Botelho & Costa, 2000; Benvenuto & Gherartdi, 2001; Caruso & Chemello, 2009), especially in the EMED, where data on this species is virtually non-existent (personal observation from literature review). My observations found that *C. erythropus* occupies gastropod shells of various shapes, belonging to different species (Figure 1.4). There have been a handful of studies conducted on *C. erythropus* populations in Italy (Gherartdi, 1990; Caruso & Chemello, 2009; Tricarico *et al.*, 2009), France (Dressler *et al.,* 2021), the Azores (Botelho & Costa, 2000), and the UK

Figure 1.4- Image of the diversity in gastropod shells used by a population of *C. erythropus* located north of Shikmona Marine Reserve*;(a) Cerithium scabridum (b) Pisania striata (c) Ergalatax junionae (d) Conomurex persicus (e) Phorcus (f) Phorcus (g) Naticarius hebraeus*. Images from marinespecies.org.

(Patterson & Early, 2022). Additionally, there was a single study (Warburg & Shuchman, 1984) that investigated the thermal response of *C. erythropus* from the Israeli coast along an artificial thermal gradient in the lab, concluding that hermit crab activity decreased with increasing temperatures. However, there is an absence of publications regarding the general biology or ecology of this species for this area of the world.

1.5 Thermal tolerance

Due to climate change and global warming, thermal tolerance has become a topic of increasing interest in recent decades (e.g. Madeira *et al*., 2012; Rezende *et al.* 2014; Vinagre *et al.* 2015; Vinagre *et al*., 2019; Amsalem and Rilov, 2021; van der Walt *et al*., 2021; Rilov *et al.*, 2022). The thermal tolerance of a species is ecologically important information given that temperature largely influences species' global distribution, phenology, and food web structure (Walther *et al*., 2002; Madeira *et al*., 2012; Amsalem and Rilov, 2021; Rilov *et al.*, 2022). Understanding the thermal limits of a species can help to evaluate local vulnerability or resilience, in the context of climate change (van der Walt *et al*., 2021) and make projections about their fate in the face of continuous warming under different scenarios. Even though this information is the foundation for understanding the effects of climate change, the thermal tolerance of most species is still largely unknown (Vinagre *et al.* 2015).

The studies that have been conducted on thermal tolerance reveal distinct trends based on latitudinal group and organism size. Tropical species tend to exhibit greater heat tolerance than their temperate counterparts, yet they are also generally at a higher risk of potential temperature increases since most tropical species may already be living close to their upper thermal limits (Somero, 2010). Notably, variations tend to exist in the thermal tolerance within species (e.g. intraspecific differences at a physiological and molecular level) and this is important to take into account when considering how the different cohorts of a species may react to the same change in temperature (Madiera *et al*., 2012). Somero (2010) also explains that size is shown to have a significant effect on the intraspecific differences of thermal tolerance, highlighting that smaller individuals generally show a greater tolerance to heat than their larger counterparts. Nevertheless, further studies are needed, and more investigations should be done on the physiological responses of a species to both acute and chronic thermal stress instead of looking at the two responses in isolation (Magozzi and Calosi, 2014). There are various ways to assess an organism's thermal vulnerability, but two common sublethal methods are: 1) Critical Thermal Maximum (CTMax) and 2) metabolic rate analysis across a range of temperatures.

CTMax is a dynamic method in which the temperature of an experimental container (e.g., a bath) is increased at a stable rate, until the organisms reach a critical point where it loses some critical functions (Mora & Ospina, 2001; Vinagre *et al.* 2015; van der Walt *et al*., 2021). It is a sublethal method that defines the limits of the ability of a species to live under extreme acute thermal events (Magozzi and Calosi, 2014). This assessment provides a more conservative upper thermal limit since it reflects temperatures at which organisms typically lose equilibrium and cannot escape predators or forage for food, but it does not stretch the range to include temperatures at which organisms experience mortalities (Vinagre *et al*., 2015).

Metabolic rates are sensitive to temperature and can be determined by using respiration rates as a proxy (Rilov *et al.*, 2022). This method can reflect the energetic costs that are placed on a species when it experiences a particular thermal environment (Magozzi and Calosi, 2014). Typically, the rate of metabolism will increase as temperature increases and then it will rapidly decline at higher temperatures (Schulte, 2015). This method is often used in stress ecology since it can be a good indicator of an organism's physiological state (Rilov *et al.*, 2022) and display the resilience of a species when placed under chronic exposure to warming (Magozzi and Calosi, 2014).

1.6 Relevance of the study and its application

This study is intended to add to the scientific knowledge on the thermal vulnerability of marine organisms living in a climate change hotspot under the present and future effects of climate change. Such information is essential for conservation and management purposes since it will contribute to the understanding of how these species may be responding to climate change. This study will focus primarily on an intertidal population of the species *Clibanarius erythropus*, along the Israeli coast. However, the CTMax of three other intertidal species (*Eriphia verrucosa, Phorcus turbinatus,* and *Pisania striata*) were tested as well so that a their thermal responses could be compared among community members found in this zone.

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Chapter 2- Manuscript

Shell Shocked: A Peak into the Thermal Tolerance and Shell Utilization of *Clibanarius erythropus*

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Keywords
climate change; thermal tolerance; *Clibanarius erythropus*; rocky intertidal, shell utilization

Abstract

Thermal tolerance of species has become a topic of increasing interest due to climate change and global warming. This type of data is important for identifying vulnerable species and establishing management strategies. In this study, the thermal tolerance and shell utilization were investigated for the Mediterranean intertidal hermit crab, *Clibanarius erythropus* in the fast warming southeastern Mediterranean Sea. The thermal tolerance was determined using two methods 1) the critical thermal maximum (CTMax) and 2) physiological performance, with the change in respiration rates used as a performance parameter. Additionally, CTMax was tested for three other intertidal species: the crab *Eriphia verrucosa,* topshell snail *Phorcus turbinatus*, and snail *Pisania striata* in order to make a comparison between the hermit crab and other members of its habitat. It was hypothesized that *C. erythropus* will have a higher thermal tolerance than the other species in their habitat, and that the size of the hermit crab will correlate with the size of the occupied shell. As expected, the CTMax of *C. erythropus* (38.8 °C) was found to be higher than the other species. This was followed by *E. verrucosa* at 37.93 °C, *P. striata* at 37.48 °C, and *P. turbinatus* which had a considerably lower value at 34.19 °C. Additionally, *C. erythropus* was the only species that was found to have a positive thermal safety margin (TSM). The weight of the occupied shell was found to have a significant correlation with the size of the hermit crab. These findings indicate that this species of hermit crab may be better equipped to handle continuous regional warming and intensification of extreme events than the other tested native species. Future work should focus also on other proxies for thermal performance such as degree of mobility and juvenile thermal tolerance.

2.1 Introduction

Due to climate change and global warming, thermal tolerance of different organisms has become a topic of increasing interest in recent decades (e.g. Madeira *et al*., 2012; Rezende *et al.* 2014; Vinagre *et al.* 2015; Yeruham *et al*., 2015; Madeira *et al*., 2018; Vinagre *et al*., 2019; Amsalem and Rilov, 2021; van der Walt *et al*., 2021; Rilov *et al*., 2022). ". Increased warming of the world's oceans is an unavoidable outcome in the near term (2021-2040) since cumulative CO_2 emissions are predicted to increase in nearly all considered scenarios (IPCC, 2023). The most recent IPCC (2023) report warns that "every region in the world is projected to face further increases in climate hazards".

The thermal tolerance of a species is ecologically important information given that temperature largely influences species' global distribution, phenology, and food web structure (Walther *et al.*, 2002; Madeira *et al.*, 2012; Amsalem and Rilov, 2021; Rilov *et al.*, 2022). Understanding the thermal limits of a species can help to evaluate local vulnerability or resilience, in the context of climate change (van der Walt *et al.*, 2021). Even though this information is the foundation for understanding the effects of climate change, the thermal tolerance of most species is still largely unknown (Vinagre *et al.* 2015). There are various ways to assess an organism's thermal vulnerability, but two common sublethal methods are: 1) Critical Thermal Maximum (CTMax) and 2) thermal performance evaluation under a range of temperatures where metabolic rate is one of the most important performance measures. CTMax is a dynamic method in which the temperature of a bath is increased, at a stable rate, until the organisms reach a critical point where it loses equilibrium that hampers its functioning, but does not reach temperatures that lead to mortality (Mora & Ospina, 2001; Vinagre *et al.* 2015; van der Walt *et al.*, 2021). Measurement of metabolic rates across a range of temperatures including lethal ones can be determined by using respiration rates as a proxy (Rilov *et al.*, 2022). Typically, the rate of metabolism will increase as temperature increases and then it will rapidly decline at higher temperatures (Schulte, 2015). This method is often used in stress ecology since it can be a good indicator of an organism's physiological state (Rilov *et al.*, 2022).

Climate warming will impact every marine habitat, but the rocky intertidal might be one of the most vulnerable ecosystems (Vinagre *et al.* 2016) because intertidal organisms are exposed to the full force of climate change impacts in both the air and shallow water mediums. However, because such organisms are experiencing strong and fast thermal fluctuations in both water and air temperatures (Benvenuto & Gherartdi, 2001; Madeira *et al.*, 2012; Vinagre *et al.* 2016; Madeira *et al.*, 2018; Amsalem and Rilov, 2021; Rilov *et al.*, 2022) these organisms seem to have evolved the ability to cope with thermal shock better than other marine organisms (Vinagre *et al.* 2015; Madeira *et al.*, 2018). Nonetheless, they can also be exposed today to extreme temperatures close to their thermal limits and crossing them with additional warming may put them in danger.

This study aims to test the thermal vulnerability of rocky intertidal hermit crabs on the southeastern Mediterranean coast, an understudied group in the context of climate change impacts. The Mediterranean Sea has been regarded as one of the leading hotspots for climate change (Rilov *et al.*, 2022) and the collapse of thermally sensitive species as well as several mass mortality events have already been recorded for marine biota in the region (Coll *et al.*, 2010; Garrabou *et al.*, 2022), primarily due to its exceptionally high ocean warming rates (Amsalem and Rilov, 2021). The eastern Mediterranean, and specifically the Levantine basin, is the fastest warming region (Pastor *et al.*, 2020) as it swiftly responds to atmospheric variability and thus is inherently extreme, boasting the hottest, saltiest, and least productive conditions in the Mediterranean Sea (Coll *et al.*, 2010). As a result, this basin can serve as a suitable natural laboratory since it may be considered a relevant model of the world's oceans (Coll *et al.*, 2010; Amsalem and Rilov, 2021; Garrabou *et al.*, 2022) and could provide insight into ecosystem resilience in climate change hotspots (Rilov *et al.*, 2022).

Among rocky intertidal and tidepool species, hermit crabs are one group that have achieved remarkable success in this zone by adapting their biology and behavior to the characteristics of this habitat (Warburg & Shuchman, 1984; Benvenuto & Gherartdi, 2001). Many hermit crab species are omnivorous detritivores and nutrient availability is suggested to rarely be a limiting factor, even among closely related sympatric species (Hazlett, 1981). These decapod crustaceans, belonging to the Anomura clade, are typically characterized by their asymmetrically coiled, soft abdomen (pleon) which they protect using a hallow object (Tsang *et al.*, 2011). While various objects can be used, most hermit crab species prefer to occupy vacant gastropod shells (Botelho & Costa, 2000; Caruso & Chemello, 2009), meaning that they are dependent upon the gastropods in their habitat (Benvenuto & Gherartdi, 2001).

We use the Mediterranean intertidal hermit crab, *Clibanarius erythropus* (Latreille, 1818) as our test species. This hermit crab is known to inhabit the Mediterranean Sea, Black Sea, and Atlantic coast, from Brittany to the Azores (Botelho & Costa, 2000; Benvenuto & Gherartdi, 2001). However, in 2016, the species was also found to have expanded up into the southwest UK (Patterson & Early, 2022). There is a significant lack of information on the biology and ecology of this species of hermit crab (Gherartdi, 1990; Botelho & Costa, 2000; Benvenuto & Gherartdi, 2001; Caruso & Chemello, 2009), especially in the eastern Mediterranean where it was found to occupy gastropod shells of various shapes (personal observation along the Israeli coast). *C. erythropus* populations have been studied in Italy (Gherartdi, 1990; Caruso & Chemello, 2009; Tricarico *et al.*, 2009), France (Dressler *et al.,* 2021), the Azores (Botelho & Costa, 2000), and the UK (Patterson & Early, 2022). Additionally, there was a single study (Warburg & Shuchman, 1984) that investigated the thermal response of *C. erythropus* from the

Israeli coast on a temperature gradient produced in a water tank, generally concluding that hermit crab activity decreased with increasing temperatures.

This study aimed to I) examine the correlation between crab size and shell size in an intertidal population of *C. erythropus*, II) produce a thermal performance curve (TPC) for the species analyzing the metabolic rate response of *C. erythropus* to a range of water temperatures, and III) evaluate acute thermal performance by determining the CTMax for *C. erythropus* in comparison to other species within the Israeli intertidal zone. It was hypothesized that hermit crabs will be better equipped to withstand increasing ocean temperatures than other species within the same habitat.

2.2 Materials and methods

2.2.1 Sampling and maintenance

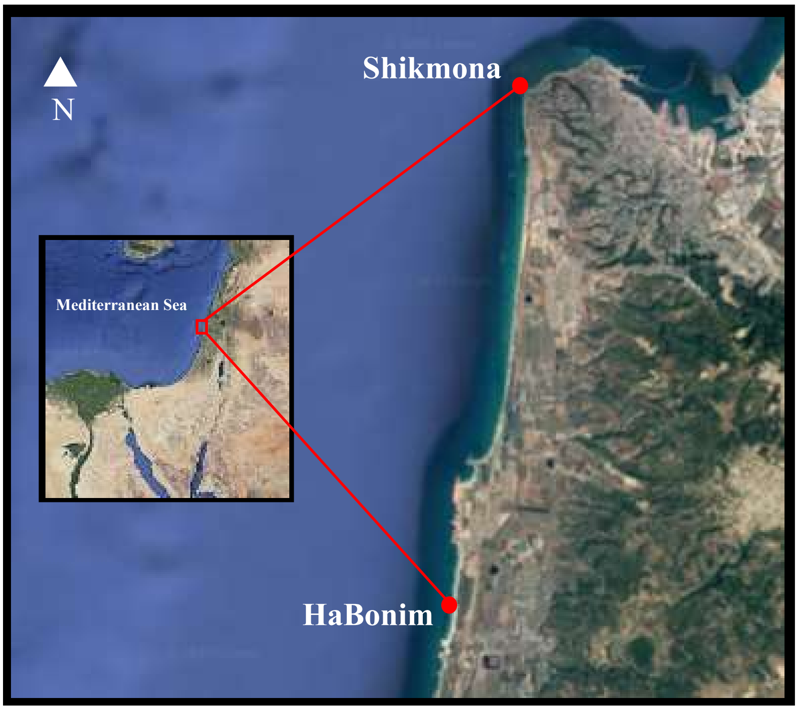

Fig. 1- Map of the study area, showing sampling sites. Images from GoogleEarth, version 2023.

In February of 2023, adult individuals of *C. erythropus* were collected, by hand, for a TPC metablic rate experiment. These hermit crabs were collected from the tide pools found just north of Shikmona Marine Reserve (32.8252014 N, 34.9554569 E), Haifa, Israel (Figure 1) and kept in an indoor flow through 2,000 L tank for 3 days (d) before they were individually placed into 300 mL glass jars (5 replicate jars per temperature treatment) within a custom-made outdoor thermobath microcosm system described in detail by Guy-Haim et al. (2016). This outdoor microcosm allowed for natural photoperiodicity and controlled temperatures. A NOVUS N1020 temperature controller (±1.5 °C) was used for independent control of the temperature within each thermobath. Air tubes were placed into each tank and every 24 h, 50%-100% of the water was replaced manually in each jar with filtered (5-10 micron) Mediterranean seawater, which was pumped from 1 m depth. Prior to the beginning of the experiment, the hermit crabs were acclimated to the microcosm for 7 d at the average ambient nearshore temperature (17 °C), for the middle of February 2023, along the Shikmona shore. The water conditions within the indoor flow through tanks and the experimental microcosm followed the natural fluctuations occurring in the site of collection. *C. erythropus* were fed a pellet of Tropical Crusta Sticks, crustacean food, every other day and allowed to feed ad libidum, for the duration of their 10-day acclimation period.

In May of 2023, a second sampling effort was conducted for the CTMax experiments. Adult individuals of *C. erythropus,* the topshell snail *Phorcus turbinatus*, and the snail *Pisania striata* were collected, by hand, from the tide pools found at the same site in Shikmona while adult individuals of the crab *Eriphia verrucosa* were collected, by hand, from the intertidal zone that is located just north of HaBonim Beach Nature Reserve (32.6608404 N, 34.9286428 E), Haifa, Israel (Figure 1). All individuals belonging to the same species were kept together in 2 L glass aquaria, except for *E. verrucosa* which were placed in separate 2 L glass aquaria due to the size and cannibalistic tendencies of this species. A plastic mesh was secured to the top of the glass aquaria to prevent their escape. Air tubes were placed into each aquaria and every 24 h, 50%-100% of the water was replaced manually in each glass with filtered (5-10 μm) Mediterranean seawater. Prior to all experiments, the individuals were acclimated to the microcosm for a minimum of 5 d at the average ambient nearshore temperature (21 °C), for the beginning of May 2023, on the Shikmona shore. The water conditions within the experimental microcosm followed the natural fluctuations occurring in the site of collection. Every other day, the crustaceans were given their respective food items and allowed to feed ad libidum. *C. erythropus* were fed a pellet of Tropical Crusta Sticks and *E. verrucosa* were given frozen shrimp. The gastropods, *P. turbinatus* and *P. striata,* which are herbivores, were given algae covered rocks from their site of collection and allowed to feed ad libidum.

2.2.2 Thermal performance of metabolic rates experimental setup and timeline

Mean rate of oxygen consumption (MO_2) was used as a proxy for Standard Metabolic Rate (SMR), as in Magozzi and Calosi (2014). After the acclimation period, an initial incubation was done before the temperatures were ramped at a pace of 1°C every 12 hour (h), for 8.5 d, until the target temperatures were reached (Figure 2). The individuals were exposed to thermal treatments held at one of seven water temperatures ranging between current winter temperature and future smmer temperatures that might be expected in coastal waters in the region in several decades under continuous ocean warming: (a) 17 °C, (b) 18 °C, (c) 19 °C, (d) 24 °C, (e) 26 °C, (f) 31 °C, and (g) 34 °C. Air tubes were placed into each jar and every 24 h, 50%-100% of the water was replaced manually in each jar with filtered (5-10 micron) Mediterranean seawater, and either warmed or cooled to the target temperature before exposure. Salinity followed the natural variation found in coastal water (39.0 +/- 0.1). Survivorship was 100% in all treatments.

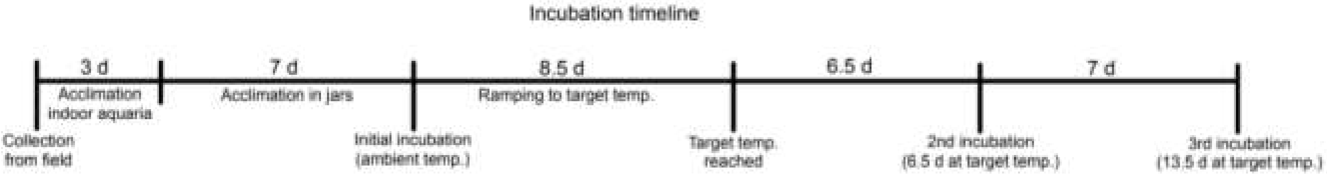

Fig. 2 Experimental timeline for the incubations

2.2.3 Respiration measurements

Respiration was measured after acclimation to the microcosm (at ambient temperature), again 6.5 d after the experiment reached target temperatures, and finally 13.5 d after the experiment reached target temperatures. The shells of the crabs were cleaned with a wire toothbrush 24 H prior to the incubations in order to remove any algae that might have developed on the shells which might affect the O_2 measurements during the incubation (Fontanini et al., 2018). As an extra precaution, the incubations were also started an hour after the sun had set. The specimens were individually placed in 300 mL plastic cups containing freshly changed water, hermetically sealed with a lid, and submerged into their respective outdoor thermobaths for approximately 5 h with water previously warmed to the same temperature of the bath. Dissolved oxygen (DO) was measured at the beginning and end (~5 h) of the incubation using an optode (Oxi3315, WTW Weilheim, Germany). A control container, with seawater only, was also placed within each bath and measured in order to account for any natural "drift" in oxygen concentration. Initial and final measurements of each incubation were used to calculate the consumption rate, standardized to the crabs' wet weight which was recorded at the conclusion of the third (and final) incubation.

2.2.4 Acute thermal tolerance

The dynamic, fast ramping method, used to determine a species CTMax, as described by Mora and Ospina (2001), was used to compare the thermal tolerance of four species (*C. erythropus, E. verrucosa*, *P. striata*, and *P. turbinatus).* All species have a distribution outside of the Mediterranean Sea as well as within and are found in the intertidal zone (Table 1). CTMax is defined as the "arithmetic mean of the collective thermal points at which the end-point is reached" (Mora and Ospina, 2001). To determine this, the organisms were supplied with constant aeration with air tubes and exposed to a rapid constant rate of 1 °C/15 min for all species (Vinagre *et al*., 2019) of water temperature increase, in a thermobath, described above. The method of determining the end-point can vary among species, but it is generally defined as the point at which the individuals experience "a loss of equilibrium" (Vinagre *et al*., 2014). For *C. erythropus*, the end-point was recorded as the temperature of shell emersion (van der Walt *et al*., 2021). The end-point of *E. verrucosa* was recorded as the temperature at which the crabs were unable to flip themselves right side up after being placed upside down with lab tweezers (Vinagre *et al*., 2014). The snails *P. striata*, and *P. turbinatus* were placed in a transparent container and observed for movement. All inactive individuals were removed from the trial and then the end-point was recorded as the temperature at which the remaining gastropods lost their attachment to the walls, upon tipping the container (Vinagre *et al*, 2018). The individuals were prompted with the tweezers or the container flipped every 15 minutes, prior to ramping the bath by 1 °C. A slide caliper was used to measure length (to the nearest 0.1 cm) of all individuals at the end of the trials. All experiments followed a natural (12L: 12D) photoperiod during acclimation.

Table 1 Species, common name, world distribution, environment, sample size and size range of the individuals (mm)

Species	Common name	Distribution	Environment	Sample size	Length (mm)
Clibanarius erythropus	Mediterranean intertidal hermit crab	East Atlantic + Mediterranean, Red, & Black Sea	Intertidal/Subtidal	10	5-10
Eriphia verrucosa	Warty crab	East Atlantic + Mediterranean & Black Sea	Intertidal/Subtidal	6	31-41
Phorcus turbinatus	Turbinate monodont	East Atlantic + Mediterranean & Red Sea	Intertidal	14	20-28
Pisania striata	Spotted pisania	East Atlantic + Mediterranean Sea	Intertidal	8	14-19

This table was constructed with information from Encylopedia of life (www.eol.org) and World Register of Marine Species (www.marinespecies.org).

The CTMax was calculated for each species using the following equation:

$$\mathrm{CTMax} = \frac{\sum(\mathrm{Tend} - \mathrm{point}n)}{n}$$

Where Tend-point is the temperature at which the end-point was reached for the individuals in the study and *n* stands for the sample size (Vinagre *et al.*, 2016). Additionally, intraspecific variability (coefficient of variation) of the CTMax was calculated for each species using the following equation:

$$\%\mathrm{CV} = \left(\frac{SD}{Mean}\right) \mathrm{x}\ 100$$

The thermal safety margin (i.e. warming tolerance) was calculated using the following equation:

$$\mathrm{TSM} = \mathrm{MHT} - \mathrm{CTMax}$$

Where MHT is the maximum habitat temperature that was determined through the field measurements done by Rilov *et al.* (2022), using temperature loggers (HOBO Tidbit, Onset, USA) that were deployed near the seaward edge of the vermetid reef platform of Shikmona, Haifa between 2009 and 2017.

2.2.5 Shell size to body size comparison

When we set up the metabolic rate experiments there were initial technical issues that resulted in hermit crab mortalities in several tanks that were eventually not used in the experiment. The 34 dead crabs were used to investigate the relationship between shell size and body size of individuals from this species. The bodies of the crabs and their respective shells were measured using a slide caliper (to the nearest 0.05 cm) and weighed using a scale (to the nearest 0.01 g). Once the bodies had been weighed and measured, 16 individuals were randomly selected for genetic testing with barcoding and PCR methods to confirm that these specimens were indeed *C. erythropus.*

2.2.6 Statistical Analysis

All statistical tests were done using RStudio (2022.02.1, 461). Specific metabolic rates (SMR, µmole oxygen consumption per hour per gram) were plotted against temperature and fitted with a model that is commonly used to describe performance curves. One-way ANOVA (p-value <0.05) was used to test if temperature affected metabolism and then a Tukey *post-hoc* test was used in cases where the null hypothesis was rejected.

The relationships between crab sizes and shell size variables were identified by plotting the data on a scatterplot and then calculating the correlation coefficient, linear regression, and r-squared values. Spearman rank correlation was chosen for the test.

2.3 Results

Table 2- p adj values of a Post-hoc test, following a One-way ANOVA test for different exposure durations to different temperatures

Temperature Sets	**Exposure Time**	
	6.5 days	**13.5 days**
17-18	0.9996819	0.4234063
17-19	0.9999278	0.9909955
17-24	0.9521238	0.7041849
17-26	0.5947232	**0.0390409**
17-31	**0.0015246**	**0.0000277**
17-34	**0.0019995**	**0.0008343**
18-19	1.0000000	0.8886727
18-24	0.9981440	0.9993484
18-26	0.9312303	0.9758222
18-31	**0.0175697**	**0.0091314**
18-34	**0.0176334**	0.1399319
19-24	0.9961435	0.9856464
19-26	0.9046316	0.3490309
19-31	**0.0150444**	**0.0008075**
19-34	**0.0152197**	**0.0140891**
24-26	0.9990166	0.8250256
24-31	**0.0514941**	**0.0037385**
24-34	**0.0489419**	0.0628974
26-31	**0.0359854**	**0.0100383**
26-34	**0.0375981**	0.2625534
31-34	0.9999758	0.7023098

Thermal performance curve experiments: Survival was 100% in all thermal treatments. The hermit crabs were able to withstand 13.5 d at average temperatures ranging from as low as 17 °C to as high as 34 °C, but their metabolic rate was different among some temperature treatments and also slightly changed between exposure periods (Figure 3). A One-way ANOVA and *Post-hoc* test from the initial incubation revealed that there were no significant differences in the metabolic rates between individuals after their acclimation to the microcosm and before the temperatures were ramped. After 6.5 days at the target temperatures, the analysis (Table 2) revealed that the hermit crab metabolism rates were significantly higher (by almost two-fold) at both 34 °C and 31 °C compared to all lower temperatures, meaning that the optimal temperature (T_{opt}) could be somewhere between these two temperatures. After 13.5 days, the hermit crab metabolism rates were only significantly different between 31 °C and all lower temperatures ($P<0.05$) which suggests that the T_{opt} may be somewhere around 31 °C since the metabolism rates began to drop at 34 °C. The only temperatures found to have a significant difference with individuals held at 34 °C were those held at 17 °C and 19 °C ($P<0.05$). Furthermore, the individuals held at 17 °C also had a significantly different metabolism from those held at 26 °C ($P<0.05$). It is also visible that the variability in rates among individuals at specific test temperatures was higher at the higher temperatures.

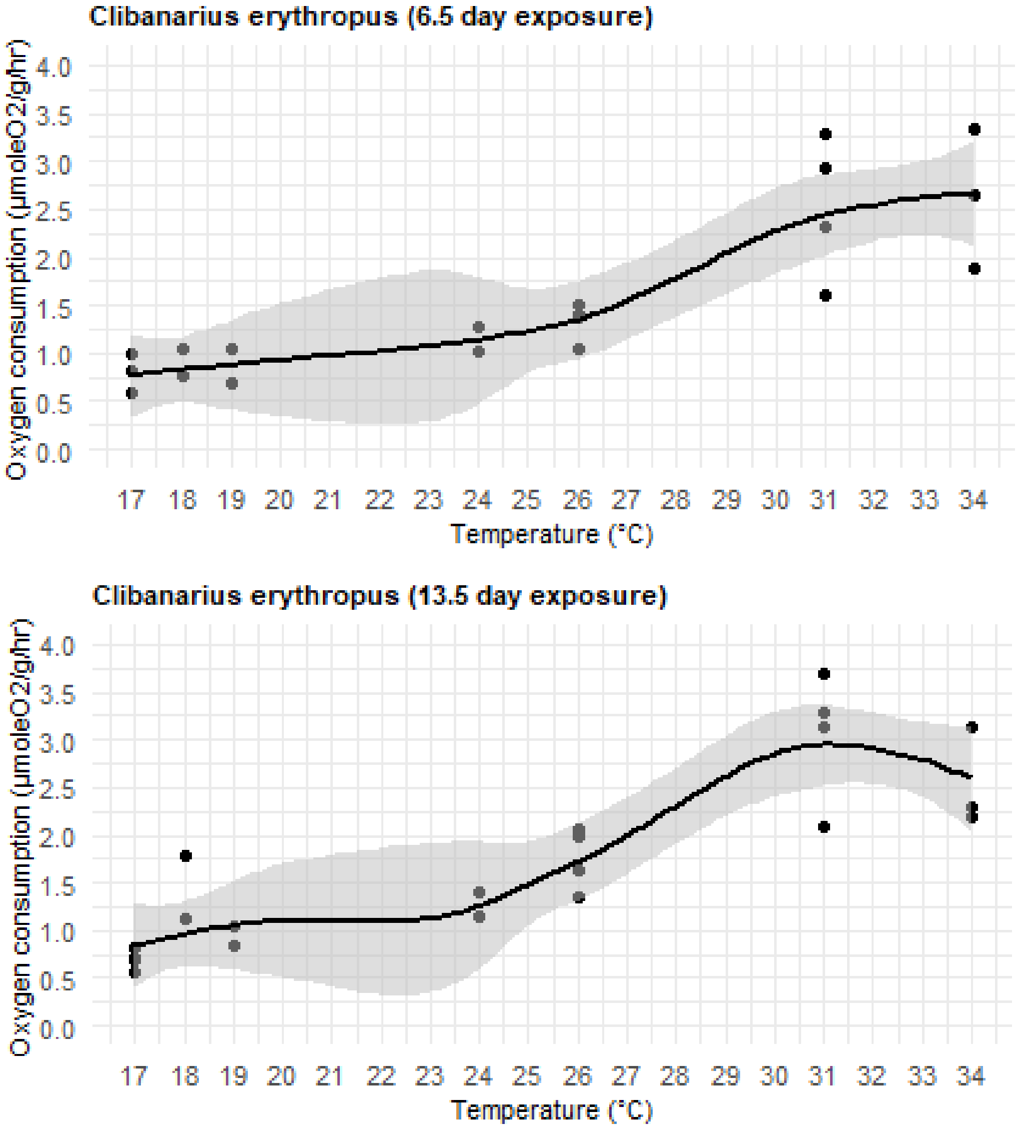

Fig. 3 The effect of temperature on hermit crab metabolic rates after a) 6.5 days and b) 13.5 days of exposure to the target temperature ranges. Gray areas show 95% confidence interval.

Acute thermal exposure experiment: Table 3 shows the CTMax values and TSM for the species tested. The highest CTMax recorded was that of *C. erythropus*, at 38.8 °C with lower value measure for *E. verrucosa* at 37.93 °C followed by *P. striata* at 37.48 °C while *P. turbinatus* had a much lower value at 34.19 °C. Additionally, the least amount of variation in CTMax was found in *E. verrucosa* (CV= 0.52%) while the highest variation was recorded for *C. erythropus* (CV= 4.46%). Figure 4 shows CTMax values for all species tested and the SD bars as well.

Table 3- Species, sample size, CTMax, SD, CV, and thermal safety margins in tide pools for the present (CTMax – maximum habitat temperature) for two species of crabs and two species of gastropods from the Israeli intertidal waters.

Species	**Sample size**	**CTMax (°C)**	**SD**	**CV**	**Thermal Safety Margin**
					Tide Pools
Clibanarius erythropus	10	38.8	1.734	4.458%	0.80
Eriphia verrucosa	6	37.93	0.197	0.518%	-0.07
Phorcus turbinatus	14	37.48	1.200	3.510%	-0.52
Pisania striata	8	34.19	1.053	2.809%	-3.81

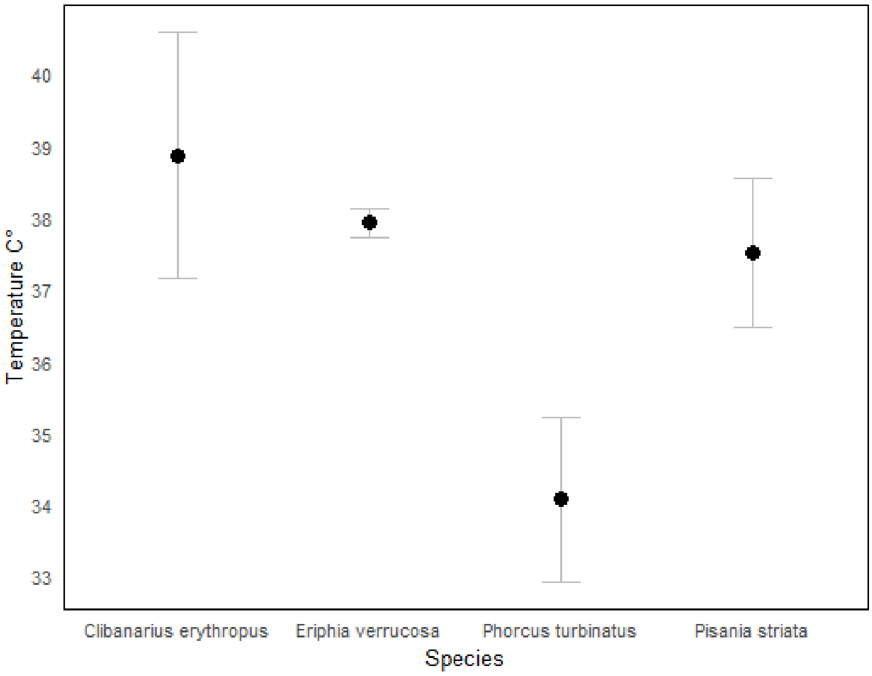

Fig. 4 Critical Thermal Maxima (+/- SD) of two crab species and two gastropod species from the Israeli intertidal waters.

The range of cephalothoracic shield length (CL) in *C. erythropus* ranged from 0.5 cm to 1.25 cm, with an average size of 0.82 cm. Crabs mainly occupied the shells of the invasive snail *Conomurex persicus*. The weight of shells occupied by hermit crabs appeared to have low but significant correlation with the crab's length ($r^2 = 0.37$; p-value <0.05) and weight ($r^2 = 0.26$; p-value <0.05, Figure 5). However, shell length did not correlate with crab length ($r^2= 0.02$; p-value >0.05) or weight ($r^2 = 0$; p-value >0.05).

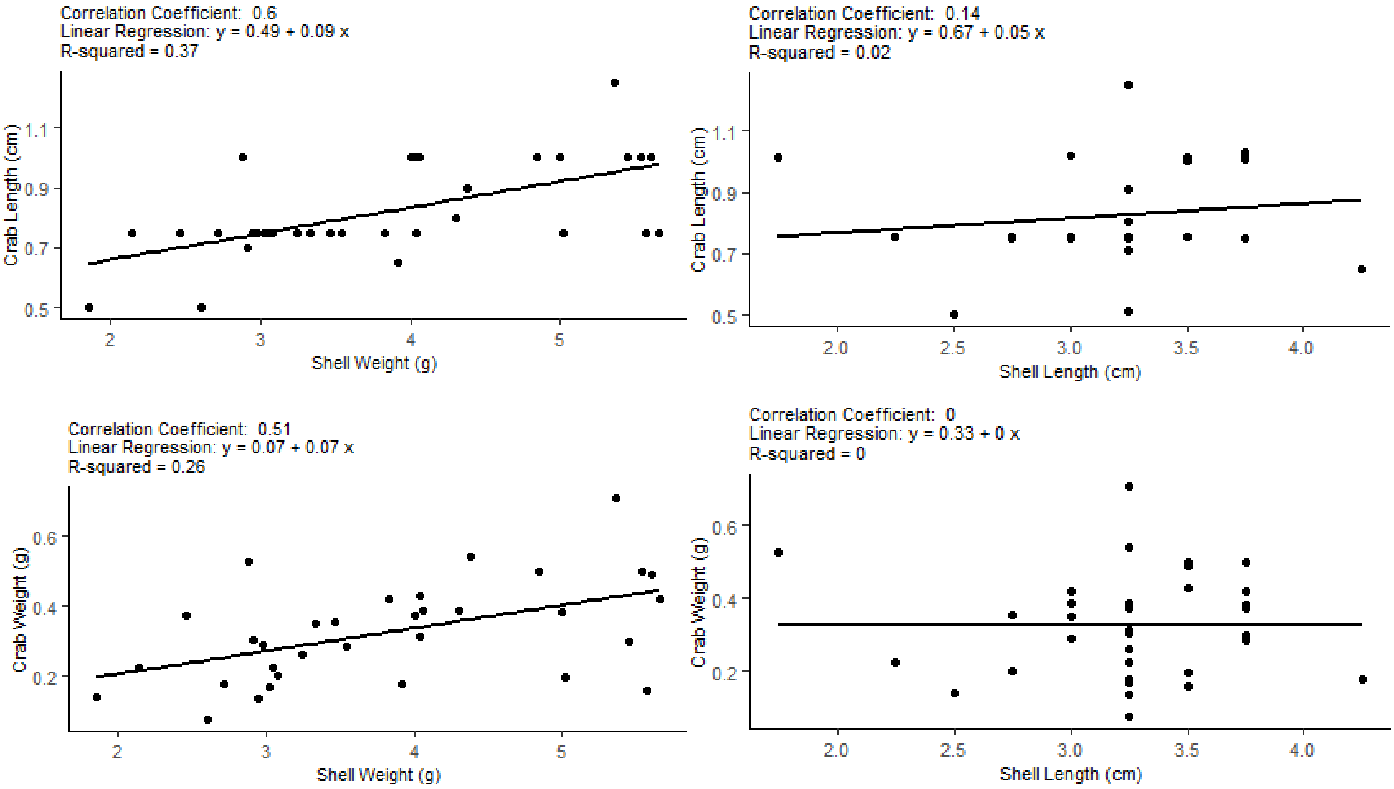

Fig. 5 A comparison of hermit crab size in relation to shell size from the Israeli intertidal waters

2.4 Discussion

2.4.1 *Respiration response*

C. erythropus living on the shores of the southeastern Mediterranean was found to have high thermal tolerance to current and future coastal water temperatures under further warming. The respiration rate of the hermit crabs increases with temperature until it reaches a point above 30 °C at which it levels off or starts to decrease with longer exposure. Similar results were found for other intertidal invertebrates, a tidepool shrimp and for intertidal snails, from the same shore (Amsalem and Rilov, 2021; Rilov *et al.*, 2022), indicating that most invertebrate species still living today in this area can handle the warming that already occurred but may suffer from future warming. Warburg and Shuchman (1984), in their simple experiment, suggested that the activity of *C. erythropus* decreased with increasing temperatures and claimed that the activity rate can be displayed by a bell-shaped curve in which the peak of the curve is an indication of an "optimal region" for the organism. The shift from a T_{opt} that is greater than 31 °C to a T_{opt} that is somewhere around 31 °C, after 7 additional days at the target temperature indicate the impact of prolonged exposure time which may impact their performance and overall fitness. This shift was also seen in the other tested intertidal invertebrates on this coast (Amsalem and

Rilov, 2021; Rilov *et al*., 2022). Prolonged exposure to constant high temperatures causes stress to accumulate, shifting the thermal breadth toward the left since it likely has high demands on the hermit crabs' metabolic functions.

The results of this metabolic rate experiment as well as the others from this coast support the hypothesis presented by Rezende *et al.* (2014) in which a trade-off seems to exist between an individual's tolerance for acute and chronic thermal stress. Rezende *et al.* (2014) stated that "the temperature range that an organism can tolerate is expected to narrow down with the duration of the thermal challenge". Similarly, Vinagre et al. (2016) found that the tropical intertidal species in their study were able to acclimate to a temporary increase of 6 °C above their current habitat temperature, yet they were unable to acclimate when exposed to a chronic increase of 3 °C above their current habitat temperature.

2.4.2 *Critical thermal maximum (CTMax)*

Short exposure to very high water temperatures can also occur in the intertidal zone within tidepools during hot and dry days when the tidepools get disconnected from the open water, causing their water to heat to extreme (up to 38 °C) temperatures (Rilov *et al*., 2022). The most isolated tidepools in this habitat have been found to have a wide daily variation of 10°C and a seasonal variation of more than 32°C within the smaller, isolated pools (Amsalem and Rilov, 2021). Therefore, currently, *C. erythropus* may be experiencing maximum temperatures slightly below its recorded CTMax. However, the intraspecific variability in the thermal endpoint of this species was found to be quite broad (CV= 4.46%) meaning that some individuals may be at risk on the days where tidepools reach their maximum temperature but others may withstand short exposure to even higher temperatures in the future. On such extreme days, the snail, *P. turbinatus* has been seen just above the waterline, in the moisture zone of the shaded tidepool sides (Rilov *et al*., 2022). It would be interesting to examine whether the hermit crab also uses behavior to thermoregulate itself in extreme weather conditions.

Rilov et al. (2022) found that the optimum temperature of *P. turbinatus* was between 31-33°C while all organisms died at 35°C and 37°C. My findings further support this finding as the CTMax falls between their recorded optimum and mortality point. In the case of the tidepool shrimp*, Palaemon elegans*, Amsalem and Rilov (2021) found the winter CTMax to be 36.46°C and the summer CTMax to be 38.1°C indicating seasonal adaptability. Such adaptability should be examined also for the hermit crab.

Undoubtedly, the intertidal organisms that have been tested here may occasionally experience temperatures either above or slightly lower than their thermal maximum even today. This suggests that these species could be vulnerable to current and future climate change impacts as projected coastal sea surface temperatures along this coast may reach close to 36 by the end of the century under business-as-usual IPCC scenarios (unpublished projections) and we know that many invertebrate species in this region have already gone extinct (Coll *et al.*, 2010; Rilov *et al.*, 2020; Garrabou *et al.*, 2022). However, some species may survive in the future through the persistence of subtidal populations (as in the case of *C. erythropus*), where short term fluctuations are milder than in tidepools (Rilov *et al.,* 2022) while other species may be able to thermoregulate through behavior (e.g. adjusting their thermal microclimate by hiding in crevices or moving to shady areas) or physiological mechanisms (e.g. Hsp70 or CAT) or by selection for more resilient genotypes.

2.4.3 *Shell size to body size comparison*

Our measurement of *C. erythropus* revealed that there is a weak but significant relationship between crab size and the size of their occupied shell when the weight of the shell is concerned. However, the length of the shell appears to have no correlation with the length or weight of the crab inside of it. The weak correlation with shell length may indicate that there are other factors influencing the selection of shells. Hazlett (1981) proposed that crabs frequently choose shells that are not their preferred size or species, speculating that the difficulty in finding a perfect-sized shell often leads them to select larger shells. Dressler *et al.* (2021) suggest that the opportunistic behavior they observed in a population of *C. erythropus,* in France, was likely influenced by a lack of availability in empty gastropod shells. In the Azores, Botelho and Costa (2000) study of 849 crabs found that the linear regressions between shell measurements and *C. erythropus* size were not significant for most gastropod species in their study, suggesting that the shell of the examined species are likely sub-optimal since there is a broad range of crab size occupying the same shell size. However, for two gastropod species with a large number of samples there was a well-fitted linear regression (Botelho & Costa, 2000). It is possible that with a sample size larger than 34 tighter correlations could be found for specific shells also in Israel. In the present study, it was found that *C. erythropus* size was better correlated with shell weight, but Botelho and Costa (2000) found that crab size was better correlated with shell length. Interestingly, Gherardi (1990) found that different sexes of *C. erythropus* occupied different species of shells. Further studies should be done to determine the criteria by which hermit crabs select shells since shell size has been shown to directly affect the fitness of the organism (Hazlett 1981). A larger sample size should be considered in a future study, with a heavy focus on the shell utilization of the different sexes and inclusion of shell choice experiments within the lab.

In conclusion, the present study adds more evidence to the concern that native southeastern Mediterranean marine species are gradually losing their thermal safety margin. Further research should be done to investigate the influence of season on thermal performance and tolerance since Amsalem and Rilov (2021) documented seasonal variability in the thermal optimum in the tidepool shrimp, *P. elegans*. While the increase in sea surface temperature is a growing topic of discussion, the critical thermal minimum should also be explored for these species, in future studies, since temperatures in isolated tide pools can drop considerably during cold nights, causing cold stress and even mortality. Additionally, further research should be done to test the thermal tolerance of individuals at different developmental stages and come from different tidal zones (e.g. intertidal vs. subtidal).

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