

## Visualizing hotspots: Applying thermal imaging to monitor internal temperatures in intertidal gastropods

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### Abstract

Investigating the impacts of climate change highlights a need to rapidly quantify an organism's thermal environment. We investigated the reliability of non-contact thermal imaging for measuring temperatures in an intertidal gastropod. Thermal maxima from images of either dorsal or ventral surfaces correlated strongly with invasive temperature-probe readings, producing highly significant regression models to predict mantle temperatures from thermal images. Thermal imaging was then field-tested to non-invasively examine temperature changes of snails relative to their substrate: those exposed to sunlight had a mean temperature 4–8°C above the substrate during the day but 2–4°C below at night. Thermoregulation was also tested in the laboratory: when exposed to 45°C for 24 hours, snails reached 35–44°C, significantly higher than those (18°C to 25°C) held at 25°C. Thermal imaging is reliable for rapidly measuring tissue temperatures in a shelled gastropod typical of intertidal environments, thus providing a powerful tool for testing hypotheses about thermal responses in the changing global environment.

**Key words:** climate change evaluation, field measurement, *Nerita atramentosa*, novel methodology, rocky seashore, southern Australia, temperature trends, thermal ecology, Gastropoda

### Introduction

Global climate change is a major threat to the sustainability of the marine environment. It is generally agreed that the earth's sea and air temperatures have warmed by 0.6°C over the past century (Walther *et al.* 2002) and this change in climate is threatening marine environments throughout the globe (Hughes 2000; Przeslawski *et al.* 2008). Many studies have reported changes in the distribution of species, with increasing sea surface temperatures resulting in the poleward migration of intertidal communities (Helmuth *et al.* 2002; Menge *et al.* 2008), sea urchins (Ling 2008) and marine fish species (Todd *et al.* 2008). Climate-mediated range shifts of key habitat-modifying species may lead to negative and disproportionate impacts on marine biodiversity (Hughes 2000; Ling 2008). These impacts can manifest as disruptions to community composition and species interactions (Walther *et al.* 2002; Menge *et al.* 2008) and may also impact important biological processes, such as larval dispersal, recruitment success (Prezslawski *et al.* 2008) and sex determination (Walther *et al.* 2002). Models predicting range shifts and changes to ecological communities under future climate conditions require innovative approaches for defining physiological and/or behavioural responses at the species and community levels.

Intertidal ecosystems have been described as “harbingers of doom” (Sagarin *et al.* 1999; Helmuth *et al.* 2002) or “early indicators” (Lima *et al.* 2007; Wethey and Woodin 2008) for ecological effects mediated by climate change. Larger sized intertidal organisms can be surveyed easily because they are generally conspicuous, slow moving or sessile. Organisms living in the intertidal zone are faced with strong thermal stress given their cyclic exposure to air and immersion in water by tides (Helmuth 2002). These

species are likely to be living closer to limit of their physical capacity to cope with thermal stress than species confined to either purely terrestrial or marine environments. The magnitude of climate warming will be much greater at higher latitudes, and species living in temperate environments experience strong seasonal variations in temperatures (e.g. Tomanek and Somero 1999). Consequently, temperate intertidal zones provide a good model system for examining effects of climate change. This highlights the need for increased attention to intertidal ecology, as well as the development of methods for quantifying stressors and their potential effects.

One global challenge is to predict how intertidal organisms, especially mobile ones, will react to climate change. Unfortunately there is a paucity of data on the thermal relations of these organisms upon which to base a firm mechanistic understanding at the individual level. Reliable and repeatable field measurements of individual organisms like snails are still needed to provide input to assessments of consumer/food interactions (e.g. Rall *et al.* 2010), modelling across larger gradients of space and time (e.g. Finke *et al.* 2009) and to extend perspectives gained from biomimetic sensors (such as loggers encased in mimics or dead shells, e.g. Helmuth *et al.* 2006) to include living animals that can react behaviourally to modify the effects they face.

In the face of environmental change, partitioning intertidal responses to global climate change into separate physiological, ecological and evolutionary components requires empirical data on temperatures of different species. Early research indicated that intertidal gastropods are quite sensitive to temperature (Southward 1958; Hamby 1975; Tomanek and Somero 1999). These include both laboratory (e.g. Williams *et al.* 2005; Ergonmwan 2007) and field-based

(e.g. Barry *et al.* 1995; William and Morritt, 1995) studies, showing thermal limits ranging from 30–50°C across different species (Broekhuysen 1940; Evans 1948). As ectotherms, gastropods are thought to have little physiological thermoregulatory ability, although they do have some capacity to adjust their metabolism to cope with changing temperatures (Sokolova and Pörtner 2001), as well as behavioural responses (Warburton 1973; Garrity 1984; Williams and Morritt 1995; Williams *et al.* 2005). The internal tissue temperatures of hard-shelled molluscs have often been assumed to match that of their external environment (e.g. Southward 1958; Tomanek and Somero 1999; Ergonmwan 2007). However, such assumptions are flawed as recent research quantifying temperatures in submerged individuals versus those exposed to air has shown that the internal temperatures are often decoupled from their environment (Helmuth and Hofmann 2001; Wethey and Woodin 2008 and references therein).

Temperature measurement with invasive probes is currently the most accurate method of determining gastropod internal temperatures and, consequently, a broad range of previous studies have used this approach to measure tissue temperatures directly (e.g. Southward 1958; Vermeij 1971; Hamby 1975; Garrity 1984; Williams and Morritt 1995; Chapman and Underwood 1996; Muñoz *et al.* 2007). Invasive probes are generally inserted behind the operculum, although Southward (1958) pierced a hole in limpet shells to insert a thermocouple and Hamby (1975) drilled holes in live mollusc shells to provide an access point for repeated sampling. However, these methods require much handling and potential damage to the organism, with the probability of additional sources of error from stress and dehydration from the holes drilled in the shell, with resulting impacts on underlying tissue health and survival. Thus traditional drilling techniques are unsuitable for repeated measurements over extended periods.

Thermal imaging (also known as infrared thermography) is a non-invasive means for determining temperature based on measuring the infrared light emitted from surfaces to create an image in the same way that visible light is used to create a photograph (Davis and Lettington 1988). As objects warm, they emit greater infrared radiation, which can be correlated with surface temperature (Davis and Lettington 1988) and this has been used to predict body temperature in a range of organisms (see Supporting information). Helmuth (2002) made reference to thermal imaging as a method of determining the temperature of intertidal organisms and used this technique to illustrate the much lower temperature of the seastar *Pisaster ochraceus* Brandt, 1835, relative to the mussels *Mytilus californianus* Conrad, 1837, on which it was feeding. Jost and Helmuth (2007) compared an infrared image with a digital photograph to illustrate temperature differences between two different species of gastropod. Thermal imaging thus offers a sampling method that is potentially far more rapid than invasive probes, as well as removing the need for handling disturbances. However, to date there has been no attempt to assess whether thermal image readings taken from the

surface of gastropod shells can be correlated with internal tissue temperatures.

This study aimed to determine the effectiveness of thermal imaging as a non-invasive temperature measurement system for intertidal gastropods, using *Nerita atramentosa* Reeve, 1855, as a model species. *N. atramentosa* is a black intertidal gastropod that ranges from the cold temperate waters of Tasmania to the subtropical waters of Southern Queensland. It is common along the southern coast of Australia (Underwood 1975; Spencer *et al.* 2007), where air temperatures can range from below zero to above 45°C, and sea surface temperature varies from 12 to 21 °C (BoM 2008). It is easily distinguishable from other species (Spencer *et al.* 2007) and is often present in high densities (>44 m<sup>-2</sup>) in the mid to upper intertidal zone (Underwood 1975). The black pigmentation of *N. atramentosa* is suitable for investigation of thermoregulation, because it should absorb much solar radiation. In this study, surface temperature readings from a thermal imaging camera were correlated with internal temperatures using invasive probes. The thermal imaging camera was then tested for its ability to detect temperature differences in nerites between day and night in the field, as well as under experimental heat stress in the laboratory. This will provide an empirical basis for application of thermal imaging to the investigation of hypotheses concerning physiological responses to climate change in intertidal communities.

## Materials and methods

### *Nerita* sampling

*Nerita atramentosa* were collected from Marino Rocks (35.04436°S, 138.50819°E), southwest of Adelaide city, South Australia. Collection times were during low tide between 11:00 and 15:00, with individuals selected haphazardly across the accessible rock platform. All individuals were sized between 15–22 mm shell height, and species identity was confirmed using the diagnostic feature of operculum pigmentation highlighted in Spencer *et al.* (2007).

### Invasive temperature measurement

To allow easy sampling, holes were drilled in the shells of experimental organisms and then plugged to prevent further stress and desiccation (see Supporting information). The survivorship and attachment strength of the snails was monitored daily for one week prior to further use. A death rate of only 1% was recorded for drilled and plugged snails within the first week and the remainder survived for a further three months in recirculating aquaria after the experiment, indicating no adverse effects on the snails that may have altered their physiological responses. For internal temperature measurements, a 0.8mm-diameter negative temperature coefficient thermistor was selected because of its small size and accuracy of ±0.1°C (Baker 1999).

### Thermal imaging

Thermal images were taken using a Fluke Ti20 thermal imaging camera (Fluke Australia Pty Ltd, Castle Hill, NSW 2154, Australia) and analysed using InsideIR 4.0 software (Fluke Corporation). For laboratory readings, nerites were placed on a freezer block cooled to  $\sim -3^{\circ}\text{C}$  immediately ( $\sim 2$  seconds) prior to taking the thermal image, in order to maximize the contrast between the background substrate and the shell. Once thermal images were recorded, they were uploaded into the InsideIR software package, which identified the pixel with the highest temperature in the nerite image. The hottest pixel on the thermal image was chosen due to its rapid and reliable determination, while mean or median values across a section of the thermal image required extensive manipulation of multiple markers that may have been inconsistent between images.

### Comparison of thermal imaging and invasive temperature measurement

To determine the accuracy of thermal imaging as a means of determining mantle tissue temperature, thermal image maxima were correlated with mantle temperatures measured with thermistors in the laboratory. Nerites ( $n = 50$ ) were heated using heat lamps to a variety of temperatures consistent with those that would be expected in the field ( $\sim 15\text{--}55^{\circ}\text{C}$ ). Internal thermistor readings were taken, followed by thermal images of the ventral surface of heated nerites. The thermal maxima from the imaging camera were then correlated (Pearson product-moment coefficient) against thermistor readings.

The ventral surface of the nerite was initially chosen as the surface to image because the operculum is the thinnest external surface (unpub. obs.). However, to avoid the need to dislodge snails and turn them over to image the ventral surface, the dorsal surface was also tested, thus trialing the effectiveness of thermal imaging for non-contact sampling in the field. The same method as for sampling the ventral surface was used, except that nerites ( $n = 50$ ) were placed with their operculum facing down and ventral shell on the freezer block for imaging. Again Pearson correlations (measured internal temperatures against thermal image values) were calculated. Model II regression (Laws and Archie 1981) was used to generate a calibration equation from the line of best fit.

### Thermal imaging of exposed nerites in the field

Thermal images were used to establish any difference between nerites and their substrate during both day and night at Marino Rocks. Images were taken of 50 randomly-selected but unshaded nerites on flat rock substrates exposed to full sun between 12:00 and 14:00, which includes the hottest part of the day (11:00 to 13:00, as identified by Helmuth *et al.* 2002). Thermal images of 50 randomly-selected nerites in similar microhabitats were taken between 20:00 and 22:00, at least two hours after sunset of the same date as the daylight readings. This was repeated on three occasions (8<sup>th</sup>, 14<sup>th</sup> and 24<sup>th</sup> April, 2008) during the austral

autumn, with air temperatures at nearby Noarlunga recorded within 30min of the sampling time as 14.2, 16.8 & 15.5 $^{\circ}\text{C}$ , respectively (BoM 2008).

Thermal images were analysed using the InsideIR software. The highest temperature point within the nerite shape was used for comparison with the lowest temperature point on the surrounding image, which was assumed to be a substrate temperature (see Supporting information). Thermal imaging values of the nerites were corrected to internal temperatures using the predictive model II regression from the dorsal correlation analysis (i.e. internal temperature = thermal image maximum \* slope + intercept). The corrected data were then used as the dependent variable in a 2-factor orthogonal ANCOVA in SPSS 15.0, with substrate temperature as the covariate, diurnal period (day or night) treated as a fixed factor and sampling date treated as a random factor. The data were square-root transformed to satisfy the assumption of equal variances.

### Laboratory heat-stress experiment

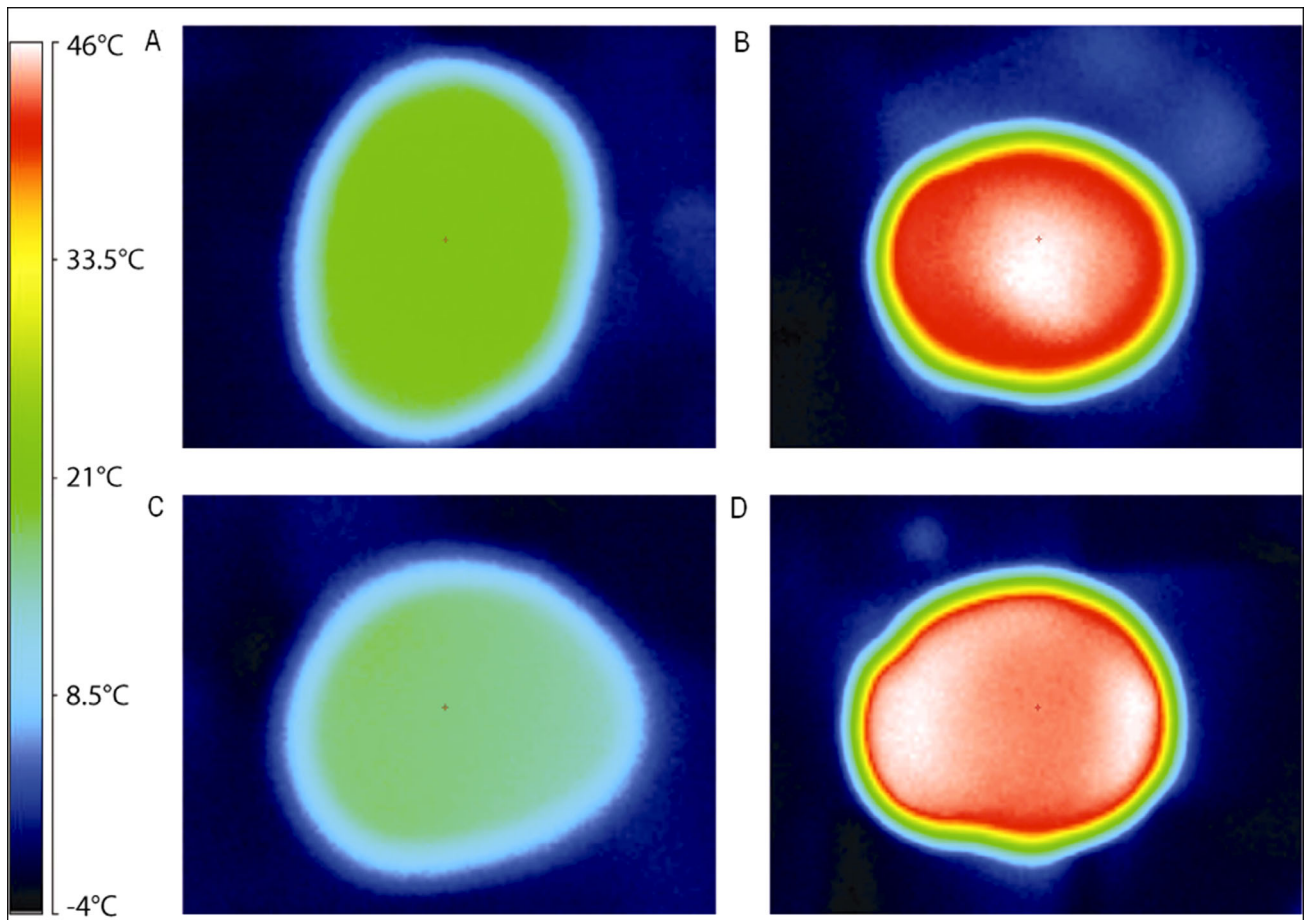
To determine the temperature response and survivorship of *N. atramentosa* to heat stress, a laboratory trial was constructed to compare nerites at temperatures similar to those found in the field, i.e. at a moderate temperature versus under heat stress. In order to provide a stable environment for experimental manipulations, four 285mm x 235mm x 235mm tanks were placed in a 16 $^{\circ}\text{C}$  controlled temperature room. To each, 250mL of sea water ( $\sim 35\%$  salinity) from the nerite holding tanks was added to provide a film of water on the base of the tank. Heat lamps suspended above the tanks were used to increase air temperatures to experimental levels of 25 $^{\circ}\text{C}$  (control) or 45 $^{\circ}\text{C}$  (thermal stress), modulated by type K thermocouples attached to BrainChild digital controllers (see Supporting information).

To allow repeated-measures analysis to be undertaken, snails were marked individually using white acrylic paint, then 10 snails were introduced into each of the duplicate tanks held at the two treatment temperatures (25 $^{\circ}\text{C}$  or 45 $^{\circ}\text{C}$ ). Temperatures were measured at 0, 1, 2, 6, 12 and 24 hours using thermal images of the dorsal surface of snails and corrected according to the predictive model II regression for internal temperature readings. These data were analyzed using a repeated-measures two-factor nested ANOVA, with the individual nerites considered to be replicates nested within replicate tanks, nested within temperature treatments (25 $^{\circ}\text{C}$  or 45 $^{\circ}\text{C}$ ).

## Results

### Thermal imaging of *Nerita atramentosa*

Clear thermal images were obtained of *N. atramentosa* in the laboratory, with high resolution possible at the maximum close focus (Fig. 1). Images taken from both the ventral (Fig. 1A, B) and dorsal (Fig. 1C, D) surfaces show clearly different thermal maxima when exposed to room temperature ( $\sim 20^{\circ}\text{C}$ ) (Fig. 1A, C) or 45 $^{\circ}\text{C}$  (Fig. 1B, D). In thermal images

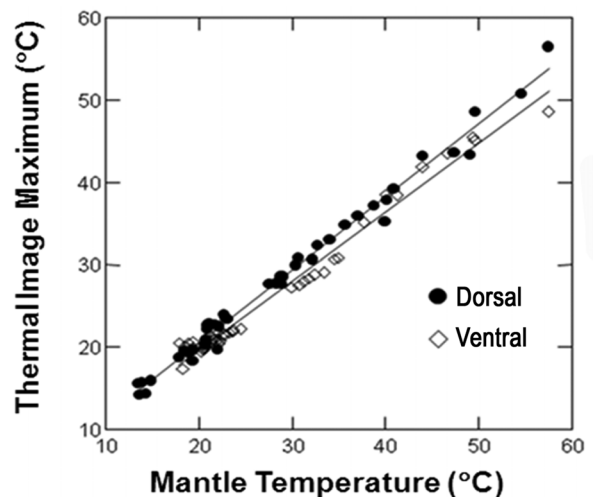


**FIGURE 1:** Thermal images obtained of the ventral (A, B) and dorsal (C, D) surfaces of *Nerita atramentosa* in the laboratory at room temperature (~20°C) (A, C) or at 45°C (B, D). Thermal maxima ( $t_m$ ) obtained from the images were A) 20.7°C, B) 45.2°C, C) 17.0°C, D) 43.4°C.

of the ventral surface, it was often possible to distinguish the operculum as being cooler than the shell surface (Fig. 1B).

**Correlation between thermal imaging and internal tissue temperature**

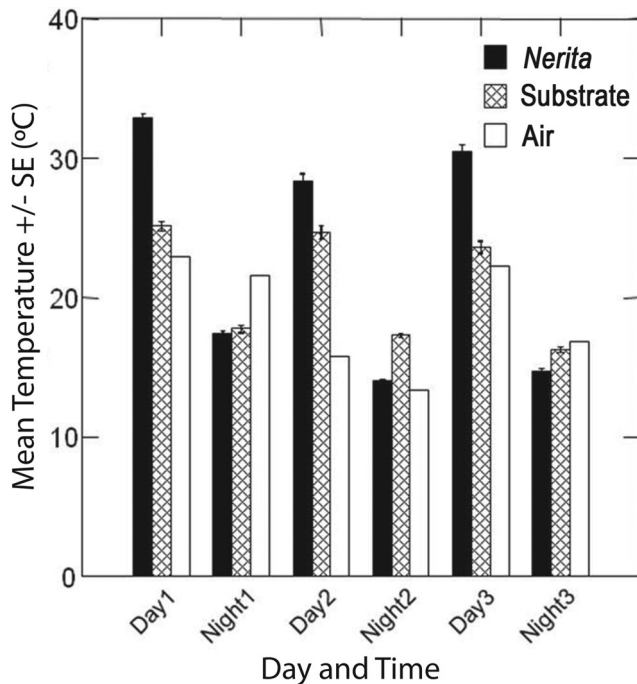
Thermal images of the ventral surface of *N. atramentosa* yielded a significant positive correlation with internal thermistor measurements ( $r^2 = 0.9818$ ;  $p < 0.001$ ) (Fig. 2). Imaging of the dorsal surface yielded a similarly strong linear correlation ( $r^2 = 0.9883$ ;  $p < 0.001$ ) (Fig. 2). The equations derived from the lines of best fit (Fig. 2) provide a calibration for measuring internal body (mantle) temperature ( $t_m$ ) from the thermal image maximum ( $t_i$ ). Both the ventral and dorsal surfaces are suitable for this but imaging the dorsal surface requires no handling of the nerites and also yielded a slightly higher correlation coefficient ( $r^2 = 0.988$ ) for the model  $t_i = 0.8875t_m + 2.7044$ . An operational regression model was generated by reversing the x & y data sets to predict the internal mantle temperature ( $t_m$ ) of *N. atramentosa* from the dorsal thermal image maximum ( $t_i$ ), where  $t_m = 1.121 * t_i - 2.884$ .



**FIGURE 2:** Temperatures derived from thermal images of the ventral and dorsal surface of *Nerita atramentosa*, correlated with mantle tissue temperatures as measured directly using a thermistor. The calibration regression model derived for the ventral surface (open diamonds) was  $t_i = 0.8376t_m + 2.8335$  and for the dorsal surface (filled circles) was  $t_i = 0.8875t_m + 2.7044$ , where  $t_i$  represents the maximum temperature recorded on the thermal image and  $t_m$  the mantle temperature as measured with an internal probe.

### Thermal imaging of exposed nerites in the field

When exposed to direct insolation, nerite temperatures were always above that for the substrate during the day, on average by 4–8°C (Fig. 3). At night, nerites were usually cooler than the substrate by 1–5°C (Fig. 3). A consistent pattern was evident across three sampling dates in April (austral autumn) (Fig. 3). ANOVA revealed a statistically significant covariation between nerite and substrate temperatures (Table 1,  $F = 75.23$ ,  $p < 0.0001$ ), as well as day values being significantly warmer than night ( $F = 928.55$ ,  $p < 0.001$ ). Significant differences were found among sampling dates ( $F = 98.85$ ,  $p < 0.001$ ) but there was no interaction between dates and diurnal period ( $F = 2.92$ ,  $p = 0.056$ ).



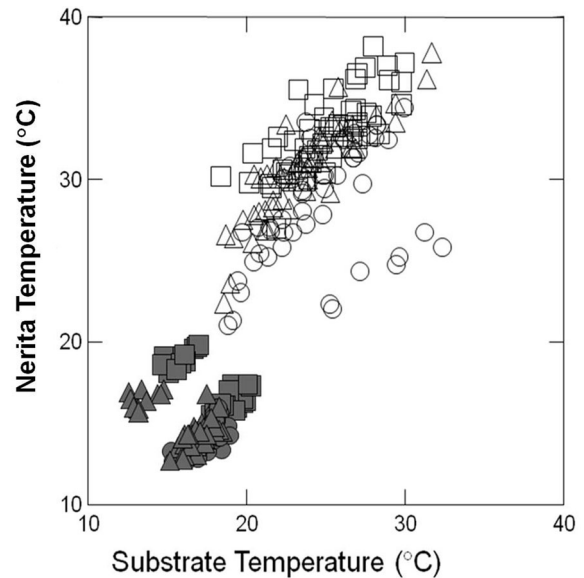
**FIGURE 3:** Temperatures of *Nerita atramentosa* and the substrate, as measured in the field with a thermal imaging camera. Nerite values have been corrected to internal temperatures using the predictive regression model for dorsal surfaces. Error bars show standard error of the means ( $n = 50$ ).

**TABLE 1:** Analysis of covariance testing the difference in diurnal *Nerita atramentosa* temperatures (square root transformed) across three sampling dates in relation to the substrate temperature.

Source	Sum of Squares	df	Mean Square	F-ratio	p-value
Diurnal period	46.747	1	46.747	928.547	<0.0001
Sampling day	9.953	2	4.976	98.850	<0.0001
Period x day	0.294	2	0.147	2.920	0.056
Substrate (covariate)	3.787	1	3.787	75.227	<0.0001
Error	14.600	290	0.05		

Statistically-significant positive correlations between substrate and nerite temperature were found for exposed nerites across all three days (Day 1:  $r^2 = 0.563$ ; Day 2:  $r^2 =$

0.21; Day 3:  $r^2 = 0.67$ ,  $p < 0.001$ , Fig. 4). However, variable results were found at night, with significant negative correlations on Night 1 ( $r^2 = 0.58$ ) and Night 3 ( $r^2 = 0.22$ ) but a significant positive correlation on Night 2 ( $r^2 = 0.55$ ). Nights 1 and 3 both contained clusters with some nerite temperatures that were much higher than would be expected from the substrate temperature (Fig. 4).



**FIGURE 4:** Correlation between the temperature of *Nerita atramentosa* and the substrate from field data as derived from dorsal thermal imaging model in exposed microhabitats during the day and night. Symbols: filled = night, open = day; square □ = day 1, circle ○ = day 2, triangle △ = day 3, filled square ■ = night 1, filled circle ● = night 2, filled triangle ▲ = night 3.

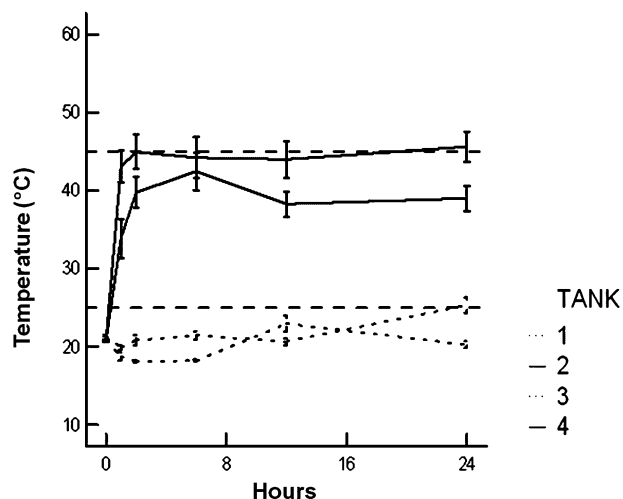
### Laboratory heat-stress experiment

Nerite temperatures in 25°C aquaria remained reasonably stable over 24 hours (Fig. 5). By comparison, nerite temperatures in the 45°C aquaria rose quickly, with the means in both replicate aquaria reaching a plateau after two hours (Fig. 5). Nerite temperatures at 45°C were also more variable than at 25°C, as shown by the larger error bars (Fig. 5) but still reached a fairly constant mean temperature after two hours, which was close to or just below the ambient temperatures recorded (~45°C in tank 2 and ~40°C in tank 4). Repeated-measures ANOVA showed a significant difference in nerite tissue temperature according to temperature treatment ( $F = 325.5$ ,  $p < 0.001$ ). Significant differences were also found among time points ( $F = 24.4$ ,  $p < 0.001$ ), with nerite temperatures significantly increasing within the first two hours at 45°C (Fig. 5).

### Discussion

This study shows that thermal imaging is a flexible, field-based alternative to using invasive techniques (as contrasted here), biomimetic loggers (e.g. Helmuth *et al.* 2006) or modeling approaches (e.g. Finke *et al.* 2009) to determine

the internal temperature of individual intertidal organisms. Measuring realised temperature without invasion is crucial for mobile species, where any disruptive behaviour could affect the immediate results, as well as subsequent movements. Thermal imaging also allows routine comparison of organisms with their substrates in terms of temperatures being experienced.



**FIGURE 5:** Temperature response of *Nerita atramentosa* in 25° (Tank 1 and 3) and 45°C (Tank 2 & 4) controlled environments over 24 hours in the laboratory. Nerite temperatures taken from the thermal imaging camera have been corrected to internal temperatures using the predictive regression model for dorsal surfaces. Error bars show standard error of the means ( $n = 10$ ).

Here we provide an efficient empirical method for investigating internal thermal responses in intertidal gastropods. Effective non-invasive techniques were developed and applied both in the field and in laboratory experiments to measure nerite tissue temperatures. In particular, the Fluke Ti20 thermal imaging camera proved to be a useful and user-friendly piece of field equipment. As body temperature of intertidal organisms can rise to over 10°C above sea surface temperatures (e.g. Southward 1958; Vermeij 1971), it cannot be assumed that either air or sea surface temperatures provide a good proxy for body temperature (Wethey and Woodin 2008). Thermal imaging provides an efficient means for examining directly the actual body temperatures of gastropod snails in their natural environment and thus provides opportunities for directly monitoring the physiological responses of intertidal organisms to ensuing climate change. Traditional techniques (e.g. Southward 1958) either measure tissue temperature (with invasive thermistors) or some average of the shell surface contacted by the probe and the air temperature (external thermocouples). Thus, thermal imaging represents a superior non-invasive and non-destructive technique for measuring the thermal profile of an intact live organism in its environment.

Thermal imaging allowed more detailed examination of nerite temperatures than traditional measurement techniques

previously applied in the field. The images allowed the surface temperature of the entire nerite to be viewed, illustrating the fine-scale temperature gradient often present (e.g. hot at the point of insolation trajectory and cooler on the shaded side, see Fig. S1 in Supporting information). By facing the sun, *N. atramentosa* would be able to expose the narrowest profile of its body to insolation, potentially minimising its heat absorbance. Field studies have demonstrated that *Nerita plicata* Linnaeus, 1758, a tropical nerite, uses the sun as a means of orienting itself in Kenya (Warburton 1973). The impact of shell angling for behavioural thermoregulation should be investigated in future studies of intertidal gastropods.

*Nerita atramentosa* and its very similar eastern Australian congener *Nerita melanotragus* Smith, 1884 (Spencer *et al.* 2007) that were, until recently, considered to be the same species, could be adopted as effective indicator species for intertidal thermal monitoring on temperate Australian rocky shores. The highly-significant correlation found between body temperatures taken from invasive probes and the temperature maxima in thermal images enabled the development of a reliable model for predicting body temperature from thermal images in this species. Thermal imaging thus estimated internal temperature of *N. atramentosa* effectively over the range of temperatures that are likely to be encountered in southern temperate zones. In the field, nerite temperatures did vary amongst individuals and measurement times but were generally correlated with substrate temperatures in sun-exposed conditions during the day. Some unexpected temperature responses were recorded for nerites at night during our study: a significant positive correlation occurred on one night, but a negative correlation with substrate temperature was detected on the other two nights, where the snails grouped into two distinct clusters, one which was much warmer than predicted from the substrate temperatures. These results are difficult to explain without further research but indicate that nerite behavior is likely to influence their thermal properties according to their specific micro-condition, such as has been recorded for the tropical limpet *Cellana grata* (Gould, 1859) (Williams and Morrill, 1995).

To demonstrate the wider applicability of thermal imaging for gastropod temperature measurement, correlations should be obtained for a variety of species of different sizes and shell morphologies. The surface area-to-volume ratio is likely to affect rates of heating and cooling of internal tissue. Shell thickness will impact upon the rate of heat absorption and re-radiation and, in terrestrial pulmonates, has been shown to be greatest for species exposed to high temperatures (Alyakrinskaya 2005). Shell pigmentation and lustre could influence the surface emissivity for infrared wavelengths, with low-reflective surfaces likely to register as being cooler on a thermal image than in reality, while surfaces with a high reflectivity are likely to register a higher temperature (Webb 1991). Due to different shell morphologies and reflectivities to UV radiation, it is likely that a new model for deriving internal tissue temperatures from thermal images will need to be

developed for each intertidal species, especially if used in comparative studies at the community level. Nevertheless, thermal imaging could still be applied directly to test species-specific hypotheses regarding thermal responses to different environmental conditions without models for internal calibration by examining differences in observed shell-surface temperatures, rather than predicting absolute internal body temperatures.

*Nerita atramentosa* was found to maintain body temperatures above substrate temperatures under mild conditions in the field and laboratory, suggesting some thermoregulatory capacity. However, under heat stress in the laboratory, their body temperatures equilibrated to the ambient air temperature (45°C) within one hour in one of the treatment tanks, which was under the lethal temperature of local *N. atramentosa* (four hours at 48°C, pers. obs., see Supporting information). *N. atramentosa* is black, and therefore darker than many other intertidal molluscs. Our findings that *N. atramentosa* is routinely warmer than its substrate during the day suggests that this dark pigmentation may be a liability for this species during the summer months, especially under climate change. However, the distribution of *N. atramentosa* extends into tropical regions along the west coast of Australia, and *N. melanotragus*, likewise extends well into the tropics on the east coast, suggesting that both species are able to cope with extended periods of high temperature. Furthermore, nerites are highly speciose in tropical regions, where they most likely evolved (Garrity 1984), thus it seems likely that many members of this family are well adapted to coping with high temperatures. Further research into the physiological and behavioural thermoregulatory abilities of these black nerites across their latitudinal range would be of interest.

In conclusion, this study demonstrates that thermal imaging is a useful innovation for visualizing the thermal interaction between intertidal organisms and their environment. By determining a strong correlation for nerite temperatures between thermal images and invasive probes, we demonstrate that it is possible to predict gastropod tissue temperatures from surface images of the shells. By applying this method in the field, we were able to document diurnal changes in *N. atramentosa* temperatures and higher body temperatures than both the air and the substrate under cool autumnal conditions. However, under extreme heat stress in the laboratory, *N. atramentosa* became thermo-conformant with the ambient environment. Due to its thermosensitivity and ease of monitoring in the field with thermal imaging, *N. atramentosa* (and its eastern congener *N. melanotragus*) would be appropriate bioindicators of thermal stress in temperate Australian intertidal ecosystems. Other species of nerites are easily identified with ubiquitous distributions on intertidal rocky reefs, making them ideal for ecological monitoring. This study furthers our understanding of thermal behavior in nerites and details novel methodologies by which thermoregulation can be explored in intertidal ectotherms by monitoring temperature responses. Our approach provides an effective framework for integrating physiological responses into ecological studies. With further validation, thermal

imaging could be applied to temperature measurements across a range of intertidal molluscs, including limpets, littorinids and mussels, thus facilitating future research aimed at investigating mechanistic hypotheses in the light of global climate change.

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