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RESEARCH ARTICLE

Survival and arm abscission are linked to regional heterothermy in an intertidal sea star

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SUMMARY

Body temperature is a more pertinent variable to physiological stress than ambient air temperature. Modeling and empirical studies on the impacts of climate change on ectotherms usually assume that body temperature within organisms is uniform. However, many ectotherms show significant within-body temperature heterogeneity. The relationship between regional heterothermy and the response of ectotherms to sublethal and lethal conditions remains underexplored. We quantified within-body thermal heterogeneity in an intertidal sea star (*Pisaster ochraceus*) during aerial exposure at low tide to examine the lethal and sublethal effects of temperatures of different body regions. In manipulative experiments, we measured the temperature of the arms and central disc, as well as survival and arm abscission under extreme aerial conditions. Survival was related strongly to central disc temperature. Arms were generally warmer than the central disc in individuals that survived aerial heating, but we found the reverse in those that died. When the central disc reached sublethal temperatures of 31–35°C, arms reached temperatures of 33–39°C, inducing arm abscission. The absolute temperature of individual arms was a poor predictor of arm abscission, but the arms lost were consistently the hottest at the within-individual scale. Therefore, the vital region of this sea star may remain below the lethal threshold under extreme conditions, possibly through water movement from the arms to the central disc and/or evaporative cooling, but at the cost of increased risk of arm abscission. Initiation of arm abscission seems to reflect a whole-organism response while death occurs as a result of stress acting directly on central disc tissues.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/216/12/2183/DC1

Key words: autotomy, body temperature, intertidal ecosystem, lethal temperature, *Pisaster ochraceus*, sublethal effect, thermal ecology, thermal heterogeneity.

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INTRODUCTION

The pervasive effects of an organism's body temperature have long attracted the attention of biologists. Temperature is among the most important abiotic factors driving the physiology and ecology of organisms (Hochachka and Somero, 2002), and a large amount of literature has accumulated on the ways that the physical environment interacts with an organism's physiology to drive its fundamental niche space (Kearney, 2006). The recognition of the ongoing and future impacts of climate change has motivated the application of concepts from thermal ecology and physiology to predicting the likelihood of changes in the distribution of organisms based on their thermal sensitivities and adaptations (Chown and Nicolson, 2004; Kearney and Porter, 2009; Buckley et al., 2010). Nevertheless, a conceptual mismatch has often occurred between thermal biologists, who typically recognize body temperature as the interface between organism physiology and environment, and most ecologists working on climate change issues, who generally use ambient air or sea surface temperature as an (often unrealistic) proxy of body temperature. The growing awareness of the importance of considering the actual body temperature experienced by organisms rather than simply relying on environmental correlates (Helmuth et al., 2010) has resulted in the development of heat budget models to predict body temperature variability over a wide range of spatial and temporal scales (Porter and Gates, 1969; Pincebourde et al., 2007; Kearney et al., 2009; Kearney et al., 2010). Biophysical studies have emphasized that body temperature varies not only as a function of microhabitat (Denny et al., 2011), but also with factors such as body size, which can affect an organism's thermal inertia (Turner, 1988; Helmuth, 1998; Pincebourde et al., 2009). These studies have also been used successfully to explore the impacts of environmental change in terms of growth and reproduction (Kearney et al., 2010; Schneider et al., 2010; Sarà et al., 2011), and rates of predation and thus interaction strengths between species (Sanford, 2002; Yamane and Gilman, 2009; Monaco and Helmuth, 2011).

However, while coupled heat budget and physiological models of large endotherms such as mammals have recognized the importance of within-body temperature heterogeneity (e.g. Porter and Gates, 1969), biophysical models of ectotherms often assume that temperature is uniform within the organism, or else that core temperature is the most important variable (e.g. Helmuth, 1998; Szathmary et al., 2009). However, for many organisms, body temperature can be heterogeneous at the within-organism scale, a phenomenon called regional heterothermy. Temperature deviations between body regions have been observed in a wide variety of ectothermic organisms, including many insects (May, 1995; Coelho and Ross, 1996; Woods et al., 2005), some intertidal invertebrates (Fyhn et al., 1972) and reptiles (Garrick, 2008; Dubois et al., 2009). Within-body thermal heterogeneity has important physiological and ecological consequences. For example, some butterflies depend on regional heterothermy to 'pre-warm' flight muscles (Kingsolver, 1983), with significant implications for predator avoidance (Srygley and Chai, 1990). Some organisms have evolved sophisticated mechanisms to generate regional heterothermy. For example, in large ectotherms such as some reptiles, blood flow to appendages can be modified to control for warming or cooling rates (Dzialowski and O'Connor, 1999; Dzialowski and O'Connor, 2004). Flying insects such as bees or bumblebees keep their thorax hot and their abdomen relatively cool *via* continuous circulation of hemolymph between the two body regions through a counter-current heat exchanger, with heat being exchanged between outgoing and ingoing fluids (Heinrich, 1996). Liquid drop exudation to lower a body region temperature is another strategy used by mosquitoes (Lahondère and Lazzari, 2012) and some hymenopterans (Coelho and Ross, 1996; Heinrich, 1996). More generally, strategies involving heat exchangers (including exudation drops) to cope with sublethal temperatures might be widespread and are associated with within-body thermal heterogeneity.

Regional heterothermy can also be 'passive' in ectotherms, corresponding to the temperature difference between various regions of an organism's body due to variations in heating rates resulting from the specific mass, color or morphology of the body parts in interaction with the environment. The proximal causes for passive regional heterothermy are diverse. Differential patterns of wind flow over the surface of an organism can cause the appendages to remain cooler than the core (Tsuji et al., 1986). The orientation angle of different body regions to solar radiation can also generate a temperature gradient across the body (Kingsolver and Watt, 1983). Passive regional heterothermy has been studied notably in butterfly species during sun-basking behavior (Kingsolver and Moffat, 1982). Overall, however, regional heterothermy is likely to result from an association of both passive and active processes for most ectotherms. Recently, for example, the major claw of the fiddler crab was suggested to serve as a heat emitter, collecting the heat (active way) from the body exposed to solar radiation, and releasing it (passive way) to the environment *via* convection (Darnell and Munguia, 2011).

Within-body thermal heterogeneity raises important physiological and ecological questions that heretofore have received relatively little attention for most ectotherms. For example, how do we measure and model body temperature if temperature varies across the organism's body? Do upper lethal temperatures always correspond to the tolerance level of the most sensitive body region? What are the sublethal consequences of variation in body temperature? Can organisms regulate their within-body thermal heterogeneity to improve their resistance to high temperatures? Do mobile organisms decide to leave an unfavorable microhabitat based on the temperature of a specific body region? The rise of thermography technology, which is now accessible at moderate cost, offers a promising tool in this context. For example, infrared cameras can be used to measure directly, non-invasively and simultaneously the surface temperature of all body regions for a single or multiple individuals (e.g. Pincebourde et al., 2009; Lahondère and Lazzari, 2012), and biomimetic sensors (e.g. Lima and Wethey, 2009) could potentially be designed to record temperatures of different body regions over prolonged periods in the field, although this has yet to be attempted.

Here we investigate the within-body thermal heterogeneity in the sea star *Pisaster ochraceus* (Brandt 1835), a keystone predator known to exhibit behavioral and physiological responses to body temperature during aerial exposure at low tide (Pincebourde et al., 2008; Pincebourde et al., 2009; Pincebourde et al., 2012). Intertidal organisms are alternatively exposed to aerial and underwater conditions during tide cycles. At low tide, the body temperature of intertidal ectotherms can differ substantially from ambient air or surface temperature, and can fluctuate drastically over short time intervals (minutes), as it is driven by the interaction of multiple environmental factors (Helmuth, 1998; Wethey, 2002). Thus, intertidal organisms experience large thermal variations and their body temperatures can frequently reach sublethal levels (Somero, 2002). The predatory sea star *P. ochraceus* is quite sensitive to both submerged (Sanford, 2002) and aerial body temperature (Fly et al., 2012; Pincebourde et al., 2008; Pincebourde et al., 2009; Pincebourde et al., 2012). Transplant experiments in central Oregon reported high mortality when this species was experimentally kept high on the shore (Petes et al., 2008). Previous studies have shown an upper lethal body (central disc) temperature of 35°C and have demonstrated a significant negative influence of temperature on feeding rates at an aerial body (central disc) temperature of 23°C and above (Pincebourde et al., 2008). However, *P. ochraceus* can exert behavioral control over its body temperature in two ways. First, animals may avoid thermally stressful environments by remaining low in the intertidal zone, despite the presence of a significant food source (mussels) higher on the shore (Robles et al., 1995), or by exploiting shaded microhabitats (Fly et al., 2012). Studies along the west coast of North America showed that this species only rarely forages in environments that would lead to a low-tide body temperature above 29°C (Sanford, 2002; Pincebourde et al., 2008; Pincebourde et al., 2012; Broitman et al., 2009; Szathmary et al., 2009; Fly et al., 2012). This suggests that the exposure to sublethal body temperatures in the range of 29–35°C may incur high energetic costs or lead to significant physiological damage. Second, the sea star shows an interesting thermoregulatory feature in that it increases the internal fluid volume following exposure to aerial body temperatures of ~27°C. By increasing the thermal inertia of the body cavity, this behavior slows down heating rate during the next low tide exposure (Pincebourde et al., 2009). We investigated the hypothesis that exposure to sublethal body temperatures in the range of 29–35°C alters the sea star morphology through arm abscission, with the arms reaching higher temperatures than other body regions (central disc).

Previous studies of this sea star have focused on measuring (Pincebourde et al., 2008) and modeling (Szathmary et al., 2009) the temperature of the central disc, because it houses critical organs (e.g. nerve ring, stomach and core of water vascular system) and it is essential for regeneration of lost arms. Arm loss in *P. ochraceus* has been documented, although the causes remain unclear (Lawrence, 1992) (supplementary material Table S1). Asteroids (Phylum Echinodermata) have the ability to regenerate arms that are damaged by natural causes (e.g. predation) and lost *via* abscission (Lawrence, 1992). However, losing an arm represents a huge cost for the sea star in terms of energy and reproductive output (Lawrence and Larrain, 1994; Barrios et al., 2008), because arms contain both stored resources (pyloric caeca) and the gonads (Lawrence and Lane, 1982; Sanford and Menge, 2007). The relative importance of arm and central disc temperatures to lethality and arm abscission remains unclear, as does the possibility that trade-offs exist between prevention of arm loss and risk of mortality. We measured the within-body thermal heterogeneity of sea stars and compared the temperature of arms with that of the central disc under extreme aerial conditions at low tide. We performed laboratory temperature assays to test whether sea star survival depends primarily on the temperature of the arms or the central disc, and specifically tested the hypothesis that arm abscission would be more closely associated with aerial arm temperatures than with central disc temperature. Then we focused on the survivors that lost one or several arms in these assays to determine whether trade-offs exist between short-term survival probability and arm abscission.

MATERIALS AND METHODS

Study system and organisms

The sea star *P. ochraceus* is common on rocky intertidal shores along the west coast of North America (Menge et al., 2004). All individuals were collected at low tide at a single study site near the Bodega Marine Reserve, California (38°19′N, 123°4′W). Wet body mass of individuals fell in the range 150–250 g (mean ± s.d.=193.1±30.9 g, *N*=70), which is the most common wet body mass range for this species in California (Menge et al., 2004). Animals were brought to the Bodega Marine Laboratory, where they were maintained submerged in a large tank with flow-through seawater. Animals were kept at a water temperature of ~13°C for 20 days before the experiment started. This acclimation period was set to homogenize the physiological state of all individuals.

Experimental design

The experiment was designed to measure the sublethal (arm loss) and lethal effects of aerial body temperature during low tide. Some of the data collected during this experiment (lethal central disc temperature) have been published elsewhere for a different purpose (Pincebourde et al., 2008). In this study, we tested whether arms can be damaged by elevated temperatures before the central disc (vital region) reaches the upper lethal temperature, and whether arm loss is most closely related to a whole-organism response or to the temperature of the arm itself. A single aquarium (75 l) was used to reproduce tidal conditions. Two heat lamps (150 W each) were positioned above the aquarium, and their height relative to the sea stars was adjusted to vary body temperatures during aerial exposure. Seawater was run continuously into the bottom of the tank beneath a flat, elevated platform that prevented sea stars from contacting the water, while keeping the relative humidity constant and high. Sea stars were placed in a flat position and were not overlapping with each other. They were exposed to aerial conditions for 6 h, corresponding to a typical aerial exposure during low tide at the study site (Pincebourde et al., 2008). The heat lamps were switched on after 1 h of exposure. The height of the lamps was then gradually decreased until experimental body temperature was reached after 3 h of aerial exposure. The experimental temperature (i.e. central disc temperature) was maintained during the last 3 h of the simulated low tide. Body region temperatures were checked every 15 min throughout the treatment using an infrared camera (ThermaCAM 695, FLIR Systems, Boston, MA, USA; thermal sensitivity <0.05°C, accuracy <1°C within the temperature range tested, spatial resolution 1.36 mrad). The infrared camera measures surface temperature of the aboral (dorsal) side, which is close (~1°C) to internal body temperature (~center of the disc) in the sea star *P. ochraceus* (Pincebourde et al., 2012), although gradients of several degrees between the aboral and oral surfaces have been observed (S.P., E.S. and B.H., personal observation). This setup generated significant thermal heterogeneity in different body regions, with the arms positioned close to the normal of the heat lamps reaching higher temperature than the other arms. We verified that this situation was representative of sea stars exposed to solar radiation by taking infrared photographs of sea stars under these conditions at low tide in the field. We found that mean arm temperature was 0.8 to 2.1°C higher than mean central disc temperature (*N*=10 individuals, in the central disc temperature range 14.0–23.7°C), which corresponds to the range of temperature deviations in our experiments. After 6 h of aerial exposure, a stand pipe was fixed in the aquaria to fill it completely with seawater, thereby simulating the high tide with water temperature ~13°C.

Experimental procedure

Ten groups of seven individuals each (*N*=70) were exposed to different experimental aerial body temperatures. Body temperatures varied slightly among individuals of the same group according to their position relative to the heat lamps. The heat lamp was set at a different height for each group, to ensure that sampling was evenly distributed across the thermal range, with a 2°C increment from 26 to 42°C. For each sea star, the central disc temperature was measured as the average of six spot measures taken with the infrared camera over the central disc during the last 3 h of aerial exposure. We also recorded the temperature of each arm by targeting the middle of the arms with the infrared camera. Arms were identified according to their position relative to the madreporite (the opening to the water vascular system on the aboral surface).

The water balance of individuals was also measured as it was expected to be influenced by variation in overall body temperature. An electronic scale was used to record wet body mass of sea stars (to the nearest 0.1 g), as an indirect measurement of variation in fluid volume (Pincebourde et al., 2009). Wet body mass was measured at both the start and the end of the experimental low tide (except for sea stars that were already dead at the end of treatment), to estimate the amount of water lost during the treatment. Subsequently, wet body mass was measured again after an 18 h period of submergence on sea stars that were alive at this time. This last measure was used to estimate the amount of body fluid recovered during the submergence period (Pincebourde et al., 2009).

Survival was assessed for all individuals at the end of the treatment and after a 24 h submersion period immediately following the experimental low tide. Each animal was placed on its dorsal (aboral) surface and a sea star was considered alive only when movement of the tube feet was observed 24 h post-treatment. Finally, the sea stars were put back into a holding tank for 3 weeks and the number and identity of arms lost were recorded. All individuals alive after 24 h were still alive after 3 weeks spent in the holding tank.

Data analysis

Six responses were measured for each individual: (1) survival, (2) temperature of the central disc, (3) temperature of each arm, (4) number and position of lost arms, (5) amount of water loss during the experimental low tide and (6) amount of water recovered at the end of the following high tide. The analysis followed three steps.

Firstly, we quantified the influence of body region temperatures on the overall water balance. Pearson correlations were performed to analyze the influence of central disc and arm temperatures on water loss and water recovery. Each variable of the water balance was expressed as a percentage of wet body mass. Three groups of individuals were distinguished: alive with no damage, alive with arm abscission, and individuals that were alive at the end of the experimental low tide (for water loss), or 18 h after this exposure (for water recovery), but which died later on.

Secondly, we analyzed survival rate as a function of arm and central disc temperatures and water balance to determine which variable was a better predictor of survival. We also examined the relationship between arm and central disc temperature in animals that survived, animals that died and animals that survived with arm loss. Pearson correlations were performed to analyze the association between these variables. Then, logistic dose–response regressions were performed to quantify the effect of central disc temperature, arm temperature and water loss on survival.

Finally, we focused on the survivors (both with and without arm loss) to explore the sublethal effect of temperature. Dead individuals were therefore excluded from this analysis. We estimated the range of the sublethal zone in terms of mean arm temperature and mean central disc temperature by plotting the number of arms lost against these two temperature variables. A LOESS spline estimation analysis was performed using smoothed data to estimate the temperature threshold initiating damage to arms. Then, every arm of each individual was considered as a separate entity to perform a logistic dose–response regression in order to study the global relationship between the temperature of an arm and its probability of 'survival', i.e. whether the arm was lost. Next we considered the fate of arms at the intra-individual scale. For each individual, arms were ranked according to their temperature, from the coldest (rank=1) to the hottest (rank=5). We separated the individuals into three groups according to the number of arms they lost (one, two or three arms lost). A one-sample Kolmogorov–Smirnov test using uniform distribution was employed to determine whether the arms lost were consistently the hottest. All statistical tests were performed in SYSTAT 10 (Systat Software, Chicago, IL, USA) while regression analyses and LOESS procedures were performed using TableCurve 2D 5.01 (Systat Software).

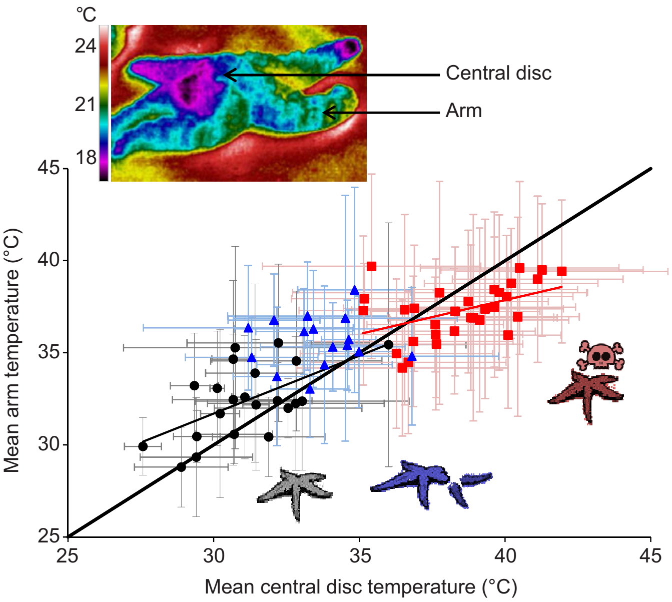

Fig. 1. Mean (±s.d.) arm temperature as a function of mean (±s.d.) central disc temperature for all *Pisaster ochraceus* individuals tested including the survivors with intact arms (black circles) and arm abscission (blue triangles), as well as the individuals that died (red squares). The linear regression lines are shown when correlations are significant. The black line indicates the equality of the two variables. The inset is an infrared image of a sea star during the exposure to experimental heating, illustrating the thermal heterogeneity of the sea star body surface (the color scale indicates temperature range).

RESULTS

Within-body thermal heterogeneity

The body temperatures of sea stars were heterogeneous with large deviations between body regions. The temperature deviation between the central disc and arms showed a distinct pattern depending on the temperature range (Fig. 1). For animals that survived with no arm loss, arms were on average warmer (29–35°C) than the central disc (27–33°C), but the temperature of the two body regions increased concomitantly (Pearson correlation: $r=0.58$, $P=0.005$). A similar correlation was also found in sea stars killed by the high temperature exposure (Pearson correlation: $r=0.46$, $P=0.008$), but in this case the arms were on average colder (34–40°C) than the central disc (36–43°C). However, the relationship did not hold for sea stars within the temperature range inducing arm abscission (Pearson correlation: $P=0.83$). In this category, mean arm temperature remained relatively constant (~35°C) over a range of central disc temperatures that were on average cooler (~31–35°C; Fig. 1). Overall, relatively large temperature deviations were found among arms and even within the central disc at the intra-individual scale (Fig. 1). This variability can be explained mostly by the various positions of the body relative to the heat source (lamps). Intra-individual variability increased slightly with both increasing central disc and arm temperatures throughout the temperature range tested (Pearson correlation on coefficients of variation; on arms: $r=0.27$, $P=0.02$; on central discs: $r=0.41$, $P=0.001$).

The effect of temperature on water balance

As expected, water loss during the experimental low tide was clearly influenced by temperature. Individuals with the warmest body lost up to 30% of their wet body mass during the treatment (Fig. 2A,B) and rate of water loss was significantly correlated with both central disc and arm temperature (Pearson correlation: $r=0.80$ and 0.64, respectively, $P<0.001$ for both). However, central disc temperature was a better predictor of overall water loss when comparing the residuals from a linear regression (mean-square of residuals from linear regression: 14.84 and 24.04 for central disc and arms, respectively). Similarly, the amount of water recovered during the following high tide was inversely related to central disc temperature (Pearson correlation: $r=-0.82$, $P<0.0001$) and arm temperature (Pearson correlation: $r=-0.73$, $P<0.0001$) (Fig. 2C,D). Central disc temperature was only a slightly better predictor of water recovery than arm temperature (mean-square of residuals from linear regression: 9.04 and 13.21 for central disc and arms, respectively).

Survival and arm loss rate were related to rates of water recovery. Most of the individuals that survived with no arm abscission increased their wet body mass by 2–6% following resubmersion, which is in agreement with the values given in Pincebourde et al. (Pincebourde et al., 2009). By contrast, individuals that lost one or several arms were not all able to recover initial wet body mass during the initial 18 h before they lost arm(s), with a change down to −7% (Fig. 2C,D). Finally, the individuals that subsequently died (between 18 and 24 h after treatment) showed the most dramatic decrease in wet body mass after the high tide, between −6 and −11% of initial wet body mass. The effect of water loss during aerial exposure was not as straightforward. Animals that lost the least amount of water (5–15%) were most likely to survive (Fig. 2A,B). However, there was no difference observed in the amount of water lost (10–30%) between animals that lost arms and animals that died, although both lost more water than survivors without arm loss.

The lethal effect of temperature

The logistic dose–response model using central disc temperature as the explanatory variable provided an estimate of the lethal temperature at 35.3°C ($R^2=0.83$, $F_{2,66}=172.5$, $P<0.001$), as has been

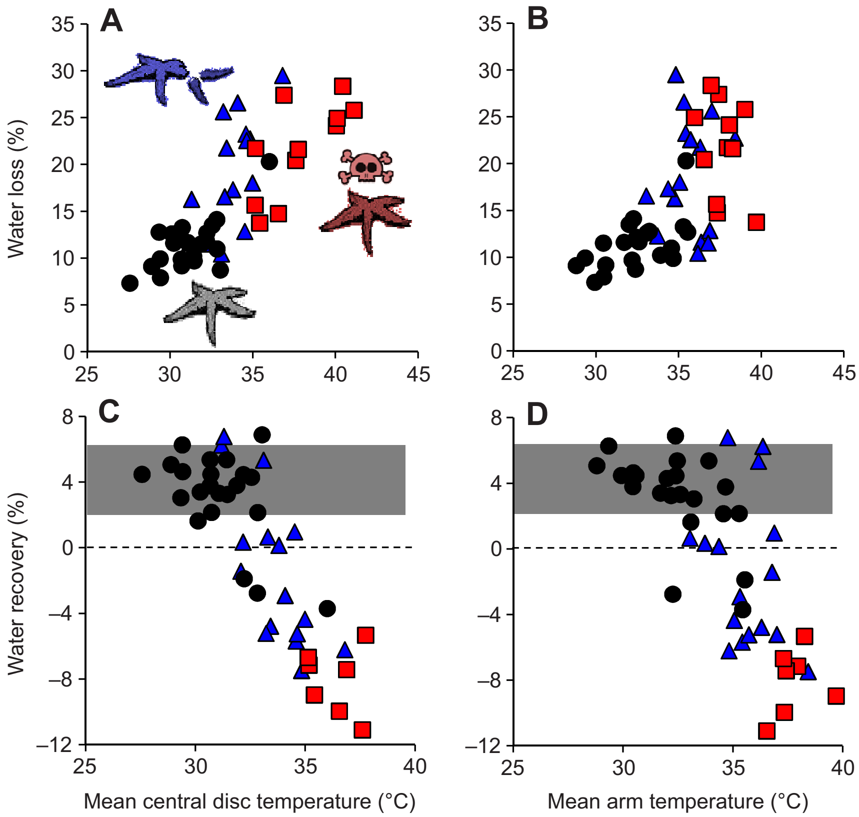

Fig. 2. Temperature influence on water balance in *Pisaster ochraceus.* (A,B) Water loss (% of initial wet body mass) during low tide as function of mean central disc temperature (A) and mean arm temperature (B). (C,D) Water recovery (% of initial wet body mass) during following high tide as function of mean central disc temperature (C) and mean arm temperature (D). The relationship is shown for survivors with no arm damage (black circles), survivors with arm abscission (blue triangles) and the individuals alive at the time of measurement that died subsequently (red squares). The gray bars indicate the expected water recovery portion (4.5±2.3% of initial wet body mass; mean ± s.d.) according to Pincebourde et al. (Pincebourde et al., 2009).

previously reported in Pincebourde et al. (Pincebourde et al., 2008). This thermal limit was very clearly identified, as there was little overlap between the temperatures at which animals survived and those at which they died (temperature range of the overlap: 1.6°C; Fig. 3A). This overlap was much greater, however, when the model used mean arm temperature (R^2=0.41, $F_{2,66}$=25.5, P<0.001; temperature range of the overlap: 4.5°C; Fig. 3B). The mean arm temperature regression model estimated the lethal temperature limit at 36°C. Overall, the logistic dose–response regression analysis showed that central disc temperature was a better predictor of survival probability than mean arm temperature (as the regression model using central disc temperature and mean arm temperature explained 83% *versus* 41% of the variability, respectively; Fig. 3A,B). Mortality was clearly caused by a direct effect of temperature rather than by a direct effect of water loss. Indeed, survival was not related to water loss during the experimental low tide ($F_{2,45}$=1.38, P=0.26; Fig. 3C), which is in accordance with a previous study that found that *P. ochraceus* is quite tolerant of desiccation (Landenberger, 1969).

The sublethal effect of temperature

The pool of survivors (N=38) was used to analyze the effects of exposure to sublethal temperatures on arm damage. Among these 38 survivors, five, six and five individuals lost one, two and three arms, respectively. Arm abscission was initiated at a mean arm temperature of ~32.5°C (LOESS spline estimation from smoothed data: tension 0.40, R^2=0.60, s.e.m.=0.76; Fig. 4A), corresponding to a mean central body temperature of ~30.5°C (LOESS spline estimation from smoothed data: tension 0.40, R^2=0.67, s.e.m.=0.67; Fig. 4B). The logistic dose–response model with arm temperature as the explanatory variable described poorly the loss of an arm when considering each arm as an independent entity (R^2=0.29, $F_{2,187}$=40.8, P<0.001, temperature range of the overlap: 13.1°C; Fig. 5). This model estimated a ‘lethal’ temperature for arms at 38.8°C. Although the specific temperature experienced by individual arms was a poor predictor of arm loss, the arms that were lost by an individual sea star were consistently among its hottest arms, whatever the total number of arms lost (one-sample Kolmogorov–Smirnov test using uniform distribution: P=0.03 for one and two arms lost, and P=0.001 for three arms; Fig. 6).

DISCUSSION

Exposure to sublethal temperatures can induce morphological responses, mostly temporary and reversible changes in morphology (e.g. posture or shape), to decrease body temperature in slow-moving organisms that cannot readily relocate to a new microhabitat (Garrity, 1984; Williams et al., 2005). Here we show that exposure to sublethal temperatures can lead to dramatic and long-lasting effects on sea star morphology by inducing arm abscission. Our results provide experimental evidence, for the first time in an echinoderm, of a direct relationship between temperature of body regions and arm abscission. All arm abscission events were observed with a separation plane located at the ~10th pair of ambulacral ossicles. Abscission always involved basal detachment of arms and we never observed distal damage on arms due to the heating treatment. This observation supports the hypothesis that arm abscission caused by high temperature exposure corresponds to a mechanism of autotomy in *P. ochraceus* initiated as a whole-organism response as opposed to local damage (but see below). However, arm abscission was delayed following exposure to thermal stress and was usually observed >2 days after the treatment. In general, in echinoderms (including asteroids), autotomy involves rapid (within at most a few minutes) abscission of a body part mediated by the nervous system, followed by a long regeneration

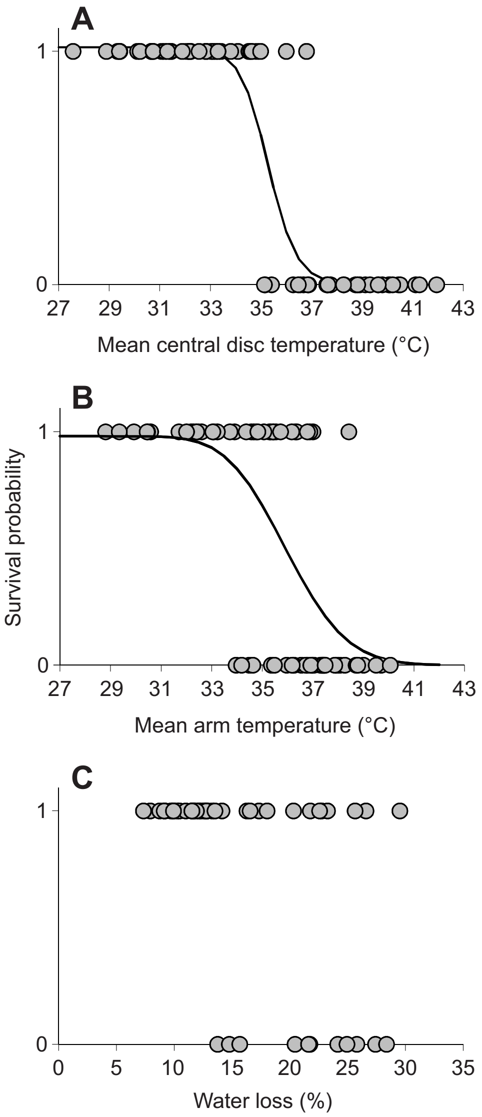

Fig. 3. Lethal effects of temperature on *Pisaster ochraceus*. Survival probability (1: survived, 0: died) of individuals according to mean central disc temperature (A) (from Pincebourde et al., 2008), mean arm temperature (B) and water loss (C). Curves indicate the logistic dose–response regression model.

process (Mladenov et al., 1989; Marrs et al., 2000; Wilkie, 2001). Indeed, the incidence of arm loss in nature is rather infrequent in *P. ochraceus* (Lawrence, 1992), although regenerating individuals can be found in every population. Field surveys of *P. ochraceus* populations at five sites on the central Oregon coast revealed that 0.44 to 5.2% of the individuals at these sites were regenerating arms (supplementary material Table S1). Although the majority of *P. ochraceus* that were regenerating arms at these sites were juveniles (mean ± s.e.m. wet mass=65.6±9.66 g), 33% weighed >90 g and thus were likely sexually mature (Menge, 1974). In general, arm abscission by autotomy is thought to have evolved as a mechanism to escape from predation or as a means of renewing damaged or infected body tissues (Lawrence, 1992). To the best of our knowledge, the role of temperature as an elicitor or proximal cue of arm abscission and/or autotomy has not been tested experimentally before.

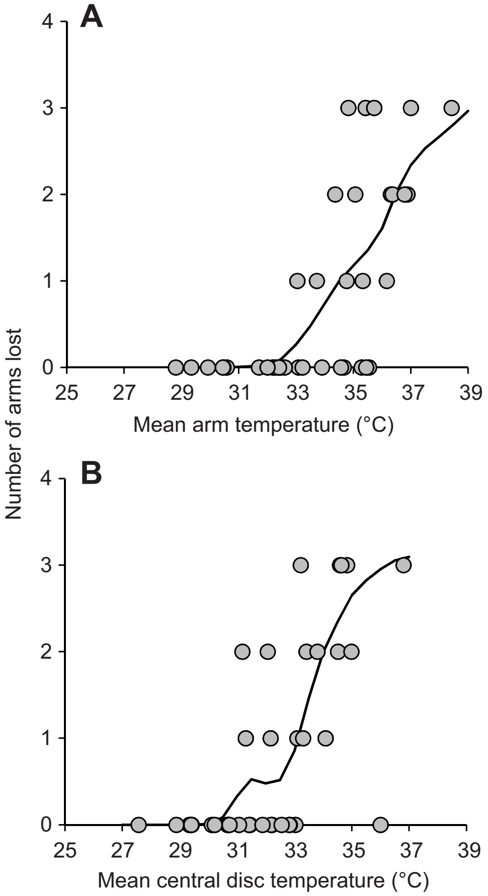

Fig. 4. Sublethal effects of temperature on *Pisaster ochraceus*. Number of arms lost in survivors as a function of their mean arm temperature (A) and mean central disc temperature (B). Curves illustrate the LOESS spline estimation from smoothed data with a tension of 0.40.

The distribution of heat throughout the sea star body shows that arms are generally warmer than the central disc within the temperature range that *P. ochraceus* can tolerate. From a biophysical standpoint, one might expect arms to heat up and cool down more rapidly than the central disc due to a higher surface area to volume ratio and a lower thermal inertia. Asteroids have a relatively large body cavity (coelom) that can be filled with fluid, thereby altering the heating rate of the central disc especially when the seawater is colder than the body temperature of the sea star in air, which is typically the case along much of the geographic range of this species. Indeed, the water balance of *P. ochraceus* is primarily altered in response to the central disc temperature, likely because the volume of fluids within the coelomic cavity is playing an important role in adjusting the thermal inertia of the sea star (Pincebourde et al., 2009). The central disc is the vital region of the sea star, and it is expected that survival is primarily related to temperature of this body region. Furthermore, appendages or peripheral body regions are usually cooler than the core in animals because of higher convective loss, although the reverse can be true when appendages act as solar collectors, as in butterflies. In *P. ochraceus*, arms are therefore expected to be cooler than the central disc at thermal equilibrium when assuming that heat is exchanged only passively between body regions and the environment. But our observations contradict this expectation.

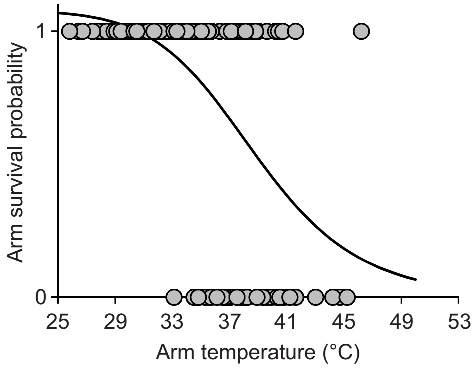

Fig. 5. Sublethal effects of temperature on *Pisaster ochraceus*. Survival probability of an arm when pooling all arms from survivors (1: intact arm, 0: arm loss) as a function of their temperature. The curve indicates the logistic dose–response regression model.

An intriguing hypothesis to explain the temperature deviation between body regions is that the arms might function as heat sinks, as proposed for the major claw in fiddler crabs (Darnell and Munguia, 2011). Such a mechanism implies a capacity to transfer heat between the central disc and the arms, where the heat can be exchanged more easily with the environment due to the comparatively higher surface area to volume ratio. Under this scenario, directional movement of fluid within the body might facilitate the transfer of heat from right below the dorsal integument, where solar energy is collected, to the arms where heat can be released to the environment *via* convective heat transfer. Directional flow can occur in the water vascular system (Prusch, 1977), but it is not known whether directed flows occur within the coelomic body cavity, although it is lined by ciliated epithelium. Another possible mechanism, not mutually exclusive from the hypothesis of arms acting as heat sinks, involves the potential for evaporative cooling. Evapotranspiration can occur across the dermal papulae, which are thin surfaces in the integument that are extensions of the fluid-filled coelomic body cavity. These structures explain why the integument of *P. ochraceus* is often wet at low tide. If fluid loss occurs disproportionately across papulae located on the central disc, then evaporative cooling might be greater in this vital region. The massive water loss we observed in individuals under high thermal stress (Fig. 2B) supports such a link between heterothermy and water balance, and can be interpreted as an emergency measure to cool down the body as its temperature approaches the tolerance threshold. Overall, whatever the mechanism, the observation that arms were cooler than the central disc in animals that died, and conversely that arms were warmer than the central disc in animals that survived without damage, supports the hypothesis of an active mechanism underlying the heterogeneity of body temperature. Nevertheless, little is known regarding such potential mechanisms in sea stars, and more detailed studies are needed to confirm the physiological and/or behavioral mechanisms that generate within-body thermal heterogeneity.

These hypothetical strategies, however, come with the risk of arm abscission, which incurs extremely high costs in terms of both energy and reproductive output (Lawrence and Larrain, 1994; Barrios et al., 2008), because the pyloric caeca and gonads are held within each of the arms. Although no wounds were observed externally on abscised arms, it is possible that arm loss was influenced by internal and irreversible damage caused by temperature, directly or indirectly. Indeed, abscised arms were more likely damaged by local desiccation rather than by a direct effect of temperature as we did not find a strong relationship between abscission and arm temperature (Fig. 5). Abscission might be favored by the advantages of retrieving fluids from a given arm (i.e. to be used for water balance management in the central disc), rather than triggered by a stress signal directly linked to temperature. This notion of sacrifice remains to be explored.

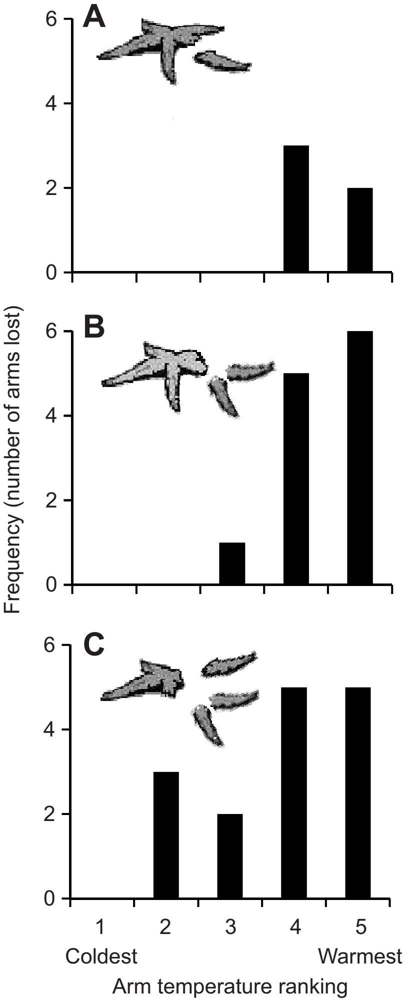

Fig. 6. Sublethal effects of temperature on *Pisaster ochraceus*. Temperature ranking of arm lost at the intra-individual scale (rank 1, coldest of the five arms, to rank 5, the hottest arm) for the groups that lost one (A), two (B) or three arms (C) after exposure to the heating treatment (*N*=5, 6 and 5 individuals in each group, respectively).

In general, beyond butterflies and some hymenopterans, little is known regarding how most ectothermic organisms regulate the thermal heterogeneity of their body regions during sublethal temperature exposure. We found large temperature deviations (5–10°C) between the central disc and the five arms in the sea star *P. ochraceus*. Our setup simulated the various angles of solar exposure to different body regions that can be found in the field. Thus, the thermal heterogeneity we describe can be initiated just by the presence of a heat source (solar radiation). Indeed, our infrared photographs confirm that within-body thermal heterogeneity occurs in *P. ochraceus* in the field at low tide. The within-body thermal heterogeneity of *P. ochraceus* is comparable to, or greater than, that of lizards [~4°C (Garrick, 2008)], bees [~10°C (Roberts and

Harrison, 1999)], mosquitoes [~4°C (Lahondère and Lazzari, 2012)] and triatomine bugs [~8°C (C. Lahondère, personal communication)] when feeding on hot blood, and even plant leaf surfaces [~4°C (Jones, 1999)]. Taken globally, these comparisons suggest that body surface thermal heterogeneity occurs over a wide range of body sizes and morphologies. The mechanistic link between this thermal heterogeneity and the physiological or behavioral response of many ectotherms to environmental stress is largely unexplored, but potentially important. The heat sink function of appendages is relatively frequent in vertebrates such as the enlarged ears of jackrabbits (Hill et al., 1980) and elephants (Weissenböck et al., 2010), or the bill of some birds (Tattersall et al., 2009), but this phenomenon remains understudied for most ectotherms (e.g. Darnell and Munguia, 2011).

Ecologists are now beginning to integrate body temperature into ecological niche models (Kearney and Porter, 2009; Kearney et al., 2010; Monaco and Helmuth, 2011). We suggest that understanding and characterizing within-body temperature heterogeneity is also quite important for at least three reasons. First, the effect of thermal exposure can potentially be a body-region-specific response to temperature or a whole-body response. Temperature can act directly on specific organs, cells, proteins or membranes, which are then identified as ‘weak links’ (Somero, 2002), or it can induce a global (physiologically adaptive) response. In *P. ochraceus*, the lethal effect appears to be a central-disc-specific response of temperature acting directly on this body region. By contrast, the sublethal effect (arm abscission) is a whole-organism response to temperature because there is not a specific temperature threshold for arm abscission induction and also because the identity of the arm that will be lost depends on its temperature relative to the others. Thus, the sublethal effect cannot be understood without knowledge of the within-body thermal heterogeneity. The way organisms integrate temperature changes in their environment, relative to their vital body regions, cannot be elucidated without this knowledge. Second, physiological rates measured in the laboratory might be only weak estimates of actual rates in the field unless a realistic heat source is used (Marshall et al., 2010). The presence and the quality of the heat source are crucial to mimicking the within-body thermal heterogeneity observed in the field. The metabolic rate of ectotherms, at the cellular or tissue scales, depends primarily on temperature. Therefore, temperature deviations between body regions could potentially translate into differences in their specific metabolism (e.g. assimilation and respiration). Different physiological functions can be optimized at different body temperatures; this is well known in reptiles (Huey and Kingsolver, 1989). Nonetheless, it is not clear whether ectotherms can adjust their within-body temperature heterogeneity to maintain several physiological functions near their optimal temperature in different body compartments. Third, ecologists have developed physical models that are useful in mapping body temperatures across space and time (Bakken and Gates, 1975; Fitzhenry et al., 2004; Dzialowski, 2005; Angilletta, 2009). These biomimetic loggers give estimates of body temperature, i.e. an average temperature over the entire body. Such a logger has been developed for the sea star *P. ochraceus* and has been shown to estimate the average temperature of the central disc (Pincebourde et al., 2008; Szathmary et al., 2009). Nevertheless, these loggers are unlikely to capture the thermal properties of peripheral body parts such as arms, legs or other appendages (e.g. crab claws), unless they are specifically designed with this question in mind. We recommend careful analysis of within-body thermal heterogeneity when building and testing such physical models. Overall, we suggest that integrating within-body thermal heterogeneity into biophysical models of ectotherm energy budgets will likely provide new insights into the responses of organisms to environmental stress and climate change.

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AUTHOR CONTRIBUTIONS

All three authors contributed significantly to conception, design and execution of the study, interpretation of the findings, as well as drafting and revising the article.

COMPETING INTERESTS

No competing interests declared.

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