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## High Heat Tolerance is Negatively Correlated with Upper Thermal Tolerance Plasticity in North Eastern Pacific Nudibranch Mollusks

### Comments

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# High Heat Tolerance Is Negatively Correlated with Heat Tolerance Plasticity in Nudibranch Mollusks

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## ABSTRACT

Rapid ocean warming may alter habitat suitability and population fitness for marine ectotherms. Susceptibility to thermal perturbations will depend in part on plasticity of a species' upper thermal limits of performance ( $CT_{max}$ ). However, we currently lack data regarding  $CT_{max}$  plasticity for several major marine taxa, including nudibranch mollusks, thus limiting predictive responses to habitat warming for these species. In order to determine relative sensitivity to future warming, we investigated heat tolerance limits ( $CT_{max}$ ), heat tolerance plasticity (acclimation response ratio), thermal safety margins, temperature sensitivity of metabolism, and metabolic cost of heat shock in nine species of nudibranchs collected across a thermal gradient along the northeastern Pacific coast of California and held at ambient and elevated temperature for thermal acclimation. Heat tolerance differed significantly among species, ranging from  $25.4^{\circ} \pm 0.5^{\circ}C$  to  $32.2^{\circ} \pm 1.8^{\circ}C$  ( $\bar{x} \pm SD$ ), but did not vary with collection site within species. Thermal plasticity was generally high ( $0.52 \pm 0.06$ ,  $\bar{x} \pm SE$ ) and was strongly negatively correlated with  $CT_{max}$  in accordance with the trade-off hypothesis of thermal adaptation. Metabolic costs of thermal challenge were low, with no significant alteration in respiration rate of any species 1 h after exposure to acute heat shock. Thermal safety margins, calculated against maximum habitat temperatures, were negative for nearly all species examined ( $-8.5^{\circ} \pm 5.3^{\circ}C$ ,  $\bar{x} \pm CI$  [confidence interval]). From these data, we conclude that warm adaptation in intertidal nudibranchs constrains plastic responses to acute thermal challenge and that southern warm-adapted species are likely most vulnerable to future warming.

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*Keywords:* gastropod, thermal physiology, acclimation, thermal sensitivity, trade-off hypothesis.

## Introduction

Temperature is a critical abiotic driver in determining distribution patterns of ectothermic organisms (Angilletta 2009; Somero et al. 2017). Predicting how climate change will shift these patterns requires an understanding of physiological sensitivity to increased mean and extreme temperatures. Beyond the scope of basic temperature-driven effects on physiological processes (i.e.,  $Q_{10}$  effects), which can pose significant ecological challenges for an organism (e.g., increased energetic demands, reduced oxygen supply capacity, etc.), two factors determine a species' relative vulnerability to severe temperature exposure: its inherent heat tolerance ( $CT_{max}$ ) and its ability to reversibly adjust heat tolerance (plasticity; Angilletta 2009; Verberk et al. 2017).

Inherent heat tolerance ( $CT_{max}$ ) is often defined as an ecologically significant thermal threshold (e.g., a temperature triggering the loss of motor control) after which survival is unlikely or time limited (Angilletta 2009). In thermally challenging environments such as the intertidal zone, where habitat temperatures regularly or frequently exceed tolerance thresholds, thermal selection around the upper heat tolerance limit can be strong. Accordingly, inherent heat tolerances in many intertidal ectotherms often closely correspond with maximal habitat temperatures, a pattern suggestive of thermal adaptation (Tomanek and Somero 1999; Stillman and Somero 2000). It is important to note, however, that whereas these evolutionary adjustments in tolerance limits can be adaptive, their maintenance is often energetically demanding. For example, maintenance of high inherent heat tolerance limits requires sustained or mobilized expression of chaperonins and damage repair proteins that have associated life-history costs (Feder et al. 1992; Krebs and Feder 1997b), including reduced reproductive potential (Krebs and Feder 1997a; Silberman and Tatar 2000; Elbaz et al. 2011) and lowered feeding activity and energy gain (Giomi et al. 2016). These costs, in addition to basic evolutionary constraints (e.g., low heritability of thermal response traits under strong but fluctuating selective pressures), leave open the question of whether thermal adaptation can offset the current pace of habitat warming (Hoffmann and Sgró 2011; Bay et al. 2017).

Adaptive shifts are not the only way to modify tolerance thresholds, however. Short- or long-term reversible responses (e.g., acclimation or acclimatization) can also significantly adjust thermal tolerance limits (Somero 2010; Huey et al. 2012) and may be

important in reducing the risk of overheating posed by rising global temperature. However, phenotypic plasticity of this nature can incur costs to an organism in at least two ways: costs of expressing suboptimal phenotypes under shifting environmental conditions and costs of maintaining the capacity to respond to environmental change (Auld et al. 2009). Both costs can impose trade-offs on energy allocation with consequences for organismal fitness and population persistence (Auld et al. 2009; Sokolova et al. 2012). This raises an important ecological question: given limited resources and the costs inherent in mounting responses to thermal extremes, must organisms “choose” between thermal response strategies; that is, does the energetic investment in maintenance of a high tolerance limit negatively impact a species’ ability to respond plastically to a thermal challenge, or are the two responses mutually reinforcing? Addressing this question is vital for understanding how organisms may respond to increased habitat warming.

The evolutionary relationship between heat tolerance and plasticity remains elusive. In some ectotherms, the maintenance of high heat tolerance comes at the cost of (i.e., trades off against) plastic response capacity. This trade-off hypothesis of thermal adaptation has been demonstrated in *Petroliastes* porcelain crabs (Stillman 2003) and shrimp (Magozzi and Calosi 2015). However, in other species, most notably *Deronectes* diving beetles, heat tolerance and plasticity are positively correlated (Calosi et al. 2008), suggesting that no trade-off exists. However, recent work examining the confounding role of oxygen supply structures has put the physiological basis of this pattern into question (Verberk et al. 2017). Complicating these results, recent meta-analyses failed to provide global support for either pattern of thermal adaptation among a diverse set of taxa including fish, amphibians, crustaceans, insects, and reptiles (Overgaard et al. 2011; Gunderson and Stillman 2015). Those analyses, however, did not include estimates for several ecologically important taxa, including mollusks.

Whereas inherent heat tolerance has been quantified extensively in mollusks (Read and Cumming 1967; Stirling 1982; Clarke et al. 2000; Compton et al. 2007; Re et al. 2013; Marshall et al. 2015; Chen et al. 2016), heat tolerance plasticity has only rarely been surveyed in this group (McMahon and Payne 1980; Clarke et al. 2000; Seebacher et al. 2015; Gleason et al. 2018) and never in some lineages such as nudibranch gastropods. Thus, predictions of responses to warming in these taxa are currently hampered by limited data. Indeed, relatively few studies have explicitly investigated thermal effects at any level of organization in nudibranchs, and thermal limits of performance have been reported only twice previously in this group (Clark 1975; Pires 2012). This lack of data is surprising given that, in many ways, nudibranchs are an ideal system in which to assess the relationship between inherent tolerance limits and thermal tolerance plasticity.

Nudibranchs represent a species-rich and plastic lineage that includes several eurythermal members (mostly intertidal species) able to cope with wide and frequent temperature fluctuations. However, unlike other highly mobile ectotherms (e.g., reptiles, crustaceans, and insects), slow-moving nudibranchs have a limited capacity to escape thermally stressful environ-

ments and are therefore likely to be reliant on physiological rather than behavioral mechanisms of compensation (Marshall et al. 2011; but for a discussion of behavioral limits, see Monaco et al. 2017). One common thermal compensation strategy adopted by other limited-mobility mollusks is the energy conservation tactic of metabolic suppression (McMahon et al. 1995; Sokolova and Pörtner 2001, 2003; Marshall and McQuaid 2011; Sokolova et al. 2012). Whereas this strategy confers several physiological benefits during heat stress (e.g., reduced ATP demand, reduced production of damaging reactive oxygen species, avoidance of decoupling of rates in linked processes, etc.; Hochachka and Guppy 1987), it also poses significant ecological costs (e.g., reduced time for foraging and/or increased vulnerability to predation) and may be less effective for nudibranchs, which lack a protective shell or water-impermeable cuticle and therefore have a significantly reduced capacity to shelter in place relative to other mollusks.

Taken together, these features suggest that thermal response in intertidal nudibranchs is likely to be under strong physiological and ecological constraint, making nudibranchs an ideal system in which to investigate evolutionary trade-offs among physiological response traits such as inherent tolerance and plasticity. Consistent with this assertion, several recent range expansions among nudibranch mollusks suggest that the group as a whole may be particularly responsive “indicator species” to climate-induced warming (Goddard et al. 2011; Nimbs et al. 2016; Sanford et al. 2019) and therefore useful models for further investigation of fundamental responses to thermal challenges.

Here, we use an integrative approach to investigate acute heat tolerance, tolerance plasticity, and relative thermal sensitivity of aerobic metabolism across eight species of closely related mid-intertidal zone nudibranchs from along the northeastern Pacific coast of North America (fig. 1) in order to assess (1) patterns of vulnerability to warming across species, including estimates of acute thermal limits and metabolic sensitivities, and (2) potential trade-offs in thermal response characteristics. We hypothesized that species with the most southerly range limits (i.e., the putative “warm-adapted” species) such as *Okenia rosacea* and *Hermisenda opalescens* (Goddard et al. 2018; Merlo et al. 2018) would exhibit lower thermal sensitivities of metabolism, higher heat tolerances, and lower heat tolerance plasticities than more northerly distributed lineages. To test these hypotheses, acute heat tolerances ( $CT_{max}$ ) were assessed as onset of neuromuscular failure and thermal sensitivities of aerobic metabolism ( $Q_{10}$ ) were examined across a range of environmentally relevant temperatures in nine species of intertidal nudibranch mollusks.

## Methods

### *Organism Acquisition and Acclimation*

Adult specimens of nine intertidal nudibranch species, *Dirona picta* (MacFarland), *Doriopsilla albopunctata* (Cooper), *Doriopsilla fulva* (MacFarland), *Doris odhneri* (MacFarland), *Hermisenda opalescens* (Berg), *Okenia rosacea* (MacFarland), *Phidiana hiltoni* (O’Donoghue), *Triopha catalinae* (Cooper), and *Triopha maculata* (MacFarland), were collected under California

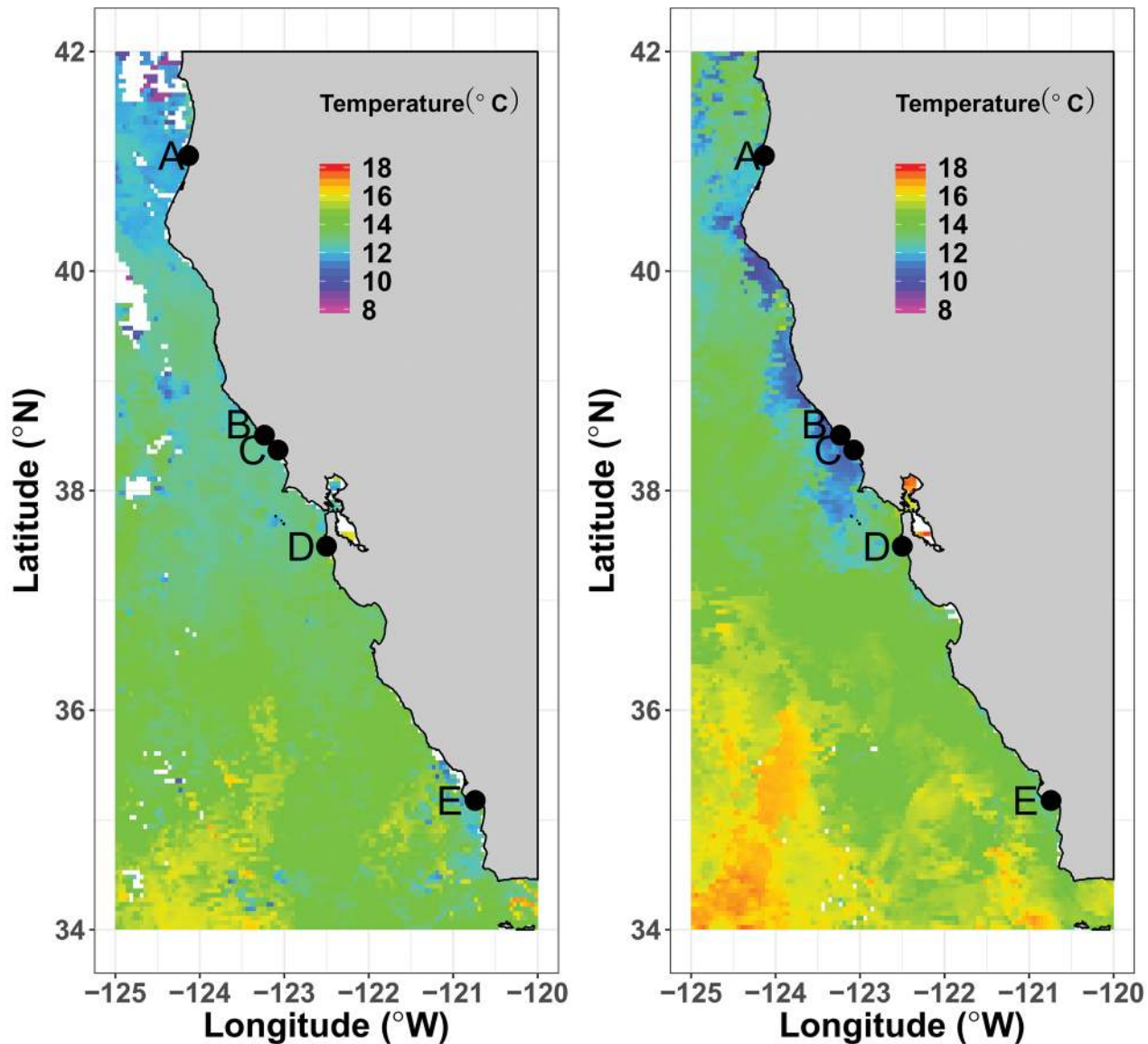


Figure 1. Maps of sea surface temperatures and collection sites for nudibranch species along the coast of California. All individuals were collected from near-shore intertidal environments. Collections were conducted at Trinidad ( $41^{\circ}03'06.2''\text{N}$ ,  $-124^{\circ}07'58.5''\text{W}$ ; A), Fort Ross ( $38^{\circ}30'20.6''\text{N}$ ,  $-123^{\circ}14'03.9''\text{W}$ ; B), Bodega Bay ( $38^{\circ}22'12.4''\text{N}$ ,  $-123^{\circ}04'29.1''\text{W}$ ; C), Pillar Point ( $37^{\circ}29'42.2''\text{N}$ ,  $-122^{\circ}29'51.4''\text{W}$ ; D), and Avila Beach ( $35^{\circ}10'40.7''\text{N}$ ,  $-120^{\circ}44'25.2''\text{W}$ ; E), California. Overlain are satellite-derived mean sea surface temperature data for February 2016 (left) and May 2016 (right) from NASA Earth Observations (<https://neo.sci.gsfc.nasa.gov>), illustrating the strong coastal sea surface temperature shifts that occur with upwelling in spring.

Department of Fish and Wildlife permit SC-13357 from sites along the Californian coast between the latitudes of  $35^{\circ}\text{N}$  and  $42^{\circ}\text{N}$  from February to June 2016 (fig. 1; table 1). Whereas geographical ranges of these species overlap around the San Francisco Bay region on the central California coast, several display predominantly southerly distributions (being rarely sighted northward of southern Oregon), including *D. albopunctata*, *H. opalescens*, *O. rosacea*, and *T. maculata* (Goddard et al. 2016). All specimens were collected from low- to mid-intertidal zones during low tide, and the vast majority were found submerged, sheltering in tidal pools.

After collection, specimens were transported back to research aquaria in Tiburon, California, and held in 26-L polyethylene flow-through seawater tanks (one tank per species per collection site) under common-garden acclimation conditions ( $13^{\circ} \pm 1^{\circ}\text{C}$ ,  $31 \pm 3.5$  psu,  $\bar{x} \pm \text{SD}$ ) for at least 3 d (maximum 14 d) before transfer to flow-through experimental acclimation tanks. Animals were acclimated to one of two temperatures,  $13^{\circ} \pm 1^{\circ}\text{C}$  or  $17^{\circ} \pm 1^{\circ}\text{C}$  ( $31 \pm 3.5$  psu,  $\bar{x} \pm \text{SD}$ ), where they were held for 11–31 d before heat tolerance measurement (table 1). Specimens from each collection site were divided as evenly as possible between acclimation treatments. Acclimation tempera-

Table 1: Nudibranch collection location and acclimation information

Species and collection details	Collection details			Acclimation period (d)
	Location	<i>n</i>	Date	
<i>Doriopsilla albopunctata/fulva</i>	Avila <sup>a</sup> /Bodega Bay <sup>b</sup> /Pillar Point <sup>c</sup>	7/19/24	June/June/February 2016	11–13
<i>Doris odhneri</i>	Bodega Bay <sup>b</sup>	3	June 2016	13–15
<i>Hermisenda opalescens</i>	Avila <sup>a</sup> /Bodega Bay <sup>b</sup>	10/19	June/February 2016	11–31
<i>Okenia rosacea</i>	Bodega Bay <sup>b</sup> /Fort Ross <sup>d</sup> /Pillar Point <sup>c</sup> /Trinidad <sup>e</sup>	32/6/24/12	June/June/February/May 2016	13–30
<i>Phidiana hiltoni</i>	Avila <sup>a</sup>	2	June 2016	11–13
<i>Triopha maculata</i>	Bodega Bay <sup>b</sup>	16	June 2016	13–15
<i>Triopha catalinae</i>	Bodega Bay <sup>b</sup>	9	June 2016	13–15

<sup>a</sup>Avila, California (35°10'40.7"N, 120°44'25.2"W).

<sup>b</sup>Bodega Bay, California (38°22'12.4"N, 123°04'29.1"W).

<sup>c</sup>Pillar Point, California (37°29'38"N, 122°30'00"W).

<sup>d</sup>Fort Ross, California (38°30'20.6"N, 123°14'03.9"W).

<sup>e</sup>Trinidad, California (41°03'06.2"N, 124°07'58.5"W).

tures were selected to reflect current annual mean habitat temperature experienced in the low-intertidal zone at Fort Ross, California ( $12.6 \pm 1.8^\circ\text{C}$ ,  $\bar{x} \pm \text{SD}$ ), and predicted future mean global temperature increase at the end of the century under the IPCC (2014) A1FI scenario. Constant acclimation temperatures were used in this study in order to permit comparison of estimates of nudibranch heat tolerance with those provided for other ectothermic taxa in recent meta-analyses (Overgaard et al. 2011; Gunderson and Stillman 2015). Specimens were starved throughout the acclimation period. For the purposes of measuring heat tolerance and tolerance plasticity, prolonged starvation was unlikely to have acted as an added stressor, as multiple individuals not used in thermal tolerance tests persisted and reproduced over a 4-mo period without feeding.

#### Habitat Temperatures

In order to accurately capture the fine-scale spatial and temporal thermal heterogeneity characteristic of intertidal zone habitats, mean daily maximum and absolute maximum habitat temperature data were calculated from data collected with iButton loggers deployed to record under-rock temperatures in the mid-intertidal zone at Fort Ross (July 1, 2015, 00:14 PST, to July 31, 2015, 23:52 PST and July 1, 2016, 00:12 PST, to July 31, 2016, 23:31 PST; Gunderson et al., unpublished data), Bodega Bay (July 18, 2004, 04:00 PST, to July 31, 2004, 23:50 PST and July 1, 2007, 12:00 PST to July 31, 2007, 23:30 PST), and Lompoc Landing (near Avila), California (July 6, 2004, 16:00 PST, to July 20, 2004, 21:10 PST; data from the Intertidal Biomimic Logger Database maintained by Dr. Brian Helmuth; Helmuth et al. 2016).

#### Experimental Protocol

**Determination of Heat Tolerance ( $CT_{max}$ ), Heat Tolerance Plasticity, and Thermal Safety Margins.** Inherent heat tolerance ( $CT_{max}$ ) was defined in this study as the body temperature at which a

specimen lost neuromuscular coordination in the foot muscle such that it could no longer maintain attachment to the substrate (McMahon 1990; Lutterschmidt and Hutchison 1997; Clarke et al. 2000; Hoefnagel and Verberk 2017). To determine  $CT_{max}$  of foot function, we used a modified version of a protocol applied previously in the sea hare *Aplysia californica* (Re et al. 2013). Each specimen was placed alone on the side of a lightly aerated 250-mL glass beaker modified with a 1-cm-wide strip of artificial turf grass installed at the water's surface to prevent escape. Beakers were placed in a 13.5-L water jacket, and individuals were held for 30 min at their respective acclimation temperature before experimentation. Water temperature was then raised at a rate of  $1^\circ\text{C}$  every 15 min using a Fisher Scientific Isotemp water bath, and temperature data were recorded with iButton ThermoChron loggers (Maxim Integrated, San Jose, CA). The time and temperature at which a specimen began to show signs of thermal stress (e.g., lost attachment to the glass, displayed contorted mantle, and entered heat coma) were recorded, after which specimens were gently prodded for a period of 2–3 s every 10–15 min until they ceased reacting to this stimulus. A specimen's  $CT_{max}$  was then recorded as the temperature at which response to mechanical stimulation had ceased. Nonresponsive individuals were immediately returned to their acclimation temperature for recovery, and all individuals successfully recovered after heat shock.

Heat tolerance plasticity was calculated as the change in upper heat tolerance limit with a given change in acclimation temperature and is hereafter referred to as the acclimation response ratio (ARR; Hutchison 1961; Gunderson and Stillman 2015):

$$\frac{CT_{max\ 17^\circ\text{C}} - CT_{max\ 13^\circ\text{C}}}{17^\circ - 13^\circ\text{C}}$$

Thermal safety margins (TSMs) were calculated as the difference between  $CT_{max}$  measured in  $13^\circ\text{C}$ -held specimens and (1) the mean daily maximum and (2) the absolute maximum habitat temperature recorded in the mid-intertidal zone at the collection site

from the warmest month of the year (July for all sites). TSMs were calculated for each population at their respective site of collection.

**Respirometry.** Rates of oxygen consumption ( $\dot{V}_{O_2}$ ) were measured twice for each specimen: once immediately before determination of  $CT_{max}$  and once approximately 1 h after heat shock. At both time points, specimens showed very little physical activity, and thus,  $\dot{V}_{O_2}$  likely approximates standard (or resting) aerobic metabolic rate.  $\dot{V}_{O_2}$  was determined using a PreSens Fibox 3 sensor (PreSens Precision Sensing; PST6v541 software) in closed-system 70-mL airtight vials fitted with planar optode spots (diameter 0.5 cm; PreSens Precision Sensing) and calibrated with seawater at 100% and 0%  $O_2$  saturation. Vials were filled with seawater at the acclimation temperature, sealed, and then placed in a circulating water bath at the corresponding acclimation temperature in the dark for 30 min before measurement of  $PO_2$ . Measurements were taken every 20 min for 2 h. The water in each vial was stirred (via lids fitted with magnetic stir bars) during measurement to prevent oxygen gradients from developing. Vials containing no nudibranchs (i.e., “blank” controls) were run in tandem with all experimental measurements of  $\dot{V}_{O_2}$ . After respirometry, all specimens were patted dry, weighed, and stored at  $-80^\circ C$ .  $\dot{V}_{O_2}$  measurements were then normalized by corresponding mass to give mass-specific estimates of standard metabolic rate for each specimen. Thermal sensitivity of mass-specific  $\dot{V}_{O_2}$  ( $Q_{10}$ ) was calculated for each species over the range of acclimation temperatures used in this study (i.e.,  $13^\circ-17^\circ C$ ; Hochachka and Somero 2001).

#### Data Analyses

**Statistical Analyses.** Our design included four predictor variables: three categorical (species, collection site, and acclimation temperature) and one continuous (body mass). Dependent variables were upper heat tolerance limit ( $CT_{max}$ ), plasticity of upper heat tolerance limit (ARR), basal metabolic rate (BMR), and  $Q_{10}$  of BMR. As some of our dependent variables (e.g., BMR) are known to be correlated with body mass, we performed correlation analyses against body mass for all dependent variables to determine which required application of covariate statistical analyses.

Differences in upper heat tolerance limits ( $CT_{max}$ ) within a species across acclimation temperature were examined separately for each species using linear mixed-effects models with acclimation temperature as a fixed factor and collection site as a random factor. Differences in upper heat tolerance limits ( $CT_{max}$ ) across species at a common acclimation temperature ( $13^\circ C$ ) were examined using a linear mixed-effects model with species as a fixed factor and collection site as a random factor. All linear models were performed using the “lme” package in the statistical software program R (ver. 3.2.2). General linear hypotheses and multiple comparisons for these nonparametric models were with the “glht” function in R. Data were tested for normality using the “shapiro.test” function of the “stats” package in R. Metabolic rate data were strongly leptokurtic (kurtosis = 56) and were transformed using a Lambert  $W \times$  Gaussian transformation via the “MLE\_LambertW” function of the “LambertW” package in R to generate a

normal distribution. In order to assess the effects of acute heat shock on aerobic metabolic rate in each species, we used a repeated-measures two-way ANOVA to compare pre- and post-heat exposure  $\dot{V}_{O_2}$  with heat stress and acclimation temperature as fixed effects and individual organism as the correlation factor.

**Testing the Trade-Off Hypothesis of Thermal Adaptation.** The trade-off hypothesis of thermal adaptation predicts that heat tolerance and tolerance plasticity should be negatively correlated such that species displaying high heat tolerance should exhibit correspondingly low heat tolerance plasticity. In order to test this assumption, goodness of fit was examined using a linear model of ARR as a function of  $CT_{max}$  for each species. However, because closely related species share a common ancestry, their physiological data are not independent and thus require phylogenetic consideration to assess species-level differences (Garland and Adolph 1994). We used the commonly applied phylogenetically independent contrasts (PICs) method to estimate the evolutionary correlation between characters and to apply phylogenetic correction to our heat tolerance and plasticity data.

The phylogeny used to generate PICs was gathered from publicly available cytochrome c oxidase subunit I molecular sequence data in the National Center for Biotechnology Information sequence database (table 2). Sequences were aligned using the program Geneious R10 (ver. 10.0.5; Kearse et al. 2012). A multiple sequence alignment using a progressive pairwise alignment algorithm was performed using global alignment with free end gaps and 65% similarity cost matrix with the Geneious R10 Aligner plug-in. Tree building was conducted in Geneious R10 by neighbor joining with a Tamura-Nei genetic distance model, and the tree was rooted with the outgroup species *A. californica*. We assessed support for the relationships by running 100 bootstrap replicates (Felsenstein 1985), and the tree was visualized using the ggtree package in R (Yu et al. 2016). PICs of ARRs and  $CT_{max}$  were generated using the multi2di and pic.x/y functions of the phytools and ape packages in R (Revell 2012). These contrasts were used in subsequent linear regression analysis, and the regressions were forced through the origin following standard procedures for analyses of independent contrasts (Garland et al. 1992).

Table 2: National Center for Biotechnology Information GenBank accession numbers for cytochrome c oxidase subunit I sequences (658 bp) used in phylogenetic analysis

Species	Accession no.	Reference
<i>Aplysia californica</i>	AF077759	M. Medina and P. J. Walsh, unpublished manuscript
<i>Hermisenda opalescens</i>	KU950161	Lindsay and Valdés 2016
<i>Triopha maculata</i>	HM162691	Pola and Gosliner 2010
<i>Triopha catalinae</i>	HM162690	Pola and Gosliner 2010
<i>Okenia rosacea</i>	KF192605	Palomar et al. 2014
<i>Doriopsilla albopunctata</i>	KR002485	Hoover et al. 2015
<i>Doriopsilla fulva</i>	KR002500	Hoover et al. 2015

## Results

### Heat Tolerance and Tolerance Plasticity

Inherent upper heat tolerance thresholds (CT<sub>max</sub>) differed significantly among species when measured at a common acclimation temperature of 13°C, and they ranged from a minimum of 25.4° ± 0.5°C ( $\bar{x}$  ± SD) in 13°C-acclimated *Doris odhneri* to a maximum of 33.6°C in 17°C-acclimated *Phidiana hiltoni* (linear mixed-effects model; table 3; fig. 2). CT<sub>max</sub> differed significantly within a species across acclimation temperatures in all but two species (*Hermisenda opalescens* and *Triopha maculata*; table 4). As there was no consistent pattern in CT<sub>max</sub> variation in relation to either maximum habitat temperature or collection site (i.e., latitude), site was treated as a random factor in all subsequent hypothesis testing by linear mixed-effects modeling. CT<sub>max</sub> (13°C acclimation group) was not correlated with body mass in any species. CT<sub>max</sub> ARR were positive for all species (0.52 ± 0.06,  $\bar{x}$  ± SE) except *H. opalescens* and *Okenia rosacea*, which displayed negative CT<sub>max</sub> ARRs of -0.17 ± 0.17 and -0.31 ± 0.11 ( $\bar{x}$  ± SE), respectively (not included in previous average; fig. S1, available online).

### Habitat Temperatures and TSMs

Mean daily maximum habitat temperatures were 28.8° ± 6.6°C ( $\bar{x}$  ± SD) at Bodega Bay, 26.6° ± 4.9°C at Lompoc (near Avila), and 22.8° ± 4.5°C at Fort Ross. Maximum habitat temperatures varied by ~8°C across the three sites, ranging from 42.5°C (July 2004, Bodega Bay), to 34.0°C (July 2004, Lompoc), to 35.5°C (July 2015, Fort Ross). In general, warmest temperatures were recorded at the northernmost site of Fort Ross. Mean daily maximum temperatures were, on average, 2.4° ± 2.3°C ( $\bar{x}$  ± SD) lower than CT<sub>max</sub> for the species examined, whereas maximum recorded habitat temperatures were almost universally higher than CT<sub>max</sub>. Thus, TSMs calculated against this latter metric were negative for nearly all species, with only two of nine species displaying positive TSMs at their respective sites of collection (fig. 3). Mean TSM calculated from maximum habitat temperatures was -8.5° ± 5.3°C ( $\bar{x}$  ± CI [confidence interval]).

### Comparative Analysis of the Tolerance-Tolerance Plasticity Trade-Off

Species with the highest inherent heat tolerance thresholds displayed the lowest plasticity, and thus, CT<sub>max</sub> and CT<sub>max</sub> ARR were strongly negatively correlated (fig. 4; linear regression,  $r^2 = 0.53$ ,  $F_{1,4} = 14.16$ ,  $P < 0.05$ ) across species. This relationship was also supported in the PICs (fig. 5; linear regression,  $r^2 = 0.88$ ,  $F_{1,4} = 34.33$ ,  $P < 0.01$  for CT<sub>max</sub> vs. CT<sub>max</sub> ARR contrast).

### Pre- and Poststress Metabolic Rates and Thermal Sensitivity of Metabolism

Prestress respiration rates differed significantly between species (linear mixed-effects model), ranging from a low of 0.74 ± 0.17 μmol O<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup> ( $\bar{x}$  ± SE) in 13°C-acclimated *Doriopsilla fulva* to a high of 4.09 ± 2.05 μmol O<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup> in 17°C-acclimated

Table 3: Linear mixed-effects model results of interspecies contrasts of inherent upper heat tolerance limits at a common acclimation temperature (13°C)

Contrast	Difference estimate (± SE)	Z	P
<i>Doriopsilla</i>			
<i>albopunctata</i> :			
<i>Doriopsilla fulva</i>	.06 ± .76	.08	1.00
<i>Doris odhneri</i>	-1.96 ± 1.16	-1.69	.71
<i>Dirona picta</i>	2.04 ± 1.84	1.11	.97
<i>Hermisenda</i>			
<i>opalescens</i>	2.60 ± .70	3.74	<b>.005</b>
<i>Okenia rosacea</i>	4.94 ± .59	8.39	<b>&lt;.001</b>
<i>Phidiana hiltoni</i>	3.67 ± 1.84	2.00	.50
<i>Triopha catalinae</i>	-1.86 ± .97	-1.93	.55
<i>Triopha maculata</i>	2.57 ± .91	1.16	.09
<i>D. fulva</i> :			
<i>D. odhneri</i>	-2.02 ± 1.16	-1.73	.68
<i>D. picta</i>	1.98 ± 1.84	1.07	.97
<i>H. opalescens</i>	2.54 ± .72	3.52	<b>.01</b>
<i>O. rosacea</i>	4.88 ± .62	7.88	<b>&lt;.001</b>
<i>P. hiltoni</i>	3.61 ± 1.86	1.94	.54
<i>T. catalinae</i>	-1.92 ± .97	-1.97	.51
<i>T. maculata</i>	2.51 ± .92	2.74	.11
<i>D. odhneri</i> :			
<i>D. picta</i>	4.00 ± 2.02	1.98	.51
<i>H. opalescens</i>	4.56 ± 1.11	4.12	<b>.001</b>
<i>O. rosacea</i>	6.90 ± 1.06	6.53	<b>&lt;.001</b>
<i>P. hiltoni</i>	5.63 ± 2.05	2.75	.11
<i>T. catalinae</i>	.10 ± 1.28	.08	1.00
<i>T. maculata</i>	4.53 ± 1.24	3.66	<b>.006</b>
<i>D. picta</i> :			
<i>H. opalescens</i>	.58 ± 1.81	.31	1.00
<i>O. rosacea</i>	2.90 ± 1.78	1.63	.75
<i>P. hiltoni</i>	1.63 ± 2.50	.65	1.00
<i>T. catalinae</i>	-3.90 ± 1.92	-2.03	.47
<i>T. maculata</i>	.53 ± 1.89	.28	1.00
<i>H. opalescens</i> :			
<i>O. rosacea</i>	2.34 ± .53	4.43	<b>&lt;.001</b>
<i>P. hiltoni</i>	1.07 ± 1.82	.59	1.00
<i>T. catalinae</i>	-4.46 ± .90	-4.93	<b>&lt;.001</b>
<i>T. maculata</i>	-.03 ± .85	-.03	1.00
<i>O. rosacea</i> :			
<i>P. hiltoni</i>	-1.27 ± 1.80	-.71	1.00
<i>T. catalinae</i>	-6.80 ± .84	-8.08	<b>&lt;.001</b>
<i>T. maculata</i>	-2.37 ± .78	-3.04	<b>.05</b>
<i>P. hiltoni</i> :			
<i>T. catalinae</i>	-5.53 ± 1.95	-2.84	.09
<i>T. maculata</i>	-1.10 ± 1.92	-.57	1.00
<i>T. catalinae</i> :			
<i>T. maculata</i>	4.43 ± 1.06	4.18	<b>&lt;.001</b>

Note. Statistically significant contrasts are displayed in bold.



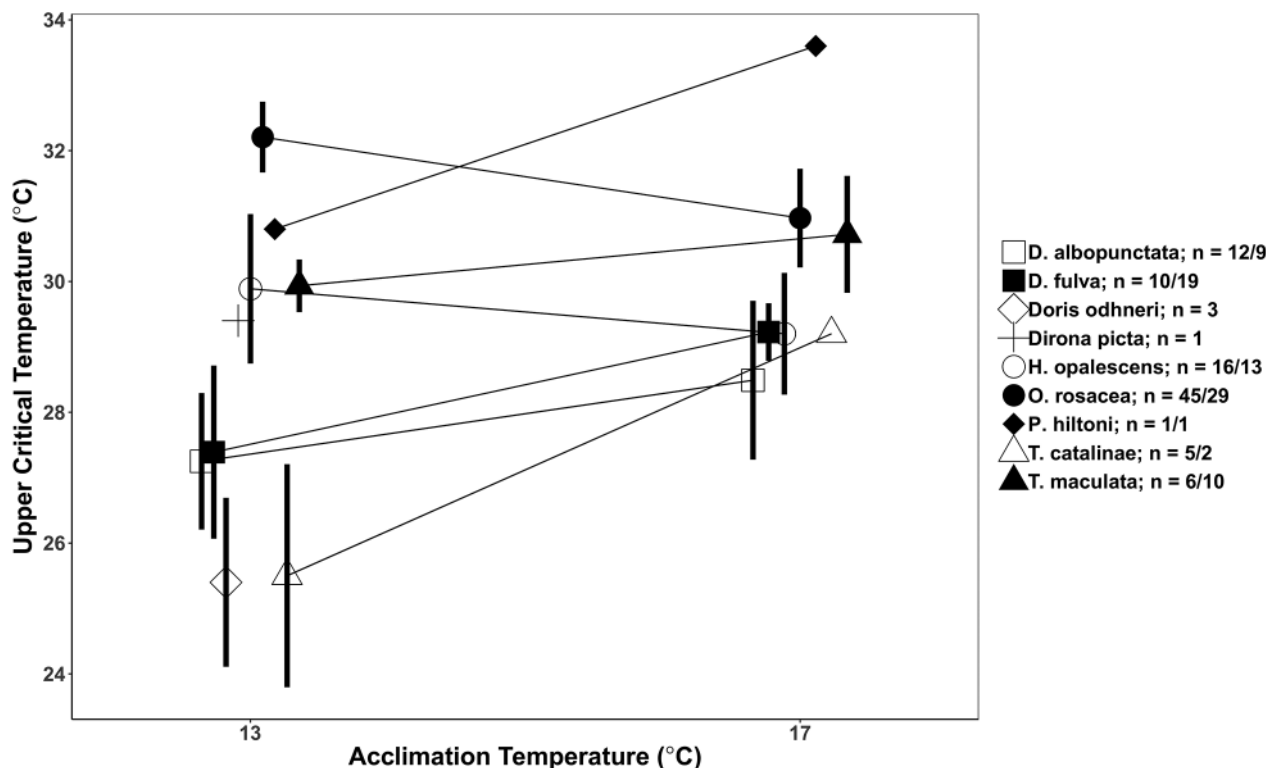


Figure 2. Heat tolerance limit of foot muscle function plotted against acclimation temperature. Sample sizes are given for 13°/17°C acclimation groups, respectively, and values are jittered for display purposes. Error bars represent 95% confidence intervals around the mean.

*T. maculata* (table 5). Averaged across all species and sites, mean metabolic rates were  $1.91 \pm 0.39$  and  $3.09 \pm 0.72 \mu\text{mol O}_2 \text{h}^{-1} \text{g}^{-1}$  ( $\bar{x} \pm \text{SE}$ ; 13° and 17°C, respectively) prestress and  $2.11 \pm 0.38$  and  $3.61 \pm 1.11 \mu\text{mol O}_2 \text{h}^{-1} \text{g}^{-1}$  poststress. There was no significant difference in metabolic rates between acclimation temperatures or between pre- and postthermal exposure treatments for any species (repeated-measures ANOVA, all  $P > 0.05$ ). Thermal plasticity of metabolism ( $Q_{10}$ ) ranged from 0.93 to 31.91 (table 5).

## Discussion

Inherent heat tolerance limits and the ability to reversibly adjust them (plasticity) remain poorly characterized in the ecologically important nudibranch mollusks. Understanding the evolutionary relationship between these traits poses an important first step in informing predictions of how these species may respond to rising global temperatures. In this study, we examined inherent heat tolerance, heat tolerance plasticity, and change in oxygen consumption in response to heat shock in eight species of nudibranch mollusks in order to assess relative thermal sensitivity among species and to determine physiological (e.g., metabolic suppression) and evolutionary (i.e., potential trade-offs between traits) patterns of thermal response.

### Ecological Significance of Heat Tolerance Physiology

Although a recent meta-analysis has demonstrated a limited capacity for acclimation in ectothermic species generally (Gunder-

son and Stillman 2015), our results demonstrate that heat tolerance plasticity among nudibranchs was comparatively high. Two of seven species displayed mean ARR values higher than previously reported for other marine ectotherms ( $0.32 \pm 0.02$ ,  $\bar{x} \pm \text{SE}$ ; Gunderson and Stillman 2015), with one species, *Triopha catalinae*, displaying near-perfect compensation over the range of acclimation temperatures investigated (fig. S1). These results reinforce findings from a second meta-analysis of ectothermic physiological plasticity that suggest that mollusks possess relatively high thermal plasticity relative to other invertebrate taxa (Seebacher et al.

Table 4: Linear mixed-effect model results of within-species contrasts of inherent upper heat tolerance limits across acclimation temperatures (13° vs. 17°C)

Contrast	Difference estimate ( $\pm \text{SE}$ )	Z	P
<i>Doriopsilla albopunctata</i>	1.61 $\pm$ .58	2.80	<b>.005</b>
<i>Doriopsilla fulva</i>	1.48 $\pm$ .41	3.61	<b>.0003</b>
<i>Hermisenda opalescens</i>	-.69 $\pm$ .71	-.97	.33
<i>Okenia rosacea</i>	-1.39 $\pm$ .41	-3.41	<b>.0006</b>
<i>Triopha catalinae</i>	3.70 $\pm$ 1.03	3.60	<b>.0003</b>
<i>Triopha maculata</i>	.79 $\pm$ .53	1.48	.14

Note. Statistically significant contrasts are displayed in bold.

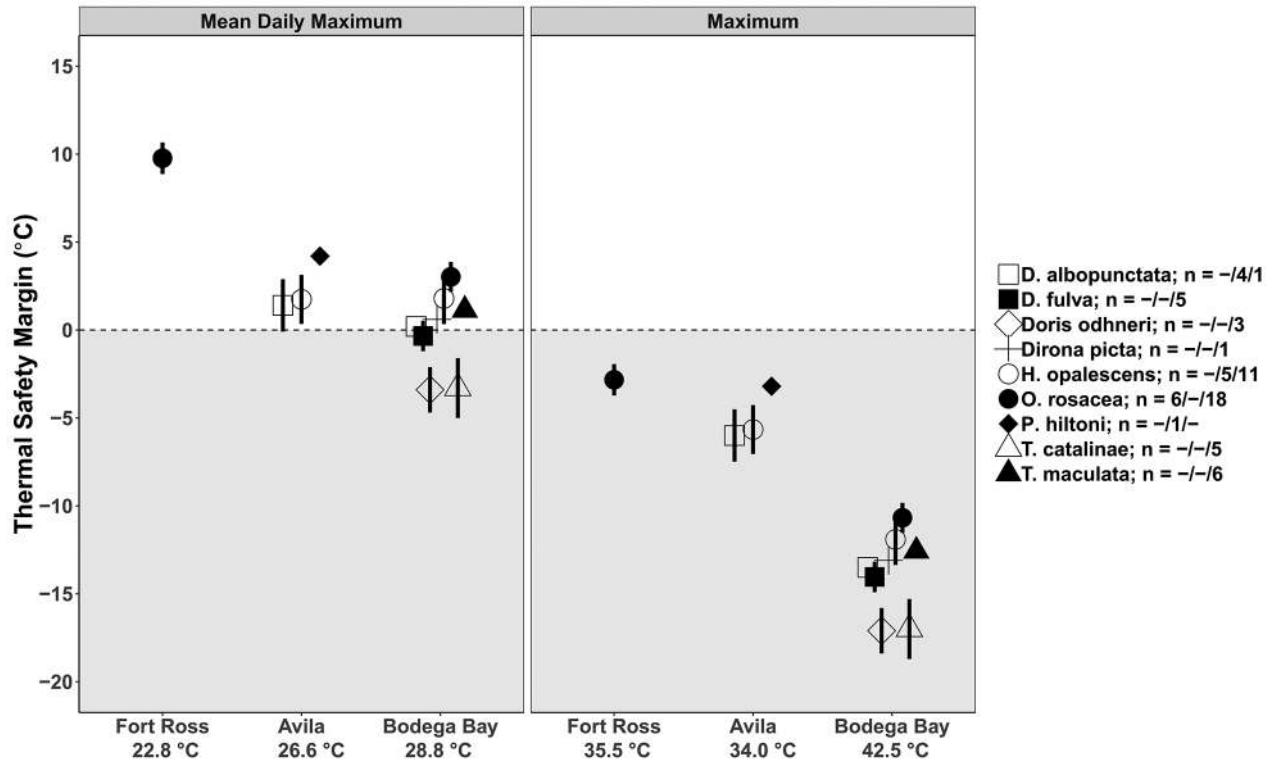


Figure 3. Thermal safety margins (TSMs) of nudibranch mollusks at three sites in California. TSMs were calculated as either the difference between heat tolerance limit and mean daily maximum habitat temperatures during the hottest month of the year (July; *left*) or the single-day maximum observed habitat temperature in the mid-intertidal zone over this same period (July; *right*). Temperatures are displayed below corresponding sites of collection. Sample sizes are given for Fort Ross/Bodega Bay/Avila, respectively, and data are plotted as mean  $\pm$  95% confidence intervals. The gray area denotes the region in which habitat temperature exceeds thermal tolerance limits and thus locations where species may already be subject to thermal selection.

2015). This pattern also holds true among the few reports of  $CT_{max}$  plasticity within gastropods specifically. For example, tropical snails of the genus *Nerita* can rapidly adjust their nonlethal upper heat tolerance limits in the face of seasonal environmental warming and display ARR of  $1.5 \pm 0.5$  ( $\bar{x} \pm SE$ ) over as little as 4 wk of acclimation (Stirling 1982). Additionally, *Littorina littorea* periwinkle snails displayed ARR of heat coma onset temperature on the order of  $0.56 \pm 0.12$  ( $\bar{x} \pm SE$ ) across an 8°C acclimation range (Clarke et al. 2000). This value is nearly identical to the average acclimation capabilities of nudibranchs we observed. Although this study adds significantly to the amount of plasticity data available for comparative analyses, additional data from several other lineages are necessary for robust comparison between gastropod mollusks and other ectothermic lineages. While limited data make further discussion speculative, the observed high ARR of gastropods relative to terrestrial ectotherms may be related to the relatively limited capacity of gastropods for behaviorally mediated thermal escape (Monaco et al. 2017).

Whereas overall mean ARR was high relative to previous reports in ectothermic taxa, there was significant variation in this trait across nudibranch species. In addition, the two most warm-adapted species displayed negative ARR values over the acclimation range surveyed (fig. S1). Analysis of this pattern within a phylogenetic framework revealed a strong negative relationship

between inherent thermal tolerance limits and thermal tolerance plasticity and provides strong evidence for thermal adaptation among these taxa in accordance with the trade-off hypothesis of thermal adaptation (Somero and DeVries 1967; Stillman and Somero 2000; Lerman and Feder 2001; Castañeda et al. 2015), with organisms displaying the highest inherent  $CT_{max}$  also exhibiting the lowest  $CT_{max}$  plasticity (Stillman 2003).

By computing safety margins alongside estimates of tolerance plasticity, we show that the most warm-adapted species/populations are potentially at greatest risk from climate change (Deutsch et al. 2008; Huey et al. 2009; Sunday et al. 2011; Diamond et al. 2012; Kellermann et al. 2012). In the two most heat-tolerant species, *Okenia rosacea* and *Hermisenda opalescens*, a +4°C increase in acclimation temperature resulted in  $-1.3^\circ$  and  $-0.6^\circ$  decreases in  $CT_{max}$ , respectively, indicating that further warm acclimation in these taxa has negative impacts on acute thermal performance. Present-day low-tide temperature extremes frequently exceed  $CT_{max}$  for all species examined, and most species have only a  $\sim 2^\circ$ C safety margin between their  $CT_{max}$  and modern average daily maximum temperatures, indicating that even a marginal increase in maximal habitat temperatures may cause strong selection in these taxa (fig. 3). This result corroborates work in other intertidal gastropods that found that of 23 species surveyed, all displayed upper thermal limits of performance significantly below current

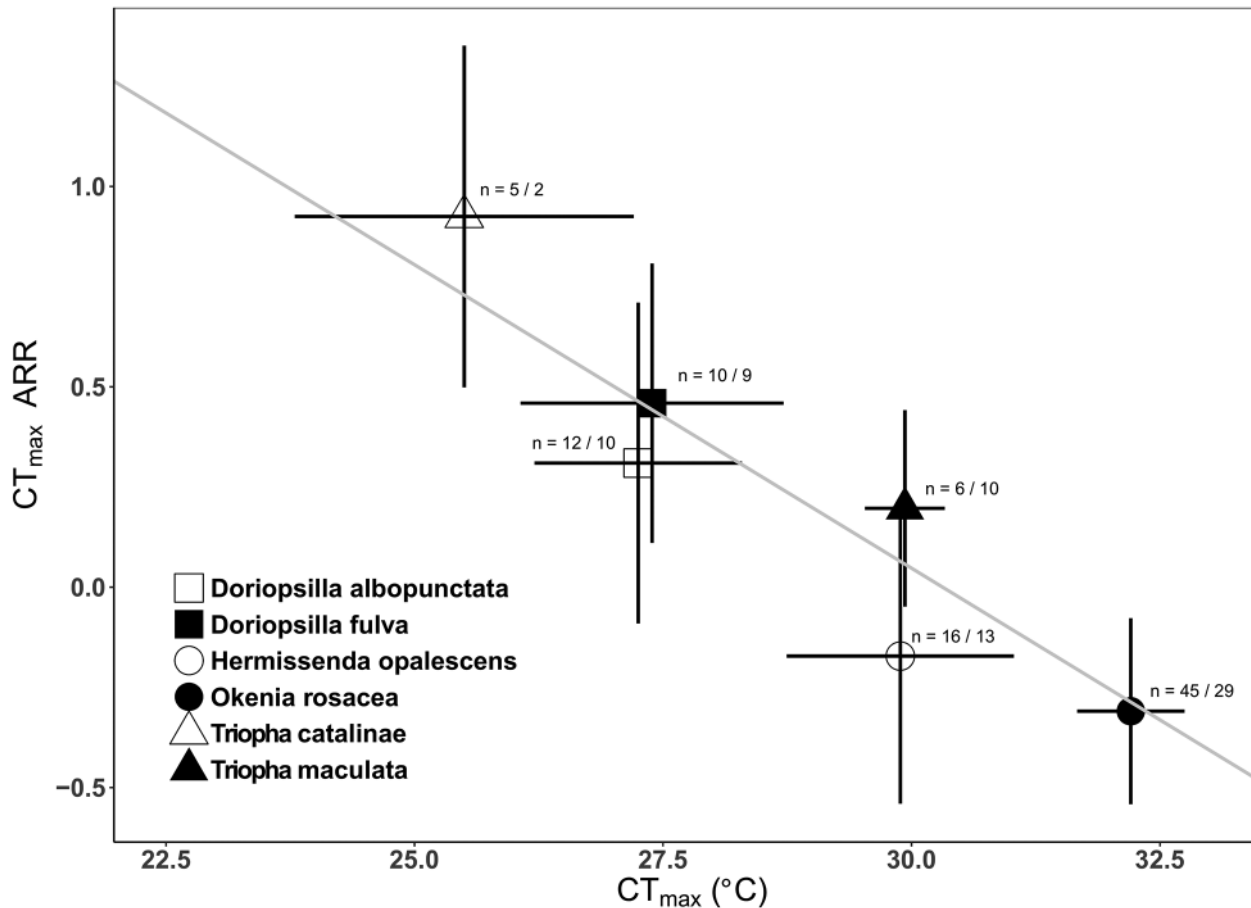


Figure 4. Heat tolerance plasticity as a function of heat tolerance limit ( $CT_{max}$ ). Mean heat tolerance plasticities ( $CT_{max}$  ARR [acclimation response ratio]) are plotted against mean heat tolerance limits ( $13^{\circ}\text{C}$  acclimation group) for each species for which data were available. Error bars (horizontal and vertical) represent 95% confidence intervals around the respective means, and number of individuals per species ( $13^{\circ}/17^{\circ}\text{C}$  acclimation groups, respectively) is also displayed. Line of best fit ( $y = 5.1 - 0.17x$ ;  $r^2 = 0.88$ ,  $P < 0.01$ ) is given in gray.

maximal temperatures in their native habitats and therefore were already potentially subject to thermal selection (Marshall et al. 2015). However, for making predictions of ecological response, the use of constant acclimation temperatures can be misleading because exposure to temperature variation or acute heat shock (environmentally appropriate conditions for intertidal organisms) can adjust heat tolerance limits (Giomi et al. 2016; Kingsolver et al. 2016). Because of this, it is likely that our measures of inherent heat tolerance are underestimates of tolerance limits exhibited in the field. However, these adjustments are usually modest: an average increase in the whole-organism heat tolerance limit of  $+0.18^{\circ} \pm 0.04^{\circ}\text{C}$  ( $\bar{x} \pm \text{SE}$ ) per degree of temperature variation range as reported across nine species, including an intertidal gastropod limpet (Threader and Houston 1983; Woiwode and Adelman 1992; Schaefer and Ryan 2006; Kern et al. 2015; Giomi et al. 2016; Kingsolver et al. 2016; Drake et al. 2017; Salachan and Sørensen 2017). At the scale of diurnal temperature variation recorded from sites in this study ( $\sim 2.2^{\circ} \pm 1.8^{\circ}\text{C}$  range,  $\bar{x} \pm \text{SD}$ ), this would amount to an increase in  $CT_{max}$  of  $0.4^{\circ} \pm 0.4^{\circ}\text{C}$ , less than the average TSM deficit reported here for intertidal nudibranchs (mean TSM  $-8.5^{\circ} \pm 5.3^{\circ}\text{C}$ ,  $\bar{x} \pm \text{CI}$ ). Even in the most compa-

table case, intertidal *Lottia digitalis* limpets acclimated to emersion at constant temperature, as compared to acclimation to stochastic variation in temperature (Drake et al. 2017),  $CT_{max}$  increased by an average of  $+2.5^{\circ}\text{C}$  (Arrhenius break temperature,  $+2.1^{\circ}\text{C}$  flat-line temperature) in response to thermal variation, which would place nudibranch heat tolerance limits at parity with modern maximal habitat temperatures. Thus, while thermal variability and previous thermal history are certainly important factors for setting heat tolerance limits, we conclude that beneficial effects of heat hardening are unlikely to significantly increase TSMs for most species in this study.

In light of these potential physiological constraints, we might predict that future warming will result in significant range shifts in eastern Pacific nudibranch species. Climate-related range expansions of this type were recently reported during sustained warm anomalies (between 2014 and 2017) in more than 52 nudibranch species, including *Doriopsilla albopunctata*, *Doriopsilla fulva*, *H. opalescens*, *O. rosacea*, *Phidiana hiltoni*, and *Triopha maculata* investigated in this study (Goddard et al. 2018; Merlo et al. 2018; Sanford et al. 2019). Tellingly, while these species showed significant northward range extensions (270 km farther north, on av-

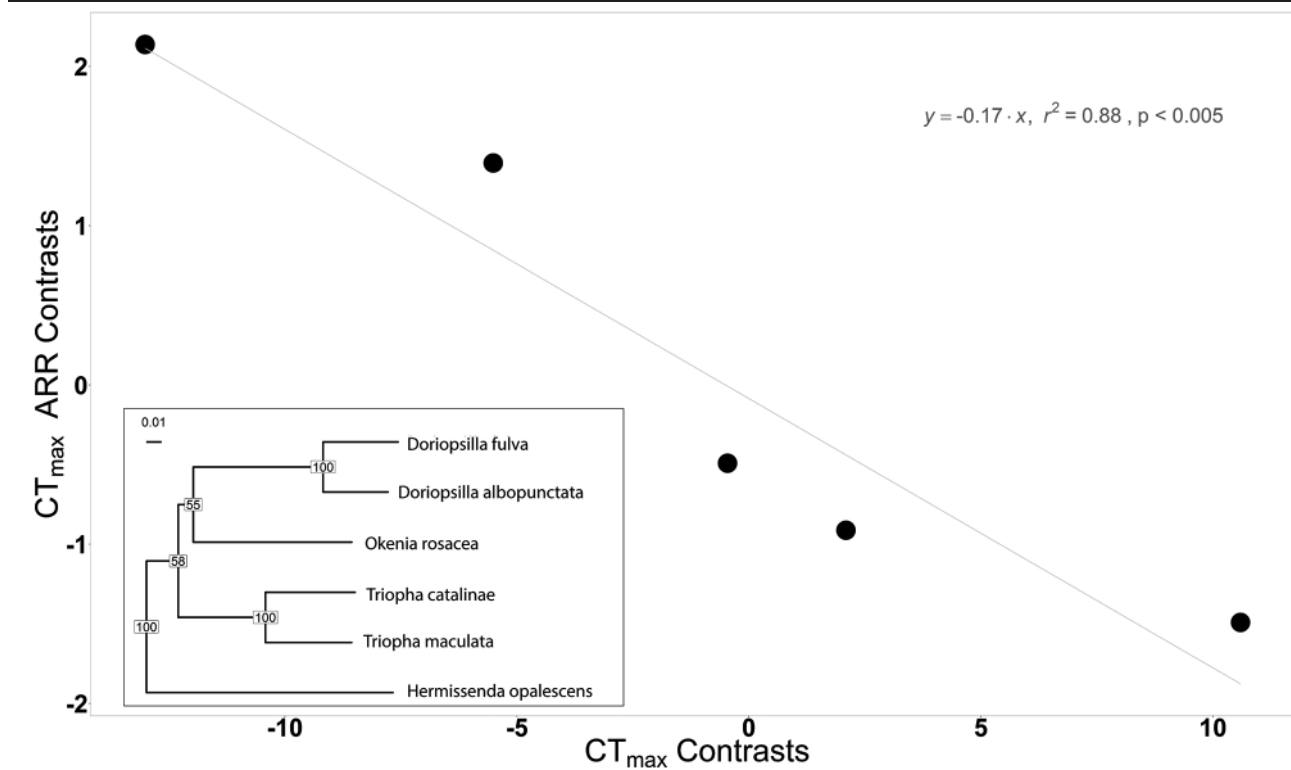


Figure 5. Phylogenetic tree and independent contrasts of physiological response variables. The relationship between heat tolerance plasticity ( $CT_{max}$  ARR [acclimation response ratio]) and inherent heat tolerance ( $CT_{max}$ ) is shown, with the regression coefficient given in the upper right. Contrasts represent standardized distances between tips and their connecting node as summed across the phylogeny based on cytochrome c oxidase subunit I (COI) sequence data (*inset*). The tree was generated from a matrix of distances created with a maximum likelihood model and constructed by neighbor-joining analysis. Distances were obtained from pairwise alignment of sequences, and values of the nodes are bootstrap values of a total of 100 replicate data sets. The tree was rooted with the outgroup species *Aplysia californica*, though this terminal node was not used to generate an independent contrast, and the scale denotes number of substitutions per nucleotide site.

erage), none were found south of their previous ranges (Goddard et al. 2018), suggesting that warmer temperatures at southern range boundaries impeded population establishment. Similarly, in California, a northward range expansion was observed in the warm-adapted species *P. hiltoni*, which from 1977 to 1992 spread northward from Monterey, California, eventually establishing a permanent population ~100 km farther north at Duxbury Reef, California (Goddard and Pearse 2011; Goddard et al. 2011). In the southern hemisphere, an inverse southward expansion (from tropical to subtropical waters) has also been documented in four tropical species of nudibranch along the eastern Australian coast, with range expansion up to 400 km southward into subtropical waters (Nimbs et al. 2015, 2016). Whereas previous work has shown that northward expansions of southern species along the western US coast can be attributed to intrusion of larvae-bearing water into coastal regions during El Niño events rather than with habitat warming per se (Goddard and Pearse 2011; Schultz et al. 2011), the lack of corresponding southward movement strongly implies that habitat temperatures do play a role in permitting population establishment. Flow-driven shifts in population range may therefore facilitate “thermal escapes” from increasingly warm southern range boundaries, allowing putative warm-adapted species such as *O. rosacea* and *H. opalescens* opportunities for rapid

northward expansion and offsetting range reductions in overly hot southern regions. However, the extent to which such large-scale biogeographic shifts may offset climate warming in eastern Pacific nudibranch species remains to be explored.

#### Temperature Sensitivity of Aerobic Metabolism

As the fundamental rate of energy turnover within an individual organism, metabolic rate is an important metric that ultimately places limitations on habitat suitability (Deutsch et al. 2015) and longevity. Recent research has also highlighted the link between metabolism and climate-associated extinction risk (Penn et al. 2018). At the population level, species with a high BMR are more likely to become extinct than similar species with lower BMRs and thus may be more susceptible to negative impacts associated with large-scale environmental change (Strotz et al. 2018). However, perhaps more important at the level of the individual organism (e.g., upper heat tolerance limit) is the relative sensitivity of BMR to rising temperature (i.e.,  $Q_{10}$ ). As Pörtner et al. (2017) suggest, species with a high  $Q_{10}$  of BMR (i.e., temperature sensitive, low capacity for oxygen supply) are likely to exhibit lower functional scope than species with a low  $Q_{10}$  of BMR (i.e., temperature insensitive, high capacity for oxygen supply). We measured BMR

Table 5: Measured oxygen consumption rates before and after heat exposure and calculated thermal sensitivities of metabolism before heat exposure

Species and temperature (°C)	Mass ( $\bar{x} \pm \text{SE}$ ; g)	Respiration rate ( $\bar{x} \pm \text{SE}$ ; $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$ )		$Q_{10}$ ( $\bar{x} \pm \text{SE}$ )
		Before exposure	After exposure	
<i>Doriopsilla albopunctata</i> :				
13 (8)	.78 $\pm$ .19	1.36 $\pm$ .41	.49 $\pm$ .20	3.26 $\pm$ .37
17 (6)	.64 $\pm$ .10	2.18 $\pm$ .81	1.46 $\pm$ .79	...
<i>Doriopsilla fulva</i> :				
13 (10)	.89 $\pm$ .18	.74 $\pm$ .17	1.32 $\pm$ .30	1.53 $\pm$ .66
17 (17)	.83 $\pm$ .15	.87 $\pm$ .29	1.12 $\pm$ .24	...
<i>Doris odhneri</i> :				
13 (2)	.68 $\pm$ .06	1.27 $\pm$ .25	.71 $\pm$ .63	...
<i>Dirona picta</i> :				
13 (1)	.24	1.13	1.78	...
<i>Hermisenda opalescens</i> :				
13 (11)	.19 $\pm$ .06	1.25 $\pm$ 1.45	4.31 $\pm$ 1.80	15.90 $\pm$ .19
17 (8)	.10 $\pm$ .04	3.77 $\pm$ .87	11.24 $\pm$ 5.34	...
<i>Okenia rosacea</i> :				
13 (36)	.28 $\pm$ .03	2.55 $\pm$ .69	2.36 $\pm$ .58	1.36 $\pm$ .73
17 (22)	.32 $\pm$ .06	2.88 $\pm$ .82	5.24 $\pm$ 2.50	...
<i>Phidiana hiltoni</i> :				
13 (1)	.22	1.22	2.42	...
<i>Triopha catalinae</i> :				
13 (5)	1.38 $\pm$ .31	1.18 $\pm$ .33	.81 $\pm$ .27	.93 $\pm$ 2.67
17 (2)	.48 $\pm$ .05	1.15 $\pm$ 1.09	1.16 $\pm$ .31	...
<i>Triopha maculata</i> :				
13 (4)	.10 $\pm$ .04	1.02 $\pm$ 1.96	2.14 $\pm$ 1.37	31.91 $\pm$ .16
17 (10)	.11 $\pm$ .02	4.09 $\pm$ 2.05	.44 $\pm$ 1.08	...

Note. Metabolic thermal sensitivities ( $Q_{10}$ ) were calculated over the range 13°–17°C. Number of individuals is listed in parentheses after each temperature.

across species to examine whether taxa with higher BMRs showed stronger temperature sensitivity (i.e., a larger  $Q_{10}$ ) and thus tested support of the previous observation of BMR as an indicator of warming-driven extinction risk.

Previous estimates of metabolic rates are sparsely reported for nudibranchs in the literature and are highly variable, spanning two orders of magnitude under similar temperature conditions. Reported oxygen consumption rates in nudibranchs range from ~0.12 and 0.17  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$  in spring- and summer-acclimated (18° and 22°C, respectively) adults of the temperate western Atlantic species *Armina maculata* (Pires 2012) to an average of 63.87 and 81.85  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$  at 13° and 18°C, respectively, in eight temperate eastern Atlantic species (Clark 1975). However, the high values reported by Clarke (1975) include estimates from newly emerged juveniles (i.e., low-mass individuals) and are therefore at least partially confounded by potential allometric effects. Reported rates for two boreal species with masses similar to the species examined in this study, *Adalaria proxima* and *Onchidoris muricata*, are somewhat intermediate to the data of Pires (2012) and Clark (1975), at 6.2 and 22.2  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$  and 8.4 and 18.1  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$  when measured at 3.5° and 13°C, respectively (Havenhand and Todd 1988). Our data for temperate eastern Pacific nudibranchs fall closer to those values reported by Haven-

hand and Todd (1988), with mean respiration rates ( $\bar{x} \pm \text{SE}$ ) of 1.9  $\pm$  0.4  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$  and 3.1  $\pm$  0.7  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$  (13° and 17°C acclimation, respectively) among the nine species examined (table 5).

In light of previous reports of strong metabolic suppression in intertidal gastropods in response to acute heat stress (Sokolova and Pörtner 2003), we expected to see a reduced metabolic rate in our study species after heat shock. However, no species showed a consistent up- or downregulation of metabolism at 1 h post-exposure to acute thermal challenge (table 5). Thus, we were unable to definitively identify a metabolic response strategy to heat stress in nudibranchs and conclude that at the integrative level of the organism, metabolic costs of exposure to extreme temperature, if they exist, are short-lived (i.e., less than 1 h in duration) in these species.

Degree of temperature compensation of metabolism (i.e.,  $Q_{10}$ ) varied significantly across taxa, with fully half of all species examined showing no thermal compensation of metabolic rate across the 4°C acclimation temperature range investigated in this study (i.e.,  $Q_{10} \gg 1$ ; table 5). Interestingly, this variation in acclimation capacity was even observed between closely related taxa, with *D. fulva* and *T. catalinae* exhibiting nearly perfect thermal compensation of metabolism ( $Q_{10}$  of 1.53 and 0.96, respectively) while

their congeners (*D. albopunctata* and *T. maculata*) showed limited or no thermal compensation across the same temperature range ( $Q_{10}$  of 3.26 and 31.91, respectively). This suggests that acclimatory capacity may be under strong selection in these groups of mollusks. The extremely high  $Q_{10}$  exhibited by *T. maculata* and *H. opalescens* suggests that prolonged exposure to the comparatively mild temperature of 17°C may have caused an increase in respiration rate beyond what would be expected by the effects of temperature alone. Such a response may be indicative of cellular stress in these species or of shifts in behavior or metabolic processes (Peck et al. 2006). In the case of *T. maculata*, the very low respiration rate of 17°C-acclimated specimens following heat shock (shift from 4.09 to 0.44  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$ ) suggests that physiological stress (even at this commonly experienced environmental temperature) may be the primary mechanism inhibiting acclimatory responses in this species.

### Conclusions

In this study we show that heat tolerance plasticity is strongly negatively correlated with inherent heat tolerance in nudibranch mollusks in accordance with the trade-off hypothesis of thermal adaptation (Stillman 2003). In addition, thermal safety margins under modern habitat thermal regimes are low or negative for nearly all nudibranch species studied, indicating that further warming may have negative selective consequences for these populations. While thermal tolerance plasticity in these species is comparatively high, plasticity alone will likely be inadequate to cope with predicted warming, and warm-adapted species are likely to be particularly vulnerable to future increases in temperature. In light of these findings, an improved understanding of the ability of nudibranchs to alter inherent thermal limits through adaptive shifts in thermal performance or to behaviorally mitigate the effects of future habitat warming is of paramount importance for informing estimates of species persistence under a changing climate.

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