Like night and day: Reversals of thermal gradients across ghost crab burrows and their implications for thermal ecology

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ABSTRACT

Ghost crabs, *Ocypode cordimanus*, inhabit relatively hostile environments subject to thermal fluctuations, including both diurnal and seasonal cycles. For many ectotherms, including ghost crabs, a major challenge is to remain cool during hot daytime temperatures. This can be achieved by adopting a fossorial lifestyle, taking advantage of thermal refuge afforded by burrows of sufficient depth. Another consideration, often overlooked, is the potential advantage associated with ready access to a thermal energy source (a "charging station") when surface temperatures are cooler. Being able to rapidly elevate body temperature during cool periods would enhance the crab's ability to maintain rate processes and carry out essential activities. We have measured ghost crab burrow temperature profiles at two times of the day with contrasting sun exposure (06:00 and 14:00), demonstrating how effective burrow depth (up to a maximum of 40 cm) provides thermal regulation below the surface of the sand (e.g., at dawn (06:00) and early afternoon (14:00) at a depth of 5 cm, temperatures ($\pm$SD) of $16.32 \pm 0.96\,°C$ and $25.04 \pm 1.47\,°C$ were recorded, respectively. Corresponding temperatures at a depth of 30 cm were $19.17 \pm 0.59\,°C$ and $19.78 \pm 1.60\,°C$, respectively). This demonstrates that while temperature conditions at the surface vary dramatically from night to day, ghost crab burrows can maintain relatively constant temperatures at the burrow base throughout the diurnal cycle, at least during winter. As a consequence, the burrow heat signatures undergo a corresponding thermal gradient reversal between night and day, as revealed by infra-red photography. Complementing these field observations, we also determined heating and cooling times/constants for *O. cordimanus* in the laboratory ($\tau = 17.54$ and $16.59\ JK^{-1}$, respectively), and analysed chemical composition of their carapace (external (with β Chitin evident) and internal (predominance of α Chitin)), which is the primary thermal interface with the environment. We find that ghost crabs both gain and lose heat relatively rapidly, which likely affects the range and duration of surface activities under different thermal conditions, and renders the thermal characteristics of their burrows vital for their persistence on beaches. Finally, we speculate that the distinctly contrasting thermal signatures of ghost crab burrows in comparison to the surrounding sand could in principle be used by crabs as spatial markers for navigation and to identify holes on return from nightly excursions, being identified either by direct thermal sensing or odours rising from the burrow base as a consequence of the thermal flux.

1. Introduction

Coastal beach-dune systems comprise a rich population of flora, fauna (Schlacher et al., 2014) and a variety of natural and anthropogenic solid matter, each with distinct, temporal and spatial thermal signatures. In many instances, these thermal signatures should be able to help researchers identify key behavioural responses of organisms and predict how such responses may evolve in response to changing environmental conditions (e.g., climate warming).

The majority of the faunal assemblage populating these beach-dune systems are cold blooded. Ectotherms are particularly

susceptible to temperature variations, as their body temperature is determined, to a large extent, by environmental conditions. Thus, key behavioural responses of ectotherms can be attributed to thermoregulation. The ability to cope with extremes rather than different mean temperatures is arguably of greatest importance for thermal adaptation and species survival in a changing climate. Ectotherms achieve this in a variety of ways including: increased circulation rates (Williams et al., 2005), orientation of the body relative to radiant heat sources (Bonebrake et al., 2014), and, utilisation of topography offering shade (Kearney et al., 2009). Indeed, many terrestrial ectotherms have some mobility and thus can behaviourally exploit temporal and spatial heterogeneity in their environment to regulate body temperatures (Kearney et al., 2009). For example, the banana fiddler crab, *Uca mjoebergi*, can escape extreme surface heat on mudflats (up to 50 °C) beyond its thermal limit (40 °C) by taking refuge in cooler habitats, such as mangrove trees (Darnell and Munguia, 2011; Powers and Cole, 1976; Smith and Miller, 1973; Thurman, 1998). They can also find thermal shelter by retreating to their burrows (Smith and Miller, 1973).

Most studies to date on burrowing crabs have focused on fiddler crabs (e.g., Klaassen and Ens, 1993; Smith and Miller, 1973), and have investigated avoidance behaviour and tolerance of daytime maximum temperatures. But burrowing can also provide buffering against extreme cold temperatures, and might even be an adaptation to extreme cold at some species' poleward range edges (e.g., Schoeman et al., 2015). Indeed, it has been shown that ghost crabs show a dramatic decline in activity during particularly cold winters, remaining confined to their burrows (Haley, 1972; Jackson et al., 1991). This, however, would only occur where winter temperatures are low relative to the thermal tolerances of the species, which would be unlikely within our study area where winters are relatively mild (see below).

For beaches of the Queensland coast, Australia, ghost crabs are a conspicuous and vital part of the food chain in the coastal ecosystem (see Lucrezi and Schlacher, 2014; Schlacher et al., 2016). These crabs inhabit small, semi-permanent burrows in the sand, which afford them refuge from a hostile environment, especially during daylight hours when they would otherwise be vulnerable both to potentially high temperatures and active visual predators. As such, they generally forage at night (Barrass, 1963; Cott, 1929; Hayasaka, 1935; Türelli et al., 2009), although contradictory results have described diurnal activity for some species (Jones, 1972; Hughes, 1966; Macnae and Kalk, 1958). Foraging entails a range of methods, including deposit-feeding (sifting of micro-fauna from the sand), scavenging and preying on other members of the beach-dune community (Robertson and Pfeiffer, 1981; Tweedie, 1950; Hughes, 1966; Jones, 1972; Hayasaka, 1935; Wolcott, 1978). The information regarding the activity profile of ghost crabs in relation to time spent residing in burrows and time utilised for out-of-burrow excursions, including foraging, is, however, limited (Schlacher and Lucrezi, 2010).

There is laboratory-based evidence to show that for certain species, activity does not correlate with either circadian or tidal periodicities (Jones, 1972). Some authors have suggested that the activity periods may be related to different methods of feeding and availability of potential prey (e.g., Jones, 1972; Hughes, 1966), as well as human activity in or around the crab habitat (Hughes, 1966). Nevertheless, carrying out activities at night (repairing existing burrows, digging new burrows, foraging for food) provides the crab with the cover of darkness and presumably reduced risk of predation. This time of day, however, is not the most energetically favourable to provide heat to this ectotherm. This is especially true during cold periods, including winter, and has further ramifications for crabs living close to the poleward edge of their distributional range, where surface temperatures might drop far enough to be

lethal (Schoeman et al., 2015) in the absence of a ready refuge. With this in mind, the current study (1) records the thermal signatures of burrow entrances, (2) determines the temperatures of their burrows at a range of depths at specific times in the day (i.e., mid-afternoon, after extensive irradiation by the sun, and early morning, before irradiation has been received), (3) examines rates of temperature change for ghost crabs, and (4) determines the chemical composition of both the internal and external surfaces of the carapace.

2. Materials and methods

Studies were conducted in the field at Warana beach, in the Sunshine Coast region of South East Queensland, Australia (Fig. 1), within 100 m in either direction along the beach shoreline of 26° 43′ 34.73″ S 153° 08′ 05.50″ E. Summer temperatures are hot (mean maximum air temperature: 28.4 °C) and winters, warm (mean maximum air temperature: 21.2 °C).

The Sunshine Coast region is bordered by long, east-facing sandy beaches, punctuated sporadically by estuaries, and broken only briefly by rocky headlands (or Points). The study site of Warana beach is typical of the region, comprising an intertidal beach face of reflective-intermediate morphodynamic type. On the landward side, the beach face grades into low embryo dunes developing around clumps of Spinifex grass and other low dune vegetation, with higher, vegetated dunes further back. Sediments comprise medium sands (250–500 μm; 1–2 Ø), which are dynamically reworked by the action of prevailing winds and waves.

2.1. Thermal properties of ghost crab burrows

Crab (*O. cordimanus*) burrows were randomly selected from the upper beach within 15 m of the vegetation zone. This is where the highest density of crab burrows were located. Measurements were collected from a period between April 17th and September 18th, 2016. This comprised measurements recorded on 16 different days for this period.

As anticipated, we rarely observed ghost crab activity during early morning or late afternoon hours, but those individuals observed during these times were juveniles (8–18 mm carapace width). We chose two discrete times at which to investigate trends in thermal properties of the ghost crab holes, based on heating cycles associated with solar radiation. Thus, temperatures of the burrows were recorded predominantly early in the morning before sunrise (06:00 (or at 08:00 once cloud cover had cleared)) and later during the day (between midday (12:00) and afternoon (16:00)) when the beach sand had been exposed to thermal radiation for several hours.

An EasIR-4 instrument was used to simultaneously collect data from both thermal and optical sensors, thereby providing paired thermal and optical images. Optical images were collected at a resolution of 1600 × 1200 pixels, with burrows briefly illuminated to acquire optical images at night. The thermal images could profile depths from 5 to 40 cm, depending on the orientation of the camera relative to the burrow profile (i.e., straightness of the burrow). The thermal detector has a resolution of 160 × 120 pixels, which captures a spectral range of 8–14 μm. The operating temperature and humidity of the instrument is between −10° - 40 °C and 10–95%, respectively. The sensitivity of the thermal sensor is < 0.1 °C at 30 °C, and its accuracy is ±2 °C. Resulting thermal images were analysed using the program Guide IrAnalyzer System. To determine general trends, temperature profiles were measured from a total of 387 burrows using the thermal camera. Measurements were collected on days with minimal cloud cover.

The surface sand temperature was also measured with a dual-

Fig. 1. Warana beach, located in the Sunshine Coast region of South East Queensland, Australia.

temperature meter HT-L13 with thermocouples (type K) and an infrared thermometer MT300. The thermocouple wire was marked at 5-cm intervals and used to probe the burrow temperature at depth. Depths up to 40 cm were measured in burrows; however, 20–30 cm was typically the furthest distance measured due to burrow termination or obstruction (often in the form of a sharp bend in the burrow). As the burrows are not orthogonal to the sand surface, and are not exactly straight in profile, the burrow depth recorded is the length along the actual burrow and not the depth from the surface of the sand. The difference, however, is marginal, as most burrow angles were over 80° relative to the surface plane.

2.2. Rates of temperature change in crabs

Ocypode cordimanus (Fig. 2) were collected from the field site, either by excavating them from their burrows, or by hand collection at the surface after dark. There was no selection process for crabs caught; all specimens retrieved on the day of capture were removed to the laboratory, with a maximum of 35 individuals held at one time. In the laboratory, the carapace width of each crab was measured to the nearest mm using electronic Vernier callipers. Thereafter, specimens were placed individually into plastic boxes

Fig. 2. Photograph of a juvenile ghost crab, *Ocypode cordimanus*, taken early in the morning.

containing a small volume of seawater taken from the same site of collection and acclimated overnight at a room temperature of 22 °C (±1.5 °C, maintained by air conditioning). The following day, 18 of the captive individuals were selected at random for use in experiments.

Ocypode cordimanus, and close relatives in the genus, commonly have critical lower thermal limits in the region of 12 °C (Darnell et al., 2015), and air temperatures at the study site in summer regularly reach 30 °C and above. We therefore tested temperature changes of 10 °C relative to the acclimation point at 22 °C (i.e., from 22 °C, warming to 32 °C, and cooling to 12 °C). These temperatures were achieved by partially submerging plastic containers in a water bath at the pre-selected temperature, and allowing them to equilibrate for an hour. Water temperature in the bath was controlled using a TK1000 tank-chilled line (closed-loop temperature-controlled system, range 5 °C–35 °C), supplemented by a Ratek TH7100 digital immersion heater with high-flow circulator (range 0 °C–100 °C).

Crabs acclimated at 22 °C (a reasonable approximation of burrow temperature – see later) were exposed to each of these temperatures in batches of six for 90 min. During this period, thermal images were captured every minute using the EasIR-4 instrument described above, mounted above the water bath. The thermal images were analysed providing estimates of the temperature at the centre of each crab's carapace as well as that of the corresponding background (i.e., the water temperature).

We tested two batches of six crabs at each temperature in a random sequence, with each individual crab used in one experiment only, after which it was released at the field site (i.e., in total we measured temperature change in 24 individual crabs).

Using a simple resistance-capacitance first-order model of cooling and rewarming (*sensu* Turner, 1997), it is straightforward to estimate the energy costs associated with periodically cooling and rewarming a crab. This approach relies on Newton's Law of Cooling and Heating, that is, the rate (over time t) of cooling/heating ($\frac{dQ}{dt}$) is proportional to the excess temperature, that is, the difference in temperature of the crab prior to cooling/heating (T) and the ambient temperature (T_{amb}):

$$\therefore \frac{dQ}{dt} = -k(T - T_{amb}) \tag{1}$$

where k is the proportionality constant. The negative value is included to describe both cooling and heating scenarios, that is, a negative rate of change for the case when heat is leaving the system (i.e., crab is cooling), or $T \geq T_{amb}$, or a positive rate of change when the crab is warming (i.e., $T < T_{amb}$). During each cycle of entry and exit from the burrow (the warming and cooling cycle) the crab temperature will oscillate between the return and exit temperature. The energy involved in heating the crab can be approximated by the product of the difference between the two temperatures and the thermal capacity of the crab (C).

Letting $Q = mcdT$, where m = mass of the crab, c = specific heat of the crab and dT is the change in temperature, and solving for ($T - T_{amb}$) via separation of variables (cooling scenario), yields:

$$|T - T_{amb}| = (T_0 - T_{amb})e^{-t/mc} \tag{2}$$

where T_0 is the initial temperature of the crab at time = 0, i.e., prior to entry or leaving the burrow after having cooled down or warmed up, respectively.

Letting the time constant equal the product of mass and specific heat,

$$\tau = mc \tag{3}$$

thus yields

$$|T - T_{amb}| = (T_0 - T_{amb})e^{-t/\tau} \quad \text{for cooling } (T \geq T_{amb}), \text{ and}$$
$$|T_{amb} - T| = (T_{amb} - T_0)e^{-t/\tau} \quad \text{for heating } (T < T_{amb})$$

(4)

The time constant τ for the cooling and heating scenarios can then be calculated via the gradient $(-1/\tau)$.

2.3. Chemical composition of the crab carapace

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were acquired from the crab carapace (external and internal surfaces) using a FTIR spectrometer (Spectrum™ 100, Perkin-Elmer, USA) equipped with an internal Globar™ IR source, a deuterated triglycine sulphate (DTGS) detector and a single-reflection diamond ATR accessory (UATR, Perkin-Elmer, USA) with 45° angle of incidence and a $2 \times 2 \text{ mm}^2$ active sensing surface. A background spectrum was individually acquired on a clean surface of the diamond crystal prior to each sample measurement. Sample measurements were conducted by pressing the carapace against the sensing surface of the diamond ATR crystal using a metal clamp, and a few high-quality spectra were collected from each side of the carapace using 16 co-added scans. Reproducible contact was achieved by the use of an electronic sensor plate available with the ATR unit.

2.4. Data analysis

In order to further investigate the crabs' rates of changes in temperature, we used mixed-effects linear modelling to investigate temperature change in captive crabs over the first 30 min of the observation period (temperature change decayed rapidly after this point at both temperatures tested). We first fit exponential decay curves by modelling ln(Crab carapace temperature) as a function of ln(time), background temperature (factor) and crab size (carapace width, mm). Next, we modelled ln(|difference between crab and environmental temperature|) as a function of time, background temperature (factor) and crab size (carapace width, mm). In both cases, we started with the maximal model including all main effects and their interactions, as well as a random effect for individual (to

account for within-individual autocorrelation). We simplified each maximal model as per Bolker et al. (2009) by dropping terms that revealed no significant deterioration in model fit on the basis of log-likelihood ratio tests ($\alpha = 0.05$). In each case, our decision to drop model elements was verified by changes in Akaike Information Criterion (AIC). Although there was substantial variability among individual crabs, the temperature trajectory for a crab of mean size in the sample (12.7 mm) fit very well, with the proportion of deviance explained by the fixed effects (all main effects, plus interactions for ln(time) × background temperature and crab size × background temperature) being 0.97. Fits for the model from which thermal constants were extracted were weaker, explaining only half the deviance of the null model (terms in the minimum adequate model were a common intercept, time, crab size, background temperature, and interactions for time × background temperature and time × crab size).

3. Results

3.1. Thermal properties of ghost crab burrows

Thermal profiles showed a distinct reversal in the thermal contrast between early morning and mid-afternoon (Fig. 3): burrows recorded at 06:00 demonstrated a higher temperature than surrounding sediment (Figs. S1 and S2), while those recorded at 14:00 were cooler than the surrounding sand surface. This reversal was observed to take place at the burrow entrance after direct irradiation from the sun terminated or significantly reduced (generally from 15:00 onwards; Fig. S3). Topographical features of the foredunes (and erosion scarp, when this was present) also affected this reversal time, especially when the burrow entrance was shaded from direct irradiation. Regardless, this contrast was evident in all comparisons of thermal profiles collected at the two ambient temperature extremes of early morning (prior to sun irradiation) and afternoon (during the heat of the day).

We investigated the depth profile of temperatures using a thermocouple to measure temperature within the burrows at 5 cm intervals, up to 40 cm in depth. The data for a haphazard selection of 50 ghost crab burrows taken at 14:00 and another 50 taken at 06:00 show that the surface temperature (±standard error) of the sand (depth = 0 cm) at 14:00 was 29.65 ± 0.12 °C, but was only
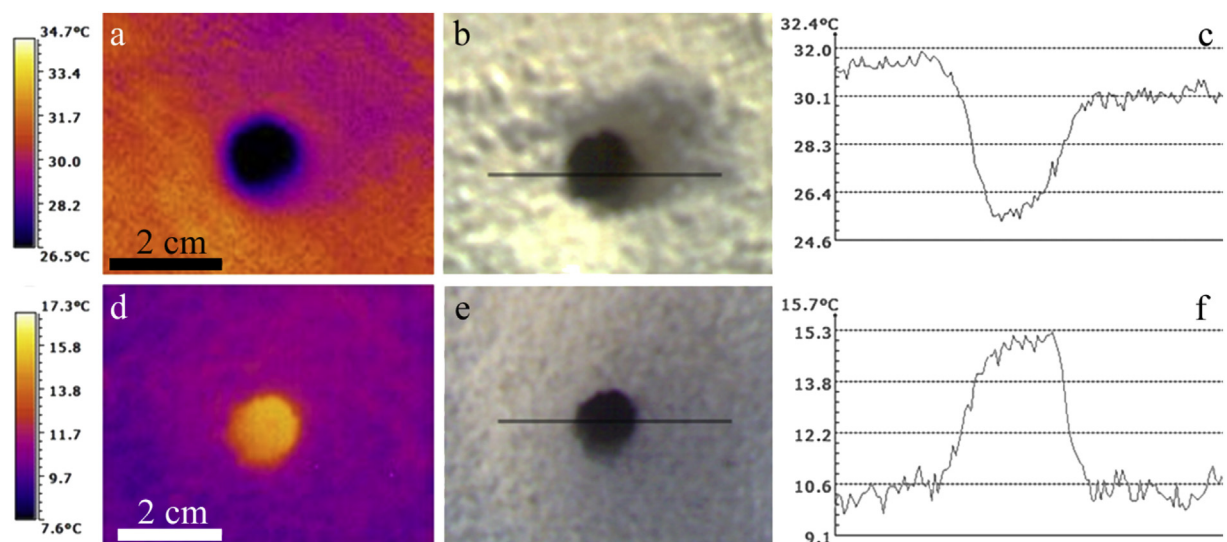

Fig. 3. Representative thermal (a, d) and optical (b, e) images, and thermal profiles (c, f) of ghost crab burrow entrance at 12:00, June 18th and 06:00, July 13th. Minimum and maximum temperatures for these days were 14.0/23.3 °C and 12.9/22.1 °C, respectively.

13.95 ± 0.05 °C at 06:00. In contrast, the temperature at a depth of ≥30 cm was 19.33 ± 0.09 °C at 06:00 and 19.14 ± 0.42 °C at 14:00 (Fig. 4). Importantly, however, the data show a rate of change in temperature decreases as depth increases. A quantitative description of the two temperature trends shown in Fig. 4 are best represented by a 3rd-order polynomial: (06:00) - Depth $(cm) = 0.2029x^3 - 8.823x^2 + 129.63x - 642.3$ where x is the temperature in Celsius; $R^2 = 0.9965$, $p < 0.001$. (14:00) - Depth $(cm) = 0.0019x^3 + 40.5093x^2 - 24.783x + 335.48$; $R^2 = 0.9992$, $p < 0.001$.

Corresponding thermal images of burrows for the same time periods as shown in Fig. 4 corroborate the thermal reversal
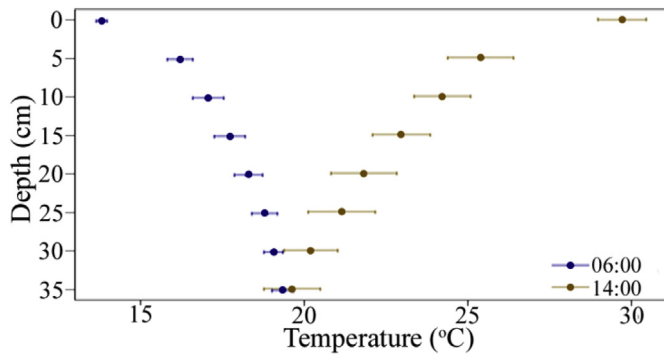

Fig. 4. Thermal gradient reversal of ghost crab burrows - temperatures measured from a sample of 50 holes mid-afternoon (14:00) July 12th, 2016 and an independent sample of 50 holes early in the morning (06:00) July 13th, 2016. Ambient air temperatures were 25.3 °C and 13.8 °C for the two collection periods, respectively, with corresponding reported minimum/maximum temperatures of 13.5/27.1 °C and 12.9/22.1 °C.

observed at the sediment surface, and the thermal stability at depth (Fig. 5(a) and (d)). Humidity measurements collected for the two burrows shown in Fig. 5 showed a humidity of 100% at all depths exceeding 10 cm below the burrow entrances. Humidity measured in other burrows (13 in total; 6 during midday temperatures and 7 during early morning/late afternoon) to a depth of 25 cm all confirmed 100% humidity at such depths.

The sediment temperatures were also measured using thermal imaging during both the early morning and afternoon. Thermal images were collected at various depths after excavation of sand layers (e.g., Fig. 5 (b), (c) and (e), which shows sediment excavated to a depth of 20 cm). These images reflect the same patterns evident in Fig. 4: sediment temperatures were within 2 °C of the burrow temperature at the corresponding depth, suggesting this to be the principal factor which dictates the thermal gradient of burrows.

3.2. Rates of temperature change in crabs

A thermal image of a ghost crab which had emerged from its burrow was captured in the field in the evening (Fig. 6 and Fig. S4). The animal emerged in very close proximity to where the thermal camera was recording, thus allowing an image to be captured. The recorded observations (and several witnessed but unrecorded events) illustrated that the body temperature of the emergent crab more closely matches that of the burrow than that of the surface sand (Fig. 6), with this particular animal having resided in its burrow for at least a 6-min period whilst we were recording burrow data. The images were captured rarely due to the swift nature of the crabs, quickly retreating into their burrows, undergrowth or surf. However, all observed but unrecorded sightings
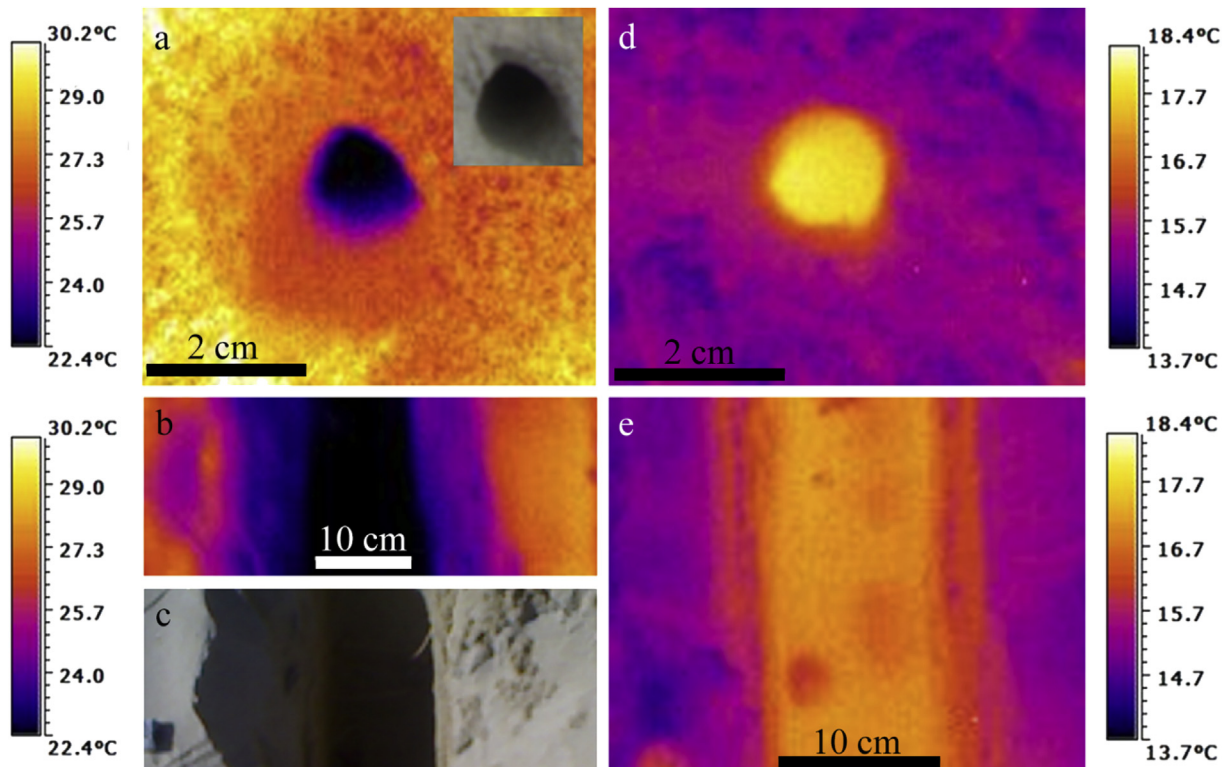
Fig. 5. (a) Thermal image of a ghost crab burrow entrance at 14:00 (inset optical image). (b) Thermal image of sediment immediately after removing 20 cm from the surface (14:00, July 12th). (c) Optical image corresponding to the sand excavation illustrated in (b). (d) Thermal image of a ghost crab burrow entrance at 06:00, July 13th, 2016. (e) Thermal image of sediment after removing 20 cm from the surface (06:00). Ambient temperatures were 13.8 °C and 25.3 °C for July 13th, 06:00 and 14:00, respectively. Min/Max for the two dates were 12.9/22.1 °C July 13th, and 13.5/27.1 °C July 12th).
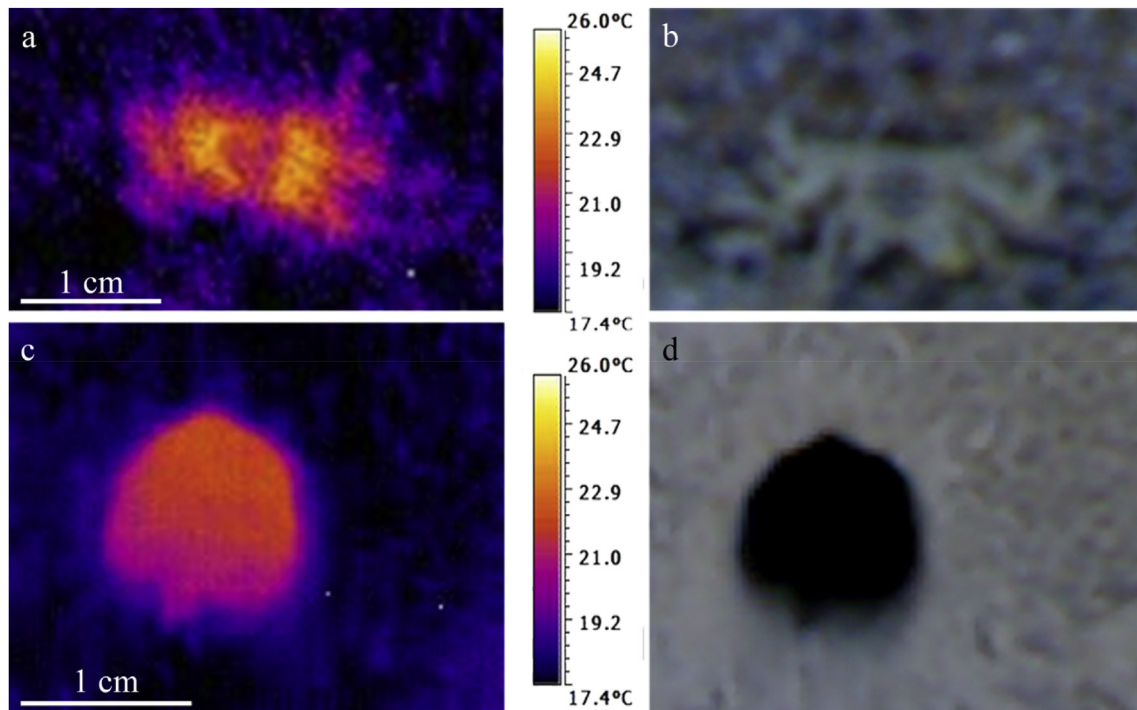
Fig. 6. (a) The thermal signature of a ghost crab which had recently left a burrow in the early evening (the crab was observed after emergence from its burrow before the measurement was collected). (b) Optical image of crab from (a). The thermal image shows a substantially higher temperature of the crab (22–25 °C) in relation to the surface of the sand (17–20 °C). (c) Thermal image of a crab burrow recorded at the same time as (a). (d) Optical image of crab burrow from (c). April 17th, 20:30, Min/Max temperatures: 16.5/27.5 °C.

revealed a similar trend in contrast.

The exponential scenario in Equation (2), for both the cooling and heating of the crab is demonstrated in Fig. 7 (a) and (b), respectively. Fits shown in Fig. 7 (c) & (d) were determined in order to extract thermal constant values (Equation (4)). Note that if the crab moves down to the burrow base quickly, it can be assumed that T_0 will be equal to the temperature achieved by the crab whilst exposed to the environment outside the burrow, as the crab will not have had sufficient time to heat up/cool down during its descent.

Importantly, from our laboratory experiments (Fig. 7 (a) & (b)) crab temperatures changed rapidly immediately after exposure to the test temperature, but the rate of change slowed dramatically after 20–30 min. This suggests that the temperature of a crab emerging from or returning to a burrow could change by as much as 5–7 °C within 30 min, but that subsequent change might be slow.

Utilising the data from Fig. 7 we can calculate the thermal heating and cooling constant. From established relationships, a crab of carapace width 12.7 mm (the mean in the sample) would have a body mass of approximately 0.001 kg (several crabs of those dimensions were weighed in the laboratory). The slope from Fig. 7 yields an estimate of $\tau = 16.59$ JK^{-1} at T = 12 °C (= 285.15K) and $\tau = 17.54$ JK^{-1} at 32 °C = 305.15K. The average value for the specific heat (from Equation (3)) $c = 16950$ J/(kg.K) and 17540 J/(kg.K), respectively.

3.3. Chemical analysis of the crabs' carapace

ATR-FTIR spectra of ghost crab carapace displayed bands typical of chitin and CaCO$_3$ as calcite. However, differences between external and internal surface chemistries were revealed (Fig. 8, Table 1). A split peak in the amide I band at 1650 and 1620 cm^{-1}, as well as a medium intensity band at 3105 cm^{-1} corresponding to NH stretching vibration, indicates a predominance of α-chitin at the internal surface (Jang et al., 2004; Wang et al., 2013). Conversely, a single peak in the amide I band at 1645 cm^{-1}, characteristic of β-chitin, is evident on the external surface (Jang et al., 2004).

4. Discussion

Ghost crabs populate coastal beach-dune systems making them subject to a much wider variation of ambient temperatures than their marine counterparts. Thermoregulation, therefore, is potentially a key factor in ensuring its survival. Although the literature suggests that burrows provide ghost crabs with a refuge from visual predators during daytime hours (Chakrabarti, 1981), Chan et al. (2006) also demonstrated that burrows provide refuge from extreme daytime heat for *Ocypode ceratophthalma*, which is conspecific with (but which grows to much larger sizes than) *O. cordimanus* on the beach studied here. In that study, 54 burrows were examined during daytime temperatures in Hong Kong, where the sand-surface temperature measured 45–48 °C. Within the crab burrows, temperatures dropped to ~36 °C at a depth of 15 cm, and ~32 °C at the deepest part of the burrow measured (25 cm below the sand surface), a decline of ~13 °C relative to surface temperatures. Our results confirm this pattern for the smaller *O. cordimanus* in winter, with a ~11 °C decrease in temperature between the sand surface and a depth of ~30 cm during the heat of the day (Fig. 6).

Our observations of burrows at the times outside of direct solar irradiation shows a remarkably different profile, however, confirming that as well as providing protection from hot temperatures during the day, the burrow also provides buffering from cold temperatures during the night (Fig. 6). The higher temperature of the burrow seen in the thermal images (Figs. 3(d), 5(d) and 6(c), Figs. S1(a), S2(a) and (c)) also suggests thermal venting (hot air rising from the bottom of the burrow to the top). We have also occasionally observed ghost crabs simply resting above the burrow
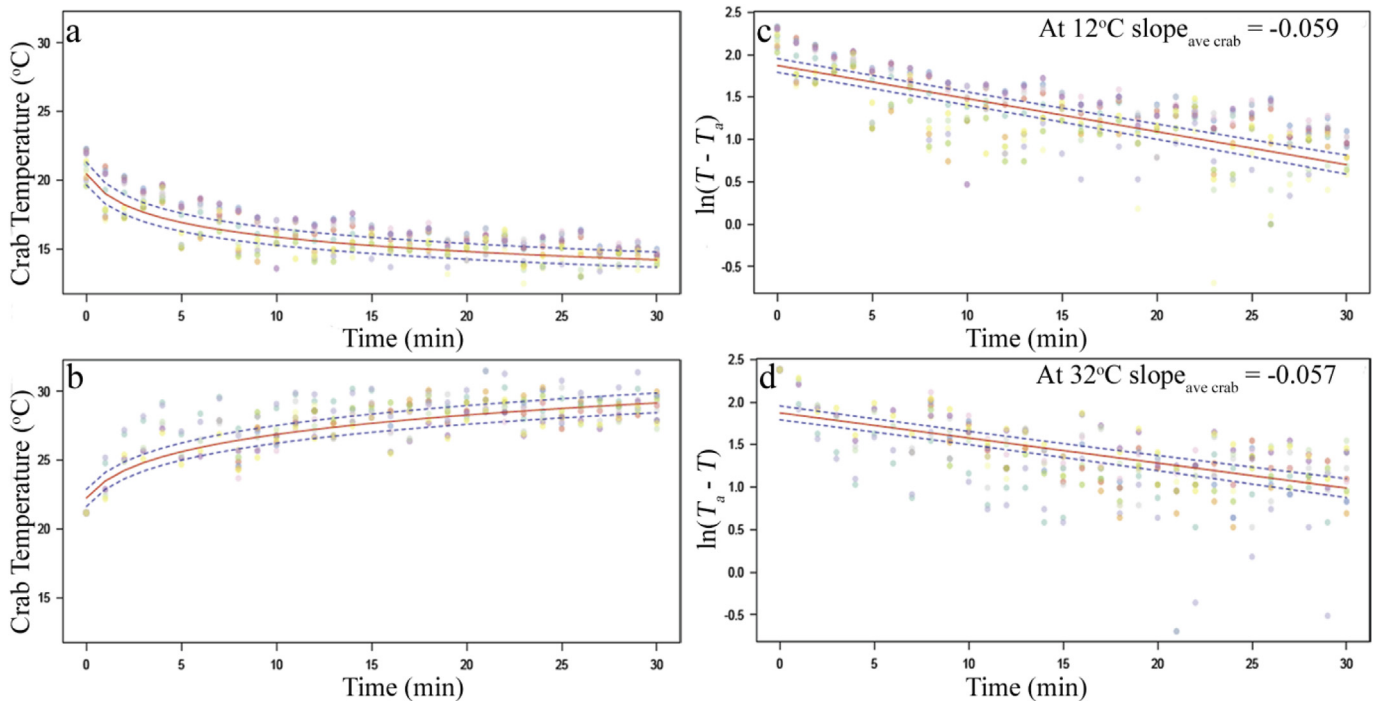
Fig. 7. Crab temperature as a function of time when cooling (a) and warming (b), with two crab temperature extremes of 12 °C (c) and 32 °C (d), fitted using a linear mixed-effects model.
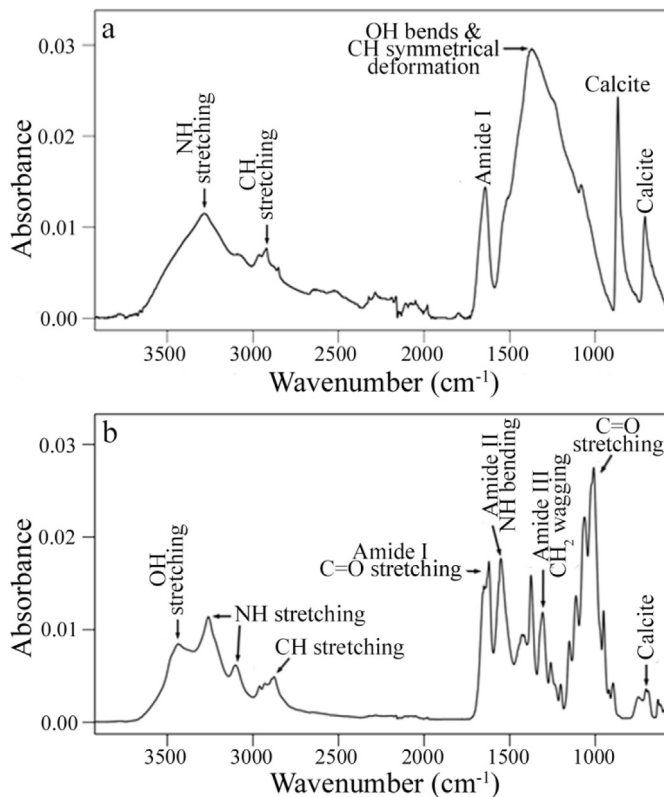
Fig. 8. ATR-FTIR absorbance spectra of ghost crab carapace on external (a) and internal (b) surfaces, highlighting molecular absorption bands and peaks of interest.

Table 1
ATR-FTIR absorbance peaks on external and internal surfaces of ghost crab carapace and vibration mode/assignments.

Wavenumber (cm^{-1})	Assignment
External	
3289	NH stretching
2958	CH stretching
2923	CH stretching
1798	Calcite
1645	C=O stretching (amide I)
1373	OH bending, CH symmetrical deformation
869	Carbonate out-of-plane bending (calcite)
710	Carbonate in-plane bending (calcite)
Internal	
3442	OH stretching
3264	NH stretching
3105	NH stretching
2924	CH stretching
2874	CH stretching
1650	C=O stretching (amide I)
1620	C=O stretching (amide I)
1550	NH stretching (amide II)
1427	Calcite
1377	CH bending
1305	CH2 wagging (amide III)
1007	C=O stretching
706	Carbonate in-plane bending (calcite)

for several minutes before retreating due to our presence, with their body temperature matching that of the vent (see Fig. S5). The early-morning temperature profiles also suggest that the rate of change in temperature decreases with depth, but there are diminishing returns with further burrow excavation beyond 30 cm in terms of reaching a suitable temperature (Fig. 6). Indeed, this temperature gradient for both decreasing (daytime) and increasing (night time) temperatures provides the crab with a habitat where a specific temperature can be accessed simply by controlling its position within the burrow profile. The burrow temperature at depth is closely aligned with the surrounding sand temperature at that

depth (Fig. 5) and thus appears to be the primary factor dictating thermal gradients within burrows. Schoeman et al. (2015) has previously noted that as ghost crab burrows are beyond the reach of most waves, sediment temperatures will play a key role in the distributional range of these species.

Whilst the thermal stability provided by semi-permanent burrows has received some attention in the literature (summarised in Lucrezi and Schlacher, 2014), we believe that there are additional novel insights to be gained. For example, we speculate that the warmer temperatures towards the burrow base may increase metabolic processes and provide the crab with a thermal energy source for nocturnal activities. Because a decline in body temperature decreases aerobic capacity and slows the kinetics of exercise-to-rest transitions in ectotherms (Florey and Hoyle, 1976; Weinstein and Full, 1998), reduced body temperatures might impede nocturnal crab activities and/or efficiency. Allen et al. (2012) have shown that body temperatures of the sand fiddler crab, Uca pugilator, can significantly affect their physiological performance. In this species, locomotor performance of individual crabs (motorised treadmill experiment) declined at lower temperatures, meaning that crabs suffered fatigue earlier and were also unable to maintain sprint speeds attained at warmer temperatures. For example, crabs with a body temperature of 10 °C fatigued three times faster than crabs at 20 °C, and sprint speeds were over double for crabs at the higher temperature. Performance relating to intermittent exercise is more complex, but has been studied for crabs such as Ocypode quadrata (Weinstein and Full, 1998). In general, higher body temperatures translate to faster speeds (avoiding predators, catching prey) and greater stamina (ability to venture further afield). This dependence on temperature would be especially problematic for an animal that is primarily nocturnal, and it did not have a ready source with which to replenish thermal energy.

The relatively high temperatures we observed during cold periods, particularly towards the bottom of the crab burrow (in comparison to sand temperatures at the surface during early mornings), suggests that the burrows might provide the crabs with a mechanism with which to acquire their ideal amount of thermal energy prior to venturing onto the cool sand surface. Larger crabs presumably would have more thermal inertia than smaller crabs and would be able to maintain surface activities for a longer period during the night. By contrast, smaller crabs might have an advantage during the heat of day by virtue of their greater surface-area:volume ratio, which might allow them to shed excess thermal energy faster than larger crabs when in the cool of their burrows. This could explain more frequent observations of juvenile crabs during the day, and larger crabs after dark (Lucrezi and Schlacher, 2014).

Chan et al. (2006) suggested that juvenile crabs venture out of their burrows more frequently than adults as they have smaller gill areas and must renew their respiratory water more often. Our humidity measurements (n = 15) showed that the burrow conditions are moist below 10 cm (100% humidity), suggesting that gill surfaces may not require additional wetting. Thus, based on juvenile crabs having lower thermal inertia than larger crabs (Allen et al., 2012), it might be that juveniles frequently require burrow return at night to recharge their thermal content in addition to water replenishment. Regardless, intermittent burrow retreat might simultaneously provide both water and thermal solutions.

The energy exchange between the crab and the environment can be represented by the energy flow (as with other ectotherms) comprising the sum of solar radiation, infrared radiation (long wave radiative heat), convective heat flow, conduction and energy loss through water respiration and water evaporation (Fei et al., 2012). In general, the total energy transfer, ignoring possible contributions from metabolism, can be summarised as:

$$\Delta Q_{Transfer} = \Delta Q_{Solar} + \Delta Q_{Longwave} + \Delta Q_{Convec} + \Delta Q_{Conduc} - \Delta Q_{Waterloss}$$

Intermittently changing temperature can influence organisms in many ways. For example, it has been shown that the time and energy requirements of intermittently warming and cooling an organism's egg, are thought by some (White and Kinney, 1974; Vleck, 1981) to noticeably shape the behaviour and energetics. Thus, crab behaviour may also be subject to such constraints dictated, in part, by cooling and heating time constants. Thus, understanding the changes in thermal energy between the crab and its surroundings, and the consequent time constant (inversely proportional to mass), allows predictions to be made regarding rates of crab responses influenced by temperature changes. Because thermal energy is not only a function of temperature and mass, but also of the type of a material, it is therefore useful to understand the chemical composition of the crab carapace.

The crab carapace is the primary interface for both heating and cooling. The chemical analysis of the carapace structure shows that the external and internal chemistry of the shell are composed mainly of chitin and CaCO$_3$ (Fig. 8 and Table 1). Some IR spectrophotometric studies of crab shell have indicated a predominance of either α-chitin (Cárdenas et al., 2004; Wang et al., 2013), or β-chitin (Sanka et al., 2016), but it appears that chitin polymorph differs between external and internal surfaces in the carapace of O. cordimanus.

Absorption peaks on the external surface at 1792, 869 and 710 cm^{-1} are characteristic of CaCO$_3$ as calcite (Gbenebor et al., 2016). An expected associated peak around 1476 cm^{-1} was not detectable as it was likely obscured by the broad peak of OH bending at 1374 cm^{-1}, due to hydration of CaCO$_3$. Indication of CaCO$_3$ is less clear on the internal surface, with peaks at 1427 cm^{-1} and 706 cm^{-1} being associated with calcite (Chuanqiang et al., 2016; Sanka et al., 2016). However, peaks in these regions have also previously been assigned as CH and NH bending, respectively (Cárdenas et al., 2004; Gbenebor et al., 2016).

Other studies have focussed on the carapace of some crabs in relation to their structural properties. For example, Boßelmann et al. (2007) found that the crab (Cancer pagurus) carapace was more highly mineralized (i.e., more CaCO$_3$ and thus stiffer) than that of a lobster. They speculated that the carapace of the lobster would need to be more flexible because they escape predators by hiding between rocks whereas crabs walk on land and thus need a more rigid carapace. It could also be argued that the crab carapace (in our case the land-dwelling ghost crab) may be more highly mineralized as an adaptation to thermoregulation in a more variable spatial and temporal thermal environment. The thermophysical properties for a calcareous layer for a crustacean: density = 2.7 × 10^3 kg/m^3; specific heat 806.62 J/(kg K) and thermal conductivity = 3.89 W/(m K) contrast in relation to the tissue/muscle component of a crab, density = 966 kg/m^3, specific heat = 3758 J/(kg K) and thermal conductivity = 0.56 W/(m K) (Dima et al., 2011).

The higher thermal conductivity of the calcium carbonate component of the carapace may be advantageous because it would allow the crab to shed or gain heat more quickly. In the context of retaining heat for nocturnal activities this may not seem advantageous. However, an optimal balance between the competing requirements of heat transfer (losing and gaining heat quickly) would presumably serve the organism well for all activities. Although the contrasting presence of α and β chitin on the inner and external parts of the carapace may also have a thermal purpose, it is unclear at present what this may entail and would necessitate a second-order analysis of the system which is beyond the scope of this

study.

Beach ecosystems, of course, provide an alternative stable source of thermal energy: the ocean. By comparison with the safety of burrows, though, the swash zone is a risky proposition, being patrolled by surf-zone fish in search of prey. We therefore speculate that crabs will elect to control their internal temperature by returning to a burrow in preference to entering the surf zone, except in the case of last resort (e.g., when being actively pursued by a terrestrial predator) or for reproductive purposes. Thermal transfer to the crab body would therefore presumably take place by conductance from sand present at depth in the burrow. Our preliminary data (observational, recorded thermal images in Fig. 6 (and Fig. S4) and quantitative in Fig. 7) demonstrate that crabs exiting burrows can have a higher temperature than the sand surface (e.g., Figs. 3–7), supporting our conjecture that thermal transfer from burrow environments takes place for nocturnal activities. Despite the carapace temperature changing rapidly (Fig. 7(a) and (b)), it remains warmer than the surrounding sand surface for several minutes, which is vital during surface excursions when predation is most likely.

The thermal landscape of the ghost crab environment at night is lit up by the numerous holes peppering the beach face (Figs. S1(a), S2(a) and (c), Figs. 3(d), 5(d), 6(c)). This provides a strong thermal signature at the entrance of each crab burrow. In principal, this sort of marker (sign stimulus) could be used by organisms (crabs) to navigate across the beach and identify burrows in general, or even individual burrows. Furthermore, the thermal gradients in crab burrows might aid in the formation of convectional air currents. Thermal convective venting has been observed for other organisms where a natural thermal gradient creates a density gradient within the burrow that induces air flow (Kennerly, 1964; Olszewski and Skoczeń, 1965; Roper and Moore, 2003). Because ghost crabs are known to be sensitive to olfactory stimuli (Wellins et al., 1989), it is possible that chemical cues might aid crabs in locating burrows during the night. Conversely, during the day when temperatures are reversed, cooler air will rest predominantly at the burrow base and with it, the characteristic odours identifying the occupier and residence status. This pattern might confer additional protection from diurnal predators hunting by smell, but might make crabs vulnerable to nocturnal invasive predators, like the red fox, which are known to affect beach food webs (Brown et al., 2015).

5. Conclusions

Adaption to varying thermal habitats depends on the temporal variations and patterns of environmental changes and the physiological tolerance of the species involved. To meet the challenge of temperature changes in their environment, crabs have been shown to demonstrate a range of physiological, behavioural and ecological adaptions (see review by Lucrezi and Schlacher, 2014). In the case of the ghost crab, thermal control is somewhat guaranteed by depth of burrows. Hotter average temperatures in summer associated with climate change might require deeper burrowing to ensure protection from daytime heat, especially at the equatorward range edge of a species. Paradoxically, protection from extreme winter cold at the poleward range edge of a crab's distribution might also be conferred by deeper burrowing, with crabs taking advantage of thermal stability at depth within the sediment matrix. Nevertheless, because the thermal gradient is reduced with depth (i.e., the deeper the burrow, the lower the temperature rate of change), individual crabs are faced with trading off the metabolic cost of digging and burrow maintenance against the benefits of ensuring a suitable temperature. Higher average temperatures may, however, offer crabs a more efficient recharging station at night, with corresponding increased activity. The chemical structure of the carapace seems to facilitate this rapid exchange of thermal energy. Indeed, burrow exit times are critical periods (first exposure from burrow to potential predators) where thermal optimisation would provide the crab with greater energy resources for evasion.

Our work also highlights numerous interesting areas for further investigation including: the relationship between burrow diameter and temperature profile; duration of crab activity at night and crab temperatures upon return and exiting of burrows (temperature profiling by miniature data loggers attached to individual crabs (e.g., male and female temperature preferences, responses and patterns)); crab navigation on return to burrow; detection and identification of chemical cues emanating from burrows during the day and night, and their correlation with the thermal gradient reversal cycle. More subtle areas are also worthy of investigation such as burrow architecture, shape (e.g., I, Y, J, U and S), as well as the temperature of the burrow 'bottom' wall as opposed to the 'top' wall (as the burrows have inclined angles to the sand surface) and their contribution, if any, to burrow properties such as thermal air currents.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ecss.2018.01.023.

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