Investigating how differences in field acclimation during early development affect thermal tolerance of free-swimming larvae in *Melanochlamys diomedea*

Jannine D. Chamorro[1]
Mahsa Alidoostsalimi[2]

FHL 548: Larval Biology of Marine Invertebrates
Summer B 2019

[1]University of California Santa Barbara, Santa Barbara, CA 93106
[2]Science and Research University, Tehran, Iran

Contact Information:
Jannine Chamorro
jdchamorro@ucsb.edu

Mahsa Alidoostalimi
m.alidoustsalimi@gmail.om

Keywords: Melanochlamys diomedea, thermal tolerance, thermal acclimation, larvae

Abstract

Marine invertebrates exhibit complex life cycles comprised of various stages, each of which face varying abiotic and biotic challenges. Understanding how environmental stressors affect species during developmental stages is important, as conditions experienced early in their life history can influence stages later on in life. For our project we examined whether differences in field environmental conditions during early development influence thermal tolerance at the larval stage of the cephalaspidean snail, *Melanochlamys diomedea*. Our results indicate that larvae which developed in the upper intertidal zone did not differ with regard to thermal tolerance from those which developed in lower zone. This study suggests that the thermal differences of the developmental environment do not impact the early free-swimming larval stage of *M. diomedea*.

Introduction

Marine invertebrates have complex life cycles comprised of various stages, each of which face varying abiotic and biotic challenges (Werner, 1988). Understanding how environmental conditions influence different life history stages is important since some stages can be more vulnerable to stress, and exposure to stress at one stage can influence the next (Marshall et al., 2016; Emlet and Sadro, 2006). While many studies attempt to understand how abiotic and biotic stress influence different stages, much of the research has been conducted in a laboratory setting (Wang et al., 2017). To get a better understanding of how natural conditions influence traits at different life history stages we must turn our attention toward field experiments.

The intertidal zone is a natural laboratory for understanding of how differences in abiotic factors can affect performance at various life history stages (Wang et al., 2017). Thermal fluctuations and desiccation, due to tide cycles, play an important role in determining species distributions in the intertidal zone (Perterson, 1979). Differences in temperature regimes of high and low intertidal sites can set boundaries for closely related species. For example, congeneric species of porcelain crabs within the genus *Petrolisthes* demonstrate thermal tolerance limits correlated with maximum microhabitat temperatures (Stillman and Somero, 2000). Additionally, individuals of the same species can experience greatly different temperature regimes and physiological responses based on position in the intertidal zone (Gleason et al., 2017).

For our project we examined if exposure to high and low intertidal temperature regimes during early development affects the subsequent free-living larval stages in *Melanochlamys diomedea*. *M. diomedea* (Bergh, 1894) is a small opisthobranch mollusc that can be found in tidal flats from southern California to Alaska (Breslau et al., 2016). As a part of their reproduction *M. diomedea* deposit egg masses into surrounding sediment. Egg mass are about 98 um in diameter and are composed of a gelatinous matrix which contains strings of embryos that are in small fluid-filled capsules within the gel matrix (Woods and DeSilets, 1997) .The eggs develop into hatching veligers lacking eyespots in 8–20 days at 8–11 °C. Shells of recently hatched veligers are 180 microns long; the minimum larval period of *M. diomedea* is unknown but >40 days. Based on this developmental information *M. diomedea* is considered a planktotrophic developer (Cooke et al., 2014). Masses can be deposited in intertidal and shallow subtidal zone where they are exposed to a wide range of abiotic conditions (Woods and DeSilets, 1997).

Our project explored whether differences in field acclimation of *M. diomedea* egg masses during early development influenced thermal tolerance of newly released veliger larvae. We hypothesized that larvae from egg masses located higher in the bay would have a higher thermal tolerance than those from the lower in the bay. We expected egg mass higher in the bay had acclimated to greater heat stress during development.

Material and Methods

Field Collection and Larval Culturing

M. diomedea egg masses were collected from False Bay, WA on July 31st, 2019. A total of 36 individual egg masses were collected, 18 from higher on the shore (high stress zone) and 18 from lower on the shore (low stress zone). We predicted high stress in the higher sites because masses were farther from the mouth of the bay where they experience longer emmersion time and higher temperatures. Low sites (low stress zone) were closer to the mouth of the bay, where egg masses are more consistently found in pools of water, experiencing less emmersion and less temperature stress. In-field temperatures of egg masses were taken to determine differences in temperature exposure of each group. The average temperatures of egg masses found in high and low sites was, 22.6 and 20.6 (n=5) °C, respectively.

Following collection, egg masses were brought back to Friday Harbor Laboratories, WA. Egg masses were kept individually in glass finger bowls in a flow-through seawater table at 12.8 °C. The predicted stage of development of each mass was determined based on observing the presence of a 'kidney spot' in developing larvae, masses were divided into those containing pre-veliger, veliger, or hatched larvae (Woods and DeSilets, 1997). The stages of larvae were checked daily to get an approximation of the developmental stage of each egg mass. Water changes were conducted daily until the end of the experiment.

Thermal Tolerance Trial

In order to determine if differences in developmental environment affect the tolerance of *M. diomedea*, hatched veliger larvae were exposed to a thermal tolerance trial. We tested newly released veliger larvae from three high (H1, H2, H3) and three low (L1, L2, L3) egg masses. All larvae were two days post hatch and had spent a total of four days in the laboratory. Four heat-blocks, each set at different temperatures (33, 34, 35, 36°C), were used to conduct the thermal tolerance experiment. Temperature treatments were determined using previously published data and preliminary experiments (Podolsky, 2003). Fifteen larvae from each mass were placed in 1.5 microcentrifuge tubes with 1ul of .45 um filtered seawater. Three replicates for each mass were placed in each heat block for 1hr. Following 1hr exposure, larvae were allowed a 1hr recovery period before scoring. Larvae were scored either alive of dead based on movement velar cilia.

Statistical Analysis

Statistical analyses were conducted using RStudio. The LT50, lethal exposure temperature at which 50% of the larvae died, was calculated from mortality in thermal tolerance trials. To determine LT50 of each group, a logistic regression was calculated from the binary mortality data. An ANOVA was used to determine if high and low larvae differed significantly in thermal tolerance.

Results

A thermal tolerance trial was conducted to determine the LT50 for high and low intertidal larvae. A linear regression was used to calculate the temperature at which each population reached the thermal limit. Larvae from high and low intertidal regions did not differ in LT50s, both reaching %50 mortality at 32.5°C. While larvae differed slightly in percent mortality with regard to treatment temperatures, difference in mortality high and low intertidal zones was not significant $p > 0.05$ (Figure 1).

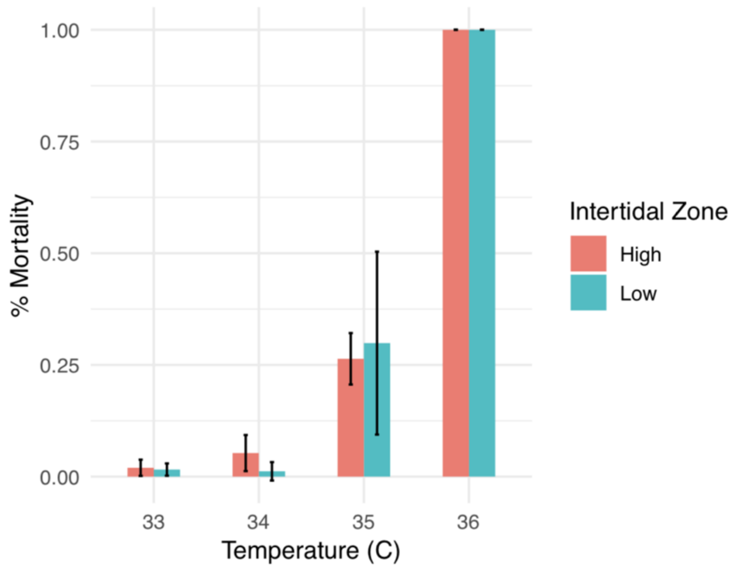

Figure 1. Thermal tolerance plot showing percent mortality at each experimental temperature for larvae from high and low intertidal origin. Mortality did not differ with regard to zone $p > 0.05$.

Discussion

Temperature is one of the major factors influencing the physiology and distribution of marine invertebrate species (Pörtner et al., 2005). Therefore, determining thermal tolerance across marine invertebrate life histories is an important key for a comprehensive understanding of how temperature can have ecological impacts (Wang et al., 2017). In this study, we compared the thermal tolerance of larvae of *M.diomedea* collected from two different tidal zones. LT50 for *M.diomedea* 2-days post hatching was 35.2 °C for larvae from both sampled zones. Our results are consistent with prior experiments examining thermal tolerance in developing *M.diomedea.* Podolsky's 2003 study, demonstrated that embryos within the egg masses began to exhibit decreased survival at 34°C. Our results may suggest that hatched larvae may have a slightly higher thermal tolerance than embryos. Larvae are able to withstand heat stress during early development due to the induction of a heat-shock response (Podolsky and Hofmann, unpublished data). However, survival at temperatures exceeding ~ 35°C may decrease since synthesis of heat-shock proteins is disrupted (Podolsky, 2003).

Apart from physiological mechanisms used to deal with heat stress, adults *M. diomedea* are predicted to have behavioral responses to heat. Podolsky's prior study looking at thermal tolerance of *M. diomedea* suggests that reproductive timing may have a great influence on fitness consequences. Field surveys of adult reproduction indicated that there was a decline of deposited egg masses during time of higher heat stress. Therefore, timing may have a larger influence on when adults decide to reproduce rather than spatial dynamics (Podolsky, 2003).

Our study demonstrated that difference in field acclimation during early development did not influence thermal tolerance of larvae for *M. diomedea.* While differences in acclimation during early development did not influence later stage thermal tolerance, we did observe that egg masses collected in the high zone hatched earlier than those from the low. While no statistical analyses were conducted on these observations, this could suggest that egg masses tethered higher in the intertidal zone may experience faster development due to environmental temperature. Or perhaps, the differences in time to hatch may simply be attributed to when they were deposited. These observations may indicate that although position in the intertidal zone may not affect later thermal tolerance, it does lead to differences in time to develop. Moreover, while we did not see significant differences in thermal tolerance of larvae from high and low origin, it may be that the environment stressor experienced by egg masses were not different enough to induce a response. Further studies on species that experience a greater difference in environmental conditions during development are needed to better determine how conditions experience in early stages can influence later stages.

References

Breslau, E., Valdés, Á., & Chichvarkhin, A. (2016). A new cryptic species of Melanochlamys Gastropoda: Heterobranchia: Cephalaspidea) from the Northwestern Pacific. *American malacologica bulletin*, *34*(2), 103-109

Chaffee, C., & Strathmann, R. R. (1984). Constraints on egg masses. I. Retarded development within thick elgg masses. *Jou*rnal of Experimental Marine Biology and Ecology, 84(1), 73-83.

Cohen, C. S., & Strathmann, R. R. (1996). Embryos at the edge of tolerance: effects of environment and structure of egg masses on supply of oxygen to embryos. The Biological Bulletin, 190(1), 8-15.

Emlet, R.B & Sadro, S. (2006). Linking stages of life history: How larval quality translates into juvenile performance for an intertidal barnacle (Balanusglandula). Integrative and Comparative Biology 46(3): 334-346. doi: 10.1093/icb/icj023.

Marshall, D. J., Burgess, S.C., & Connallon, T. (2016) Global change, life-history complexity and the potential for evolutionary rescue. Evolutionary Applications, 9, 1189-1201.

Pörtner HO, Langenbuch M, Michaelidis B. (2005). Synergistic effects of temperature extremes, hypoxia, and increases in CO2 on marine animals: from earth history to global change. J Geophys Res. 110:C09S10. doi: 10.1029/2004JC002561.

Peterson, C. H. (1979). The importance of predation and competition in organizing the intertidal epifaunal communities of Barnegat Inlet, New Jersy (1979). Oceologia, 39(1),1-14.

Podolsky, R. D. (2003) Integrating Development and Environment to Model Reproductive Performance in Natural Populations of an Intertidal Gastropod. Integrative and Comparative Biology, 43 (3) 450–458.

Wang, W., Ding, M., Li, X., Wang, J., & Dong, Y. (2017). Divergent thermal sensitivities among different life stages of the pulmonate limpet Siphonaria japonic. Marine Biology. 164, 125.

Werner, E. E. (1988). Size, scaling, and the evolution of complex life cycles. In. B. Ebenman and L. Persson (eds.), Size-structured populations, pp. 60-81. Springer-Varlag, Berlin.

Woods, H. A. & DeSilets, R. L. (1997). Egg-Mass Gel of Melanochlamys diomedea (Bergh) Protects Embryos From Low Salinity. The Biological Bulletin, 193(3), 341-349.