UNLV | UNIVERSITY LIBRARIES

UNLV Retrospective Theses & Dissertations

1-1-2003

Thermoregulatory behavior of the crayfish Procambarus clarkii in a burrow environment

Aaron Lawrence Payette
University of Nevada, Las Vegas

Follow this and additional works at: https://digitalscholarship.unlv.edu/rtds

Repository Citation

Payette, Aaron Lawrence, "Thermoregulatory behavior of the crayfish Procambarus clarkii in a burrow environment" (2003). *UNLV Retrospective Theses & Dissertations*. 1502.
http://dx.doi.org/10.25669/uzxs-df0r

This Thesis is protected by copyright and/or related rights. It has been brought to you by Digital Scholarship@UNLV with permission from the rights-holder(s). You are free to use this Thesis in any way that is permitted by the copyright and related rights legislation that applies to your use. For other uses you need to obtain permission from the rights-holder(s) directly, unless additional rights are indicated by a Creative Commons license in the record and/or on the work itself.

This Thesis has been accepted for inclusion in UNLV Retrospective Theses & Dissertations by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact digitalscholarship@unlv.edu.

THERMOREGULATORY BEHAVIOR OF THE CRAYFISH *PROCAMBARUS CLARKII* IN A BURROW ENVIRONMENT

by

Aaron Lawrence Payette

Bachelor of Science
Pacific University
2000

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science Degree
Department of Biological Sciences
College of Sciences

Graduate College
University of Nevada, Las Vegas
May 2003

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

UMI Number: 1414543

UMI®

UMI Microform 1414543

Copyright 2003 by ProQuest Information and Learning Company.

All rights reserved. This microform edition is protected against unauthorized copying under Title 17, United States Code.

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

UNLV
UNIVERSITY OF NEVADA LAS VEGAS

Thesis Approval

The Graduate College
University of Nevada, Las Vegas

February 11, 2003

The Thesis prepared by

Aaron Lawrence Payette

Entitled

Thermoregulatory Behavior of the Crayfish Procambarus clarkii in a Burrow Environment

is approved in partial fulfillment of the requirements for the degree of

Master of Science

Examination Committee Chair

Dean of the Graduate College

Examination Committee Member

Examination Committee Member

Graduate College Faculty Representative

PR/1017-53/1-00

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ABSTRACT

Thermoregulatory Behavior of the Crayfish *Procambarus clarkii* in a Burrow Environment

by

Aaron Lawrence Payette

Dr. Iain McGaw, Examination Committee Chair
Assistant Professor of Biology
University of Nevada, Las Vegas

The behavioral thermoregulation of the red swamp crayfish, *Procambarus clarkii*, was investigated and the environmental parameters of crayfish burrows were measured *in situ*. Although temperatures within burrows fluctuated less than surface temperatures in the Mojave Desert, crayfish can experience sub-optimal temperatures inside the burrow. In the laboratory, *P. clarkii* heated and cooled more rapidly in water than in air, selected a water temperature of 22°C, and avoided water temperatures above 31°C and below 12°C. *P. clarkii* displayed three main shuttling behaviors between water and air and the relative amounts of these behaviors and the time spent in air were significantly greater at 34°C than at 12°C, 16°C, 22°C or 28°C. This reflects an increased use of behavioral thermoregulation at critical temperatures. These periods of emersion were interspersed with frequent dipping in the water, allowing the crayfish to gain the benefits of evaporative cooling, without the physiological costs incurred by long-term exposure to air.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE OF CONTENTS

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF FIGURES

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ACKNOWLEDGMENTS

First and foremost, I wish to thank my parents, Lawrence and Barbara Payette. Thank you Mom and Dad for always being supportive of me and for encouraging me to pursue my interests. You have always provided me with opportunities that have allowed me to grow as a person. I am very fortunate to have such wonderful, caring parents.

I also wish to thank a number of people and organizations that have provided me with the intellectual, emotional, monetary, and logistical support that I needed to make this project possible. I am extremely grateful for the mentoring I received from my major advisor, and friend, Dr. Iain J. McGaw. Working with you has been a very positive experience, Iain. I could not have completed this project without all of your input and countless hours of revising. Thank you for helping me to become more self-sufficient, by not always giving me the answer. Oh, and thank you for lending me your clothes when my luggage got lost in Canada. It was the middle of summer, so I left Vegas wearing a t-shirt, shorts, and sandals.

Next I wish to thank my thesis committee members, Dr. Carl L. Reiber, Dr. Stephen P. Roberts, and Dr. Shawn L. Gerstenberger for their time and intellectual input. The permit to collect my specimens was provided by the United States Fish and Wildlife Service. The following organizations provided me with partial funding for this project: The UNLV Department of Biological Sciences, the UNLV Biological Association of Graduate Students (BIOS), and the UNLV Graduate Student Association (GSA).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

I would like to thank Dr. Jonathon H. Stillman and Dr. Louis E. Burnett for helpful discourse and suggestions regarding my experimental design. I would also like to thank Jutta A. Guadagnoli for helping me to revise this document.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 1

INTRODUCTION

Many members of the animal kingdom are able to build physical structures that benefit their makers in some way. These structures often provide their architects the means to inhabit extreme environments that they would otherwise be unable to tolerate (Turner, 2000). The burrows of decapodan crustaceans may benefit their inhabitants by providing a thermal refuge from extreme surface conditions, in addition to providing a source of standing water and humid air (see Atkinson and Taylor, 1988; Eshky *et al.*, 1995). Although burrowing allows crustaceans to avoid adverse surface temperatures and desiccation, these animals must overcome the challenges associated with the switch in ventilatory media as they migrate between the standing water and the air in the burrow.

Migrating into air may be physiologically costly to crustaceans, and several studies have examined the effects of emersion stress on decapod species that voluntarily breathe air. Amphibious decapods such as the shore crabs, *Carcinus maenas* and *Hemigrapsus nudus*, and the crayfishes, *Cherax destructor* and *Austropotamobius pallipes* are able to maintain both oxygen consumption rates and heart rates, after migrating from water and remaining in air of the same temperature for 3h (Taylor and Wheatly, 1979, 1981; Greenaway *et al.*, 1996; Morris and Callaghan, 1998). However, CO_2 excretion is reduced in air (Taylor and Wheatly, 1979, 1981; McMahon and Wilkes, 1983; Morris and Callaghan, 1998), which results in a marked acidosis (Taylor and Wheatly, 1981;

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

McMahon and Wilkes, 1983; Johnson and Uglow, 1985; Tyler-Jones and Taylor, 1988; Stillman and Somero, 1996; Morris and Callaghan, 1998). In addition, the blood and muscle concentrations of stored nitrogenous compounds increase during air exposure, due to a decrease in ammonia excretion (Durand and Regnault, 1998). An increase in tissue ammonia levels has been shown to affect the regulation of hemolymph sodium, calcium and chloride ions (Rebelo *et al.*, 1999), which may ultimately affect nervous function.

Even though the burrows of decapod crustaceans are considered to be thermal refuges, animals may encounter sub-optimal temperatures while inhabiting them (Eshky *et al.*, 1995). Exposure to sub-optimal temperature regimes may result in the alteration of certain metabolic parameters in decapod crustaceans. Oxygen consumption rates are positively correlated with water temperature for *C. maenas* (Taylor and Wheatly, 1979) and the lobster, *Homarus gammarus* (Whiteley *et al.*, 1995a). The Q_{10} values for MO_2 of semi-terrestrial decapods typically vary between 1.3 and 2.1 (Taylor and Wheatly, 1979; Burggren and McMahon, 1981; Reiber and Birchard, 1993). Heart rates, and presumably cardiac output, of many decapod species are also positively correlated with temperature (Taylor and Wheatly, 1979, 1981; Eshky *et al.*, 1995, 1996; Stillman and Somero, 1996; De Pirro *et al.*, 1999).

Temperature extremes have deleterious effects on various physiological processes in decapod crustaceans. An increase in temperature causes a reduction in the oxygen carrying capacity of hemolymph and oxygen binding to hemocyanin (Taylor, 1981; Truchot, 1983; Eshky *et al.*, 1996), which ultimately affects the delivery of oxygen to tissues. Acid-base balance is also affected by temperature. The hemolymph pH of 1°C-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

acclimated crayfish (*A. pallipes*) is higher than in 5°C-acclimated crayfish, and tissue pH decreases when these animals are heated from 1°C to 12°C (Whiteley *et al.*, 1995b). The responses of decapod crustaceans to increases in temperature are even more pronounced in air. Acute exposure (15min) to air above 15°C causes a thermal acidosis and compensatory increases in cardiac output to maintain adequate oxygen uptake (Morris *et al.*, 1996a, b). Prolonged exposure to extremely high temperatures leads to an increased mortality rate due to breakdown of normal gill permeability, resulting in a loss of extracellular sodium and nervous function (Willmer *et al.*, 2000).

Because ectotherms are unable to thermoregulate physiologically, behavioral mechanisms are often used to control body temperature (Wood, 1991; Crossin *et al.*, 1998). During periods of thermal stress, decapodan crustaceans may retreat to shade or into burrows (Smith and Miller, 1973; Eshky *et al.*, 1995), or emigrate from water of sub-optimal temperature in order to regulate body temperature (Taylor and Wheatly, 1979; McGaw, 2003). Fiddler crabs (*Uca spp.*) are able to change their carapace color (blanching) to alter the amount of solar radiation absorbed (Smith and Miller, 1973). In conjunction with blanching *Uca* and other genera of crabs can lower body temperatures through evaporative cooling (Edney, 1961; Taylor and Wheatly, 1979; Thurman, 1998; McGaw, 2003).

Fully aquatic species have served as the dominant model organisms in studies of behavioral thermoregulation in decapod crustaceans (see Lewis and Roer, 1988; Crossin *et al.*, 1998). However, fewer studies have explored the behavioral thermoregulation of amphibious decapods in systems where they have the ability to migrate between water and air (Taylor and Wheatly, 1979; Eshky *et al.*, 1995; McGaw, 2003). The red swamp

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

crayfish, *Procambarus clarkii*, is adapted for life in habitats with fluctuating hydroperiods due to its ability to construct burrows and bimodally breathe (Huner and Barr, 1991). This species is native to the Mississippi River valley, but has been introduced across much of the United States and is found in the Mojave Desert. *Procambarus clarkii* is classified as a tertiary burrower (Hobbs, 1981), living in open water it retreats to burrows to avoid adverse environmental conditions such as receding water levels (Correia and Ferreira, 1995) and freezing temperatures (Hobbs, 1981). The aim of the present study is to determine how *P. clarkii* behaviorally thermoregulates in the burrow environment using the thermal properties of both air and water.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 2

MATERIALS AND METHODS

Adult male and female *P. clarkii* were collected (permit #14555-00003) from Corn Creek at the Desert National Wildlife Refuge, Nevada (N 36 26.24 W 115 21.60). Crayfish were transported to the University of Nevada, Las Vegas and were housed in 130-L tanks filled with dechlorinated tap water, maintained at 22°C ± 1°C. Male and female crayfish were separated and pieces of PVC pipe were added to the tanks for use as refugia. Animals were maintained on a 12h light-dark cycle for a minimum of two weeks before beginning experiments. The crayfish were fed frozen fish twice weekly, but were isolated from food supplies two days prior to experimentation. Only intermolt crayfish of 22.0g ± 4.0g (44.5mm ± 3.0mm carapace length) were used in experiments. Approximately equal numbers of males and females were used and individual crayfish were not used in more than one trial for any experiment.

Environmental Parameters of Crayfish Burrows *In Situ*

Although natural crayfish burrows were found at Corn Creek, they tended to be sparsely scattered in different microhabitats. Therefore, to avoid variance due to location or burrow shape, artificial burrows were constructed on the shoreline of Corn Creek using measurements of plaster casts taken from a number of *P. clarkii* burrows. The burrows were constructed by hammering sharpened pieces of 5cm-diameter PVC pipe into the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ground, until the water table was reached. The pipes were then removed, and the terminal chambers of the burrows were constructed by using a metal rod (curved at one end) to widen the burrow terminus. Three burrows were constructed for each of the following depth classes: 20cm, 40cm, and 60cm. The burrows were left for two weeks before beginning experiments. The temperature and relative humidity inside and outside each burrow (n=9) was measured once every 4h during a 24h period, and the experiment was replicated on three days with varying ambient air temperatures. The temperature profile of each burrow was measured by inserting a thermocouple (Physitemp IT-18), attached to a meter stick, into the burrow. Temperatures were recorded, with an electronic thermometer (Physitemp BAT-12), at the burrow entrance and at consecutive 5cm depths until the terminal end of the burrow was reached. The temperature of the standing water in the burrows was also recorded at this time. Burrow humidity was recorded at the midpoint of each burrow tube using an electronic hygrometer (Fisher Scientific). These measurements were compared to surface air temperature and relative humidity measured near the burrow entrances, in the shade. A two-way ANOVA with repeated measures design was used to determine if temperature in the burrow was significantly different from surface air and burrow entrance temperatures over 24h.

Body Temperature Equilibration Rates

Changes in the core temperature of *P. clarkii* were studied in both water and air. Crayfish were prepared for experiments by drilling a small hole in the carapace just dorsal to the heart, and a catheter mounted (PE90) thermocouple (Physitemp IT-80) was inserted into the body cavity until it rested against the sternal artery. The thermocouple

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

was then secured with dental wax and cyanocrylate glue. Thermocouples were connected to an electronic thermometer (Sable Systems TC-1000) and changes in body temperature were recorded with a data acquisition system (AD Instruments Powerlab/400).

To determine equilibration rates in water, crayfish (n=20) were transferred directly from water maintained at 22°C to a basin containing dechlorinated tap water maintained at either 35°C or 12°C. An air stone was placed in the basin to reduce thermal stratification (Layne *et al.*, 1987). Data recording was initiated upon transfer to the new temperature and terminated once all crayfish reached a stable body temperature.

To determine equilibration rates in air, crayfish (n=20) were removed from water of 22°C, shaken, and then towel dried. Animals were then transferred to an incubator (Thelco 32MR) maintained at 35°C or 12°C, and data recording was initiated. Recordings were terminated once all crayfish reached a stable body temperature. Incubator air temperature and relative humidity were monitored using an electronic hygrometer (Fisher Scientific). To determine if crayfish were able to control equilibration rates through active mechanisms, animals (n=10) were sacrificed by injecting 0.5ml of 10% formalin into the heart, and then body temperature changes of the dead crayfish were followed as described previously. The total time required for live and dead crayfish to equilibrate to new temperatures, in water and air, were compared using a student T-test.

Thermal Tolerance in Water and Air

The critical thermal maximum (CTMax) and critical thermal minimum (CTMin) temperatures for *P. clarkii* were determined in both water and air. To assess critical

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

thermal ranges in water, crayfish (n=30) were placed in a basin filled with 20-L of dechlorinated tap water maintained at 22°C. Water was heated or cooled at a rate of 0.2°C/min (Lagerspetz and Bowler, 1993; Korhonen and Lagerspetz, 1996) with a recirculating water bath (VWR Scientific Products). The water was aerated and both temperature and oxygen were monitored with an electronic dissolved oxygen meter (YSI 55). All crayfish were turned on their dorsal surfaces, following every 0.5°C change in temperature. The temperature at which each animal could no longer right itself within 1min was considered as the CTMax or CTMin (Lagerspetz and Bowler, 1993; Cuculescu, *et al.*, 1998).

The CTMax and CTMin temperatures of *P. clarkii* were also determined in air. Crayfish (n=30) were shaken, towel dried, and then held in 22°C air for 30min to standardize the volume of water present in their branchial chambers. The crayfish were transferred to an incubator (Thelco 32 MR) that was maintained at 22°C. The air in the incubator was then heated or cooled at 0.2°C/min. Air temperature and relative humidity were monitored with an electronic hygrometer (Fisher Scientific). Again animals were turned on their dorsal surfaces and the temperature at which each individual animal could no longer right itself within 1min was recorded.

Control experiments were conducted to determine if the physiological stress due to repeated handling affected the endpoint of thermal tolerance tests. The righting response of crayfish (n=20) was tested every 2min for 1h, in both water and air maintained at 22°C. A binomial test was used to determine if repeated handling affected the righting response of the crayfish.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Preference in a Thermal Gradient

The temperature preference of *P. clarkii* was tested in a thermal gradient constructed from a cylindrical trough (3m in length, 12cm in diameter). The trough was filled with deionized water and was heated at one end and cooled at the other end using recirculating water baths. Air stones were placed in the gradient to keep the water aerated, and to reduce vertical thermal stratification (Espina *et al.*, 1993; Kivivuori, 1994). To determine if sex and reproductive state affect temperature selection in *P. clarkii*, individual male, female, and ovigerous-female crayfish (n=15, each group) were fitted with thermocouples and then randomly introduced into different areas of the gradient. The apparatus was covered with a sheet of black plastic to minimize disturbance to the crayfish, and all experiments were carried out in constant, dim light. Crayfish were allowed 1h to acclimate to their surroundings before beginning experimentation. Body temperature was recorded continuously for 5h with a data acquisition system (AD Instruments Powerlab/400), and the temperature that the crayfish selected was calculated as a mean core temperature from each 5h recording.

To determine if crayfish preferred particular areas of the trough independent of water temperature, the apparatus water was maintained at 22°C and crayfish (n=10) were randomly introduced into one of six equal quadrants assigned to the trough. The quadrant in which a crayfish was located was recorded once every 2h, for 6h. A Chi-square test was conducted to determine if crayfish were observed in each of the six quadrants at equal proportions.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Temperature Avoidance in Water

In the laboratory, an artificial burrow chamber (Fig. 1) was used to ascertain the temperature preference range of the animals after they experienced an increase or decrease in water temperature. Individual crayfish (n=30) were allowed to settle for 30min in the water of the chamber. The water was then heated or cooled from 22°C, at a rate of 0.3°C/min, with a recirculating water bath. Air stones placed in the water maintained oxygen levels and reduced thermal stratification. The temperature and oxygen tension of the water were recorded every 2min using a dissolved oxygen meter (YSI 55). The temperature at which crayfish exited water completely and moved into the aerial environment of the burrow was recorded.

A separate series of experiments were conducted to ensure that the crayfish were exiting water due to a temperature avoidance response, and not because of a decrease in oxygen tension. Crayfish (n=15) were allowed to settle in the burrow for 30min and water was maintained at 22°C. Nitrogen was then bubbled through the water, reducing the dissolved oxygen concentration by approximately 0.3 kPa per minute. The dissolved oxygen concentration at which crayfish exited from the water was recorded. Student T-tests were conducted to determine if the oxygen tension at which crayfish exited the water was significantly different from that measured during the temperature avoidance experiments.

Behavior in Response to Temperature

The behavior of crayfish was monitored at different temperature regimes to determine the amount of time spent in air. The artificial burrow chamber (Fig. 1) was placed in an

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

incubator (Thelco 32MR) maintained at temperatures of: 12°C, 16°C, 22°C, 28°C or 34°C. In the first series of experiments, individual crayfish (n=8, each temperature) were placed in the burrow chamber and behavior was monitored over 24h using a time-lapse camera (Panasonic WV-BP120) and video recorder (Panasonic AG-RT600A). Trials were carried out in constant, dim red light. Air stones placed in the water maintained oxygen levels and reduced thermal stratification. Burrow water temperature and oxygen tension were monitored using a dissolved oxygen meter (YSI 55), while air temperature and relative humidity were monitored using an electronic hygrometer (Fisher Scientific). Videos were analyzed to determine the percentage of time spent in air by crayfish, the number of exits into air, and the time spent in air per exit as a function of temperature.

A second series of experiments was conducted to determine if the crayfish were able to control body temperature by migrating between the two media. Crayfish were fitted with thermocouples (Physitemp IT-80) in the body core and body temperatures were monitored continuously with a data acquisition system (AD Instruments Powerlab/400), while simultaneously recording behavior with the time-lapse video system. The experimental apparatus was maintained at a temperature of 32°C-34°C. The relative humidity of the incubator air was controlled using a household air humidifier, and the crayfish were exposed to both low (40-50%) and high (70-80%) humidity regimes.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 3

RESULTS

Environmental Parameters of Crayfish Burrows *In Situ*

Surface air temperatures at Corn Creek reached a minimum of 6.8°C on April 22nd, 2002. Air temperatures reached 41.4°C during the hottest times of the day on June 6th, 2002, and this day was typical of summer conditions in the Mojave Desert. For this reason, data recorded on June 6th, 2002 were analyzed. At 12:00h, the mean air temperature at the burrow entrance was 33.5°C ± 0.7°C (Fig. 2), and temperature decreased sharply with increasing burrow depth (ANOVA, F=36.563, P<0.0001). The burrow temperature stabilized at 23.0°C ± 0.9°C, at a depth of 30cm, and there was no significant change in temperature below this depth (Tukey test, P<0.05). A thermal gradient did not exist in the burrows at 00:00h; the temperature remained at 19.4°C ± 0.6 and showed no significant fluctuation with increasing burrow depth (ANOVA, F=0.362, P=0.966).

On June 6th, 2002 the mean surface air temperature of six burrows fluctuated between 33.4°C ± 0.8°C and 14.2°C ± 0.4°C during the 24h experimental period, while burrow temperature at a depth of 30cm fluctuated between 25.2°C ± 0.6°C and 17.1°C ± 0.4°C (Fig. 3). The surface air temperature and burrow entrance temperature were similar for the duration of the experimental period (Tukey test, P<0.05). The burrow temperature at 30cm depth was 10.4°C ± 0.4°C cooler than the surface air temperature at 12:00h and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

7.8°C ± 0.6°C cooler at 16:00h, and these differences were significant (Tukey test, $P<0.05$). At 20:00h and 00:00h, the air temperature in the burrow lumen was not significantly different from the outside air. By 04:00h the outside air had cooled to 14.5°C ± 0.3°C, while the burrow temperature (at 30cm) remained 2.7°C ± 0.3°C warmer. During the early morning (08:00h), surface air temperature had warmed to 30.6°C ± 1.9°C, but the temperature of the burrow lagged behind and was 7.0°C ± 1.5°C cooler. The burrow water temperature remained at 18.3°C-18.7°C during the 24h sampling period. The relative humidity in the burrows (measured at the burrow midpoint) was approximately 90% during the entire 24h period, while relative humidity at the surface was approximately 10% during the day and 20% at night. Humidity profiles were measured in two burrows, and the relative humidity increased with increasing burrow depth.

Body Temperature Equilibration Rates

The body temperature of crayfish equilibrated rapidly when they experienced a change in water temperature (Fig.4). Animals transferred from 22°C water to 35°C water reached thermal equilibrium in 2.8min ± 0.2min. The rate of heat gain in water was significantly greater than the rate of heat loss (t test = -7.267, $P<0.0001$), with animals taking 4.7min ± 0.2min to equilibrate to a 10°C decrease in water temperature.

When transferred from 22°C water to 35°C air, body temperatures failed to equilibrate to this new temperature within the 3h experimental period. The core temperatures of the crayfish only reached 30.1°C ± 0.3°C. Although crayfish in 35°C water equilibrated within 4min, animals exposed to 35°C air only reached 80% of their maximum

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

temperature within 4min. When crayfish were transferred from 22°C water to 12°C air, their body temperature equilibrated with the medium in 24.8min ± 0.6min. This was significantly longer than the 4.7min required to equilibrate in 12°C water (*t* test = -31.423, $P<0.0001$).

The core temperatures of live crayfish took less time to equilibrate to a 13°C increase or 10°C decrease in water temperature, than that of dead crayfish (*t* test = -7.452, $P<0.0001$; *t* test = -5.565, $P<0.0001$, respectively). Because all crayfish exposed to 35°C air reached a minimum core temperature of 27.5°C, the time required for live and dead crayfish to equilibrate to 27.5°C in 35°C air were compared, and there was no statistically significant difference (*t* test = -0.242, $P=0.810$). However, the body temperatures of live animals in 12°C air equilibrated in significantly less time than those of dead animals (*t* test = -2.361, $P=0.025$).

Thermal Tolerance in Water and Air

The CTMax of *P. clarkii* in water was 36.0°C ± 0.2°C, and this was not significantly different from the CTMax of 35.5°C ± 0.5°C measured in air (*t* test = 0.766, $P=0.447$). However, CTMin was affected by the medium in which animals were tested. The CTMin in water, of 8.4°C ± 0.2°C, was significantly lower than the CTMin in air, of 11.4°C ± 0.5°C (Mann-Whitney Rank Sum Test, $P<0.001$).

To determine if repeated handling affected the ability of the crayfish to right themselves, the righting response of crayfish was tested in both water and air maintained at 22°C. After the 1h period, it was apparent that repeated handling did not affect the ability of animals to right themselves as all 20 animals were able to right themselves in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

water, and 19 of 20 crayfish succeeded in righting themselves in air (Binomial test, $P<0.0001$).

Preference in a Thermal Gradient

Individual male, female, and ovigeorus-female crayfish made frequent movements in the thermal gradient during the 1h acclimation period. The core temperature of crayfish fluctuated between 14.2°C and 28.0°C during this period. The amount of movement was greatly reduced by the end of this 1h acclimation period. There was no difference in the water temperature selected between the three groups of crayfish (ANOVA, $F=0.726$, $P=0.490$). Therefore, data for the three groups was pooled and the mean water temperature selected by crayfish was 22.2°C ± 0.4°C (n=45).

When water temperature was maintained at 22°C, crayfish were observed in each of the quadrants of the water trough with equal frequencies (Chi-square test, $X^2=3.200$, $P=0.669$). Thus the crayfish did not have a preference for a certain area of the apparatus.

Temperature Avoidance in Water

None of the animals exited the water in the burrow chamber during the 30min prior to the start of the temperature avoidance experiments. When water was heated, the crayfish exited water and moved into air when the temperature reached 31.3°C ± 0.4°C. The crayfish fully migrated into air, and upon exiting the water they would usually walk up and down the length of the burrow tube. When the water was cooled at the same rate, only 16 of the 30 animals (53%) exited the water and moved into air. The remaining 14 animals eventually reached their CTMin temperatures (approximately 8.5°C), at which

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

time they became incapacitated and were unable to exit the water. Only data for animals displaying avoidance behaviors were used in the final analysis. The mean exit temperature of crayfish when the water was cooled was 12.0°C ± 0.6°C.

To determine the oxygen tension at which *P. clarkii* would exit water, the crayfish were exposed to progressive hypoxia at 22°C in the burrow chamber. The crayfish exited from the water when the oxygen tension reached 2.8 kPa ± 0.9 kPa. When compared to the oxygen tensions recorded during the temperature avoidance experiments (16.0 kPa ± 0.3 kPa and 24.9 kPa ± 0.5 kPa), the oxygen tension at which crayfish exited the 22°C water was significantly lower (t test = -17.57, $P<0.001$; t test = -21.00, $P<0.001$, respectively).

Behavior in Response to Temperature

Observations of behavior within the burrow showed that crayfish display three dominant shuttling behaviors between water and air. The first type of shuttling behavior consisted of animals making partial migrations into air, termed "emersions" (Taylor and Butler, 1973). Two types of types of emersions were observed: a) The crayfish would lie horizontally at the water-air interface, exposing one lateral aspect of their bodies and one branchial chamber to air while the other remained submerged. This was termed "unilateral emersion" (McMahon and Wilkes, 1983). b) The crayfish would position themselves vertically at the interface with the entire branchial chamber exposed to air, while the tail remained submerged. This was termed "bilateral emersion" (McMahon and Wilkes, 1983).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

In the second type of shuttling behavior the crayfish made full migrations into air. This was termed "emigration" (Taylor and Wheatly, 1979). Crayfish would completely exit the water and remain inactive, in close proximity to the water-air interface. Occasionally they would climb up and down the burrow tube before re-immersing, and this was termed "patrolling."

A third type of behavior was observed in conjunction with periods of emigration and/or bilateral emersion. After remaining completely or partially exposed to air, an animal would briefly re-submerge in water and return to air within 10s. This behavior was termed "dipping." A dipping session was defined as a period of emigration and/or bilateral emersion in which dipping occurred; some dipping sessions lasted almost 3h and consisted of 120 or more dips.

The temperature had a significant effect on the number of unilateral emersions displayed by crayfish (Fig. 5a). The crayfish made an average of 74.0 ± 17.7 unilateral emersions at 22°C; this was significantly greater than the number of unilateral emersions at the other four temperature regimes (ANOVA, F=15.324, P<0.001). The crayfish made between 1.0 ± 0.5 and 1.4 ± 0.6 unilateral emersions at 12°C, 16°C, 22°C, and 28°C, and there was no significant difference between these temperatures (Tukey test, P<0.05). Each unilateral emersion lasted between 0.2min and 0.7min (Fig. 5b) and there was no significant difference in the duration of unilateral emersions as a function of temperature (ANOVA, F=0.974, P=0.436).

Temperature affected the number of bilateral emersions made by the crayfish (Fig. 5a). Crayfish made an average of 266.9 ± 58.3 bilateral emersions at 34°C; this was significantly greater than at all other temperatures (ANOVA, F=15.557, P<0.001). No

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

differences in the frequency of this behavior existed between 12°C, 16°C, 22°C, and 28°C (Tukey test, P<0.05). At 34°C the crayfish spent an average of 2.0min ± 0.5min at the water-air interface per bilateral emersion (Fig. 5b), whereas the duration of bilateral emersions at the other temperatures were significantly lower and ranged between 0.2min ± 0.0min and 0.5 ± 0.1min (ANOVA, F= 8.923, P<0.001).

The pattern of emigration behavior (full migration into air) was very similar to that observed for the bilateral emersion response. At 34°C the animals made 94.9 ± 20.8 emigrations during a 24h period in the burrow chamber (Fig. 5a). This was significantly greater than the number of emigrations displayed at each of the remaining temperatures (ANOVA, F=26.324, P<0.001), and no significant difference in the number of emigrations between the remaining four temperatures occurred. Patrolling of the burrow tube was observed almost exclusively at 28°C, with 54 of the 61 emigrations (89%) involving patrols. In comparison, only 0.9% to 37.5% of the emigrations made at the other four temperatures were patrols. The amount of time spent in air per emigration by crayfish exposed to both 16°C and 34°C (approximately 1.8min ± 0.5min) was significantly greater than the 0.4min ± 0.2min spent in air by crayfish exposed to 12°C, but not greater than the time per emigration at 22°C and 28°C (ANOVA, F= 4.134, P<0.01; Tukey test, P<0.05). No significant differences were present between the remaining temperatures (Fig. 5b).

Temperature also had a significant effect on the dipping behavior (Fig. 6a). The crayfish made an average of 238.6 ± 79.3 dips during a 24h period when exposed to 34°C. This was significantly greater than the 0.9 ± 0.7 and 0.4 ± 0.4 dips at 22°C and 28°C, respectively (ANOVA, F= 8.226, P<0.001; Tukey test, P<0.05). Dipping behavior

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

was not observed at temperatures of 12°C and 16°C. The number of dips per dipping session was also calculated. Although the number of dips per dipping session at 34°C was almost three times greater than the values recorded at 22°C and 28°C (Fig. 6a), no statistically significant difference could be demonstrated (ANOVA, F=1.110, P=0.375).

The total amount of time spent in air by crayfish (all behaviors) was compared across the five temperature regimes (Fig. 6b). Crayfish spent 51.0% ± 9.8% of 24h in air at 34°C, while crayfish exposed to the other four temperatures spent a maximum of 3.3% ± 0.5% of their time breathing air. This difference was highly significant (ANOVA, F=23.244, P<0.0001).

The exiting behaviors were then monitored while simultaneously measuring core temperatures of crayfish. The implantation of a thermocouple did not appear to affect the ability of the crayfish to exit the water or alter their behavior, relative to the animals tested without temperature probes. The migrations from water into air were associated with a significant change in core temperature. When a crayfish migrated from 33.7°C water into air of 37.7°C (40% relative humidity; dew point temperature = 21.0°C), its core temperature decreased from 33.7°C ± 0.0°C to 31.5°C ± 0.2°C (Figure 7a). Even though the crayfish was moving into air that was 4°C warmer than its body temperature, core temperature decreased significantly by 2.1°C ± 0.2°C during the 2min period in air (t test = 12.177, P<0.0001). Thereafter, the core temperature started to increase, and the crayfish responded to this 1.0°C ± 0.1°C rise in core temperature by re-submerging in the water, where body temperature rapidly equilibrated with the medium.

When crayfish exited from 32°C water and moved into air of 33°C (75% relative humidity; dew point temperature = 27.5°C) a similar pattern was observed. However,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

even though the air temperature was only slightly greater than the crayfish body temperature, upon exiting the core temperature only decreased by 1.1°C ± 0.1°C (Figure 7b). This was significantly reduced from that experienced by crayfish in low humidity air (t test = 5.031, P<0.001). Crayfish re-submerged 3.3min ± 0.3min after entering air, and unlike the behavior observed at low humidity, the crayfish migrated back into water at approximately the time that the maximum decrease in body temperature occurred.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 4

DISCUSSION

The use of animal-built structures is a widespread phenomenon in the animal kingdom. Many mammals, birds, and invertebrates (termites, beetles, spittlebugs, spiders, and worms) derive some physiological benefit by utilizing their self-built structures (Turner, 2000). A number of decapod crustaceans utilize the protective benefits of burrows in different ways. Semi-terrestrial crustaceans construct burrows mainly to avoid adverse environmental conditions, and for access to standing water or damp conditions (Edney, 1961; Smith and Miller, 1973; Powers and Cole, 1976; Eshky *et al.*, 1995).

In the present study, temperatures within crayfish burrows were more stable than the surrounding surface air temperatures (Fig. 3), which is consistent with other studies. Measurements within and surrounding the burrows of fiddler crabs (*Uca annulipes*) reveal that while air temperature at the surface is 32.5°C and the surface sediment is 46.4°C, the temperature in the burrow lumen (at a depth of 30cm) is 26°C (Edney, 1961). The fiddler crabs, *Uca rapax* and *U. pugilator*, are able to use this thermally stable environment in the burrow to decrease their core temperatures by up to 10°C on a hot day, and raise body temperatures by up to 5°C on a cold day by periodically entering their burrows (Smith and Miller, 1973). Previous studies have reported the occurrence of temperature gradients within the burrows of a variety of fiddler crab species (Powers and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Cole, 1976; Eshky *et al.*, 1995). In the present study, *P. clarkii* burrows also possessed natural thermal gradients (Fig. 2), and this could benefit the crayfish by allowing them to select from a range of temperatures within the burrow lumen.

Because the crayfish could migrate between the standing water and the air within the burrow environment, the heating and cooling rates of crayfish body temperatures were measured in both water and air. When exposed to high and low temperatures in both water and air, crayfish body temperatures equilibrated much more rapidly in water than in air (Fig. 4). This phenomenon has also been observed in the lobster, *Homarus gammarus* (Whiteley *et al.*, 1995a) and the purple shore crab, *Hemigrapsus nudus* (Greenaway *et al.*, 1996; McGaw, 2003). In the present study, the difference in the rates of body temperature change between water and air may be attributed to several factors. Water has a much higher specific heat capacity than air (Dejours, 1981), and thus animals submerged in water will absorb or lose heat at a greater rate than organisms immersed in air. Secondly, the gills of aquatic organisms serve as the principle site for heat exchange, due to their large surface areas and small diffusion distances (Taylor, 1982). Because the gills tend to clump during emersion (deFur, 1988), which effectively reduces the surface area for diffusion, heat transfer is likely reduced in air. Finally, because decapods can retain water in their branchial chambers during aerial exposure (Burnett and McMahon, 1987), and the relative humidity was maintained at low levels (40-60%), it is likely that the crayfish experienced some degree of evaporative heat loss in air during these experiments. Previous studies of fiddler crabs, *Uca spp.* (Edney, 1961; Thurman, 1998), and the shore crab, *Carcinus maenas* (Taylor and Wheatly, 1979), show that evaporative cooling occurs in air of low relative humidity, but is drastically reduced during exposure

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

to saturated air. This was also demonstrated in the present study (Figs 7a, b). It is likely that heat loss through evaporative cooling temporarily slowed the rate of heat gain when crayfish were exposed to 35°C air, and enhanced their rate of heat loss in 12°C air (Fig.4). This would explain why crayfish failed to equilibrate when exposed to 35°C air for 3h, and why animals exposed to 12°C air reached thermal equilibrium within 25min.

Live crayfish equilibrated to changes in water temperature in less time than dead crayfish. Live crayfish were active during the experiments, and locomotion results in an increased flow of hemolymph to tissues (Jorgensen *et al.*, 1989, Reiber *et al.*, 1997), therefore it is likely that their gills were being perfused with larger volumes of hemolymph than the dead animals. As the gills are the primary site for heat exchange in crayfish (Taylor, 1982), an increase in gill perfusion would result in an increased rate of heat loss or gain for live crayfish, compared to dead animals. Also, the exchange of branchial water is probably reduced in dead crayfish due the cessation of the scaphognathite beating, and the stagnation of branchial water may slow heat transfer. There was no difference in the amount of time required for live and dead crayfish to reach core temperatures of 27.5°C, in 35°C air. This result was unexpected, as the core temperatures of live *Uca spp.* are 5°C-8°C lower than those of dead crabs in air of 75% relative humidity; this difference was attributed to evaporative cooling by the live animals (Edney, 1961). However, when transferred to 12°C air, live crayfish reached thermal equilibrium in less time than dead crayfish, presumably also due to evaporative cooling.

Because *P. clarkii* will experience large fluctuations in temperature in the Mojave Desert, the thermal survival limits for this amphibious species were determined in both

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

water and air. In water, the CTMax and CTMin for *P. clarkii* were similar to critical temperature values recorded for another crayfish. Rusty crayfish, *Orconectes rusticus*, acclimated to 22°C tolerated slightly warmer temperatures (37°C) than *P. clarkii*, while the thermal minimums of these two species were approximately equal at 8.5°C (Mundahl and Benton, 1990). Upon removing *P. clarkii* from the 36°C water, the crayfish appeared to be thermally stressed and often required several hours to recover full locomotion. However, the animals exposed to their CTMin temperature in water recovered quickly. This was expected, as exposure to high temperatures is known to disrupt nervous function in crustaceans (Lagerspetz and Bowler, 1993; Korhonen and Lagerspetz, 1996) and to alter enzyme structures and functions (Willmer *et al.*, 2000).

There are no previous reports on thermal survival limits for *P. clarkii* in air. The CTMax of crayfish in air was similar to the thermal maximum in water, but the CTMin in air was significantly elevated from that in water. However, the results of the CTMax in air experiment should be interpreted cautiously since the rate at which crayfish were heated (0.2°C/min) was very close to the rate of core temperature equilibration (approximately 0.3°C/min) that animals experienced when placed in 35°C air (Fig. 4). It was not possible to directly measure core temperatures during the experiments because the thermocouples tended to tangle around the legs of the crayfish and hinder the righting reflex. Therefore the air temperature at the surface of the animals was measured. But it is unlikely that this had any effect on the results of the CTMin in air experiment, as the rate of body temperature equilibration when animals were exposed to 12°C air (approximately 0.7°C/min) was clearly greater than the rate at which animals were cooled (Fig. 4). Evaporative cooling from the gill chambers may have occurred during the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CTMax and CTMin experiments, due to the low relative humidity regime (40-60%) maintained in the incubator. However, the effects of evaporative water loss on body temperature were probably minimized by shaking the crayfish, towel drying them, and then keeping them in air for 30min prior to experimentation.

In the control experiments, the repeated handling of crayfish in water and air did not affect the ability of animals to perform the righting reflex. This suggests that the endpoint of the CTMax and CTMin tests were due to adverse temperatures.

Although *P. clarkii* can survive a wide range of temperatures, the temperature regime selected by this species is much narrower. There was no difference in the temperature selection of male, female, and ovigerous crayfish. These findings were consistent with another study that determined the final temperature preferendum of *P. clarkii* to be 23.4°C, and this was independent of acclimation temperature, developmental stage, and body weight (Espina *et al.*, 1993). The rusty crayfish, *O. rusticus*, has a similar thermal tolerance to that of *P. clarkii* and also exhibits no preference based upon sex (Mundahl and Benton, 1990). It has been suggested that the final preferendum that crayfish select represents the optimal thermal conditions for locomotion, foraging, breeding, as well as the temperature at which survival and growth rates are maximized (Kivivuori, 1994).

Additional thermal preference experiments were conducted to determine the temperatures at which this species would migrate into air. Emigrations in response to temperature have been observed in other species of decapod crustaceans. The green shore crab, *C. maenas*, emigrates from water into air when temperature reaches 28°C (Taylor and Wheatly, 1979), while purple shore crabs, *H. nudus*, will emigrate from water when temperature reaches 26°C (McGaw, 2003). Interestingly, *P. clarkii* exited the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

water approximately 4°C prior to reaching either its CTMax or CTMin temperature, suggesting that this exiting behavior is employed in an attempt to avoid critical temperature regimes.

To ensure that the observed temperature avoidance behavior was a consequence of temperature change rather than in response to hypoxia, the oxygen tensions were maintained at a minimum of 16.0 kPa during the experiments, which is well above the exit-tensions recorded for several species of crayfish. When exposed to gradual hypoxia *P. clarkii* exited from water of 6.2 kPa, which was similar to values (7.2 kPa) recorded previously for this species (Huner and Barr, 1991). Observations of other crayfish species have determined that *O. rusticus* will remove itself from water at 4.0 kPa (McMahon and Wilkes, 1983), and the Australian Yabby, *Cherax destructor*, displays an increased frequency of emersion behavior when experiencing oxygen tensions of 2.7 kPa or below (Morris and Callaghan, 1998). Furthermore, *P. clarkii* has a high hemocyanin oxygen affinity (P_{50}=3.2-3.8 torr at 15°C, pH 7.85) and is well adapted to the hypoxic and hypercapnic conditions experienced in the burrow environment (McMahon and Hankinson, 1993). Therefore, the crayfish in the present study were exiting water due to a temperature avoidance response and not because the oxygen tensions were too low.

The ambient temperature in the burrow chamber affected many aspects of the exiting behaviors displayed by *P. clarkii*. Crayfish exhibited very little activity when exposed to temperatures of 12°C and 16°C (Figs. 5,6). This lack of activity at the lower temperatures was consistent with the temperature avoidance experiment where only 16 of 30 (53%) animals exited from cooled water. Similar findings have been reported in *H. nudus*, where only 30-45% of crabs exited from water that was cooled. As with the crayfish in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the present study, *H. nudus* that failed to enter air also reached their CTMin temperature and became incapacitated (McGaw, 2003). These responses suggest that exposure to the thermal minimum may not be physiologically detrimental to some decapod crustaceans, when compared to CTMax exposure. Because the water temperature that crustaceans are exposed to is negatively correlated with tissue pH (Whiteley *et al.*, 1995b) and positively correlated with the p50 of hemocyanin (Taylor, 1981; Truchot, 1983; Eshky *et al.*, 1996), animals exposed to their CTMin temperature in water probably experience little physiological disturbance. In support of this, *P. clarkii* recovered quickly, even after being exposed to several degrees below its CTMin temperature. *H. nudus* also recovers from exposure to their CTMin temperatures within minutes, and recovery is still rapid after several hours exposure to this temperature (McGaw, 2003). Also, the crayfish, *A. astacus* can over-winter at temperatures below 4°C (Kivivuori, 1994). The lack of activity by crayfish at low temperatures, in the present study, may also be the result of a suppression of temperature dependent metabolic processes such as heart rate and oxygen consumption rates (Taylor and Wheatly, 1979, 1981; Eshky *et al.*, 1995, 1996; Whiteley *et al.*, 1995b; Stillman and Somero, 1996; De Pirro *et al.*, 1999).

Crayfish were significantly more active at 22°C compared to 12°C and 16°C. They displayed the greatest number of unilateral emersions at this temperature (Fig. 5a). This high occurrence of unilateral emersions was unexpected because this behavior is typically a response to aquatic hypoxia, and has not been shown to be temperature dependent (McMahon and Wilkes, 1983; Huner and Barr, 1991; Morris and Callaghan, 1998). In natural burrows, *P. clarkii* probably use emersion behaviors regularly to meet their oxygen requirements, since the water present in their burrows tends to be extremely

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

hypoxic (Horwitz *et al.*, 1985; Huner and Barr, 1991). The unilateral emersions observed in the present study were not due to hypoxia, because oxygen tensions were maintained at high levels. However, emersion responses have also been observed in crayfish inhabiting small volumes of water (McMahon and Wilkes, 1983), and the volume of water accessible to crayfish in the burrow chamber was only 0.5-L. When the crayfish were observed in a larger chamber (containing 15-L of water, maintained at 22°C and 15.8 kPa ± 0.8 kPa), they did not display the unilateral emersion response. Therefore, confining crayfish to a small volume of water may have accounted for the occurrence of unilateral emersions in the burrow chamber. The significant increase in this behavior at 22°C may be explained by the fact that burrow water temperatures in the field were consistently between 19°C and 21°C (Fig. 2). Therefore, 22°C represents the experimental temperature closest to that encountered by this population of *P. clarkii* in their natural burrows, where they would frequently be eliciting the unilateral emersion response.

The number of exits displayed as well as the time spent in air by crayfish at 28°C, was similar to the levels observed at 12°C and 16°C (Figs. 5,6). However, the majority of emigrations (89%) into air at this temperature were patrols, while the majority of the emigrations made at the other temperatures were primarily those described by Taylor and Wheatly (1979), where the animals remained inactive near the water-air interface. While displaying patrols, the crayfish would climb up and down the burrow tube and would often reach the burrow entrance. This increase in patrolling at 28°C may have been the result of an increased metabolic rate due to exposure to higher temperature (Taylor and Wheatly, 1979, 1981; Eshky *et al.*, 1995, 1996; Whiteley *et al.*, 1995b; Stillman and Somero, 1996; De Pirro *et al.*, 1999). Nevertheless, while patrolling the crayfish

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

probably experienced a decrease in body temperature due to evaporative cooling in the low humidity (40-60%) air. In the natural environment the crayfish could exploit the humidity and temperature gradient in the burrows to control their core temperature. Even though crayfish may encounter warmer air temperatures when moving toward the burrow entrance (Fig. 2), because the humidity in the desert environment is very low, they would gain the benefits of evaporative cooling. Patrolling was rarely observed at 34°C. As this temperature is close to their CTMax, they would already be experiencing thermal stress, and increased exercise results in an increase in metabolism (see Taylor, 1982), which would exacerbate any physiological disturbances.

Crayfish exposed to 34°C displayed the greatest numbers of bilateral emersions and emigrations (Fig. 5a). Since this temperature regime was close to the CTMax of 36°C, the behaviors were probably employed by the crayfish in an attempt to decrease their core temperature. In support of this, the core temperatures of animals decreased immediately and significantly (2.1°C) upon moving into air of 40% RH (Fig. 7a). This decrease in core temperature occurred even though the surrounding air was 4°C warmer than the water. The magnitude of the core temperature decrease was significantly reduced in air of 75% relative humidity (Fig. 7b). Because it was difficult to exactly replicate the burrow water temperatures when switching between the two humidity regimes, water temperatures within 2°C of each other were deemed acceptable. The temperature decreases in the present study were reflective of values recorded for other crustaceans, in air of low and high relative humidity (Edney, 1961; Taylor and Wheatly, 1979; Thurman, 1998). Thus, heat loss through evaporative cooling was probably the primary thermoregulatory mechanism being exploited by crayfish. This is advantageous because

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

decreasing body temperature would result in a decrease in both oxygen demand and heart rate (Taylor and Wheatly, 1979, 1981; Eshky *et al.*, 1995, 1996; Whiteley *et al.*, 1995b; Stillman and Somero, 1996; De Pirro *et al.*, 1999).

The periods of emersion that *P. clarkii* made at 34°C were associated with frequent dipping. This was atypical of behaviors associated with periods of emersion and emigration for other amphibious decapod crustaceans. When heated in water, *C. maenas* emigrates and remains quiescent in air for the duration of experiments (3-4h), and does not return to the water (Taylor and Wheatly, 1979). Likewise, after *H. nudus* emigrates into air, it can remain there for up to 5h without re-submerging (McGaw, 2003). Exposure to warm air has a drastic effect on acid-base status in decapod crustaceans. For example, in the purple shore crab, *H. nudus*, increases in PCO_2 and decreases in pH are more pronounced after 15min in 25°C air, than during 15min exposure to 10°C air (Morris *et al.*, 1996b). Therefore, the dipping behavior displayed by *P. clarkii* is potentially advantageous in that it provides the crayfish with the benefits of evaporative cooling, without requiring them to endure the physiological costs associated with longer emersion stress. It is likely that crayfish received the maximum benefits of evaporative cooling during these excursions, because the core temperature decreased by over 2°C, however, once the body temperature began to increase, this triggered the crayfish to re-submerge. Dipping may aid in preventing the accumulation of potentially harmful byproducts of metabolism such as carbon dioxide and ammonia, acquired during air-exposure. No difference in the hemolymph pH of the rusty crayfish, *O. rusticus*, exists between animals making trips of up to 6.5min into air compared to that of submerged animals (McMahon and Wilkes, 1983). Also, the Australian yabby, *C. destructor*, can

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

remain in air for up to 30min without experiencing any significant changes in blood pH or PCO_2 (Morris and Callaghan, 1998). Therefore, this frequent dipping may be a way to release carbon dioxide from the system, and thus maintain acid-base homeostasis. Finally, dipping may enable the maintenance of aerobic respiration during aerial exposure. Although lactate levels were not measured for *O. rusticus*, hemolymph oxygen tensions are not different from control values after 6.5min excursions into air (McMahon and Wilkes, 1983). There is no significant accumulation of lactate in the muscle of *C. destructor* in the first 30min of aerial exposure, and although the MO_2 of *C. destructor* is significantly elevated from submerged values after 5min in air, careful analysis of oxygen consumption traces indicate that MO_2 is probably not elevated prior to 3min exposure to air (Morris and Callaghan, 1998).

This work suggests a mechanism that may allow crayfish to survive the extreme temperatures experienced in the Mojave Desert. Discernment of the thermoregulatory behaviors of *P. clarkii* may allow us to better understand this species an invader. It was not possible to monitor behavior in the natural burrow environment and these thermoregulatory mechanisms could be modulated in response to oxygen or pH status of the burrow water. Imposed upon this, endogenous activity rhythms and feeding history could also influence behavior. This is an exciting area that will be pursued in future studies.

The present study has shown that the burrow environment of *P. clarkii* is a thermally stable refuge from adverse surface conditions (Fig. 3), and provides the crayfish with a source of standing water and humid air during periods of drought (Huner and Barr, 1991). Thus this animal-built structure provides physiological benefits to its inhabitant. Indeed

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

many other organisms are able to exploit the properties of their structures in order to control physiological parameters (see Turner, 2000). In the artificial burrow chamber, *P. clarkii* used a complex series of behaviors to exploit the thermal properties of water and air, which allowed them to survive exposure to temperature regimes close to their thermal limits.

These experiments provide evidence that the use of behavior may compensate for physiological disturbances and emphasize the importance of exploring the physiological parameters of organisms, in conjunction with behavior. Clearly neither can be considered in isolation.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

REFERENCES

Atkinson, R.J., and A.C. Taylor. 1988. Physiological ecology of burrowing decapods. Symp. Zool. Soc. Lond. 59: 201-226.

Burggren, W.W., and B.R. McMahon. 1981. Oxygen uptake during environmental temperature change in hermit crabs: adaptation to subtidal, intertidal, and supratidal habitats. Physiological Zoology 54(3): 325-333.

Burnett, L.E., and B.R. McMahon. 1987. Gas exchange, hemolymph acid-base status, and the role of branchial water stores during air exposure in three littoral crab species. Physiological Zoology 60: 27-36.

Correia, A.M., and O. Ferreira. 1995. Burrowing behavior of the introduced red swamp crayfish *Procambarus clarkii* (Decapoda: Cambaridae) in Portugal. Journal of Crustacean Biology 15(2): 248-257.

Crossin, G.T., S.A. Al-Ayoub, S.H. Jury, W.H. Howell, and W.H. Watson III. 1998. Behavioral thermoregulation in the American lobster *Homarus americanus*. Journal of Experimental Zoology 201: 365-374.

Cuculescu, M., D. Hyde, and K. Bowler. 1998. Thermal tolerance of two species of marine crab, *Cancer pagurus* and *Carcinus maenas*. Journal of Thermal Biology 23(2): 107-110.

deFur, P.L. 1988. Systematic adaptations to air exposure in intertidal decapod crustaceans. American Zoologist 28: 115-124.

Dejours, P. 1981. Principles of comparative physiology. North Holland Publishing Co., Amsterdam.

De Pirro, M., S. Cannicci, and G. Santini. 1999. A multi-factorial experiment on heart rate variations in the intertidal crab *Pachygrapsus marmoratus* 135: 341-345.

Durand, F., and M. Regnault. 1998. Nitrogen metabolism of two portunid crabs, *Carcinus maenas* and *Necora puber*, during prolonged air exposure and subsequent recovery: a comparative study. Journal of Experimental Biology 201: 2515-2528.

Edney, E.B. 1961. The water and heat relationships of fiddler crabs (*Uca* spp.). Trans. Roy. Soc. South Africa 36: 71-91.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Eshky, A.A., R.J. Atkinson, and A.C. Taylor. 1995. Physiological ecology of crabs from Saudi Arabian mangrove. Marine Ecology Progress Series 126: 83-95.

Eshky, A.A., A.C. Taylor, and R.J. Atkinson. 1996. The effects of temperature on aspects of respiratory physiology of the semi-terrestrial crabs, *Uca inversa* (Hoffmann) and *Metopograpsus messor* (Forskal) from the Red Sea. Comparative Biochemistry and Physiology 114A(4): 297-304.

Espina, S., F.D. Herrera, and L.F. Buckle. 1993. Preferred and avoided temperatures in the crayfish *Procambarus clarkii*. Journal of Thermal Biology 18(1): 35-39.

Greenaway, P., S. Morris, B.R. McMahon, C.A. Farrelly, and K.L. Gallagher. 1996. Air breathing by the purple shore crab, *Hemigrapsus nudus* (Dana). I. Morphology, behavior, and respiratory gas exchange. Physiological Zoology 69(4): 785-805.

Hobbs, H.H. 1981. The crayfishes of Georgia. Smithsonian contributions to zoology 318: 1-549.

Horwitz, P.H., A.M. Richardson, and A. Boulton. 1985. The burrow habitat of two sympatric species of land crayfish, *Engaeus urostrictus* and *E. tuberculatus* (Decapoda: Parastacidae). Victorian naturalist 102(6): 188-197.

Huner, J.V., and J.E. Barr. 1991. Red swamp crawfish: Biology and exploitation. Louisiana Sea Grant College Program, Louisiana.

Johnson, I., and R.F. Uglow. 1985. Some effects of aerial exposure on the respiratory physiology and the blood chemistry of *Carcinus maenas* and *Liocarcinus puber*. Journal of Experimental Marine Biology and Ecology 52: 151-165.

Jorgensen, D.D., C. Asay, T.L. Daniel, and A.P. Farrell. 1989. Circulatory responses to pedal locomotion the lobster. American Zoologist 29(4): 54A.

Kivivuori, L.A. 1994. Temperature selection behavior of cold- and warm-acclimated crayfish [*Astacus astacus* (L.)] Journal of Thermal Biology 19(5): 291-297.

Korhonen, I.A., and K.Y. Lagerspetz. 1996. Heat shock response and thermal acclimation in *Asellus aquaticus*. Journal of Thermal Biology 21(1): 49-56.

Lagerspetz, K.Y., and K. Bowler. 1993. Variation in heat tolerance in individual *Asellus aquaticus* during thermal acclimation. Journal of Thermal Biology 18(3): 137-143.

Layne, J.R., D.L. Claussen, and M.L. Manis. 1987. Effects of acclimation temperature, season, and time of day on the critical thermal maxima and minima of the crayfish *Orconectes rusticus*. Journal of Thermal Biology 12(3): 183-187.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Lewis, D.H., and R.D. Roer. 1988. Thermal preference in distribution of blue crabs, *Callinectes sapidus*, in a power plant cooling pond. Journal of crustacean biology 8(2): 283-289.

McGaw, I.J. 2003. Behavioral thermoregulation in the amphibious purple shore crab, *Hemigrapsus nudus*. Biological Bulletin 204 (in press).

McMahon, B.R., and J.J. Hankinson. 1993. Respiratory adaptations in burrowing crayfish. Freshwater Crayfish 9: 174-182.

McMahon, B.R., and P.R. Wilkes. 1983. Emergence responses and aerial ventilation in normoxic and hypoxic crayfish *Orconectes rusticus*. Physiological zoology 56(2): 133-141.

Morris, S., and J. Callaghan. 1998. The emersion response of the Australian Yabby *Cherax destructor* to environmental hypoxia and the respiratory and metabolic responses to consequent air-breathing. Journal of Comparative Physiology B 168: 389-398.

Morris, S., P. Greenaway, B.R. McMahon. 1996a. Air breathing by the purple shore crab, *Hemigrapsus nudus* (Dana). II. Respiratory gas and acid-base status in response to emersion. Physiological Zoology 69(4): 806-838.

Morris, S., P. Greenaway, B.R. McMahon. 1996b. Air breathing by the purple shore crab, *Hemigrapsus nudus* (Dana). III. Haemocyanin function in respiratory gas transport. Physiological Zoology 69(4): 839-863.

Mundahl, N.D., and M.J. Benton. 1990. Aspects of the thermal ecology of the rusty crayfish *Orconectes rusticus* (Girard). Oecologia 82: 210-216.

Powers, I.W., and J.F. Cole. 1976. Temperature variation in fiddler crab microhabitats. Journal of Experimental Marine Biology and Ecology 21: 141-157.

Rebelo, M.F., E.A. Santos, and J.M. Monserrat. 1999. Ammonia exposure of *Chasmagnathus granulata* (Crustacea, Decapoda) Dana, 1851: accumulation in haemolymph and effects on osmoregulation. Comparative Biochemistry and Physiology A 122: 429-435.

Reiber, C.L., and G.F. Birchard. 1993. Effect of temperature on metabolism and hemolymph pH in the crab *Stoliczia abboti*. Journal of Thermal Biology 18: 49-52.

Reiber, C.L., B.R. McMahon, and W.W. Burggren. 1997. Cardiovascular functions in two macruran decapod crustaceans (*Procambarus clarkii* and *Homarus americanus*) during periods of inactivity, tail flexion, and cardiorespiratory pauses. Journal of Experimental Biology 200: 1103-1113.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Smith, W.K., and P.C. Miller. 1973. The thermal ecology of two south Florida fiddler crabs: *Uca rapax* Smith and *U. pugilator* Bosc. Physiological Zoology 46: 186-207.

Stillman, J.H., and G.N. Somero. 1996. Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (Genus *Petrolisthes*): correlation of physiology, biochemistry and morphology with vertical distribution. Journal of Experimental Biology 199: 1845-1855.

Taylor, E.W. 1981. Some effects of temperature on respiration in decapodan crustaceans. Journal of Thermal Biology 6: 239-248.

Taylor, E.W. 1982. Control and co-ordination of ventilation and circulation in crustaceans: responses to hypoxia and exercise. Journal of Experimental Biology 100: 289-319.

Taylor, E.W., and P.J. Butler. 1973. The behaviour and physiological responses of the shore crab, *Carcinus maenas,* during changes in environmental oxygen tension. 7th European Symposium on Marine Biology 7: 496-505.

Taylor, E.W., and M.G. Wheatly. 1979. The behavior and respiratory physiology of the shore crab, *Carcinus maenas* at moderately high temperatures. Journal of Comparative Physiology B 130: 309-316.

Taylor, E.W., and M.G. Wheatly. 1981. The effect of long-term aerial exposure on heart rate, ventilation, respiratory gas exchange and acid-base status in the crayfish *Austropotamobius pallipes.* Journal of Experimental Biology 92: 109-124.

Thurman, C.L. 1998. Evaporative water loss, corporal temperature and the distribution of sympatric fiddler crabs (*Uca*) from south Texas. Comparative Biochemistry and Physiology 119A: 279-286.

Truchot, J.P. 1983. Regulation of acid-base balance. In The Biology of the Crustacea. Academic Press, New York.

Turner, J.S. 2000. The extended organism. Harvard university press, Cambridge.

Tyler-Jones, R., and E.W. Taylor. 1988. Analysis of haemolymph and muscle acid-base status during aerial exposure in the crayfish *Austropotamobius pallipes.* Journal of Experimental Biology 134: 409-422.

Whiteley, N.M., A.H. Al-Wassia, and E.W. Taylor. 1995a. The effects of sudden changes in temperature on aquatic and aerial respiration in the lobster *Homarus gammarus* (L.). Mar. Fresh. Behav. Physiol. 27(1): 13-27.

Whiteley, N.M., J.K. Naylor, and E.W. Taylor. 1995b. Extracellular and intracellular acid-base status in the freshwater crayfish *Austropotamobius pallipes.* Journal of Experimental Biology 198: 567-576.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Willmer, P., G. Stone, and I. Johnston. 2000. Environmental physiology of animals. Blackwell science, London.

Wood, S.C. 1991. Interactions between hypoxia and hypothermia. Annual Review of Physiology 53: 71-85.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

APPENDIX I

FIGURES

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

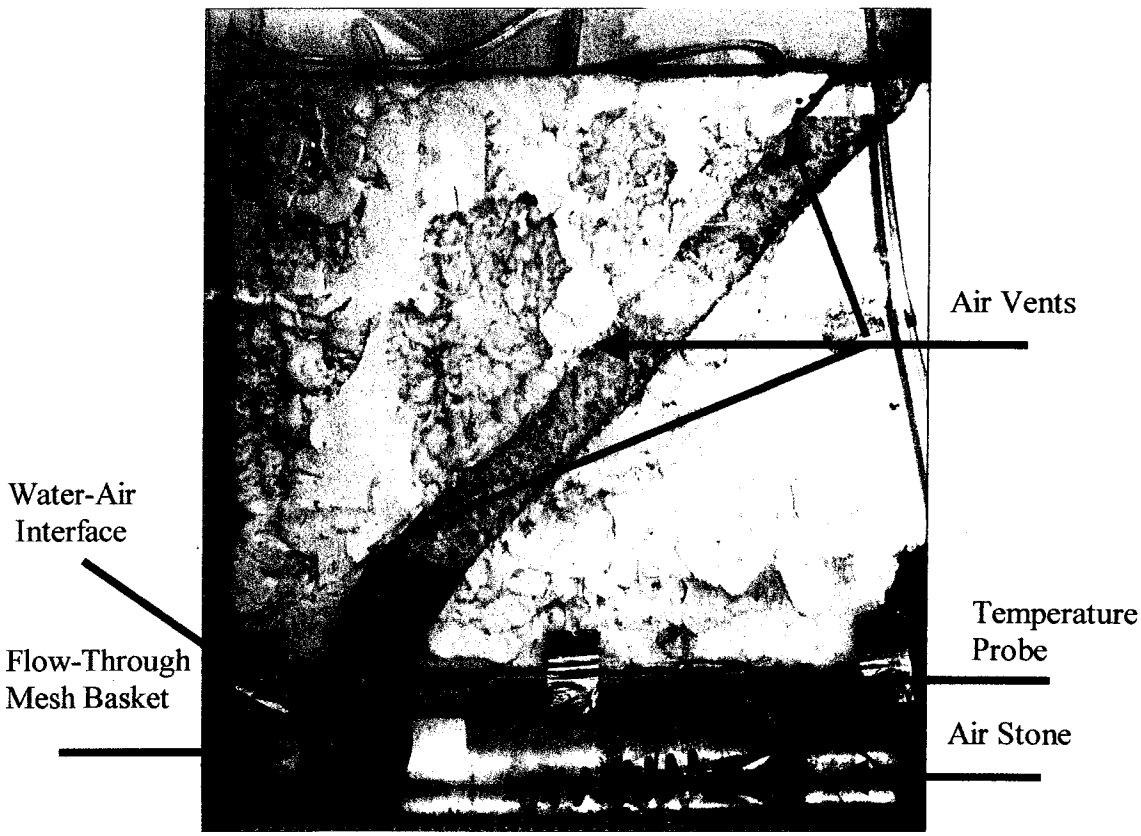

Figure 1. Artificial burrow chamber of dimensions: 90cm H x 75cm x 15cm L.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

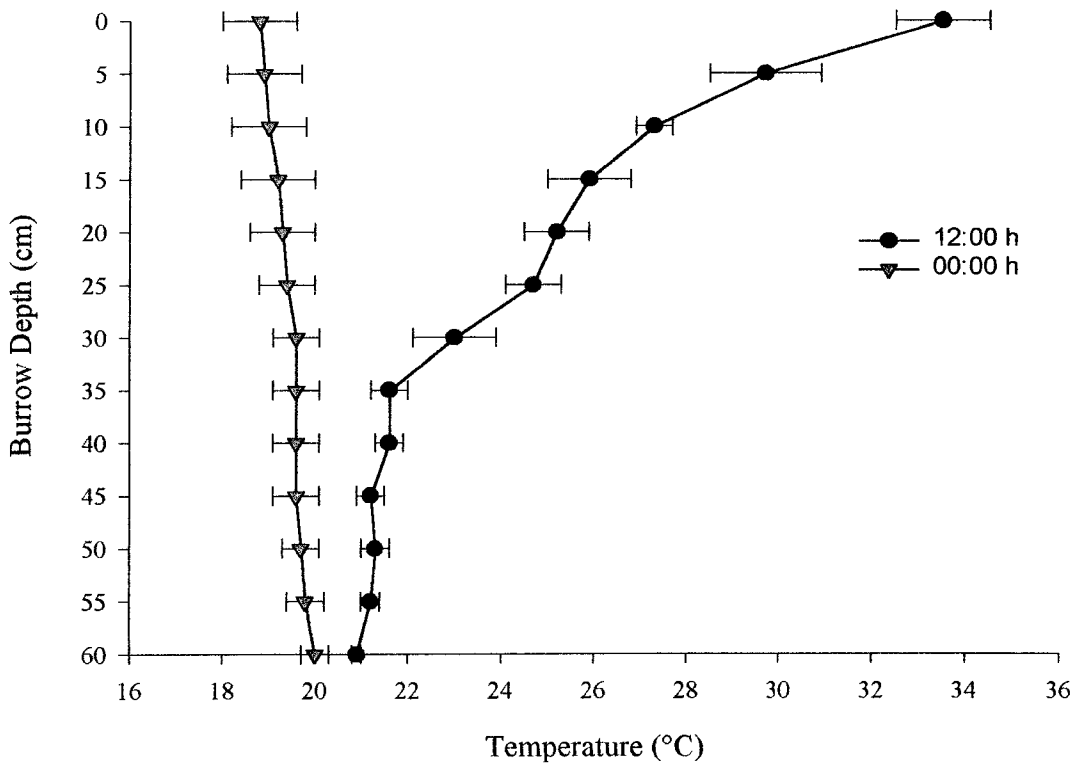

Figure 2. Air temperature profiles (mean ± SEM) for the 60cm-deep crayfish burrows measured at 12:00h and 00:00h on 6/28/02. Temperatures were recorded at 5cm intervals, from the burrow entrances to the terminal ends. Surface air temperature was 33.4°C at 12:00h and 17.6°C at 00:00h.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

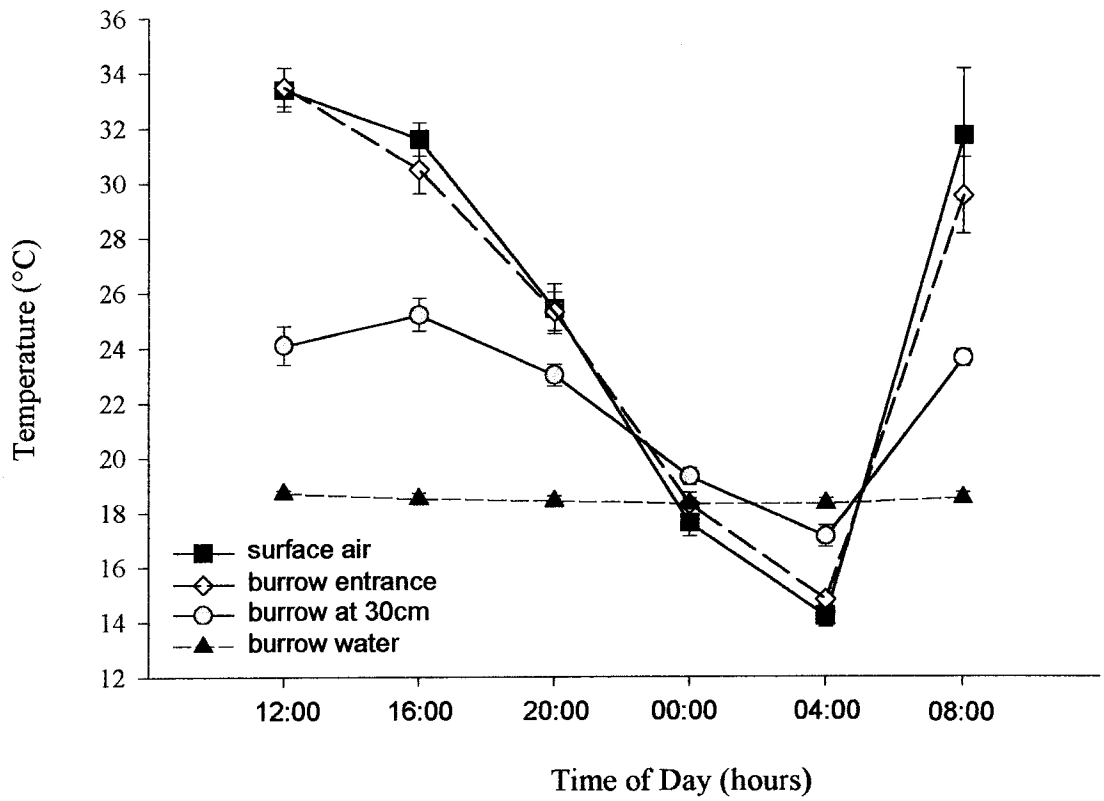

Figure 3. Diel variations (mean ± SEM) in the temperatures surrounding and within burrows at Corn Creek, on 6/28/02 (n=6). Temperatures of the surface air, air at the burrow entrance, burrow air (at a depth of 30cm), and burrow water were recorded.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

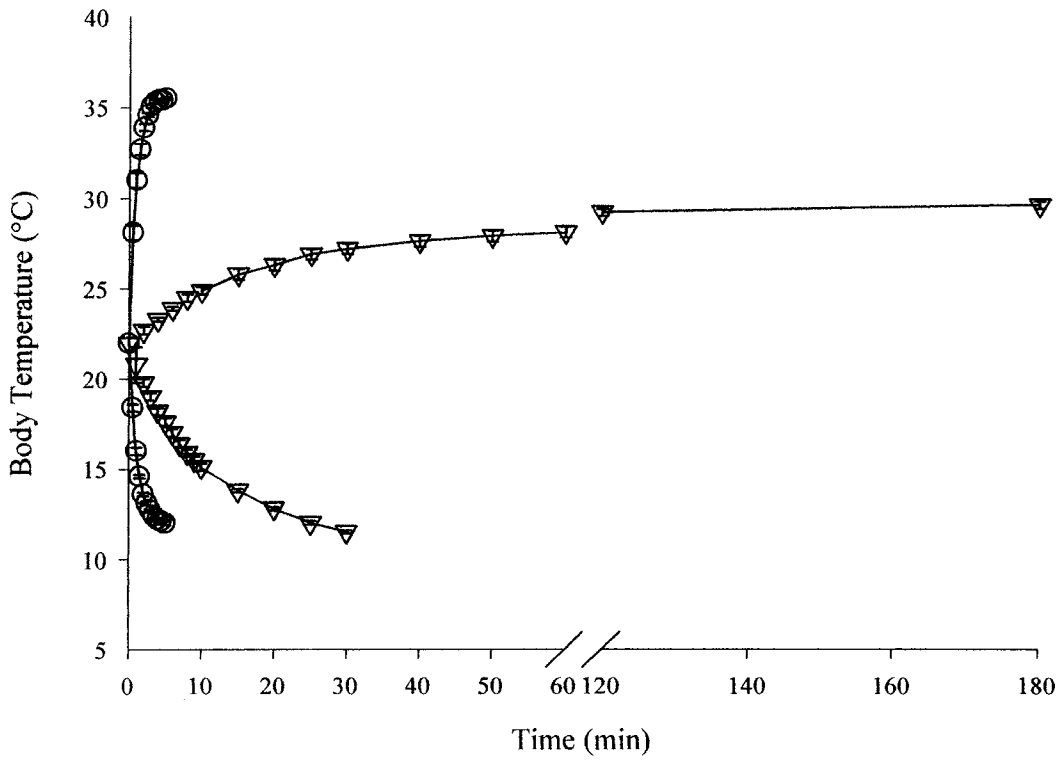

Figure 4. Changes in body temperature (mean ± SEM) of 20 *Procambarus clarkii*, upon transfer from 22°C water to water (circles) and air (diamonds) of 35°C and 12°C. Data recording was initiated once the crayfish were transferred to the new temperature (time 0). Standard errors were low, making them difficult to discern on the graph.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

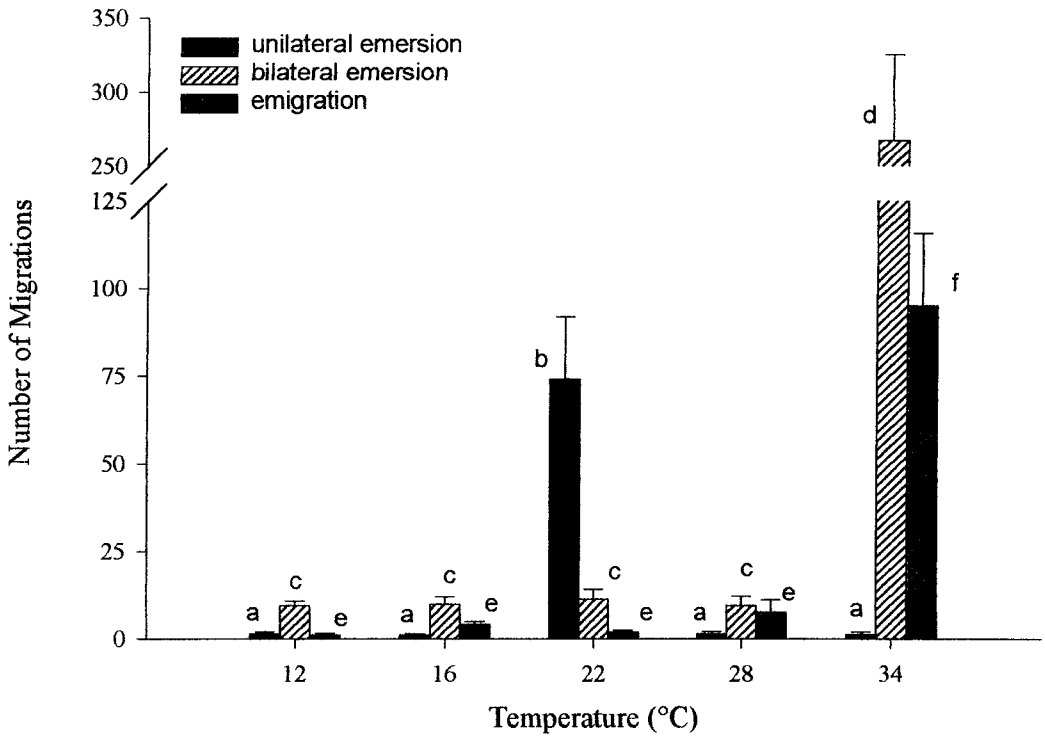

Figure 5a. The number of observed migrations from water (mean ± SEM) made in response to five temperatures. Trials were carried out in 24h periods in an artificial burrow chamber (n=8, for each temperature regime). Differences in the occurrences of the respective behaviors are represented by the letters: (a,b) unilateral emersions, (c,d) bilateral emersions and (e,f) emigrations. Bars that do not share the same letter are significantly different from each other.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

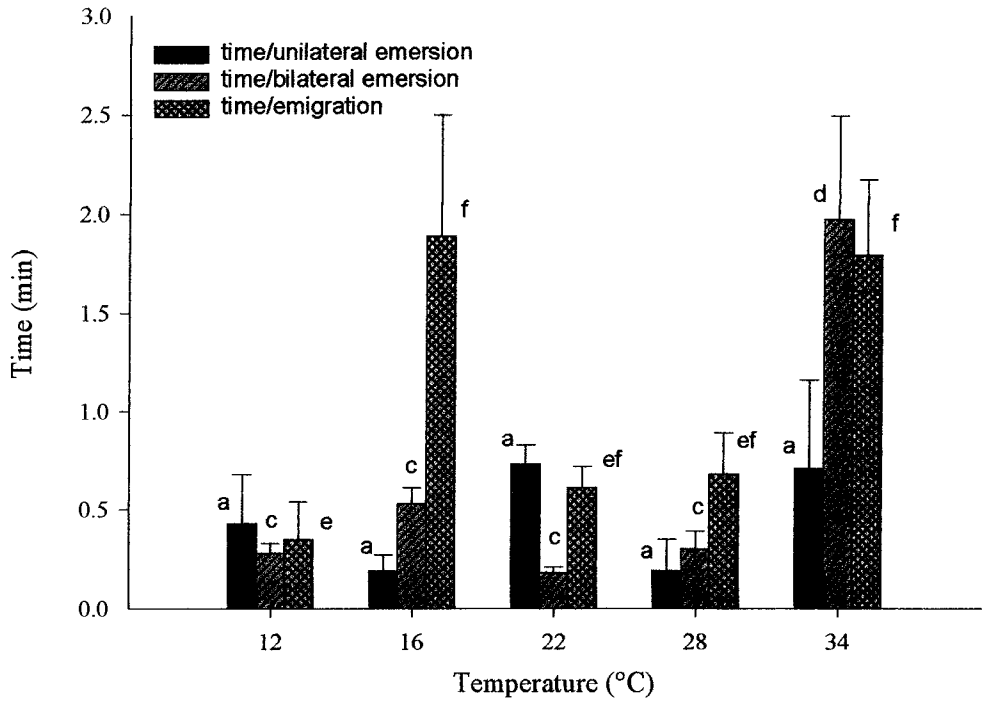

Figure 5b. The amount of time spent in air per emersion or emigration (mean ± SEM) in response to five temperatures. Trials were carried out in 24h periods in an artificial burrow chamber (n=8, for each temperature regime). Differences in the durations of the respective behaviors are represented by the letters: (a,b) unilateral emersions, (c,d) bilateral emersions and (e,f) emigrations. Bars that do not share any of the same letters are significantly different from each other.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

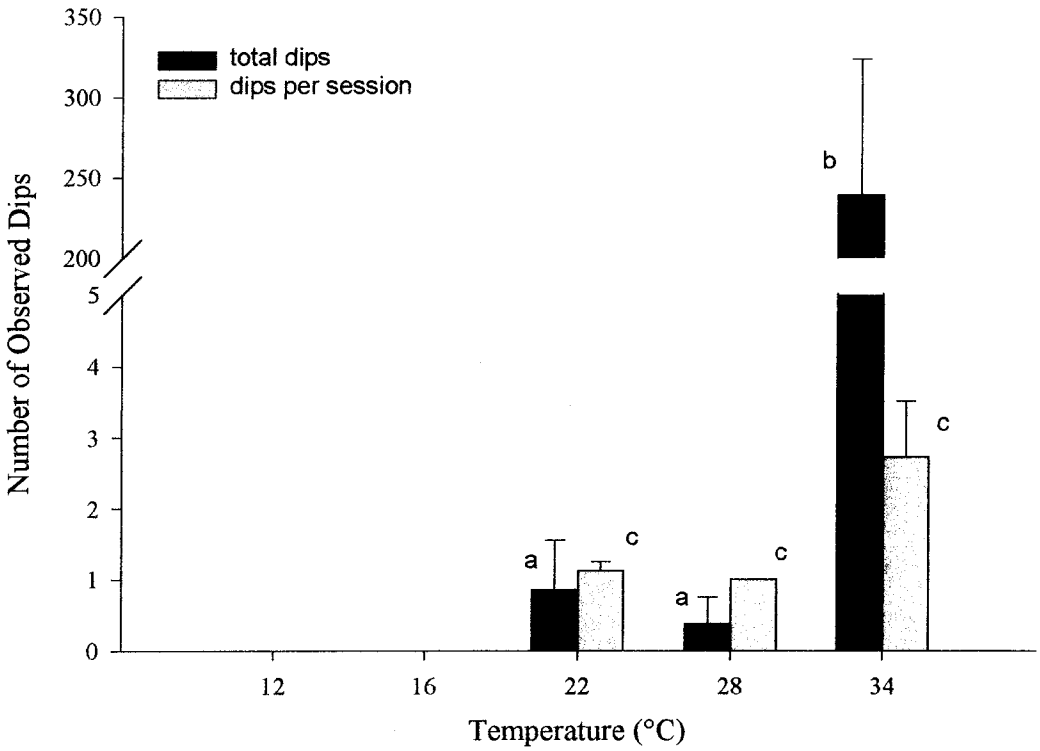

Figure 6a. The number of dips made in response to five temperatures (mean ± SEM). Trials were carried out in 24h periods in an artificial burrow chamber (n=8, for each temperature regime). The letters (a,b) represent differences in the total number of dips made (solid bars), and the letters (c,d) represent differences in the number of dips made per dipping session (shaded bars). Bars that do not share the same letter are significantly different from each other.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

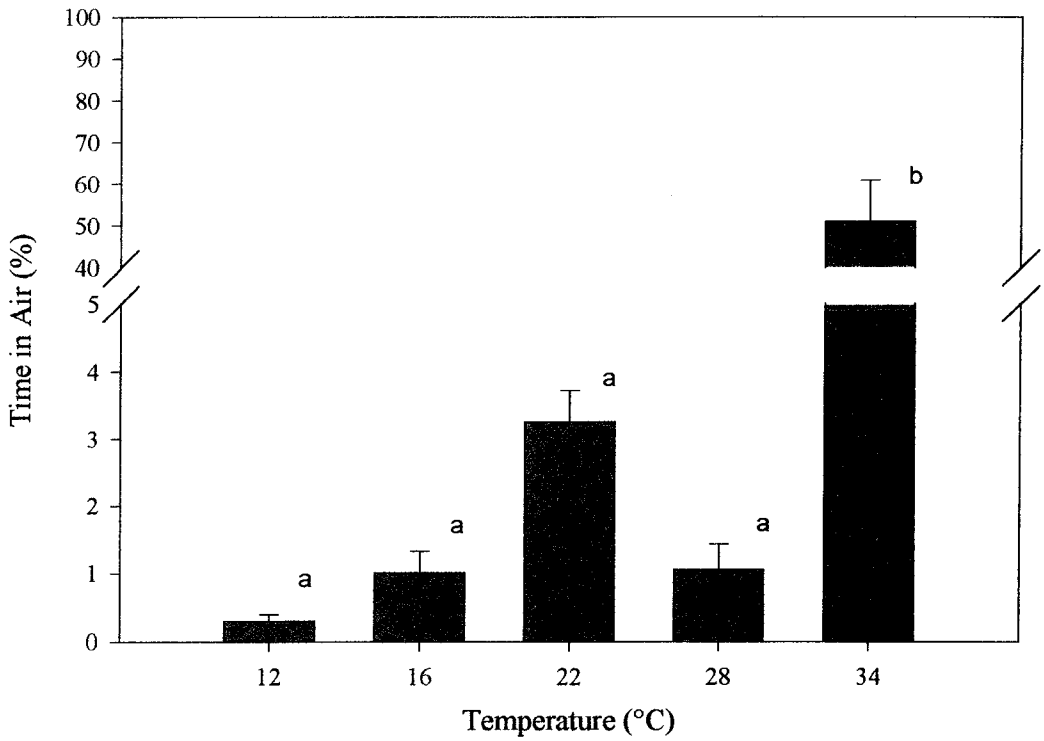

Figure 6b. The total percentage of time (mean ± SEM) spent in air in response to five temperatures. Trials were carried out in 24h periods in an artificial burrow chamber (n=8, for each temperature regime). Differences in the percentage of time spent in air are represented by the letters (a,b). Bars that do not share the same letter are significantly different from each other.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

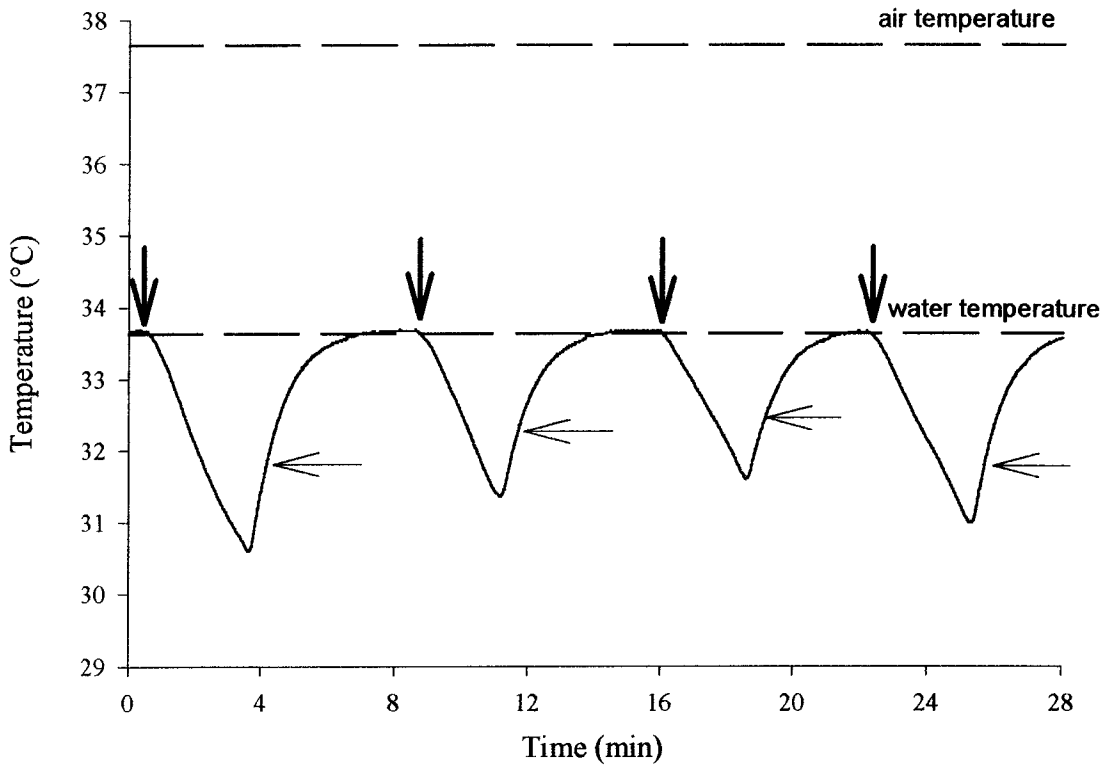

Figure 7a. Change in core temperature of an individual crayfish upon migrating from water and moving into air of 40% relative humidity (dew point temperature = 21.0°C). The bold vertical arrows represent the point of emigration and the light horizontal arrows represent the point of re-submergence. Experiments were carried out in an artificial burrow chamber.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

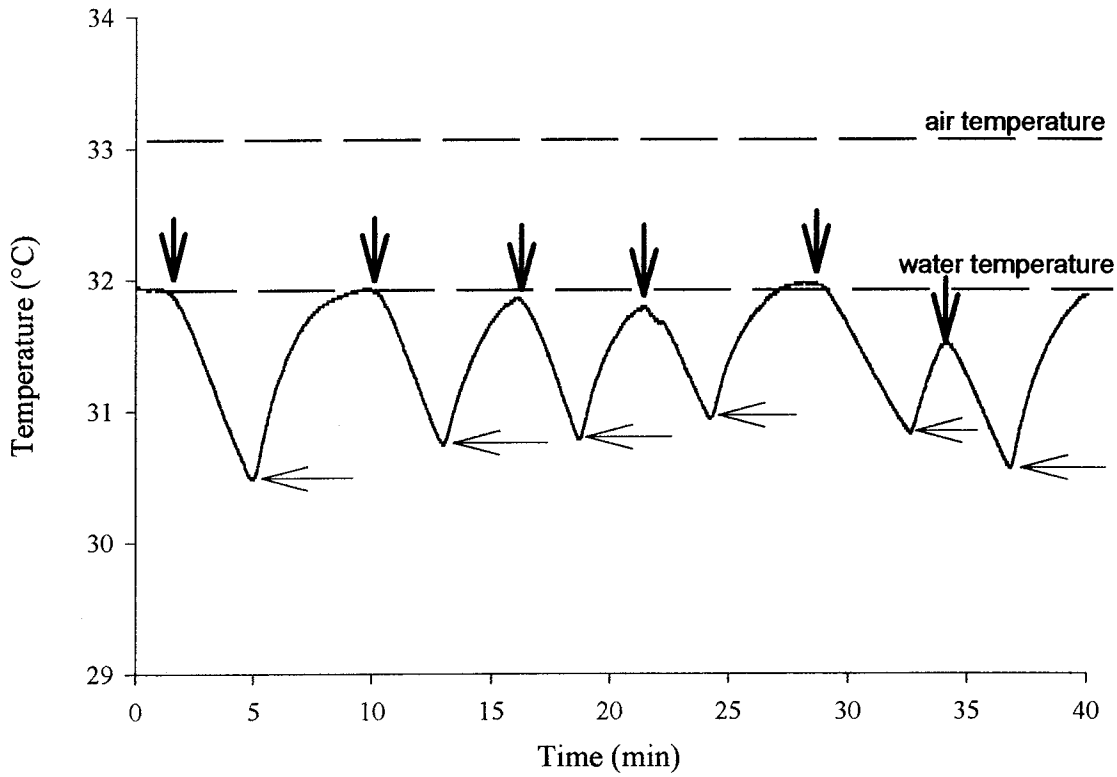

Figure 7b. Change in core temperature of an individual crayfish upon migrating from water and moving into air of 75% relative humidity (dew point temperature = 27.5°C). The bold vertical arrows represent the point of emigration and the light horizontal arrows represent the point of re-submergence. Experiments were carried out in an artificial burrow chamber.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

VITA

Graduate College
University of Nevada, Las Vegas

Aaron Lawrence Payette

Home Address:
P.O. Box 113
Sandy, OR 97055

Degrees:
Bachelor of Science, Biology, 2000
Pacific University, Forest Grove, OR

Special Honors and Awards:
2002 DBS Scholarship for Graduate and Undergraduate Students ($200)
2001 GSA Research Forum, Science Platform Presentations, 1st Place ($150)
2001 DBS Scholarship for Graduate and Undergraduate Students ($400)
2000 GSA Spring Grant ($500)

Publications:
Payette, A.L., and I.J. McGaw. 2002. Emersion behavior of the crayfish *Procambarus clarkii* in response to temperature. Proceedings of the Arizona-Nevada Academy of Science. Vol. 37.
Payette, A.L., and I.J. McGaw. 2001. Can ovigerous crayfish (*Procambarus clarkii*) control their brooding environment through behavior? Proceedings of the Arizona-Nevada Academy of Science. Vol. 36.

Thesis Title: Thermoregulatory Behavior of the Crayfish *Procambarus clarkii* in a Burrow Environment

Thesis Examination Committee:
Chairperson, Dr. Iain McGaw, Ph. D.
Committee Member, Dr. Carl Reiber, Ph. D.
Committee Member, Dr. Stephen Roberts, Ph. D.
Graduate Faculty Representative, Dr. Shawn Gerstenberger, Ph. D.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.