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High thermal stress responses of *Echinolittorina* snails at their range edge predict population vulnerability to future warming

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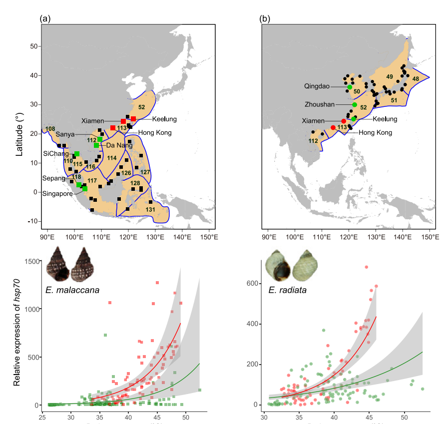
HIGHLIGHTS

- Range edge populations of *Echinolittorina* snails had higher temperature sensitivity as compared to central populations.
- The mean *hsp70* expression level was higher in range edge than range center populations.
- Highest temperature sensitivity was detected at the northern edge for *E. malaccana* and the southern edge for *E. radiata*.
- Strong heat shock responses will lead to higher energetic costs for thermal defense.
- Populations living at their species' range edges are likely more vulnerable to future global warming.

GRAPHICAL ABSTRACT



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ABSTRACT

Populations at the edge of their species' distribution ranges are typically living at the physiological extreme of the environmental conditions they can tolerate. As a species' response to global change is likely to be largely determined by its physiological performance, subsequent changes in environmental conditions can profoundly influence populations at range edges, resulting in range extensions or retractions. To understand the differential physiological performance among populations at their distribution range edge and center, we measured levels of mRNA for *heat shock protein 70* (*hsp70*) as an indicator of temperature sensitivity in two high-shore littorinid snails, *Echinolittorina malaccana* and *E. radiata*, between 1°N to 36°N along the NW Pacific coast. These *Echinolittorina* snails are extremely heat-tolerant and frequently experience environmental temperatures in excess of 55 °C when emersed. It was assumed that animals exhibiting high temperature sensitivity will synthesize higher levels of mRNA, which will thus lead to higher energetic costs for thermal defense. Populations showed significant geographic variation in temperature sensitivity along their range. Snails at the northern range edge of *E. malaccana* and southern range edge of *E. radiata* exhibited higher levels of *hsp70* expression than individuals

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collected from populations at the center of their respective ranges. The high levels of *hsp70* mRNA in populations at the edge of a species' distribution range may serve as an adaptive response to locally stressful thermal environments, suggesting populations at the edge of their distribution range are potentially more sensitive to future global warming.

1. Introduction

Rising atmospheric carbon dioxide levels have led to changes in global temperature, climate in general and seawater chemistry resulting in subsequent impacts on species' distribution patterns (Hofmann and Todgham, 2010; Parmesan and Yohe, 2003). Variation in environmental conditions resulting from climate change has, for example, been shown to result in broad biogeographic shifts of species, such as range retraction or expansion (Perry et al., 2005; Poloczanska et al., 2013), as well as localized changes in species which are distributed in spatially patchy habitats (Somero, 2012). As many species occur over wide latitudinal or altitudinal gradients, populations in different locations of a species' distribution range may experience very different environmental challenges (Buckley and Huey, 2016; Mathiasen and Premoli, 2016; Vergeer and Kunin, 2013). Typically, populations at the edge of a species' range are located at the extreme of the environmental stress gradient that the species can tolerate (Gaston, 2009; Mägi et al., 2011; Sexton et al., 2009). Such populations are, therefore, assumed to be constrained by current environmental conditions and subsequently are predicted to be more sensitive to future climate change (Sagarin and Somero, 2006; Sorte and Hofmann, 2004). Understanding the divergent responses between different populations along their distributional range is, therefore, important to inform predictions of the ecological impacts of climate change.

Physiological adaptation is a key response for species to cope with environmental variations, and the physiological responses of different populations are closely related to their local environments (Helmuth, 2009; Seabra et al., 2015; Somero et al., 2016). The heat shock response (HSR), which is highly conserved across almost all taxa (Feder and Hofmann, 1999; Somero et al., 2017), is a homeostatic response that maintains the correct protein-folding environment in the cell (Guisbert et al., 2008) and, as such, heat shock proteins (Hsps) are considered to be one of the most important cellular defense mechanisms against environmental stresses (Sørensen et al., 2003; Tomanek, 2010). As a consequence, Hsp expression is believed to reflect both the environmental variability associated with different microhabitats and its interaction with large-scale environmental gradients across the whole distribution range of a species (Dutton and Hofmann, 2009; Lima et al., 2016; Sagarin and Somero, 2006; Wang et al., 2018). Environmental stress is also an evolutionary driver for local adaptation in natural populations (Kawecki and Ebert, 2004). Generally, natural selection acts on the HSR in different populations by changing the expression of *hsp* genes, rather than by gene mutations that affect the properties of the heat shock proteins themselves (Bettencourt et al., 2002; Dutton and Hofmann, 2009; Sagarin and Somero, 2006; Sorte and Hofmann, 2004). As a result, geographic variation of expression of Hsps as well as plasticity of expression are both heritable (Somero et al., 2017), and consequently, divergent HSRs to local thermal environments have been identified in various marine invertebrates (Gleason and Burton, 2015; Schoville et al., 2012), showing the evolutionary adaptation of the HSR to local environmental conditions (Tedeschi et al., 2016) and its suitability to be used as a measure of thermal sensitivity (Tomanek and Somero, 1999a).

Rocky shores are one of the most physically challenging habitats on earth, both on a local scale (as a result of tidal changes; wave action etc. Helmuth and Hofmann, 2001; Little et al., 2009) but also with highly variable thermal environments over biogeographic scales (Harley, 2008; Helmuth et al., 2002). Activation of the HSR within the typical range of body temperatures organisms experience is part of the strategy of intertidal species to cope with thermal stress. Distinct strategies of Hsp70 expression can be employed by intertidal species occupying different heights along the intertidal zone. High-intertidal species, for example, tend to have higher constitutive levels of Hsp70 than low- and mid-intertidal species. By contrast, lower-occurring species maintain low levels of Hsp70, but are capable of rapidly inducing high levels of synthesis when exposed to heat stress (Dong et al., 2008; Nakano and Iwama, 2002). Different populations of widely distributed species, thus, can face divergent thermal regimes at different locations along their distribution range (Helmuth et al., 2002; Dong et al., 2017). As a result, persistent regional differences in tidal regimes, climate and other environmental factors will act as selective forces to influence the physiology of intertidal species which span broad latitudinal ranges. Upper limits of the temperature range of Hsp synthesis are close to the highest body temperatures that these organisms experience under natural conditions (Sorte and Hofmann, 2005; Tomanek and Somero, 1999a, 1999b; Tomanek, 2002). Consequently, Hsp synthesis may be a thermally sensitive weak-link that contributes to setting species' thermal tolerance limits (Somero, 2004). The HSR is thus a convenient biochemical indicator to assess levels of physiological stress among populations along their distribution range (Dutton and Hofmann, 2009; Sagarin and Somero, 2006; Sorte and Hofmann, 2004; Stillman and Tagmount, 2009).

At present, many intertidal organisms experience temperatures at or above their tolerance limits during low tides in stressful seasons (Wolcott, 1973; Williams, 1994; Williams et al., 2005; Somero, 2012; Zhang et al., 2016). High shore species suffer from the most extreme thermal stress and, as a result, have been postulated to be especially vulnerable to climate warming (Tomanek and Somero, 1999b; Somero, 2012). The littorinid snails, *Echinolittorina malaccana* and *E. radiata* are widely distributed along the NW Pacific (NWP) coast, with representatives of the two species covering over 50° of latitude from 5°S to 45°N (Fig. 1, Reid, 2007). Living on the high shore, littorinid snails experience long emersion periods when environmental (=rock surface) temperatures can exceed 60 °C (Williams, 1994; Marshall et al., 2011; Seuront and Ng, 2016). The thermal environment of intertidal species along the NWP coastline is extremely variable among different locations (Dong et al., 2015), and shows a highly non-linear relationship with latitude (Helmuth et al., 2002; Lima et al., 2016; Dong et al., 2017). Different populations of *Echinolittorina* snails, therefore, face extreme and divergent thermal stresses along their distribution ranges.

Using specifically designed primers, we can precisely detect the mRNA levels of inducible isoforms of Hsp70 (*hsp70*), which is an important biochemical indicator to assess levels of physiological stress. Up-regulation of *hsp70* in response to thermal stress has been well documented in a variety of marine taxa (bivalves, gastropods and shrimps; Clark et al., 2008; Cottin et al., 2010; Giomi et al., 2016; Han et al., 2013; Prusina et al., 2014). In a previous study, we found that Hsp70 protein levels significantly increased in response to thermal stress in *Echinolittorina malaccana* (Marshall et al., 2011) which supports the use of up-regulation of *hsp70* mRNA to predict the production of Hsp70 protein in *Echinolittorina* snails, and the positive relationship between levels of *hsp70* mRNA and Hsp70 protein. The present study was, therefore, designed to highlight the importance of estimating population-specific physiological performance when evaluating and predicting the ecological impacts of climate change on species with wide geographic distributions. Specifically, we investigated variation in heat shock response using levels of mRNA for *hsp70* to test the hypothesis that populations at the range edge of the two *Echinolittorina*