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Thermal Ecology of the Shore Crabs *Ocypode quadrata* and *Pachygrapsus crassipes* Using Infrared Thermography

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Marine Biology

by

Sonya Timko

Committee in charge:

> Professor Jennifer Taylor, Chair
> Professor Ryan Hechinger
> Professor Greg Rouse

2021

The Thesis of Sonya Timko is approved, and it is acceptable in quality and Form

for publication on microfilm and electronically.

University of California San Diego

2021

DEDICATION

This is dedicated to my wife for her sacrifices and support of my long academic

journey, to my mom for helping me be the woman that I am, and to my

grandparents for instilling a love of reading, writing and education.

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ABSTRACT OF THE THESIS

Thermal Ecology of the Shore Crabs *Ocypode quadrata* and *Pachygrapsus crassipes* Using Infrared Thermography

by

Sonya Timko

Master of Science in Marine Biology

University of California San Diego, 2021

Professor Jennifer Taylor, Chair

Temperature is crucial to the optimal functioning of most physiological and behavioral processes in animals. For ectothermic crabs that inhabit shorelines, temperature fluctuations and anomalies pose a challenge for maintaining optimal body temperature. Here we examine the thermal ecology of two shore crabs, the ghost crab, *Ocypode quadrata*, and the striped shore crab, *Pachygrapsus*

crassipes, using non-invasive infrared thermographic (IRT) technology. We tested the hypothesis that the exoskeleton helps buffer changes in environmental temperature by recording exoskeleton surface temperature in live and dead animals exposed to external heat sources. Crabs were first acclimated to room temperature in an experimental arena, then heat was sequentially added from a hot plate (conduction), IR emitter (radiation), and a heat lamp (radiation) until peak heat was reached, then all heat sources were sequentially removed. Exoskeleton surface temperature was recorded across multiple body locations (carapace, chelipeds and 5th pereopods) throughout increased and decreased heat exposure. For both species, dead crabs had significantly greater exoskeleton temperature flux than live crabs across all body locations, as well as the substrate. Thus, the exoskeleton itself does not appear to provide much thermal buffering, but the physiology and behavior of live crabs do. While *O. quadrata* spent most of the time sponging, *P. crassipes* actively avoided areas of high heat. This study demonstrates the responsiveness of the crab exoskeleton to environmental temperature changes and lays a foundation for exploring additional thermal adaptations of crabs on land using non-invasive IRT technology.

1.   INTRODUCTION

Nearly every aspect of an animal's physiology is affected by temperature. Consequently, the ability to maintain internal body temperature within a certain range regardless of environmental temperature can influence a species distribution and ecology. For ectothermic animals like crustaceans, one would expect their habitat and geographic ranges to be limited due to their conformance with external temperatures. Yet, decapod crustaceans are widespread across the global oceans, from shallow waters to the deep sea, from the tropics to the polar regions (Hall and Thatje 2011; Martin, Crandall, and Felder 2016). They have even successfully evolved to the dynamic temperature extremes of freshwater and terrestrial habitats (Yeo et al. 2007; Bliss 1968). Such a widespread distribution suggests that decapod crustaceans either have a broad physiological temperature tolerance or mechanisms to help limit internal temperature fluxes. One potential mechanism for temperature control that has not yet been explored is the insulation capacity of the exoskeleton, which provides an important physical barrier between the internal and external environment of all crustaceans. In this thesis, I examine the thermal conductance of the exoskeleton to determine its potential role in crustacean thermoregulation, and ultimately gain new insights into the nearly boundless distribution of decapods.

1.1 Thermal limits

Virtually all biochemical reactions that provide life to an organism are sensitive to temperature, and they work effectively only within a certain temperature range. These reactions, along with an animal's metabolic rate, help set the sublethal thermal tolerance (critical minimum and maximum temperature limits) of a species. For crabs (Crustacea: Decapoda: Brachyura), the preferred temperature and thermal tolerance vary with age and life cycle (Paschke et al. 2013). If the temperature drops below the critical minimum (CTmin), many crab species may delay maturity (Rao 1966), cease growth and extend the time between molts (Brylawski and Miller 2006; Hiatt 1948), or block molting altogether (Passano 1960). Critical minimum temperatures can also temporarily suspend reproduction (Bert, Gerhart, and Crawford 2016; Colpo and López-Greco 2017; Mauro and Mangum 1982) and have detrimental effects on the stages of embryonic development (Zeng 2007). At the other end of the temperature scale, temperatures higher than the critical maximum (CTmax) also negatively affect embryonic development (Zeng 2007) and correspond to decreased rates of hatching success (Chou, Head, and Backwell 2019). Females with eggs have a lower CTmax than females without eggs (Madeira et al. 2012). Exposure to temperatures outside a species' critical limits can thus have major impacts on their populations.

The effects of temperature on metabolic processes vary widely across organisms, but crustaceans routinely show increases in standard indices of

metabolic rate, such as heart rate (De Wachter and McMahon 1996; De Pirro, Cannicci, and Santini 1999) and oxygen consumption (Dehnel 1960; Newell 1969; W.B. Vernberg and Vernberg 1968; Scholander et al. 1953), when exposed to higher environmental temperatures. The magnitude of these changes are typically greater in aquatic environments because of the higher thermal conductivity and specific heat capacity of water (White 1988). Some species of crabs that access both water and air media have shown different thermal tolerances in air and water. For example, the fiddler crab *Uca pugilator* was found to have a maximum heart rate of 180 beats min^{-1} at 40°C in water and a maximum heart rate of 160 beats min^{-1} at 35°C in air; furthermore, with a 10°C increase to both media, the crab's heartbeat drops to zero in 50°C water temperature, while it plateaus at 45°C air temperatures (Levinton et al. 2020). Similarly, oxygen consumption rates rise with elevated temperatures in both media, but are higher in water versus air in other bimodal species, such as the green crab *Carcinus maenas* (Wallace 1972) and the marbled rock crab *Pachygrapsus marmoratus* (Fusi et al. 2016).

Exposure to critical temperature extremes can also have immediate kinematic impacts, such as slowing down or inhibiting locomotor skills. Some studies have used the righting reflex, or a crab's ability to turn itself over if flipped upside down, to examine the effect of temperature on motor control. The brown crab *Cancer pagurus* and the green crab *Carcinus maenas* lose their righting response at critical maximum temperatures ranging from 21.5 to 35.4°C, with

those acclimated to higher temperatures being able to right themselves at higher

temperatures (Cuculescu, Hyde, and Bowler 1998). For the rock crab *Cancer*

antennarius, increasing temperature to approximately 40°C initiated hyperactivity,

muscle spasms, a decrease in movement and a lack of righting response

(Padilla-Ramírez et al. 2015). The running speed of the Hawaiian ghost crab,

Ocypode ceratophthalma, decreased with only a ±4°C temperature change

beyond the crab's normal conditions, and the crab was rendered immobile below

10°C (Florey and Hoyle 1976).

1.2 Ecological effects of temperature

Temperature is a primary factor affecting the range of many crustacean

species and thus the community dynamics along the coasts (Lewis 1963; Miller

and Vernberg 1968; Blanchette et al. 2008; Cahill et al. 2014; Wethey and

Woodin 2008). Coastal ecosystems, such as intertidal zones, are known for

extreme fluctuations in many abiotic factors, especially in hydration and

temperature. The intertidal zone is so extreme that it can be broken down into

smaller microhabitats with predicted variations of these factors. The

biogeography of many organisms adapted to these microhabitats can be easily

seen, both vertically within and horizontally across these boundaries (Bullock

1955; Southward 1958). While some invertebrates like the shore crab

Pachygrapsus crassipes can tolerate a wider range of temperatures along the

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intertidal zone (Hiebert 2015), other invertebrates are limited to specific

microhabitats within this larger zone, including the porcelain crabs *Petrolisthes*

cinctipes and *Petrolisthes eriomerus* (Stillman and Somero 1996).

Within the oceans, temperature affects species distributions on a much

larger scale. Stenothermic crabs, or crabs that are able to occupy a limited

temperature range, can be greatly affected by minor temperature variations. For

instance, the snow crab *Chionoecetes opilio* has a preferred temperature of 0.0-

1.5°C for juvenile instar III and 1.0-4.5 °C for instar IV (Dionne et al. 2003).

Furthermore, embryos of *Chionoecetes opilio* showed a period of diapause with a

temperature difference from just 0.0°C to 1.0°C (Webb et al. 2007) and are

restricted to temperatures below 7°C (Foyle, O'dor, and Elner 1989). On the other

end of the temperature extreme, vent crabs, *Bythograea spp.,* occupy

hydrothermal vents and their population distribution and abundance have been

used to predict other vent locations (Van Dover, Franks, and Ballard 1987).

As with other animals, the distributions of crustaceans are being altered by

changes in ocean and air temperatures associated with climate change. The

Antarctic shelf has long been a cold-water barrier for crabs, but recently large

populations of king crabs were found on the continental slope of the western

Antarctic Peninsula (Aronson et al. 2015). As waters warm, it is anticipated that

crabs will expand their presence in the Antarctic, where their durophagy can

greatly affect established communities. Range expansion is not limited to the

oceans and has recently been documented in mangrove tree crabs, *Aratus*

pisonii from Florida (Riley et al. 2014). These mangrove crabs have not only moved northward at an unusual rate over the last century (63 km per decade), they have also expanded into salt marsh habitats where they climb cordgrass instead of mangrove trees. In general, range expansion of non-native species can greatly affect native communities, as observed in the invasive green crab *Carcinus maenas* , which has significantly impacted the native populations of their molluscan prey (Grosholz and Ruiz 1996).

1.3 Heat exchange and the crustacean exoskeleton

Heat exchange between animals and their environment occurs through three distinct regions: internal, boundary, and external (Moen 1973). The internal thermal region includes the internal tissues of the organism. The boundary region consists of the outermost layer of the animal that is in contact with the external environment, and may contain skin, surface feathers, fur, or hair. The last region, the external thermal layer, includes the atmosphere and the substrate. Heat exchange across these regions occurs by four main mechanisms: conduction, through contact with surfaces; convection, through the movement of the surrounding air or water; radiation, through electromagnetic waves; and evaporation, through the conversion of liquid to air. The first three mechanisms relate to heat gain and heat loss, while the fourth, evaporative cooling, relates solely to heat loss.

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Crabs have a multi-layer, highly mineralized chitin-based exoskeleton

(Chen et al. 2008; Garth and Abbott 1980) that differ in mechanical properties

between regions of the body (Lian and Wang 2011) and across terrestrial versus

aquatic species (Taylor 2018). Exoskeletons provide support and protection for

the body (Taylor and Kier 2003; Taylor 2018) and are subject to species specific

changes from ocean warming and acidification (Page et al. 2017). The shell of

the mangrove horseshoe crab *Carcinoscorpius rotundicauda* is thought to

provide thermal stability (Wardiatno et al. 2021) and the inorganic material

structure of the pincer or claw of the sheep crab *Loxorhynchus grandis* is stable

up to 250°C (Zhou et al. 2010). Deep sea hydrothermal vent crabs *Austinograea*

rodriguezensis show an increased proportion of aluminum element and organic

matter in their exoskeleton compared to the Asian paddle crab *Charybdis*

japonica, which may have improved the thermal stability of these crabs living at

the vents (Cho, Kim, and Kim 2020). Crab exoskeletons have shown amazing

mechanical, compositional and thermal properties but have yet to be explored on

a large basis.

1.4 Crab thermoregulation

Crustaceans are ectothermic, meaning the internal body temperature

varies with the temperature of the environment. Despite this, many ectotherms

can exhibit internal temperature ranges that seem independent of the

surrounding air temperatures and are capable of acclimating to highly variable climates (Bullock 1955; Dehnel 1960; McGaw 2003). This gives crustaceans a survival advantage in areas with a greater range of temperatures, such as the intertidal zone, where diel and seasonal fluctuations can disrupt normal patterns in the abundance and distribution of organisms (Kenneth M Leber 1982).

Crustaceans have multiple mechanisms of thermoregulation and reports have been published on more than 7 orders since the early 1900s (Lagerspetz and Vainio 2006). Two principal mechanisms of thermoregulation in crabs are evaporative cooling and behavioral thermoregulation techniques, such as temperature avoidance (Schlacher and Lucrezi 2014). Other methods, such as body coloration or claw mediated thermoregulation, have also been described. These thermoregulation techniques are perhaps most significant for species that spend time out of water and are thus best studied in semi-terrestrial and terrestrial species. The following discussion will therefore focus primarily on intertidal species.

Evaporative cooling

Evaporative cooling has been demonstrated in crabs and many other intertidal invertebrates across temperature and moisture gradients (Edney 1961; Lewis 1963; Bliss 1968; Thurman 1998). Intertidal and aquatic crabs have a higher degree of water loss by evaporative cooling than do terrestrial species of crabs (Herreid II 1969b; Wolcott 1992) due to the greater availability of water for

evaporation. In higher temperatures, a water permeable exoskeleton assists thermoregulation by aiding in increased evaporative loss, and some crabs have variable permeability (Herreid II 1969a; R.I. Smith and Rudy 1972; Wolcott 1992). Evaporative cooling has been shown to keep moist *Uca spp.* up to 4°C cooler than dry crabs (Edney 1961). While evaporative cooling aids in thermoregulation, crabs must be able to access water.

Evaporative cooling often requires behaviors that permit the uptake of water. While immersion is the most effective means for acquiring moisture for evaporative cooling, other methods of water uptake are also possible. For example, crabs will frequently retreat to their burrows intermittently after surface activity on hot days. While in the burrow, the permeable carapace can take in water from the damp soil and high humidity within it (Green et al. 1959; W.K. Smith and Miller 1973; Wolcott 1992, 1984). This is an effective strategy as a moist carapace was observed in *Uca subcylindrical* after emerging from burrows (Thurman 1998). After the carapace lost moisture, crabs returned to their burrows. Some crabs can take in interstitial water from damp soil using hydrophilic setal tuffs in a behavior termed 'sponging' (Bliss 1968; Wolcott 1984; Maitland 1990). Sponging can take place outside of burrows and was the primary method to reduce body temperatures by 1.3°C in the sand bubbler crab *Scopimera intermedia* (Hui et al. 2019). Crab species that do flotation feeding can take in water directly with their buccal cavity (Quinn 1980; Powers and Bliss 1983).

Behavioral thermoregulation

Behavioral thermoregulation is often used to enhance or prevent evaporative cooling. For example, the crab *Hemigrapsus nudus* selectively uses different microhabitats to aid in evaporative cooling; they move between air and water media to regulate body temperature to an exact optimum range (McGaw 2003). Such behavior enables *H. nudus* to maintain body temperature through evaporative cooling from 3 to 22°C different from the temperature of the surrounding air or water media (McGaw 2003). In temperatures below 12°C, intertidal *Scylla serrata* crabs reduce evaporative cooling by decreasing the amount of time they spend in air and even stop feeding altogether (B. Hill 1980).

Temperature avoidance behaviors are so common that they have been observed in all orders of crustaceans (Lagerspetz and Vainio 2006). The most common behavior is to seek shelter. Solar radiation can cause rapid temperature change within seconds and can even keep body temperatures 2.5°C higher than air temperatures on a cold winter day (Edney 1961). Intertidal crabs can avoid stressful high temperatures by entering water (Bovbjerg 1960) or taking shelter under rocks, in crevices or other shaded areas (Broekhuysen 1940; Lewis 1963; McGaw 2003; Hiatt 1948). Crabs that excavate burrows retreat into their tunnels during the day, when the outside temperature is at its highest (Milne and Milne 1946; F.J. Vernberg 1969). Crabs that cannot excavate sometimes use burrows made by other species (Willason 1981). Closing off, or plugging, burrows helps to maintain optimum temperature conditions inside (Milne and Milne 1946). A recent

study found that the intertidal marsh fiddler crab, *Minuca pugnax*, retreats to its burrow when air temperature reaches 24°C (Hews et al. 2021). Within burrows, crabs can remain 0.34-0.50°C cooler than the surface temperature (Hews et al. 2021).

Coloration

Color change occurs in crustaceans and is correlated with temperature, suggesting that it may have a thermoregulatory function in addition to camouflage. Dark colors absorb more light energy, and therefore more heat, while light colors reflect more light energy, and therefore absorb less heat. Similarly, dark colors emit heat more readily than lighter colors. Crabs have various pigments in chromatophore cells that, when concentrated or dispersed, can change the color or brightness of the individual (Powell 1962a). Some crabs display a normal circadian cycle of color change, but their appearance can be significantly modified over just a couple of hours through endocrine control (Powell 1962b; Little 1967; Stevens, Rong, and Todd 2013; Stevens, Lown, and Wood 2014). Temperature is known to have a blanching effect on some crabs where either lighter pigment will be dispersed, or darker pigment will be concentrated at higher temperatures (Brown Jr and Sandeen 1948; Fingerman 1956; Powell 1962b). This blanching helps to increase surface reflectance, thereby taking in less heat to maintain a cooler body temperature (Wilkens and Fingerman 1965; W.K. Smith and Miller 1973; K.R. Smith et al. 2016; Thurman 1990). In *Uca pugilator* , blanching altered the absorption coefficient, varying

body temperatures up to 2°C between light and dark pigmented crabs (Wilkens and Fingerman 1965).

Claw mediated thermoregulation

Recently it has been hypothesized that the enlarged claw of the fiddler crabs *Uca pugilator* and *Uca panacea* aids in thermoregulatory processes by transferring heat from the body to the enlarged claw (Windsor, Crowe, and Bishop 2005; Darnell and Munguia 2011). Although no mechanisms of heat transfer were explored, they proposed it could be through evaporative cooling, or physiological mechanisms (Darnell and Munguia 2011). Another study on *Uca panacea* showed that crabs with larger major claws relative to body size had a higher CTMax than those with a smaller ratio (Darnell, Nicholson, and Munguia 2015). The large claw of fiddler crabs acts as a heat sink to draw heat away from the body and vital organs; it can account for up to 10% of body temperature (Darnell, Nicholson, and Munguia 2015). Interestingly, regenerated large claws of the fiddler crab *Leptuca urguayensis* are more efficient for heat loss, comprising up to 35% more heat transfer between the body and claw than unregenerated claws (De Grande, Fogo, and Costa 2021). These regenerated claws have "less muscle mass and longer fingers" that may increase surface area to volume ratio and result in more efficient heat transfer (De Grande, Fogo, and Costa 2021). Research on the thermoregulatory capacity of crab claws is minimal and limited to one group of crabs, so this is an exciting area for further research.

1.5 Objective

The scope of thermoregulation strategies known to be used by crustaceans is relatively limited and the potential role that the exoskeleton plays, through structure and color, has largely been neglected. Additionally, most thermoregulation research is based on a single group of small crabs, the fiddler crabs. This study aims to explore the thermal ecology and heat exchange of two distinct, medium-sized intertidal crab species: the ghost crab, *Ocypode quadrata*, and of the lined shore crab, *Pachygrapsus crassipes*. Specifically, it tests the hypothesis that the exoskeleton helps moderate heat gain and heat loss of crabs in air. This hypothesis was tested by measuring changes in exoskeleton surface temperature of live and dead crabs exposed to different mechanisms of heat transfer using FLIR thermal technology.

2. MATERIALS AND METHODS

2.1 Study Species

Two semi-terrestrial, intertidal species of shore crabs were selected for this study: the Atlantic ghost crab, *Ocypode quadrata* (Fabricius, 1787) (Brachyura, Ocypodidae) and the striped shore crab, *Pachygrapsus crassipes* (Randall, 1839) (Brachyura, Grapsidae). Although both are categorized as semi-terrestrial, intertidal species, these two crabs are behaviorally different, and they occupy separate, distinct habitats within their respective zones.

The Atlantic ghost crab, *Ocypode quadrata*, is distributed throughout the Western Atlantic Ocean from the state of Massachusetts, United States of America to Rio Grande do Sul, Brazil, approximately 41°N - 32°S (Sakai and Türkay 2013). They can be found on shorelines of sandy beaches in these tropical, subtropical and temperate regions, where they make their homes by digging burrows in the sand (G.W. Hill and Hunter 1973). *O. quadrata* occupy different areas of the beach in relation to the water, forming vertical bands based on age and sex. Juveniles tend to reside in all areas, mature females stay closest to the water, and mature males make their homes in the middle and upper areas, past the females (Corrêa et al. 2014). Ghost crabs are a monotone, light sand color and blend in well with their surrounding habitat. Both mature females and males have large, asymmetrical chelae at approximately 50% equal handedness (Haley 1969). These crustaceans are most active when the air temperature is

between 13.5°C and 30°C (Vinagre et al. 2007; Rosa and Borzone 2008; Branco

et al. 2010). While their upper thermal limits have been shown to vary from 28 –

34°C (Santos and Moreira 1999; Burggren, Moreira, and Maria do Carmo 1993;

Maria do Carmo, Moreira, and Brotto 1989; Valero-Pacheco et al. 2007;

Robertson and Pfeiffer 1981), their lower thermal limits range between 10 – 16°C

(Haley 1972; Florey and Hoyle 1976; Kenneth Miles Leber 1977; Antunes et al.

2010; Weinstein and Full 1994; Weinstein, Full, and Ahn 1994). Inactivity or

remaining in burrows can last for 3-6 months and is thought to be due to

temperatures near their lower thermal limits (Haley 1972; Kenneth M Leber

1982).

The striped shore crab, *Pachygrapsus crassipes*, is distributed along the

eastern Pacific coast, from Oregon to Baja California, Mexico, approximately

45°N - 24°N (Cassone and Boulding 2006; Wicksten 2011); and along the

western Pacific coast, in Japan and Korea at approximately 34°N - 37°N (Hiatt

1948; Morris, Abbott, and Haderlie 1980). Across their distributions, they are

found in sandy and muddy estuaries as well as the rocky intertidal and in the

upper littoral zone bordering on terrestrial habitat (Hiatt 1948; Roberts 1957; Hui

et al. 2019; Hiebert 2015). These crabs spend approximately half of their time in

the terrestrial zone and can stay out of the water for as long as 70 hours (Garth

and Abbott 1980). While mostly found in rock crevices (Hiatt 1948), *P. crassipes*

has also been seen occupying muddy burrows created by other crabs (Willason

1981). Shore crabs are a mixture of darker black, green, red and purple with

lighter coloration on their chelipeds. Both females and males have symmetrical

chelae, but females are slightly smaller than males at maturity (Hiatt 1948; Garth

and Abbott 1980). *P. crassipes* can tolerate a wide range of temperature

changes and is known to have a wider thermal distribution than that of other

regional crabs (Hiatt 1948). In a laboratory setting, *P. crassipes* can acclimate in

8.5-30°C air temperatures (Roberts 1957), and in the field Hiatt (1948) observed

lowered activity and feeding levels in water temperatures below 18°C and

reduced ecdysis below 14°C.

2.2 Animal collection and maintenance

Live *Ocypode quadrata* were purchased from a supplier (Gulf Specimen

Marine Lab, FL, USA). Crabs were shipped to Scripps Institution of

Oceanography (SIO) at the University of California, San Diego (UCSD) where

they were maintained in an environmental room set at 30°C with a 12 h day, 12 h

night light cycle. Humidifiers kept the room between 80-90% humidity. Crabs

were kept individually in plastic storage containers (43 cm × 30 cm × 16.5 cm)

with lids and air holes. The bottom of each container was filled with

approximately 2.5 - 5 cm of clean play sand (<1% organic material). A large

shallow dish filled with seawater (33-35 ppt salinity) was placed in each container

so that crabs could immerse themselves. Crabs were fed carrots and lettuce

every other day. Crabs were checked daily for molts and exuvia were removed

immediately. Sea water was changed and sand was cleaned every other day. Crabs that were used for the dead trials died on their own in the experimental room.

Pachygrapsus crassipes were collected from the SIO pier inflow and were held in the experimental aquarium at SIO. The crabs were kept individually in small experimental holding tanks (2.81 L) that were floated in a large open tank, which received filtered seawater pumped in from the SIO pier at ambient pH (7.99), temperature (18.7°C), and salinity (33-35 PSU). Crabs were fed commercially available frozen squid or tilapia 5-7 times per week, with uneaten pieces removed daily. Crabs were checked daily for molts and exuvia were removed immediately. Crabs that did not die on their own were put in an experimental freezer in order to use them for the dead trials.

All animals were blotted dry, sexed, and weighed to the nearest 0.01 g (Radwag PS3500/c/2, Radom, Poland). Carapace width was measured at the widest part of the carapace using digital calipers (Mitutoyo CD-6" CS, Aurora, IL). Only non-gravid, intermolt individuals with all appendages intact were used for the experiment. A total of 11 adult *O. quadrata* (6 female, 5 male; carapace width range: 29.78 – 47.73 mm; mass range: 16.4 - 56.57 g) and 11 adult *P. crassipes* (6 female, 5 male; carapace width range: 16.15 – 29.51 mm; mass range: 1.66 - 29.83 g) were used in this study.

2.3 Experimental design

The experimental arena (Figure 1) consisted of an initial acclimation container (shown empty), experimental container, hot plate, IR emitter, heat lamp, and thermal camera. Two plastic containers (18 cm x 18 cm x 22 cm), filled with 2.5 cm (550mL) of play sand were used to carry out heat exchange experiments. Heat exchange measurements were conducted on live crabs in a temperature-controlled room maintained at 20°C and 62 ±5% humidity. Surface temperatures were measured by exposing individuals to a series of heat sources and recording temperature changes on the exoskeleton surface using infrared thermography (described below).

Initial acclimation containers were adjusted so that the temperature of the container and the sand matched the acclimation temperature of each species in their holding tanks (30°C for *O. quadrata* and 20°C for *P. crassipes*). Acclimation containers and sand were placed in the holding rooms and allowed to acclimate for days to weeks before animals were added (cold temperatures acclimated faster). *O. quadrata* were taken directly from their terrestrial habitat (30°C) and placed in the acclimation container with sand at 30°C and acclimated to the 20°C air temperature of the experimental room. *P. crassipes* were taken directly from their seawater habitat (20°C) and placed in the acclimation container with sand at 20°C and acclimated to the 20°C air temperature of the experimental room. The acclimation container was emptied, cleaned and new sand was added between individuals.

Once crabs were added to the initial acclimation container, they were given 10 minutes to acclimate before heat gain was measured by the sequential addition of three different sources of heat: conduction via hot plate, radiation via IR emitter, and radiation via heat lamp. Conduction (Figure 1, C.) was induced by transferring the crab to a second plastic container (18 cm x 18 cm x 22 cm) lined with 2.5 cm (550mL) of play sand already warmed to 27-30°C using a hot plate (x, CA) directly beneath the experimental container. Radiation via IR emitter (Figure 1, D.) was induced using an IR emitter (x, CA) on 12 amps placed 8 cm above the container, with focus on the bottom left quadrant of the container. Radiation via heat lamp (Figure 1, E.) was induced using a 25-watt reptile heat lamp (Dadypet, Shenzhen City, China) placed 8 cm directly above the container.

Conductive heat was introduced immediately following the acclimation period by transferring crabs into a separate container that had substrate with a temperature range of 27 - 30°C. Crabs were exposed to conductive heat for 10 minutes before the next heat source, radiation via IR emitter, was added. After 10 minutes of both conductive heat and radiation via IR emitter, the third source of heat was added, radiation via heat lamp. Crabs were exposed to all three heat sources for 10 minutes, which is considered peak heat gain.

Immediately following peak heat gain, heat loss was measured through the removal of all three heat sources, at 10-minute intervals, in the following sequence: conduction, radiation via IR emitter, and radiation via heat lamp. In total, crab temperature was measured over a 70-minute period (10-minute

acclimation, 30-minute heat gain, and 30-minute heat loss). Surface temperatures were recorded throughout the 70-minute experiment, as described below.

The experiment was repeated on newly deceased crabs to assess the contributions of physiological and behavioral thermoregulation. Frozen crabs were acclimated for 3-5 hours in their own environments and were checked for appropriate surface temperature before experiments were carried out.
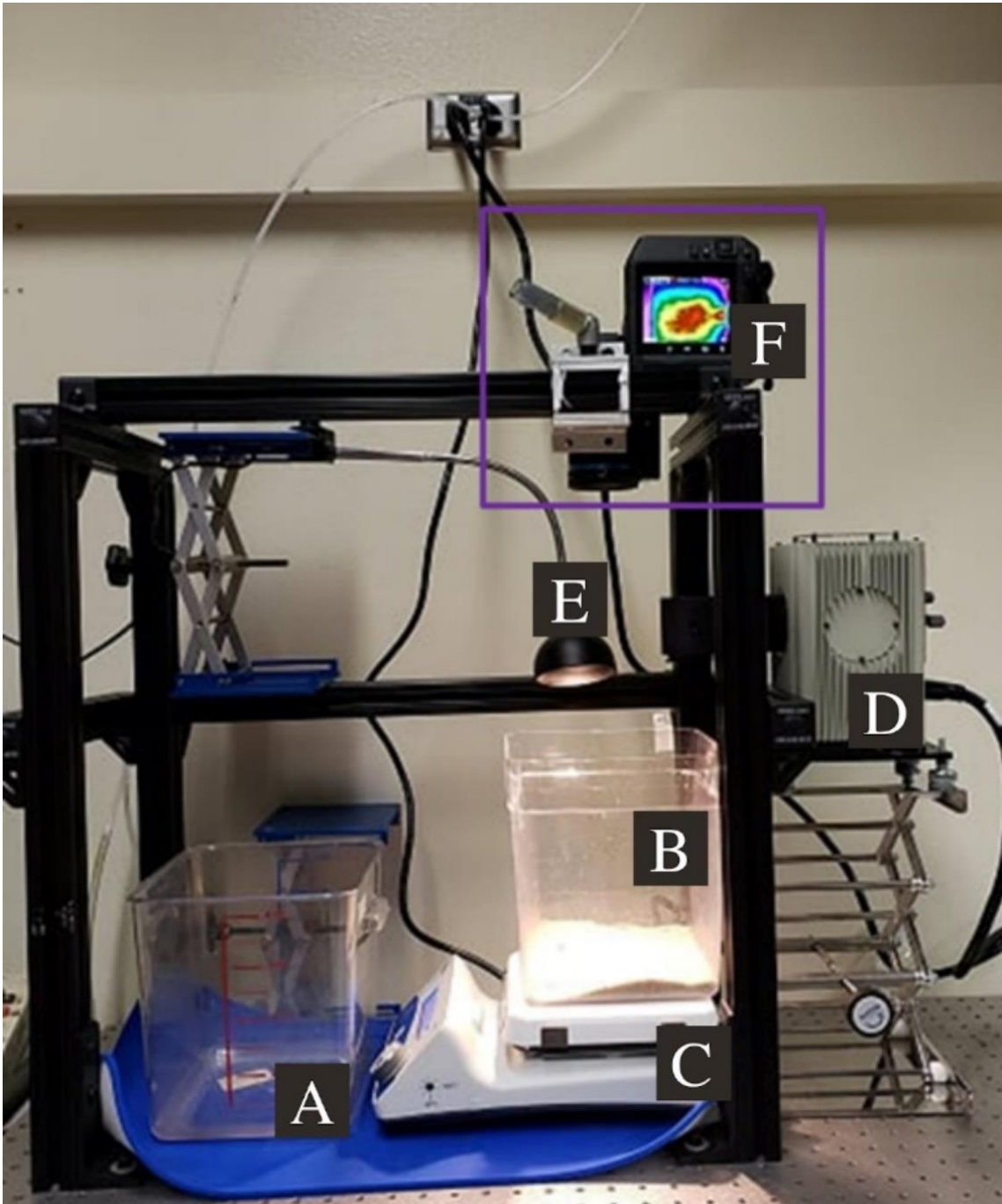
Figure 1. Experimental arena - A. Initial acclimation container (shown empty), B. experimental container, C. hot plate (conduction), D. IR emitter (radiation), E. heat lamp (radiation), F. thermal camera

2.4 Infrared Thermography

Infrared thermography (IRT) is a non-invasive biotelemetry tool used to measure the surface temperature of objects. IRT has been shown to be a suitable proxy for heat flux measurements within Steller sea lions (Willis et al. 2005) and Weddell seals (Hindle, Horning, and Mellish 2015), when compared to heat flux sensor recordings taken at the same time. Furthermore, it has been used to accurately measure surface heat or heat transfer in lizards (K.R. Smith et al. 2016), cattle (Salles et al. 2016), and even humans (Bouzida, Bendada, and Maldague 2009).

A forward looking infrared (FLIR) thermal imaging camera (T540; FLIR Systems Inc., Wilsonville, OR) was mounted directly above the experimental container (Figure 1, F.). This camera has a thermal sensitivity of 0.03°C (<50 mK, 14° at 30°C) with a spatial resolution of 0.52 mrad per pixel and a 14° field of view. True color media, false color media and radiometric data were collected in every video and image taken. True color media refers to an image, photograph, or video you might expect from a regular camera while false color media refers to a method of color rendering an image to show parts of the electromagnetic spectrum that are not visible to humans (thermal properties are shown via false color media in this experiment). Radiometric data accurately measures the temperature of each individual pixel, and with a resolution of 464 × 348 (161,472 pixels) at 30 Hz (or frames per second), this system produces near real-time monitoring and data capture.

Video recordings, image captures, and radiometric data were used to assess thermal changes in both the sand substrate and the crab body. Prior to crab measurements, a complete experiment was run on the experimental container with sand only to measure substrate temperature. Substrate temperature increased with each addition of heat source and decreased with each removal of heat source, demonstrating that each heat source contributed to substrate temperature (Figure 2).
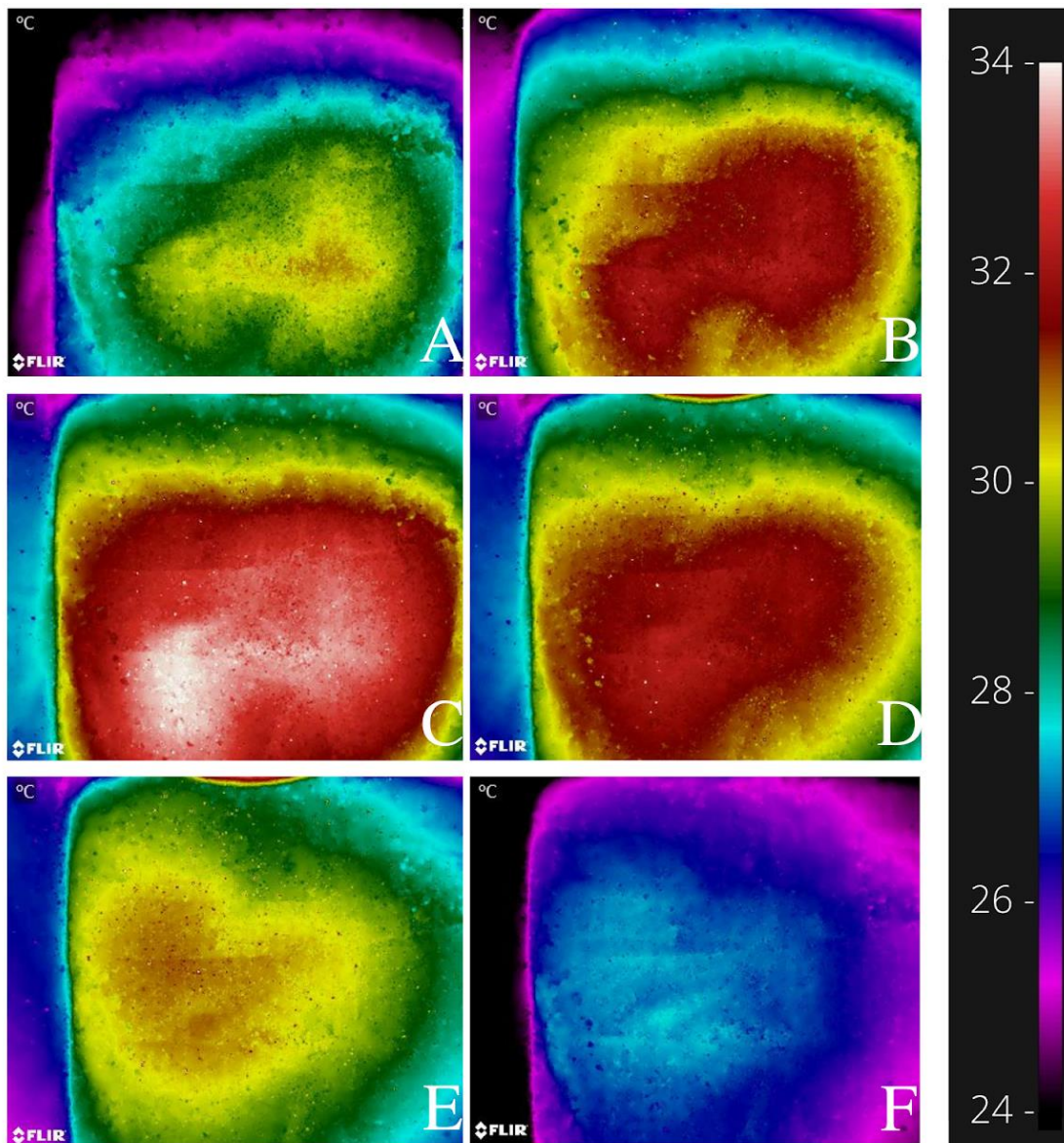
Figure 2. Thermal measurement of the substrate during heat addition and removal. A. Conduction (substrate at 30C), B. addition of radiation via IR emitter (substrate at 32°C), C. addition of radiation via heat lamp (substrate at 33.5°C), D. removal of conduction (substrate at 32°C), E. removal of IR emitter (substrate at 31°C), F. removal of heat lamp (substrate at 27.5°C).

For both live and dead crabs, thermal changes were measured in three regions of the body: dorsal carapace, both chelipeds and the fifth pair of pereopods. Temperature measurements of the dorsal carapace were taken by averaging the area of a square box that was approximately 50% of the medial carapace length (in pixels) of each individual crab (Figure 3, A.). A maximum, minimum and average temperature could be quantified from this box through the radiometric data stored in each pixel. Chelipeds and pereopods were measured by drawing lines laterally along the length of each segment near the midline, from the proximal to the distal end. *O. quadrata* has dimorphic claws, so the side of the body with the larger claw was labeled as 'major' and the other side as 'minor' so that they could be separately compared and analyzed across all specimens regardless of handedness. *P. crassipes* has symmetrical claws and was measured with the left side of the body always designated by default as the major side for easy comparison. For the major cheliped (Figure 3, B.) and the minor cheliped (Figure 3, C.), 2 lines were used: one extending from the coxa to the distal edge of the carpus and one extending from the proximal edge of the propodus to the tip of the dactyl. For the major pereopod (Figure 3, D.) and minor pereopod (Figure 3, E.), 3 lines were used: one extending from the coxa to the distal edge of the carpus, one spanning the propodus and one spanning the dactyl.

All radiometric data were exported and analyzed using FLIR Tools Software (FLIR Systems Inc., Wilsonville, OR). The FLIR camera records at 30

Hz, providing 18,000 temperature data points for each 10-minute treatment. To simplify analysis, temperature was recorded at each 1-minute interval, for a total of 10 temperature recordings per treatment. Maximum temperature was determined for each treatment as the highest temperature recording. Temperature flux was calculated as the difference in temperature between minute 1 and minute 10 recordings.
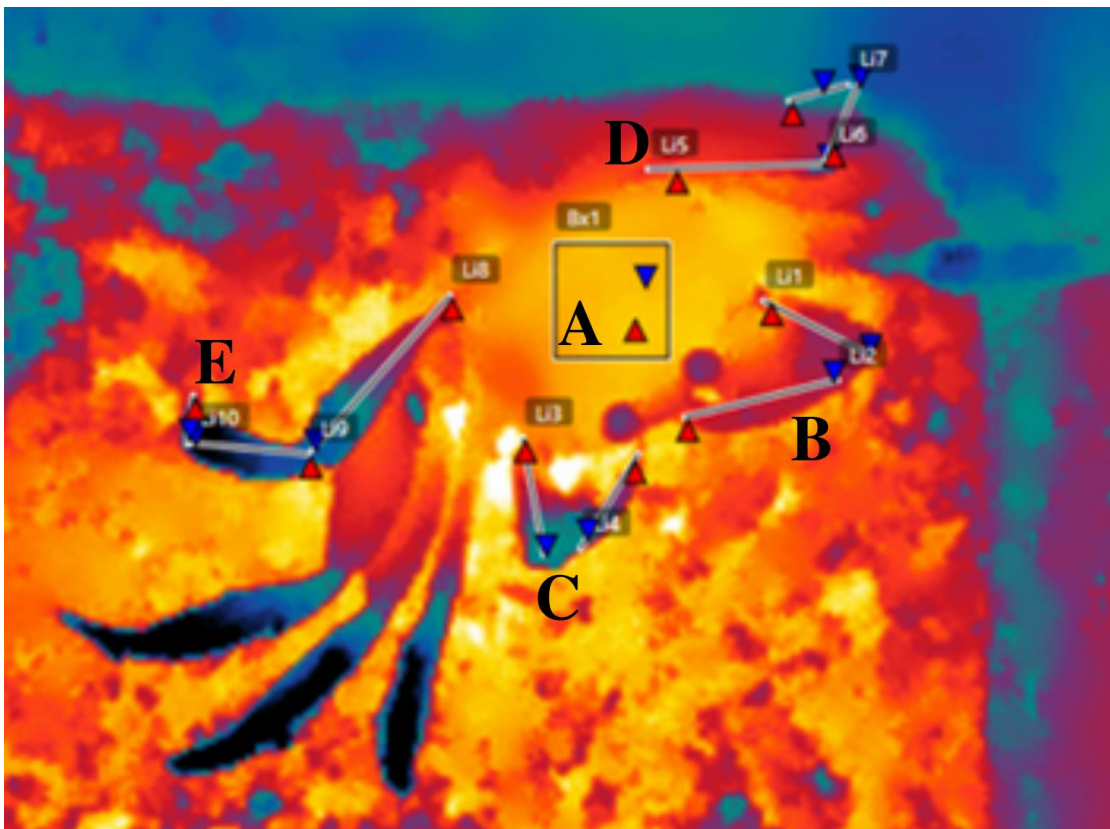
Figure 3. Thermal measurement locations - dorsal carapace (A) measured over boxed area, major (B) and minor (C) chelipeds measured along two lines, and major (D) and minor (E) pereopods measured long three lines.

2.5 Behavioral thermoregulation

Sponging and moving in between burrows are behaviors that aid in thermoregulation (Gherardi, Russo, and Anyona 1999). *O. quadrata* has setal tufts that are used to uptake interstitial fluid located between their third and fourth pereopods (Wolcott 1984). Sponging posture was defined as when the abdomen and the majority of the thoracic sterna were in contact with the substrate, therefore increasing the possibility of the setal tufts being in contact with the sand (i.e., flattened against the substrate; Figure 4). The active or non-sponging posture was defined as when the abdomen and the majority of the thoracic sterna were not in contact with the substrate, therefore decreasing the possibility of the setal tufts being in contact with the sand (i.e., body held off substrate or animal walking; Figure 4). Observations on sponging versus active posture were recorded at the beginning of each treatment and every minute throughout the experiment.

Figure 4. Representative postures of sponging (left) and active (right).

Although not much is known about behavioral thermoregulation methods for *P. crassipes* specifically, some *Pachygrapsus spp.,* including *P. crassipes* do not have setal tufts to take up water. Rather, these species actively use behavioral thermoregulation and locomotion to avoid temperature by moving to cooler microclimates or between air and water media to aid in evaporative cooling (Fusi et al. 2016; Bovbjerg 1960). Observations on direct versus avoidance position were recorded at the beginning of each treatment and every minute throughout the experiment. Direct position was classified as at least half of the crab located in the upper 50% temperature range of substrate as shown in Figure 5. Avoidance position was classified as less than half of the crab located in the upper 50% temperature range of substrate (Figure 5).
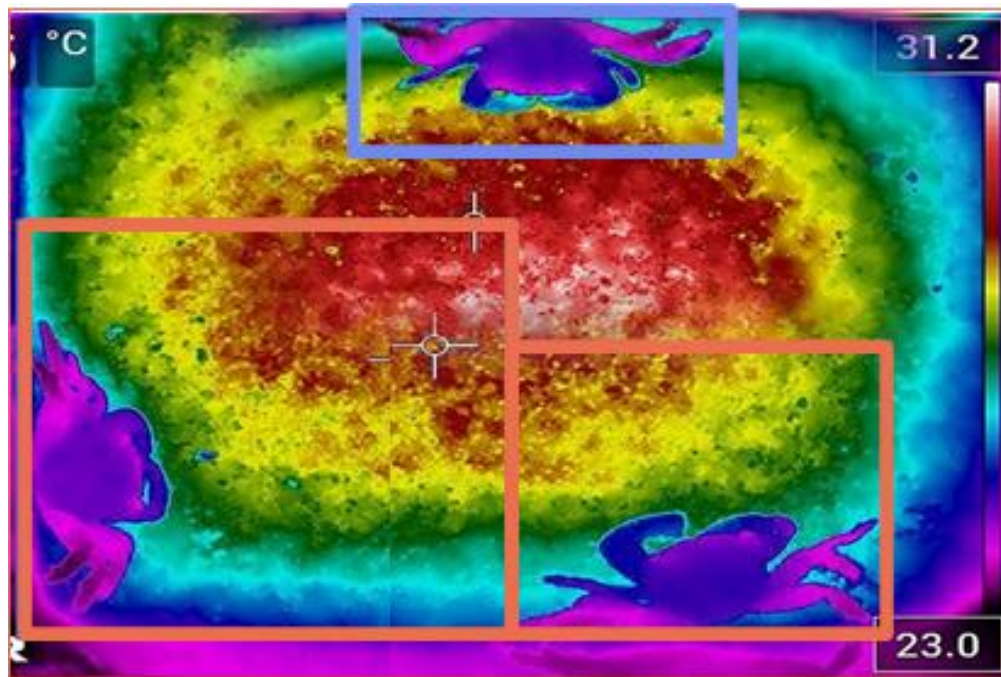
Figure 5. Direct position was classified as at least half of the crab located in the upper 50% temperature range of substrate, shown in top blue outline. Avoidance position was classified as less than half of the crab located in the upper 50% temperature range of substrate, shown in the bottom orange outline.

2.6 Statistical analysis

Maximum temperature and flux data were tested for normality using Shapiro-Wilk and homogeneity using Levene's tests. Temperature flux data were log transformed for subsequent analysis. Maximum temperature and flux were compared across body locations and between live and dead animals of each species using a linear mixed model (LMM; lmer function, lme4 package; (Bates et al. 2014) in R (v 4.0.5) due to the non-independence of data (we used multiple trials from some individuals). Our model included body location as the

independent variable and temperature as the dependent variable. Both mass and condition (live versus dead) could potentially affect temperature, therefore mass and condition were included as fixed effects. Individual crab ID was included as a random effect to account for multiple measurements from a single individual. Our resulting model was:

temperature ~ body location + mass + condition + (1|ID), REML=FALSE

P-values were acquired for the modeled variables using a Satterthwaite approximation for degrees of freedom (lmerTest package; (Luke 2017; Kuznetsova, Brockhoff, and Christensen 2017). An adjusted post-hoc Tukey test was used to account for multiple comparisons. All summary data are presented as mean ± s.d.

3.   RESULTS

3.1 Maximum crab temperatures

For live *O. quadrata* at the end of acclimation, the mean maximum temperature of the carapace was approximately 4°C warmer than that of the chelipeds and pereopods; it also had the smallest deviation (±0.31) (Table 1). All other body regions were similar, though the distal regions of the pereopods had a comparatively lower maximum temperature (Table 1). Following the addition of all heat sources (peak heat), there were no significant differences in maximum

temperature across any body location (LMM: df=53.83, t=-0.23-0.89, all p>0.05; Table 1). At final heat removal (following removal of all heat sources), maximum temperatures returned to their acclimation temperatures for all body locations, with the carapace being 2-3.5°C warmer than the chelipeds and pereopods (Table 1). Notably, the major cheliped held the second highest temperatures behind the carapace in all treatments shown.

Dead *O. quadrata* had similar maximum temperatures as live animals during acclimation (Table 1). At peak heat, dead animals had significantly higher maximum temperatures (1.5-6°C) for all body locations compared to live animals (LMM: df=59.51, t=-4.70, p<<0.001; adj Tukey: all p<0.05). Maximum temperatures for all body locations in dead animals remained up to 3°C higher than live animals following the final heat removal as well (Table 1).

Maximum temperatures in live *P. crassipes* following acclimation were 1°C higher for the carapace than the chelipeds and pereopods (Table 1). At peak heat, all recorded maximum temperatures were around 25°C and were not significantly different from one another (LMM: df=53.56, t=2.28-3.00, adj Tukey: all > 0.05;). After the final heat removal, the carapace recorded maximum temperature was up to 5°C hotter than the appendages.

Maximum temperatures of dead *P. crassipes* were similar to those of live individuals for all body locations following acclimation (Table 1). At peak heat, dead individuals had significantly higher maximum temperatures (up to 3.5°C)

than live specimens for all body locations (LMM: df=62.91, t=-6.60, p<<0.001; adj

Tukey: all p<0.05). Maximum temperatures after the final heat removal increased

slightly for the carapace but decreased for the chelipeds and pereopods by up to

3°C (Table 1). These differences in maximum temperatures mimicked those of

the live crabs, save for a larger temperature difference (up to 1°C) for the

appendages (Table 1).

Table 1. Maximum temperatures recorded from live and dead crabs during acclimation, heat gain treatment (peak heat), and final heat removal treatment. Maximum temperature (°C) for each location is the average of the highest temperature of all crabs recorded in the last minute of the treatment. STD = standard deviation.

Species	Measurement Area	Acclimation		Peak Heat		Final Heat Removal	
		Max temp	STD	Max temp	STD	Max temp	STD
O.quadrata live	Carapace	29.89	0.31	27.20	1.32	28.20	0.94
	Major cheliped, proximal	25.86	0.54	27.18	1.05	26.18	1.26
	Major cheliped, distal	25.28	0.43	27.93	1.91	26.14	1.69
	Minor cheliped, proximal	25.48	0.62	27.14	1.49	26.06	1.30
	Minor cheliped, distal	24.53	0.69	27.58	1.84	25.89	1.43
	Major pereopod, proximal	25.26	0.65	26.90	1.03	25.68	1.17
	Major pereopod, distal	23.50	0.70	27.25	1.61	24.77	1.40
	Minor pereopod, proximal	25.38	0.58	26.97	1.80	25.79	1.20
	Minor pereopod, distal	23.50	0.60	26.84	1.76	24.74	1.22
O.quadrata dead	Carapace	29.34	0.39	33.12	1.17	31.12	1.42
	Major cheliped, proximal	26.77	0.91	29.43	0.91	27.77	0.49
	Major cheliped, distal	26.10	0.62	30.56	0.62	27.19	1.10
	Minor cheliped, proximal	25.75	0.24	29.60	0.24	27.64	0.83
	Minor cheliped, distal	25.58	1.25	31.47	1.25	27.51	1.45
	Major pereopod, proximal	24.94	1.38	28.06	1.38	25.86	1.76
	Major pereopod, distal	23.81	1.68	28.96	1.68	25.17	0.50
	Minor pereopod, proximal	25.55	1.06	29.90	1.06	26.54	1.70
	Minor pereopod, distal	23.98	0.50	29.33	0.50	25.80	0.86
P. crassipes live	Carapace	20.33	0.52	25.46	1.47	27.82	5.67
	Major cheliped, proximal	18.90	0.55	25.19	1.36	23.44	1.50
	Major cheliped, distal	19.10	0.57	25.93	1.48	24.25	1.43
	Minor cheliped, proximal	18.85	0.52	25.24	1.24	23.45	1.49
	Minor cheliped, distal	19.26	0.67	25.94	1.34	24.50	1.50
	Major pereopod, proximal	19.15	0.59	24.91	1.45	22.95	1.39
	Major pereopod, distal	19.50	0.54	25.49	1.51	23.08	1.24
	Minor pereopod, proximal	19.05	0.59	24.83	1.19	22.91	1.28
	Minor pereopod, distal	19.46	0.73	25.26	1.42	23.35	1.02
P. crassipes dead	Carapace	19.76	1.25	27.96	1.85	28.58	1.73
	Major cheliped, proximal	18.94	0.57	27.74	1.82	25.28	0.68
	Major cheliped, distal	18.98	0.42	28.67	2.06	25.64	1.67
	Minor cheliped, proximal	18.90	0.57	27.79	1.60	24.93	0.60
	Minor cheliped, distal	18.90	0.48	28.45	1.61	25.43	1.12
	Major pereopod, proximal	18.91	0.48	27.56	1.82	24.82	0.83
	Major pereopod, distal	19.10	0.24	28.89	1.56	25.37	0.78
	Minor pereopod, proximal	18.90	0.59	28.25	1.83	24.99	0.65
	Minor pereopod, distal	19.14	0.37	28.77	1.47	24.70	0.76

3.2 Average Heat Flux

The substrate temperature data points (Graphs 1-5) are individualized across each species of crab because substrate measurements were taken directly adjacent to the location of the crab and averaged for each species separately. This was done to account for the preferred or active location of a crab during a specific observation or the fact that crabs altered their sand substrate by burrowing or moving it around.

Ocypode quadrata

Overall, temperature flux increased for both live and dead *O. quadrata* as each heat source was added, and then decreased as each heat source was removed for the carapace (Graph 1, A), chelipeds (Graph 2), and pereopods (Graph 3). Live and dead crabs started out with a similar negative heat flux during acclimation for the carapace (Graph 1). At peak heat, dead crabs gained significantly more heat (greater positive heat flux) than live crabs (LMM: df=73.79, t=4.02, p<0.01; adj Tukey: all p<0.05). Live crabs had a maximum temperature flux of 3.5°C while the dead crabs had more than twice that flux (8.3°C). During the final heat removal treatment, dead crabs lost significantly more heat for the carapace than live crabs.

Comparing the heat flux of the major and minor claws of live *O. quadrata* specimens through heat gain and heat loss showed that they both followed a similar pattern (Graph 2), as did the contralateral fifth pereopods (Graph 3). Heat

flux was negative during acclimation and the final heat removal for all

appendages, while heat flux was positive and at its greatest during peak heat.

The heat flux in the appendages of the dead specimens followed the same

overall rise, peak, fall pattern as for the live crabs (Graph 2-3). The major claw of

the dead crabs continued to have a negative heat flux in the first heat gain

treatment, while the minor claw and both pereopods gained approximately 1°C

each; this contrasts with the live specimens in that all of the appendages had a

negative heat flux (Graph 2). Overall, the appendages in the dead specimens

had a higher flux in most of the treatments than the live specimens, with

significant differences at peak heat for the major chelipeds, minor chelipeds,

major pereopods, and minor pereopods (LMM: df=73.79, t=4.02, p<0.01; adj

Tukey: all p<0.05). Following final heat removal, flux was similar between live

and dead crabs for all body regions.

Graph 1. Average carapace heat flux of A) *O. quadrata* and B) *P. crassipes*.
Box plots (box boundaries = the 25th and 75th quartiles, error bars = 1.5
times the interquartile distance, center line = median) show that live (gray)
and dead (white) crabs differ significantly in heat flux at peak heat and at final
heat removal for *O. quadrata* but are not significantly different for *P.
crassipes*. Dotted line = average substrate heat flux with standard deviation.
Yellow highlight = Peak heat
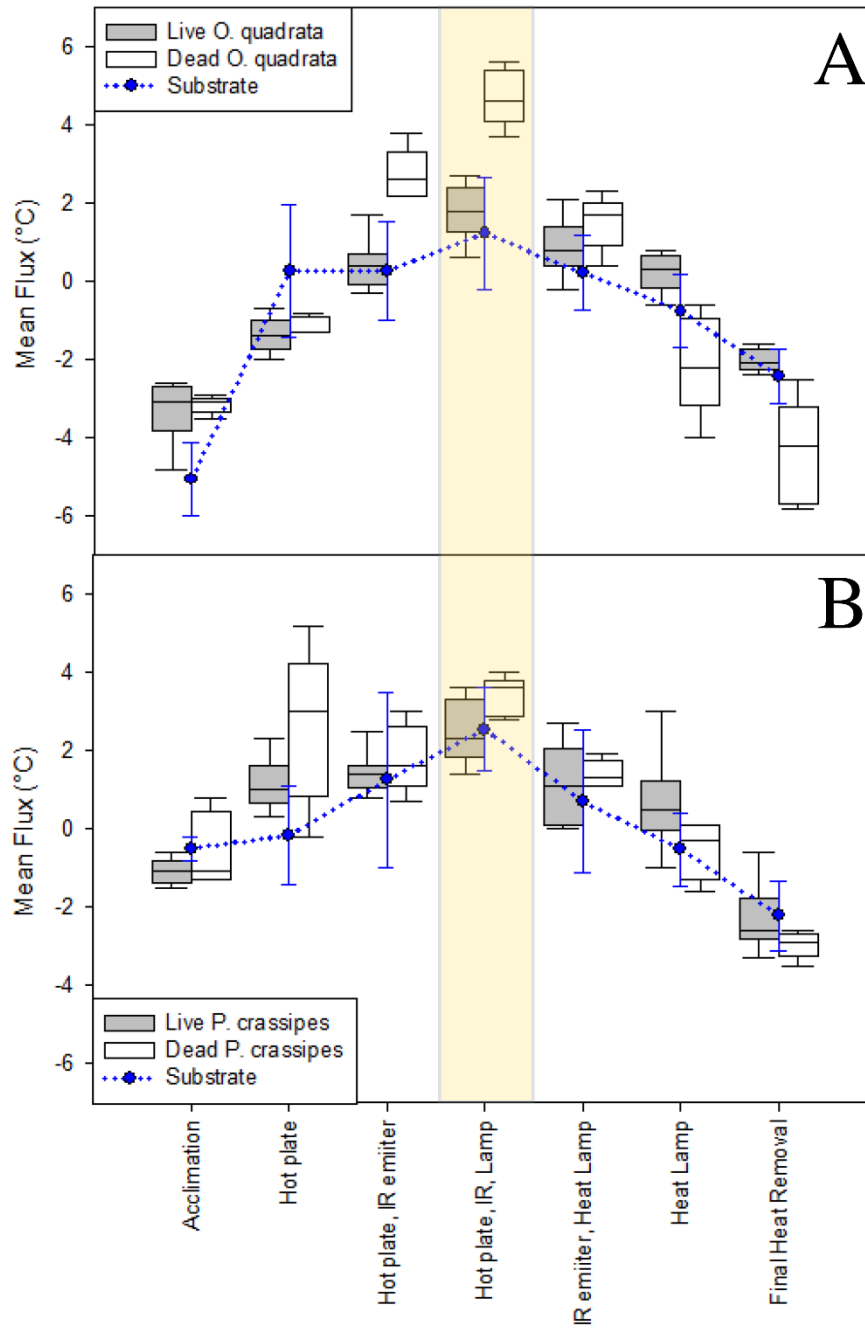
Graph 2. Average *Ocypode quadrata* heat flux of A) major cheliped and B) minor cheliped. Box plots (box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median) show that live (gray) and dead (white) crabs differ significantly in heat flux at peak heat for both the major and minor cheliped. Dotted line = average substrate heat flux with standard deviation. Yellow highlight = Peak heat
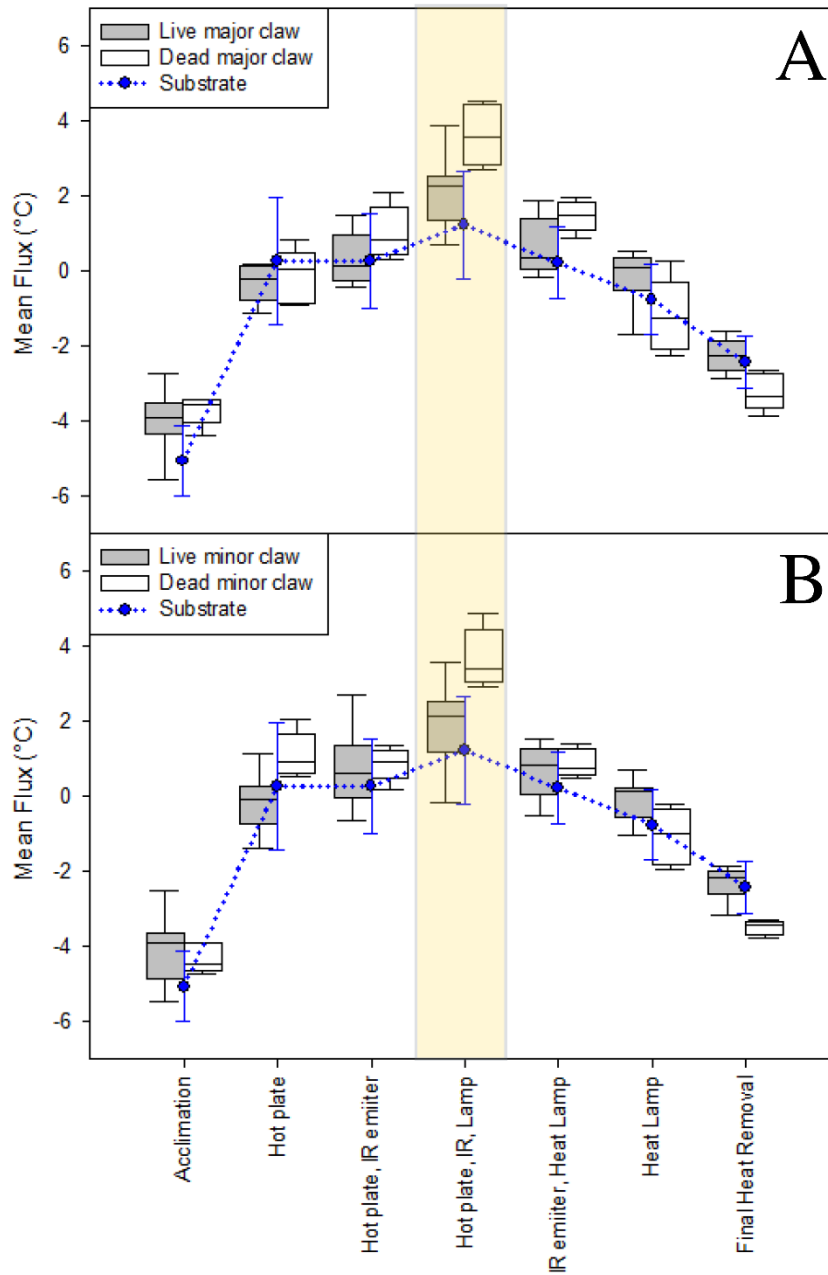
Graph 3. Average *Ocypode quadrata* heat flux of A) major pereopod and B) minor pereopod. Box plots (box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median) show that live (gray) and dead (white) crabs differ significantly in heat flux at peak heat for both the major and minor pereopod. Dotted line = average substrate heat flux with standard deviation. Yellow highlight = Peak heat
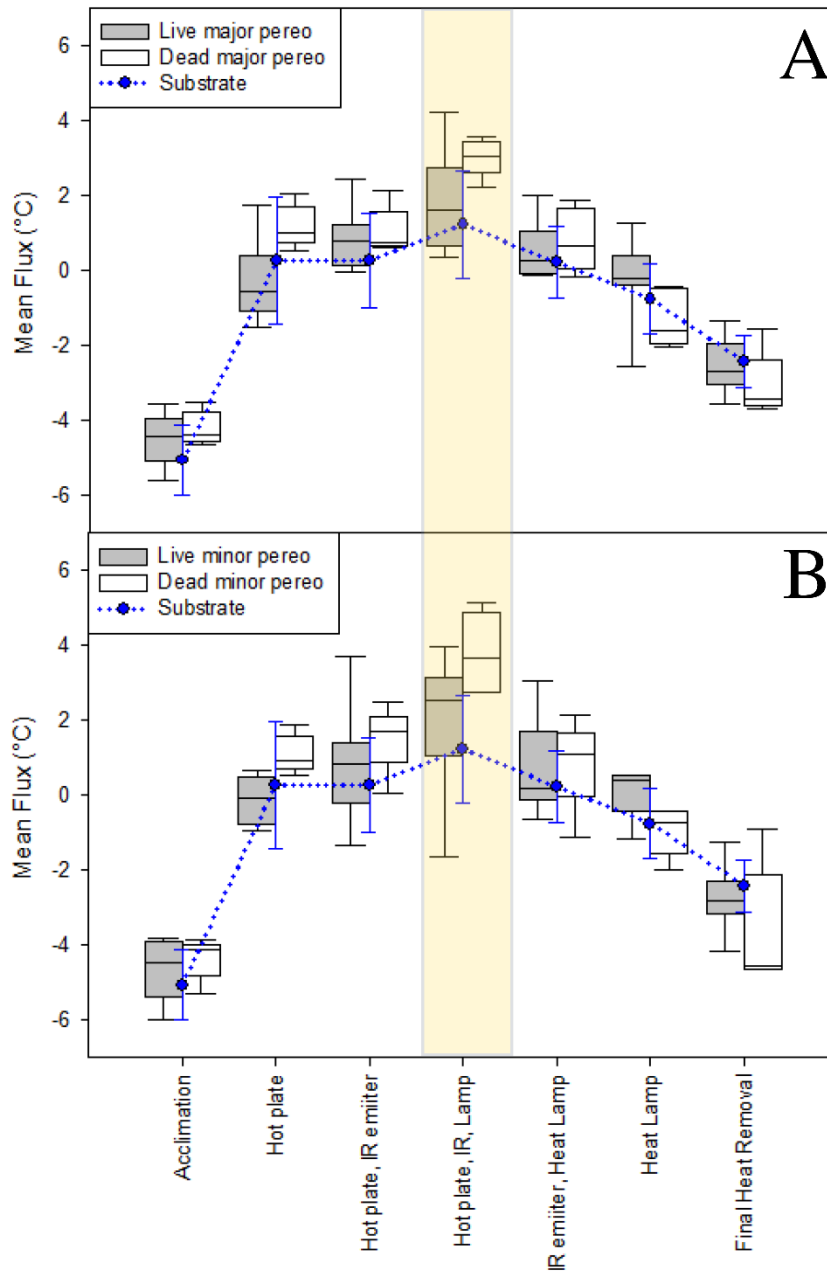
Pachygrapsus crassipes

Temperature flux increased for both live and dead *P. crassipes* as each heat source was added, and then decreased as each heat source was removed for the carapace (Graph 1, B), chelipeds (Graph 4), and pereopods (Graph 5). Live *P. crassipes* specimens lost twice the amount of carapace heat than the dead crabs in the acclimation treatment. Live crabs showed a heat flux that was 25 - 100% less at peak heat gain (LMM: df=75.09, t=5.28, p<0.05; adj Tukey: all p<0.05). The live crabs also had a positive heat flux in the second heat removal treatment with the substrate plus IR emitter turned off, while the dead crabs had a negative heat flux. Live crabs lost less heat than dead crabs in the final heat removal treatment, overall maintaining a more conservative temperature range.

P. crassipes has symmetrical claws, which showed similar temperature fluxes throughout the acclimation, heat gain, and heat removal treatments when compared between major and minor chelipeds for the live specimens and between major and minor chelipeds for the dead specimens (Graph 4). The fifth pereopods also had similar temperatures fluxes during acclimation, heat gain, and heat removal treatments when compared between major and minor pereopods for the live specimens and between major and minor pereopods for the dead specimens (Graph 5).

Comparing across live and dead crabs, mean temperature flux was approximately three to four times higher in all appendages of dead specimens

compared to the live specimens during the first heat gain treatment. It is also significantly greater at max peak heat gain between live (+2.30-2.66°C) and dead (+2.75-3.52°C) specimens (LMM: 75.09, 5.28, 0.00; adj Tukey: p<0.05 all appendages). Overall, the dead crabs exhibited a higher flux for all appendages during each of the treatments, except for the initial acclimation, where they lost less heat than the live specimens.

Graph 4. Average *Pachygrapsus crassipes* heat flux of A) major cheliped and B) minor cheliped. Box plots (box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median) show that live (gray) and dead (white) crabs differ significantly in heat flux at peak heat for both the major and minor cheliped. Dotted line = average substrate heat flux with standard deviation. Yellow highlight = Peak heat
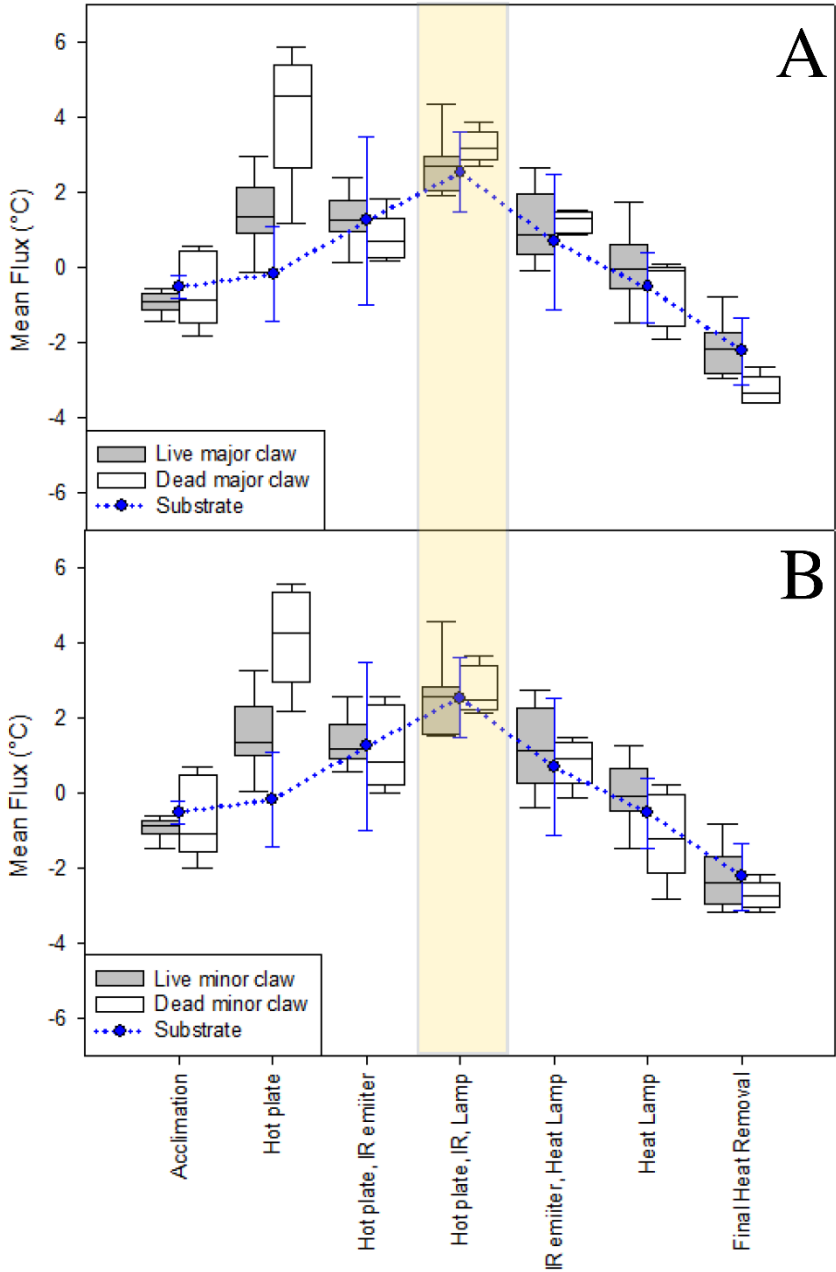
Graph 5. Average *Pachygrapsus crassipes* heat flux of A) major pereopod and B) minor pereopod. Box plots (box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median) show that live (gray) and dead (white) crabs differ significantly in heat flux at peak heat for both the major and minor pereopod. Dotted line = average substrate heat flux with standard deviation. Yellow highlight = Peak heat
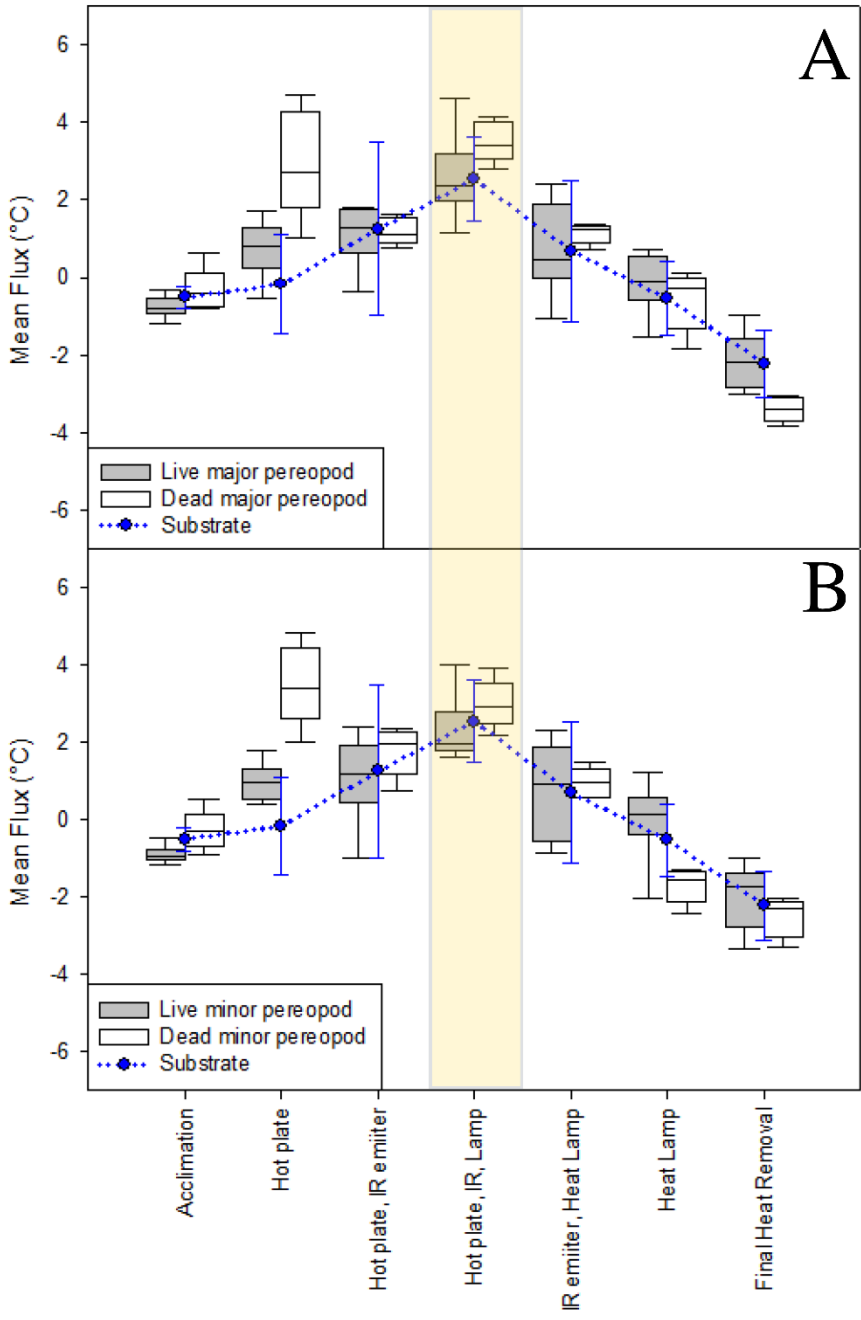
3.3 Mass specific heat flux

Individual temperature flux for carapace, chelipeds and pereopods were divided by the mass of each specimen (°C/grams) and then averaged to get the mass specific temperature flux for each species (Graph 6, A). Both *O. quadrata* (orange) and *P. crassipes* (blue) started off with a similar negative mass specific flux during acclimation (-0.13°C/g), but while *O. quadrata* recorded a more conservative mass specific flux overall (range -0.14 – 0.09°C/g), *P. crassipes* (blue) had a more extreme mass specific flux in all treatments (range -0.56 – 0.62°C/g).

    O. quadrata showed a larger negative average temperature flux in the acclimation treatment compared to *P. crassipes* (ranges -3.0 to -4.5 and -1.0, respectively; Graph 6, B), but heat fluxes both increased in the first heat gain treatment and overlapped in the rest of the heat gain and heat removal treatments. Both species had an overall similar heat flux starting with the second heat gain treatment and continuing for the rest of the experiment.

Graph 6. A) Mass specific temperature flux (°C/g) and B) Average temperature flux of *O. quadrata* (orange) and *P. crassipes* (blue) show varying mass specific rates between the species, but a similar pattern for overall heat flux.
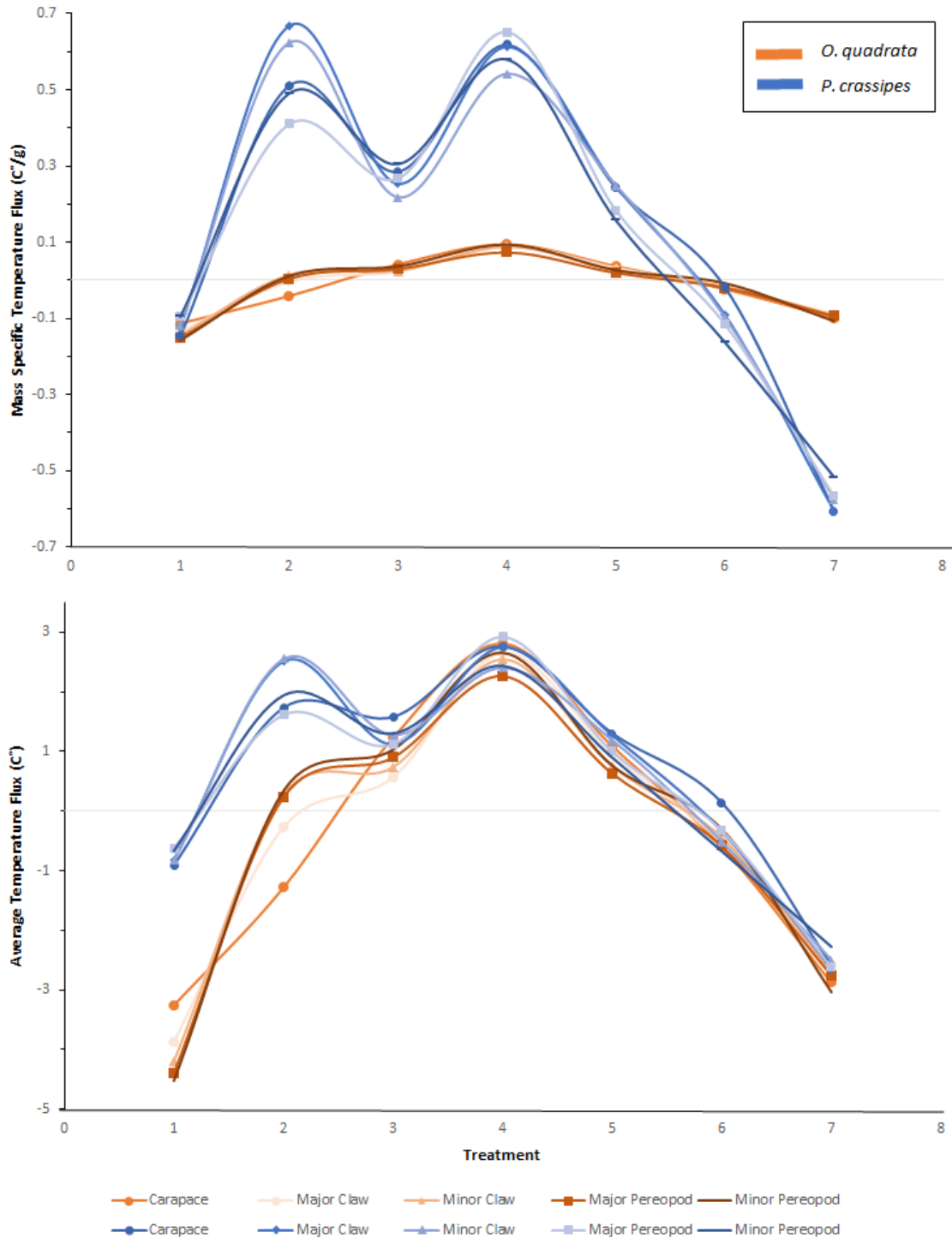
3.4 Behavioral Thermoregulation

O. quadrata spent 65-91% of each heat addition treatment in the sponging posture, that is when the abdomen and the majority of the thoracic sterna are in contact with the substrate (Graph 7, blue). In the treatments when 2 (hot plate, IR emitter) and 3 (hot plate, IR emitter, heat lamp) heat sources were added, the crabs spent approximately 10% longer in the active position (Graph 7, orange), either moving sand around to access the cooler sand below the surface or looking for a way to escape the enclosure.

Graph 7 . Percent time O. quadrata spent in sponging (blue) versus active (orange) postures in each treatment.
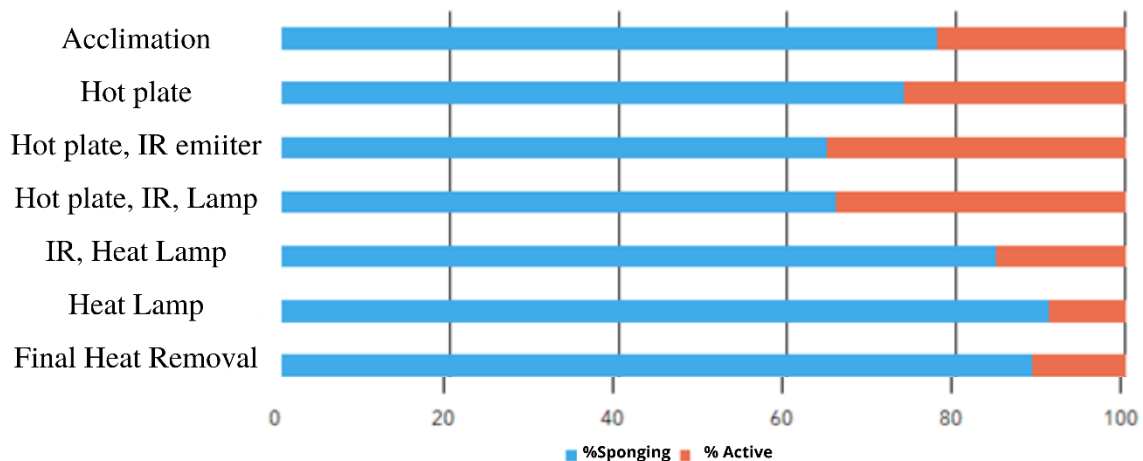
P. crassipes actively avoided the center of the experimental tank where the substrate temperature was highest. Most of the time (60-94%) crabs were outside of this zone, with only part of their bodies exposed (Graph 8, orange). This was the case for all heat addition and heat removal treatments.

Graph 8. Percent time *P. crassipes* spent in the direct (blue) versus avoidance or non-direct position (orange).
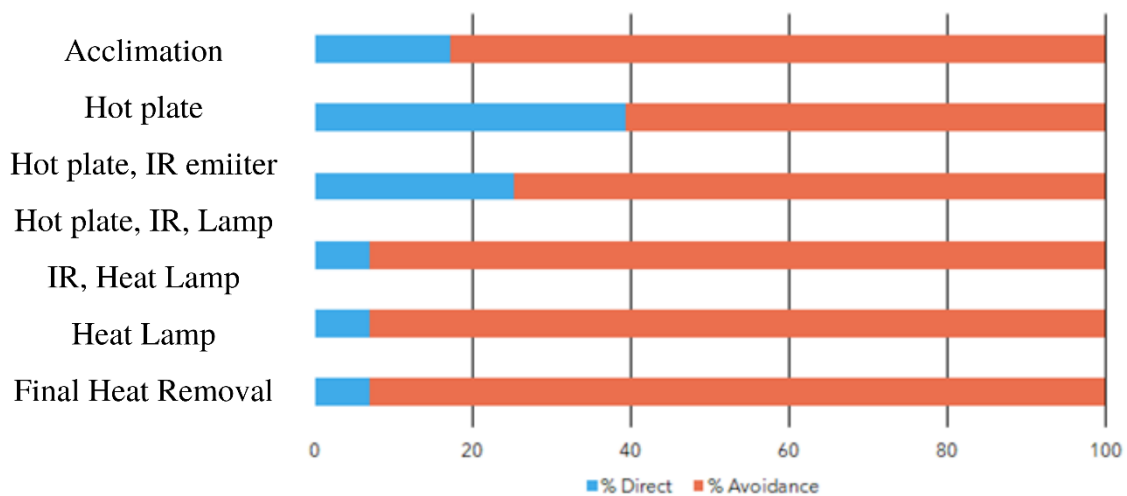
4. Discussion

The crustacean exoskeleton plays an integral role in many essential animal functions, from locomotion to feeding, yet its role in thermoregulation remains undefined. The present study investigated thermal changes over different regions of the exoskeleton surface in two shore crabs, *Ocypode*

quadrata and *Pachygrapsus crassipes*, during heat gain and heat loss using external heat sources and non-invasive IRT technology. Results show that, for both species, temperature flux increased across all body regions (carapace, chelipeds, and pereopods) when external heat was added and that these fluxes correspond to those of the substrate. Maximum temperatures differed among some body locations, indicating uneven surface heating. Dead crabs, however, showed greater maximum temperatures and heat fluxes than live crabs, with the fluxes exceeding those of the substrate. While there are multiple mechanisms through which the exoskeleton can contribute to heat flux, the exoskeleton surface is highly responsive to external temperature and behavioral thermoregulation is an essential mechanism for both species to control heat flux during environmental fluctuations.

4.1 Role of exoskeleton in thermoregulation

Exoskeleton temperature fluxes, as well as maximum temperatures for most body locations, increased and decreased in concordance with serial heat addition and removal, indicating that the exoskeleton surface is responsive to environmental temperature changes through both radiation and convection heat sources. Other animals, including the dragonfly *Libellula saturata* (Heinrich and Casey 1978) and the fiddler crab *Uca panacea* (Darnell and Munguia 2011) also exhibited increases in exoskeleton surface temperature when exposed to heat

lamps. *L. saturata* showed exoskeleton heat gain equally between live and dead specimens from 15-40°C in 4 minutes using a focused narrow beam of light (Heinrich and Casey 1978). *U. panacea* recorded 25-35°C over a 90-minute experiment using a 60 watt incandescent light (Darnell and Munguia 2011). A more natural heat system was explored in this study; *O. quadrata* increased from 23-33°C while *P. crassipes* ranged from 19-29°C for the entire 70-minute experiment. In other words, the exoskeleton does not appear to maintain a consistent temperature during environmental fluctuations.

Temperatures were recorded from dead crabs to control for physiological and behavioral temperature regulation, and their maximum temperatures and fluxes were consistently different from live crabs. Dead crabs experienced higher maximum temperatures and greater fluxes as heat was added and removed. During peak heat, the maximum temperature and temperature fluxes exceeded those of the substrate, while following heat removal, they were lower than the substrate. This indicates rapid and excessive heat loss and gain of the exoskeleton without the activity of the live animal to moderate the temperature. Differences in thermal flux between live and dead specimens have also been observed in a variety of arthropods, such as the dragonfly *Anax junius* (Heinrich and Casey 1978), the hermit crab *Coenobita scaevola* (Achituv and Ziskind 1985), and the crayfish *Procambarus clarki* (Payette and McGaw 2003). Although physiological and behavioral thermoregulation has been frequently studied in the literature, many studies do not compare thermal flux between live and dead

individuals. It appears that shore crabs do not possess exoskeleton adaptions

that provide insulation against temperature fluctuations.

4.2 Effects of body size

While the exoskeleton is responsive to external temperatures, there are

differences throughout body locations that suggest size is an important factor in

heat flux. Though the carapace exhibited changes in heat flux, it appeared to

have more consistent maximum temperatures throughout the heat gain and heat

removal treatments. Not only was the maximum temperature of the carapace

consistent, but it was also higher than the other body regions at acclimation and

following heat removal. These differences indicate uneven heating over the body

surface for both species. It is likely that this uneven heating is simply be due to

differences in the surface to volume ratio, particularly since the same pattern was

observed in live and dead specimens. The carapace, which covers the body of

the crab, has a much smaller surface area to volume ratio than the chelipeds and

pereopods. Thus, the body experienced a more conservative heat loss than the

appendages did overall. Surface area to volume ratio is an important factor for

heat exchange and thermoregulation in animals (Moen 1973) and humans

(Tilkens et al. 2007). For shore crabs, the large carapace could help maintain

internal temperatures of the organs where most physiological processes are

taking place. In this sense, the crab body plan passively permits greater

temperature variations in the appendages, a thermoregulation strategy that is common invertebrates.

Both species of shore crab, regardless of habitat, had a negative mass specific heat flux of approximately 0.13°C per gram during acclimation. On the other hand, *P. crassipes* showed a higher mass specific heat flux per gram than *O. quadrata* during the rest of the treatments. This is likely due to differences in body size of the two species, where the smaller *P. crassipes* (carapace width < 30 mm) took in more heat per gram than the larger *O. quadrata* (carapace width > 30 mm). Mass and linear size of organisms are related to the surface area to volume ratio, which known to correlate with heat flux (Moen 1973) and influence a species' behavior and distribution. For example, the brown shrimp *Crangon crangon* preferentially selects water temperatures based on body size, with smaller sized animals selecting higher temperatures (Reiser et al. 2016). Furthermore, sea surface temperature was a strong predictor for body size and resulted in significant latitudinal patterns of body size in widely distributed crustaceans such as the amphipod *Orchestoidea tuberculata*, isopods *Excirolana braziliensis* and *E. hirsuticauda*, and the crab *Emerita analoga* (Jaramillo et al. 2017).

4.2 Behavioral thermoregulation

While we did not assess physiological mechanisms of thermoregulation in this study, we did examine behaviors correlated with temperature regulation. During heat gain, *O. quadrata* did not actively avoid hotter temperatures, but instead spent most of the time in the sponging posture, or actively burying themselves in cooler subsurface level sand. After the peak heat treatment, crabs had already moved the sand around and were able to access the cooler and more moist subsurface sand. Sponging behavior helps *O. quadrata* uptake water from sand with just 5% water content at a rate of ≥1% body weight / hour (Wolcott 1984). This aids in evaporative cooling and was a primary method to reduce body temperatures by 1.3°C in the sandy shore crab, *Scopimera intermedia* (Hui et al. 2019).

P. crassipes actively avoided substrate with higher temperatures throughout the experiment. Live *P. crassipes*, lost more heat initially than the dead specimens due to evaporative cooling; the live specimens were taken directly from their seawater habitat, while the dead crabs were dry acclimated. The mass specific heat flux of the live crabs more closely mirrored the heat flux of the substrate due to the behavioral thermoregulation of the live crabs and that they actively altered the experimental enclosure. A study done on the intertidal crabs *Scylla olivacea* and *Thalamita crenata* found that these crabs increased their escape response in experimental enclosures when temperatures were close to their CTMax (Azra et al. 2020). When *P. crassipes* are in their natural habitat,

they prefer to take shelter in crevices or under rocks to mitigate the effects of high temperatures (Hiatt 1948).

4.3 Pigment effects

Although quantifiable pigmentation assays were not completed for this study, *P. crassipes* has a visibly darker pigmented exoskeleton than *O. quadrata*. The carapace of both live and dead *P. crassipes* had a greater increase in maximum temperature (5.13°C and 8.20°C, respectively) from acclimation to Peak heat than both live (-2.69°C) and dead (3.78 °C) *O. quadrata*. Pigmentation of the crabs may play a factor in the higher heat gain of the darker pigmented crabs. Heat gain differences between dark and light crabs have been reported in the fiddler crab, *Uca pugilator,* where dark crabs gained 2°C more heat than light crabs after only 5 minutes in the sunlight (Wilkens and Fingerman 1965). Of the two species measured in this study, *O. quadrata* is known to blanch, which is the lightening of the exoskeleton and increase of surface reflectance (Palmer 1971). This may explain why only the live specimens had a negative temperature flux for the carapace during this time. Coloration may play an important role in the heat flux difference between the two species measured in this study, but it is not possible to distinguish this effect from the that of surface area to volume ratio given their disparate body sizes and carapace shapes.

4.5 IRT technology

This study used non-invasive infrared thermography (IRT) to monitor heat exchange on the exoskeleton of crustaceans with a high enough resolution to distinguish heat fluxes between different locations on the crab. IRT improves on problems with sensor placement and attachment, engineering methods of the sensors themselves and limited resources for acquiring enough sensors for significant information (Hindle, Horning, and Mellish 2015; Willis et al. 2005). Furthermore, researchers have shown that data taken from IRT is comparable to data taken from heat sensors that are applied to the body of an organism when measured in air (Hindle, Horning, and Mellish 2015). One of the drawbacks to this technology is that IRT only measures surface temperatures, therefore, we cannot ascertain how much heat gets transmitted through the exoskeleton to the internal environment. Aspects of exoskeleton morphology, such as thickness and density, could potentially affect heat transfer. Furthermore, IRT does not provide sufficient resolution of organisms in a water medium, thereby rendering it ineffective at measuring heat flux in aquatic environments. Despite these limitations, the use of IRT technology in this study has increased our understanding of how the exoskeleton enables the transfer of heat throughout the body of the crab and to the surrounding environment.

4.5 Future directions

IRT has been shown to be an effective, non-invasive proxy to measure the surface body temperature of an entire organism. This technology will be a useful new approach for addressing many remaining questions in crustacean thermoregulation. For instance, it can be used to determine how the changing exoskeleton acts as a regulatory mechanism for crabs during different phases of the molting cycle, especially considering that molting is highly sensitive to environmental temperature (Stoner, Ottmar, and Copeman 2010; Brylawski and Miller 2006). This technology can also be used to identify how changes in the exoskeleton's chemical composition or mechanical properties affect heat flux of the exoskeleton. Research on the color changing effects of crabs and the thermoregulatory role it plays can be expanded to include more species of crabs from different habitats and with different body shapes and multiple color morphs. It would be especially interesting to compare the thermal properties of aquatic and terrestrial crabs to identify potential thermal adaptations of the exoskeleton to life on land. Thermal cameras are small, compact and portable enough to be taken to the field to observe and measure animals under natural environmental conditions. This could ultimately be used to frame questions about the impacts of climate change and ocean acidification on crustaceans and to gain a better understanding of the thermal ecology of crabs and their microhabitats.

4.6 Conclusions

Studies on the thermal properties of exoskeletons provides an important understanding of how species acclimate to a wide variation of physical factors in their environments, including temperature, humidity, and radiation. The exoskeleton of shore crabs exhibits heat flux when exposed to external heat sources, but it is moderated by behavioral thermoregulatory mechanisms. Differences in heat flux observed across body locations and between species can most likely be attributed to differences in surface area to volume ratios. The results from this study suggest that the exoskeletons of two species of shore crabs do not have specific thermal properties to insulate against changes in environmental temperature. This study also shows that infrared thermography can be a critical tool to aid in understanding how decapod crustaceans will adapt to future conditions under climate change.

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