Burnin' Up for You, Larvae: Larval Thermal Tolerance of the Ochre Sea Star *Pisaster ochraceus*

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Abstract

Marine heatwave (MHW) events cause extreme disturbances throughout coastal marine ecosystems. Studies on these events suggest that MHWs are increasing in frequency and intensity. Our study investigated the survival of *P. ochraceus* larvae after being acclimated to MHW conditions and estimated their upper thermal limits (LT_{50}) using acute heat exposure (25–34°C). We observed larvae in the ambient non-MHW treatment (13°C) to have a lower LT_{50} compared to the MHW (17°C) treatments which had a higher LT_{50}. Our results suggest that larvae that were acclimated to MHW conditions are more tolerant to an acute heat shock over 12 h of exposure compared to larvae that were not. As MHWs increase in frequency and intensity, it is imperative to understand how these events will impact marine invertebrates, particularly the early life stages.

1. Introduction

Marine heatwaves (MHWs) are discrete time periods of anomalously warm water temperatures exceeding the 90th percentile of local climatology for five days or more (Hobday et al., 2016; Smith et al., 2023). MHWs are becoming increasingly common and will likely further intensify in the next century as the climate warms (Oliver et al., 2018; IPCC 2021). The Northeast Pacific Ocean experienced the largest MHW event on record ("the Blob") in 2014, which traveled from the Gulf of Alaska to coastal southern California (Sanford et al., 2019). The Salish Sea, connecting the NE Pacific Ocean to the estuarine Puget Sound (WA, USA) and the Strait of Georgia (BC, Canada) experienced the largest effects of the MHW in 2015–2016 (Khangaonkar et al., 2021).

Elevated temperatures have been documented to affect growth, reproductive potential, resilience to disease and mortality in marine invertebrates (Nguyen et al. 2011; Suckling et al. 2015; Delorme and Sewell 2016). More generally, the persistence of the 2015–2016 MHW event through El Niño (Jacox et al., 2019) devastated kelp canopy cover and drove disease outbreaks, extensive toxic algal blooms, species distribution changes and mass mortality of marine invertebrates (Cavole et al. 2016; Gentemann et al. 2017; Sanford et al., 2019; Rogers et al., 2019; Harvell et al. 2019).

Sea stars in warmer ocean waters are shown to slow their foraging behavior and succumb to higher mortality (Arribas et al., 2021; Lang et al., 2022). Concerningly, *Pisaster ochraceus* (*P. ochraceus*, Brandt, 1835) were impacted heavily by sea star wasting during the 2015–2016 thermal event (Harvell et al. 2019). As influential predators of invertebrates in rocky intertidal areas of the North American Pacific coast, including the Salish Sea (Paine, 1966), impaired predation and reductions in populations have undoubtedly contributed greatly to changes in benthic community structure and localized stable states throughout their range (Alaska – Baja California; Menge et al. 2016; Traiger et al., 2022).

A necessity for stable populations, and population recovery after disturbance, is the success of all ontogenic stages. Early developmental stages of marine invertebrates, especially echinoderms, are highly sensitive to environmental stressors including temperature (Byrne 2011) and may represent a population bottleneck under warming ocean conditions (Pechenick 1999). For echinoderms with a planktonic life stage, the thermal structure of a water column may impact development rates, developmental trajectories and morphology (e.g., cloning and phenotypic plasticity, Titus and Hearther, 2019; Utsch and George, 2022), and therefore dispersal of the larval stages (Byrne 2011; Daigle & Metaxas, 2011). While climate change scenarios of thermal impacts on marine invertebrates are well studied (Przeslawski et al., 2015; Byrne et al., 2018), investigations on the impacts of extreme events such as MHWs are less understood (Gall et al., 2021).

In acute (short-term) thermal stressor experiments on echinoderms (Vergara et al. 2017), the response of organisms can be inferred to indicate thermal tolerance to spikes in marine temperatures (heat waves) (Wernberg et al. 2016). On the other hand, longer term exposures to elevated temperatures give organisms time to acclimate, allowing insight into longer term effects of warming (Delorme and Sewell 2016). This distinction is useful, because acclimation can enhance an organism's resilience to elevated temperatures (Seebacher et al. 2014).

The apparent link between sea star wasting and temperature in *P. ochraceus* (Bates et al. 2009) is a major concern for these pivotal predators in a changing, warming, ocean. Understanding larval sensitivity to thermal environments that potentially contribute to dire conditions for adults will provide insight into the potential importance of the larval stage in replenishing the benthic population.

During the spawning season of *P. ochraceus* (Spring–late summer, Strathmann, 1987) water temperature in the Salish Sea region ranges from ~10–13 °C (NVS: https://nvs.nanoos.org/Explorer). Previous work by Utsch and George (2022) showed 10 d old bipinnaria stage larvae of *P. ochraceus* are sensitive to a one-week exposures to ~ +10 °C (20.15 °C) thermal stress, with higher mortality compared to + 5 °C (15 °C mid-range temperature treatment) and the control, local San Juan Island (WA, USA) treatment (~ 9.5 °C). For species with long planktonic durations during summer months, such as *P. ochraceus* (~ 50 dpf, Pia vet al., 2012), the relative likelihood of being impacted by a MHW is high if conditions favor an extreme thermal event. To our knowledge, there is no data on how elevated thermal conditions will alter the response of *P. ochraceus* larvae to acute thermal shocks, or on the brachiolaria stage.

Using a graduated temperature block, we aim to determine the thermal tolerance of *P. ochraceus* brachiolaria larvae over 12 h of acute thermal stress and how a 5-d acclimation to elevated temperatures conditions modulates this tolerance and determine the LT50 of MHW/non-MHW acclimated larvae at three different time points under acute thermal stress. We hypothesize that larvae will have reduced survival with increasingly higher acute thermal temperatures, and that these effects will be cumulative with time. We also hypothesize acclimation to a simulated heatwave event (5 d exposure to +4 °C) will buffer the effects of exposure to an acute thermal shock (2–12 h exposure to extreme high temperatures 25–34 °C), which would be expressed by increased survival at higher temperatures. Lastly, we hypothesize LT50 will occur at higher temperatures for MHW acclimated larvae compared to non-MHW acclimated larvae, and the LT50 value will decrease with exposure time under acute thermal stress.

2. Materials and Methods

2.1. Spawning and rearing

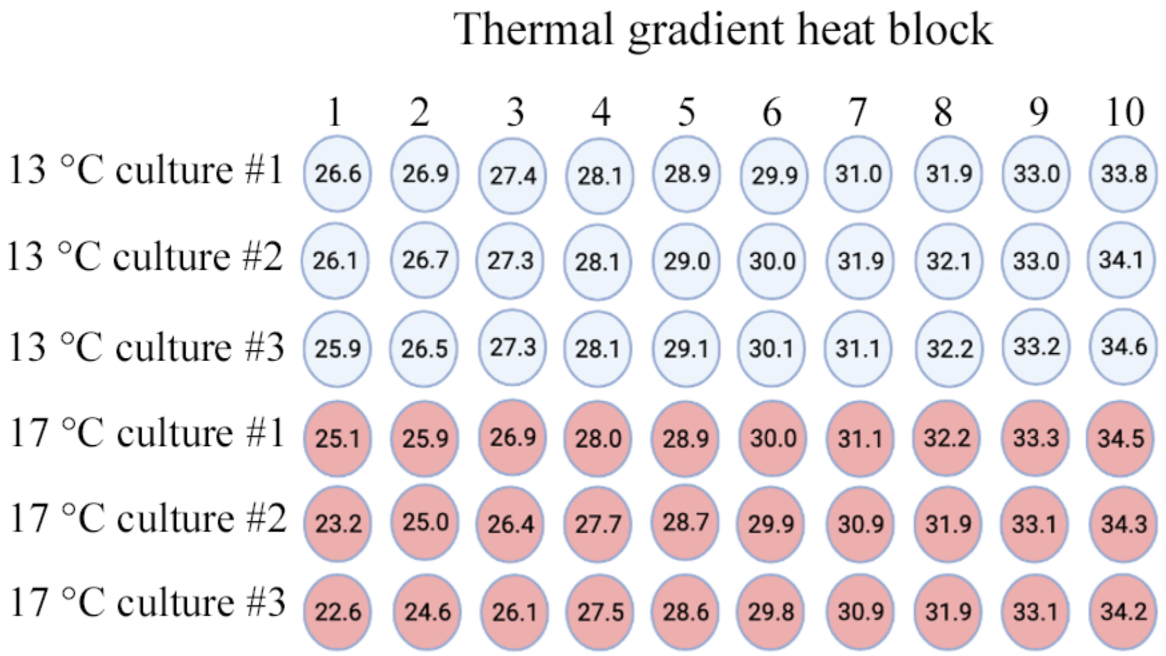
P. ochraceus adults were collected from the shoreline near Friday Harbor Laboratories, Friday Harbor, WA (FHL) in March 23, 2023 by Sophie George. Adults were sexed after gonad biopsy via small 1-cm^2 incisions at arm junctions (Hodin et al. 2021). All fertilizations occurred in March 2023. For surgical fertilizations, two females and two males were biopsied as above. Testes were stored in a tube on ice until use. Ovaries were rinsed gently in 1-mm filtered seawater (FSW) and placed in a dish of the maturation hormone 1-methyl-adenine (10^{-5} M in FSW). For both biopsy and natural spawn events, fertilizations were confirmed microscopically to have >90% success. The embryos were rinsed several times to remove excess sperm and placed in 2 L beakers of 1-mm FSW. For surgical fertilizations, gametes from all males and females were mixed to increase genetic variability. Experimental vials containing larvae from various male–female crosses and fertilizations were not monitored separately (see below).

The embryos (~1 ml^{-1}) were reared in 3 L glass beakers of 1-µm FSW and maintained at 11–13°C (± 1°C) in seatables. At 5 days post-fertilization (dpf), larvae had developed a complete digestive tract, indicating the feeding bipinnaria stage. For the first 3.5 months, larvae were subsisted on a low food diet of 2500 cells ml^{-1} of the live microalgae *Rhodomonas* sp. and fed every other day after a water change to delay development until use in experiments (*direct comms*, Jason Hodin). 2 weeks prior to use in experiments, cultures were fed a mix of 2500 cells ml^{-1} *Rhodomonas* sp. and 3000 cells ml^{-1} of *Dunaliella tertiolecta* after water changes (~80% of the water was replaced) by gentle reverse filtration (Hodin et al., 2019). Water temperature (~12°C), dissolved oxygen (~ 8.64, ± 0.4), and salinity (30 ‰, ± 1 ‰) were measured with a graduated glass thermometer, YSI ProODO, and refractometer (Tiaoyeer Brix ATC), respectively, every day and adjusted as needed. The water in culture jars was gently agitated with slow-moving stirring paddles to suspend larvae while maintaining temperature and dissolved oxygen (Strathmann, 2014).

2.2 Thermal acclimation set-up

Brachiolaria stage *P. ochraceus* larvae (n = 70), with a normal morphology (following Pia et al. 2012), were hand-picked from rearing cultures using stereomicroscopes and randomly assigned to a 3 L culture jar with 1 µm filtered sea water (FSW) at either 13°C (ambient Friday Harbor water, control, n = 3) or 17°C (+ 4 °C above ambient Friday Harbor water, high temperature, n = 3). Cultures were set up in two sea tables. Control beakers were placed in a flow through sea table with water intake from Friday Harbor. Water temperature was measured daily and varied minimally (mean = 13 °C, ± 2.5 °C, n = 5). The +4 °C cultures were maintained at 17 °C temperature in a static water bath in the laboratory sea tables. These water bath temperatures were stable (measured daily, ± 1 °C, n =5), maintained using an external Thermo Scientific PC200–A25 Arctic refrigeration bath circulator (7–12 L), that circulated heated water through a submerged radiator condenser coil. Temperatures within the sea tables were kept uniform with constant agitation by slow-moving mechanized stirring paddles (Strathmann, 2014). Temperature and DO, in culture, was measured daily with a YSI ProODO sensor and stable (DO: ± 1, Temperature: ± 0.4 °C, n = 5). Salinity was held constant at 30 ‰, measured with a refractometer (Tiaoyeer Brix ATC). Cultures were acclimated for 5 d. An 80 % water change was done on the third day.

2.3 Thermal limit estimation



Thermal gradient heat block

	1	2	3	4	5	6	7	8	9	10
13 °C culture #1	26.6	26.9	27.4	28.1	28.9	29.9	31.0	31.9	33.0	33.8
13 °C culture #2	26.1	26.7	27.3	28.1	29.0	30.0	31.9	32.1	33.0	34.1
13 °C culture #3	25.9	26.5	27.3	28.1	29.1	30.1	31.1	32.2	33.2	34.6
17 °C culture #1	25.1	25.9	26.9	28.0	28.9	30.0	31.1	32.2	33.3	34.5
17 °C culture #2	23.2	25.0	26.4	27.7	28.7	29.9	30.9	31.9	33.1	34.3
17 °C culture #3	22.6	24.6	26.1	27.5	28.6	29.8	30.9	31.9	33.1	34.2

Fig. 1. Stable static thermal gradient heat block set up with temperature of each 1.1 × 4.2 cm well.

From the 5 d acclimation cultures, 5 larvae were handpicked under a stereomicroscope and placed into microcentrifuge tubes (1.5 mL, 5 larvae/tube). Upper thermal limit assay (LT_{50}) was performed on *P. ochraceus* larvae following thermal exposure and live:dead enumeration protocol per Chan et al. (2023). The microcentrifuge tubes were randomly placed in one of the 6 × 10 milled holes (1.1 cm diameter × 4.2 cm deep) in an aluminum heat block with a stable static thermal gradient temperature ranging from 26.5–34°C (Fig. 1). *P. ochraceus* larvae were scored for survival at 2, 4, and 12 h. Mean percentages were calculated for each time point from the proportion of alive larvae versus the starting number of larvae (n = 5) at the beginning of the assay for each replicate.

2.4 Statistical analyses

Statistical analyses were conducted using *R* (version 4.0.5, R Core Team 2021). Percentage data on the survival of late-stage *P. ochraceus* larvae at 2, 4, and 12 h of exposure (exposure time) to an extreme thermal temperature gradient after 5 d in acclimation were plotted as box plots. After an arcsine square root transformation, mean percentage survival were analyzed with repeated measures 3-way ANOVA using the *aov* function (*stats* package). Levene's test validated the data was homoscedastic (Levene's test, $p > 0.05$) and the normal distribution assumption was confirmed by visual inspection of the model residual distribution on the q-q plot. Post-hoc tests were not computed for significant effects due to the difficulty of interpreting a significant 3 factor interaction (Length, 2016). To determine the lethal temperature (LT_{50}, the temperature at which 50 % mortality occurs), generalized linear models were run on arcsine square root transformed proportional survival data frames with binomial error (survival = 1, dead = 0) and logit link functions. LT_{50} values were extracted from these models using the *dose.p* function in the MASS package (Ripley et al., 2002).

Results

Overall, for the percentages of brachiolaria larvae survival, temperatures ≥ 27.5 °C were deleterious for the control acclimation group and ≥ 30 ° C were deleterious for the elevated temperature acclimation group (Figs. 1). By 2 h, survival was impacted across nearly all acute thermal treatments, with 0 % survival occurring in most acute temperatures > 32 °C regardless of acclimation temperature (Fig. 1). At this time, larval survival declined relatively gradually from 100 % at 27.5 °C to 0 % at 32 °C for larvae from control acclimations (Fig. 1). In the elevated thermal acclimation group, a far sharper decline in survival from 100 % at 31 °C to 0 % at 32 °C occurred (Fig. 1). Over time, mortality increased and the temperature at which 100 % mortality occurred in both control and elevated temperature acclimated larval groups shifted to 30 °C and 31 °C respectively (Fig. 1).

All model terms in the repeated measures 3-way ANOVA were significant (Table 2). The 3-way interaction between acclimation treatment, exposure time to the acute thermal temperature array and the acute thermal exposure temperature significantly influenced the mean percentage larval survival (Table 2). This interaction is likely occurring due to the decreasing survival % over the acute thermal temperature array with time in conjunction with the small changes in greater mean percentage survival between the acclimation groups over time at the 30 °C acute temperature (Fig 1, Table 2). The decline in survival over time is shown by the decrease of the LT_{50} for both acclimation groups (Table 3). The LT_{50} for both acclimation groups decreased by ~ 1.5 °over the 12 h exposure to acute thermal temperature, indicating a cumulative deleterious effect of high acute thermal shock on the larvae (Table 3). The LT_{50} value was consistently ~ 1 °C lower in the ambient acclimation group compared to the elevated temperature group (Table 3).

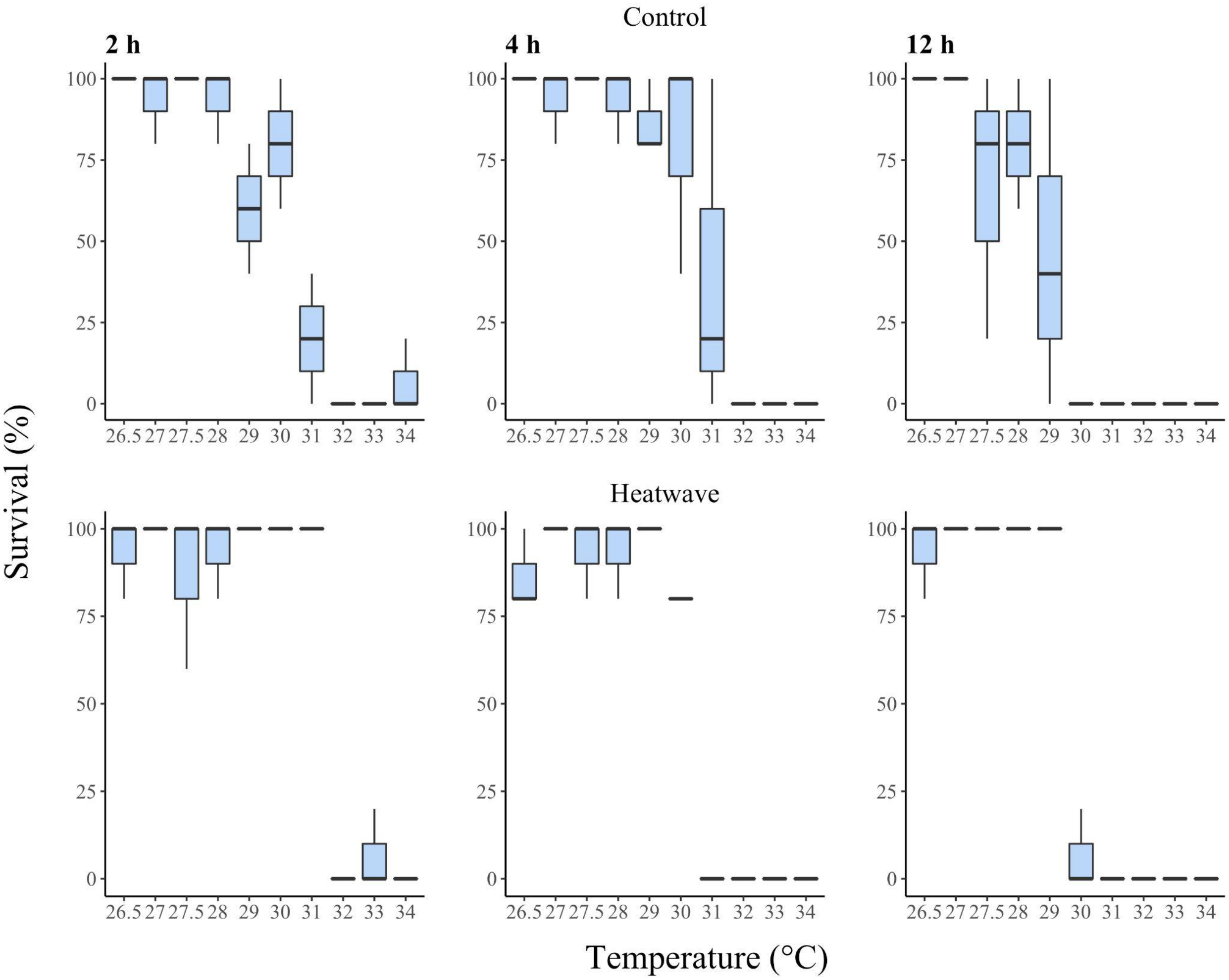


Fig 1. Percentage of brachiolaria reared at ambient San Juan Island temperature for June 2023 (13°C, top row) and a simulated +4 °C MHWtemperature (17°C, bottom row) surviving after 2 h (left), 4 h (center), and 12 h (right) exposures to temperatures ranging from 26–34°C. The box plots show treatment medians and interquartile range (25th–75th percentiles); maximum and minimum.

Table 2. Repeated measures 3-way ANOVA to test for mean differences in arcsine transformed percentages of competent brachiolaria. Acclimation treatment ("Acc temp", two levels: 13 °C, 17 °C), exposure time to the acute thermal temperature array ("Exposure time", 3 levels: 2, 4 and 12

h) and acute thermal exposure temperature ("Acute temp", 10 levels: 25 – 34 °C) were included in the analysis as fixed effects. Replicate Id was computed as a random effect to control for correlations between observations on repeated measures.

Factors	**df**	**SS**	**MS**	**F**	**p-value**
Acc temp	1	0.42	0.42	8.259	**0.006**
Acute temp	9	69.99	7.78	152.792	**< 0.001**
Exposure time	2	2.13	1.07	17.82	**< 0.001**
Acc temp* Acute temp	9	1.75	0.195	3.831	**0.001**
Acc temp*exposure time	2	0.746	0.37	6.231	**0.003**
Acc temp*Acute temp*exposure time	18	3.126	0.174	6.67	**< 0.001**
Error	80	4.790	0.06		

Table 3. The upper lethal temperature (LT_{50}) values for the temperature at which 50 % brachiolaria survival occurred for larvae acclimated at 13 °C and 17 °C at 2, 4 and 12 h of exposure to acute temperatures ranging from 26.5 – 34 °C.

Acclimation temperature (°C)	**Time of exposure to pulse temperature**	**Temperature with 50 % survival (°C, ±SE)**
13 °C	2 h	30.05 (0.44)
	4 h	30.4 (0.41)
	12 h	28.55 (0.32)

17 °C	2 h	31.3 (0.5)
	4 h	29.87 (0.41)
	12 h	29.51 (0.3)

Discussion

Both chronic climate change associated with ocean warming and acute events such as MHWs are expected to greatly impact the ability of marine organisms to endure these severe changes in environmental conditions (Byrne et al., 2022). Ecosystems affected by western boundary currents are experiencing warming which will exacerbate the extent and intensity of MHWs (Kumgai et al., 2018; Vergés et al., 2014). Organisms that are not adapted to significant changes in water temperature are likely to struggle, particularly at early, more vulnerable life stages (Balogh & Byrne, 2020; Byrne et al., 2018; Gall et al., 2021). Thermal tolerance is essential to ascertaining how species will adapt to impending acute and chronic warming events in their habitats, however, there are few. Investigating this issue has significance to the greater population as dispersive life stages largely determine the population structure and persistence of the species (Byrne et al., 2022).

We asked what is the tolerance of Pisaster larvae to acute heat shocks, and whether that tolerance was modified by prior exposure to moderate heat stress. Survival in late-stage *P. ochraceus* larvae exhibited high thermal tolerance to acute thermal shock (≥80% survival) to half-day exposures of temperatures ~14°C above the ambient temperature seen in the waters of the San Juan Islands. The larvae pretreated with a prior 5 days of simulated MHW conditions were more resilient compared to the 5 d control group.

Deleterious tipping points representing a steep decline toward 100% mortality above thermal optima are typical for marine invertebrates (Kroeker and Sanford, 2022). In the present study, late-stage *P. ochraceus* brachiolariae demonstrated a steep decline toward 0% survival at acute thermal shock (2–12 h) ≥27°C (14°C above ambient, regardless of MHW acclimation). That decline occurred in close proximity to the upper limit of the thermal optima range. For *P. ochraceus* bipinnariae after a 1 of week thermal stress >16°C (2.65°C above ambient for the San Juan Islands archipelago (Titus and Hearther, 2019), mortality approached 100%. The timing, extent, and developmental stage are therefore likely important in determining the effect of press and pulse thermal stress on *P. ochraceus* larvae, and thus their ability to recruit into the benthic population.

Acclimation to +4°C appeared to partially buffer the deleterious effects of subsequent extreme thermal shock for over 12 h. For marine invertebrates, thermal acclimation in the embryonic, pre-swimming larval stages can shift later development stage optimal temperatures (Byrne et al., 2011), including echinoderms (Fujisawa, 1995; Bingham et al., 1997). However, the mediation of deleterious effects on survival in larvae acclimated to MHW conditions suggests larvae from populations in relatively high latitude (cool) isotherms may have a capacity to tolerate shorter pulses of high temperature water if these larvae are pre-exposed to presses of higher temperatures. These results also suggest acute heat stress experiments need to reflect long natural timescales in order to truly draw a conclusion on their thermal tolerances. Thermal tolerance plasticity has been observed across the marine invertebrate phyla (Sasaki et al., 2021; Hamdoun et al., 2003; Magozzi et al., 2015), including for echinoderm embryos and bipinnariae (early stage) that were exposed to pulses of 19.4–36.5°C after 72 h (Lamare et al., 2014). The animals that we studied here originate in a relatively poleward (i.e., cooler) isotherm compared to individuals from

Baja California, Mexico, the southernmost extent of the species (Lamb et al., 2006). With potential carryover effects between generations of echinoderms (Minuti et al., 2002), accurate assessment of the thermal tolerance window of *P. ochraceus* larvae and the impact of thermal stress requires thermal tolerance assessments on larvae from different isotherms. Especially for larval populations at the upper extreme of the realized thermal niche.

The loss of top predators can alter community structure with consequences for local biodiversity, conservation efforts, and ecosystem processes (Ritchie & Johnson, 2009; Rooney et al., 2006; Estes et al., 2011). Classic examples include the removal of wolves in Yellowstone National Park, USA (Ripple et al., 2001) and the loss of *Pycnopodia helianthoides* due to sea star wasting disease (Schultz et al., 2016). Decreasing the number of successful larvae, especially for a keystone species such as *P. ochraceus*, has the potential to disrupt the predator–prey balance in a variety of habitats. The sea star wasting disease outbreak in 2013 received significant attention and highlighted the ecological importance of *P. ochraceus* as a predator. The results of this study could serve as an indicator that MHWs may not have a significant impact on *P. ochraceus* larval survival and as a result, the role as a keystone species In 2015, the predation rate on mussels was significantly lower than the long-term average (Menge et al., 2016), which could play an important role in determining the lower range of mussel beds along the West Coast (Paine, 1974). Predation rates on mussels by *P. ochraceus* help with the establishment of macrophyte and invertebrate species that are usually excluded by *Mytilus californianus* (Menge & Sanford, 2013). Therefore, a significant decline in *P. ochraceus* larval success as a result of the increased frequency of MHWs could lead to a reduction in the diversity in these lower zones dominated by *M. californianus* beds.

Conclusion

Continuous heatwave events such as MHWs are expected to increase in duration and frequency as a consequence of climate change. Therefore, it is extremely important to assess how MHWs will impact marine invertebrates, especially at life stages in which they are more vulnerable to a changing environment. This study sheds light on how larvae may respond to changes in their environment by comparison of larval thermal tolerance in MHW acclimated (17°C) and ambient acclimated conditions (13°C). Here, we observed a significant impact of temperature on the survival of *P. ochraceus* larvae as the duration of exposure to an acute heat shock reached 12 h. *P. ochraceus* larvae that were acclimated to the MHW treatment (17°C) demonstrated higher survival rates at higher temperatures (31°C) compared to larvae that were acclimated to ambient temperatures (13°C), whose survival rates were higher at lower temperatures (27°C). This study contributes important insights to the critical knowledge gap of understanding how larval stages of an important keystone species are responding to environmental changes.

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