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EFFECTS OF LIPOPOLYSACCHARIDE, ANTIPYROGEN, AND BODY CONDITION ON THE CHRONOPHARMACOLOGY OF FEVER IN *DIPSOSAURUS DORSALIS*

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EFFECTS OF LIPOPOLYSACCHARIDE, ANTIPYROGEN, AND BODY CONDITION ON THE CHRONOPHARMACOLOGY OF FEVER IN *DIPSOSAURUS DORSALIS*

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ABSTRACT

I examined the effects of two doses (2.5 mg kg^{-1} and 25 mg kg^{-1}) of a pyrogen (lipopolysaccharide, LPS) independently and in combination with an antipyrogen (acetylsalicylic acid) at two times of injection (noon and midnight) on behavioral thermoregulation of adult desert iguanas in linear thigmothermal gradients (3.60 ± 0.19 - 75.91 ± 1.14°C). I also described some aspects of the basic febrile response (latency period and duration of the response), the chronopharmacology of the febrile response, and the effects of morphological parameters on thermoregulation.

After acclimation for 4 to 5 days at 30°C with a 12:12 LD photoperiod, I recorded body temperatures in lizards that received a low or high dose of pyrogen either in the absence or presence of an antipyrogen, and whether at noon or midnight. Overall mean Tb for 48 hours after injection, for day 1, and for day 2 from these animals were compared with each other and to the Tb of control animals to elucidate the effects of these agents and time of injection on thermoregulation. Variance in Tb for these groups was analyzed to compare thermoregulatory precision under the influence of pyrogen dose, time of injection and presence or absence of an antipyrogen. Skewness of Tb response was compared among all treatment groups to compare latency period. Kurtosis of Tb response was compared among all treatment groups to compare response duration. Comparisons of Tbs within each treatment group against initial and final mass, initial and final body condition, and change in body mass revealed an influence of energy reserves on thermoregulatory decisions.

Dose and time of injection in the presence of only pyrogen affected Tb for the total run period and day 2. Animals receiving the high dose had Tbs higher than lizards

receiving the low dose. Lizards injected at noon had higher Tbs than lizards injected at midnight. All lizards that received an antipyrogen with the pyrogen exhibited Tbs similar to the Tbs of the controls on day 1 and the total run period. On day 2, lizards receiving pyrogen + antipyrogen showed a dose effect.

Time of injection affected whether or not energy reserves are the most important factor determining Tb in the face of a pyrogen + antipyrogen. Lizards injected with the low dose of pyrogen + antipyrogen at midnight exhibited positive correlations between Tb and body condition, mass, and SVL at various times during the trial. Lizards injected with the high dose of LPS and antipyrogen at midnight showed positive correlations between Tb and SVL, and lizards injected with the high dose of LPS at midnight showed positive correlations between Tb and mass change. Lizards subjected to the control treatment exhibited positive correlations between Tb and mass and body condition.

Animals that received the high dose of pyrogen + antipyrogen at midnight had higher thermoregulatory precision than those injected at noon on day 1. Control animals had higher precision than those injected with the high dose of pyrogen + antipyrogen and those injected with the high dose of pyrogen only on day 1. Midnight injections produced longer responses than did noon injections, and higher doses induced faster responses.

INTRODUCTION

Body Temperature Maintenance

> "If a definition of life were required, it must be clearly established on that capacity, by which the animal preserves its proper heat under the various degrees of temperature of the medium in which it lives. The most perfect animals possess this power in a superior degree, and to the exercise of their vital functions this is necessary. The inferior animals have it in a lower degree, in a degree however suited to their functions. In vegetables, it seems to exist, but in a degree still lower, according to their more limited powers, and humbler destination… There is reason to believe, that while the actual temperature of the human body remains unchanged, its health is not permanently interrupted by the variation in the temperature of the medium that surrounds it; but a few degrees of increase or diminution of the heat of the system, produces disease and death. A knowledge therefore of the laws which regulate the vital heat, seems to be the most important branch of physiology." (Currie, 1808)

Most terrestrial thermoregulators maintain an internal body temperature between 35°C and 42°C (Kluger, 1979a) – most endotherms consistently and many ectotherms during their active period. Ectothermic animals do not have the ability to sustain this relatively high body temperature metabolically, so they must engage in behaviors that allow appropriate heat exchange between the animal's body and the environment to preserve thermal stability. In reptiles, information derived from the thermal sensitivity of the anterior brainstem and from peripheral temperature sensors interact to determine

which thermoregulatory behaviors are needed to maintain internal body temperature at a set-point temperature (Myhre and Hammel, 1969). For example, freely thermoregulating desert iguanas, *Dipsosaurus dorsalis*, maintain a body temperature of 39°C ± 1°C during active periods (Kluger, 1979a). Because the rates of most chemical reactions are largely dependent on temperature as shown by the van't Hoff-Arrhenius law, maintenance of a precise body temperature insures that the biochemical reactions of the body proceed both optimally and efficiently in homeothermic organisms by freeing them from temperature fluctuations of the ambient temperature (Blatteis, 1998a). Because of the correlation of reaction speed and enzyme function with temperature to a certain point, higher temperatures insure faster biochemical reactions. However, enzymes denature at higher temperatures, which decreases the speed of reactions and leaves a narrow range of temperatures at which biochemical reactions can proceed optimally. By maintaining such a precise body temperature, ectotherms such as *D. dorsalis* can gain the biochemical advantages provided by constant thermal conditions without the energy cost paid by metabolically thermoregulating animals (endothermic homeotherms).

To maintain a specific internal body temperature, an ectothermic vertebrate must have a mechanism for detecting body temperature, integrating this information, comparing it to a set-point temperature, and initiating behaviors to correct for any discrepancies between body temperature and set-point temperature. In vertebrates, free nerve endings detect temperature information from the skin, abdomen, veins, hypothalamus, midbrain, and the spinal cord (Hensel, 1974) and alter their pattern of action potentials according to the temperature detected (Hensel, 1981). As temperature decreases, firing rate of cold-sensitive neurons increases; and as temperature increases,

firing rate of warm-sensitive neurons increases in primates and close relatives (Iggo, 1969) and in other mammals and lizards (Wit and Wang, 1968). This variation in firing rate reaches the hypothalamus where it is integrated with information from hypothalamic thermosensitive neurons and compared to a set-point temperature (Keller, 1933; Birzis and Hemingway, 1957; Hammel et al., 1960; Hellone, 1967; Bligh, 1973; Boulant, 1998).

Cutaneous cold-sensitive neurons depend on the rate of function of the sodium potassium pumps in the cell membrane (Boulant, 1998). Because the membrane potential of a neuron is dependent upon the maintenance of a high concentration of sodium ions on the outside of the cell and a high concentration of potassium ions on the inside of the cell, which are constantly leaking out down their concentration gradient, changes in the rate of movement of these ions from one side of the membrane to the other against their concentration gradients by the sodium-potassium pump affect resting membrane potential. Warming cold-sensitive neurons increases the rate of leakage of potassium ions to the outside of the cell and increases the rate of function of the sodium-potassium pump which leads to a higher concentration gradient of potassium ions across the cell membrane and more sodium ions on the outside of the cell. Together, these factors increase the electrical gradient across the cell membrane and make an action potential less likely to occur. Cooling cold-sensitive neurons decreases the rate of function of the sodium-potassium pump, which leads to a lower concentration gradient of potassium ions across the cell membrane and a lower rate of leakage of potassium ions to the outside of the cell. This depolarizes the cell and makes an action potential more likely to occur. Therefore, the result of warming cold-sensitive neurons is a decrease in the firing rate, and the result of cooling cold-sensitive neurons is an increase in firing rate.

Alternately, cutaneous warm-sensitive neurons depend upon the effect of temperature on the permeability of the cell membrane to sodium and potassium ions (Boulant, 1998). In warm-sensitive neurons, temperature change has a greater relative effect on the membrane permeability of sodium ions than on the membrane permeability of potassium ions (Boulant, 1998) because the resting membrane potential is already close to the equilibrium potential of potassium. Increasing the permeability of the membrane to sodium ions increases the contribution of sodium ions to the resting membrane potential as shown by the Goldman equation. Warming warm-sensitive neurons increases the permeability of the membrane to sodium ions, which increases the inward flux of sodium ions down their concentration and electrical gradients to the inside of the cell, which depolarizes the cell closer to threshold and makes an action potential more likely to occur. Cooling warm-sensitive neurons decreases the permeability of the membrane to sodium ions, which decreases the flux of sodium ions down their concentration gradient to the inside of the cell. Because fewer sodium ions are being added to the inside of the cell, hyperpolarization of the cell occurs and makes an action potential less likely to occur. Therefore, the result of warming warm-sensitive neurons is an increase in firing rate, and the result of cooling warm-sensitive neurons is a decrease in firing rate.

Hypothalamic thermosensitive neurons are responsible for gathering information about core body temperature from the blood in the vessels that run through the hypothalamus. Hypothalamic warm-sensitive neurons display a pacemaker potential or depolarizing prepotential which initiates a slow depolarization after every action potential that eventually reaches threshold and triggers the subsequent action potential (Boulant,

1998). Increases in core body temperature increase the rate of this depolarizing prepotential by inactivating potassium channels prematurely during an action potential and thus prohibiting hyperpolarization of the neuron by the outward flux of potassium ions (Boulant, 1998). Prohibition of hyperpolarization decreases the time interval between two action potentials by allowing the membrane potential to reach threshold faster, and therefore, increase the rate of action potentials in hypothalamic warm-sensitive neurons (Boulant, 1998).

Hypothalamic cold-sensitive neurons may not be intrinsically "cold-sensitive". Data suggest that hypothalamic cold-sensitive neurons may not have the ability to react to a decrease in temperature directly, but rather respond to varying degrees of inhibition from warm-sensitive neurons (Boulant, 1998). During warming, hypothalamic warm-sensitive neurons fire at higher rates, which increases the inhibition of cold-sensitive neurons and depresses their firing rates. During cooling, hypothalamic warm-sensitive neurons fire at lower rates which decreases the inhibition of cold-sensitive neurons and increases their firing rates. This thermally dependent pattern of inhibition makes this population of neurons appear to be "cold-sensitive".

During integration of thermal data from various parts of the body, not only do signals from cutaneous thermosensitive neurons affect the firing rate of hypothalamic thermosensitive neurons, but other endogenous non-thermal factors such as pyrogens, reproductive hormones, osmolality of blood, blood glucose levels, and the circadian clock affect the hypothalamic thermosensitive neurons either directly or synaptically (Boulant, 1998). Integration of these various types of information that shows body temperature to be below the set-point induces the initiation of behaviors that increase heat gain and

reduce heat loss, while integration of these various types of information that shows body temperature to be above the set-point induces the initiation of behaviors that increase heat loss and reduce heat gain. If environmental conditions allow for appropriate heat flow, a stable internal body temperature is maintained.

A variety of physiological and environmental factors influence thermoregulation in ectothermic vertebrates both by affecting the set-point temperature of an organism and the organism's perception of its set-point temperature. The concept of a set-point temperature allows for the classification of body temperature into four categories: normothermia, hypothermia, hyperthermia, and fever (Snell and Atkins, 1968). Normothermia is the condition where actual body temperature and set-point temperature coincide. Hypothermia is the condition where actual body temperature is below the set-point temperature. Hyperthermia is the condition where actual body temperature is above the set-point temperature. Fever is the condition where actual body temperature may or may not be at the set-point level, but the set-point temperature is raised. Fever is a relatively rare phenomenon in vertebrate physiology because it is an example of a regulated change in homeostasis that is not tied to circadian rhythms in a system that tolerates very little variance from the physiological set-points determined by such daily changes (Kluger, 1998).

Fever and Antipyresis

Fever-causing agents and antipyrogens act by affecting the activity patterns of thermally sensitive neurons in the hypothalamus (Kluger, 1979b). Exposure to a pyrogen causes a decrease in the firing rate of warm-sensitive neurons and an increase in the firing

rate of cold-sensitive neurons (Wit and Wang, 1968; Cabanac et al., 1968; and Eisenman, 1969) which would essentially inform the hypothalamus that body temperature is below the set-point temperature. The hypothalamus then sends messages via the nervous and endocrine systems which initiate heat gain activities to elevate the body temperature to the set-point temperature. Antipyretic drugs, including acetylsalicylate and sodium salicylate, counteract the effects of a pyrogen on the warm-sensitive neurons (Wit and Wang, 1968), which effectively negates the conveyance of information to the hypothalamus showing that body temperature is too low. Salicylates act as cyclooxygenase inhibitors in their role as cryogens, or molecules that act as mediators to attenuate fever (Kluger, 1991). Because many prostaglandins are cyclooxygenated during their conversion from a precursor molecule into a pyrogenic mediator, the presence of a salicylate decreases the ability of the cell to produce these molecules, and hence, lowers body temperature. Many prostaglandins are implicated in the down-regulation of warm-sensitive neurons (Blatteis, 1998b). Inhibition of such endogenous pyrogens production would allow for the maintenance of a higher firing rate in warm-sensitive neurons which would mitigate the effects of an exogenous pyrogen.

Most groups of ectothermic animals have members that show an increase in set-point temperature and body temperature in response to endotoxins found in the cell walls of either live or dead gram-negative bacteria (Kluger, 1979b). By phagocytosing the lipopolysaccharides (LPS) forming the endotoxin or exogenous pyrogen, the host's leukocytes trigger the release of low molecular weight proteins called endogenous pyrogens (Beeson, 1948; Bennett and Beeson, 1953) which then travel to the brain and the hypothalamus through the bloodstream to cause an increase in the set-point

temperature and ultimately an increase in body temperature, which is otherwise known as a fever (King and Wood, 1958; Cooper et al., 1967; and Jackson, 1967). In response to LPS, vertebrate mononuclear phagocytes typically release tumor necrosis factor-α (TNF-α) which stimulates the release of interleukin-1β (IL-1β) and interleukin-6 (IL-6) (Blatteis, 1998b). In addition, the release of IL-1β stimulates an increase in the release of interleukin-6 (IL-6) (Blatteis, 1998b). These cytokines are transported by the bloodstream to the brain where they directly or indirectly affect the activity of the thermosensitive neurons (Blatteis, 1998b). Because these endogenous pyrogens are large hydrophilic peptides, they are unlikely to diffuse passively across the blood-brain barrier, so many hypotheses have been proposed for their mechanism of function. These molecules may be actively transported across the blood-brain barrier (Banks et al., 1996), or may interact with sensory elements on brain structures that lack a blood-brain barrier to evoke secondary neural or chemical messages that travel to the thermosensitive neurons (Blatteis and Schic, 1997a). Microglia may have a role in amplifying and sustaining the signal from these endogenous pyrogens by producing more cytokines inside the brain (VanDam et al., 1996). In addition, afferent nerves from various places in the vertebrate body, including sensory cells and the abdomen, are implicated as a pathway of communication between circulating cytokines and the thermally sensitive neurons behind the blood-brain barrier (Blatteis and Schic, 1997b).

History of the Debate about the Adaptive Value of Fever

Human views on fever have changed over the years from reflecting a belief that fever is “good” to a belief that fever is “bad”, back to a belief that fever is “good”. The

earliest records of human attempts to understand the role of fever in disease appear in the 5th century B.C in the writings of Empedocles, Hippocrates, Plato, and Aristotle (Milton, 1998). Hippocrates suggested that fever was an attempt by the body to rid itself of the overproduction of one of the four humors (blood, phlegm, yellow bile, and black bile); and thus was the first person to suggest that fever has an adaptive value physiologically (Milton, 1998). Milton (1998) reports that the use of antipyretics did not become popular until the 19th century with the introduction of synthetic salicylates. This reflects the negative change in attitude towards the adaptive value of fever at that time, a change which has continued until very recently (Kluger, 1998).

Modern studies on fever have concentrated on elucidating the role of fever in response to infection or disease and answered many questions clinically relevant to humans such as: Is fever harmful or beneficial to the host? If fever is beneficial, what is the mechanism? Many studies show that by simply raising body temperature to a febrile level, many organisms including humans can amplify an immune response (Kluger, 1998). Benefits of an elevated body temperature during fever include enhanced phagocytosis of invading microbes, enhanced neutrophil migration to the site of infection, increased T-cell proliferation, increased oxygen radical production, increased synthesis of IFN (a cytokine that acts as an antiviral and anti-tumor factor), the decreased growth rate of iron-dependent bacteria, and the decreased viability of iron-dependent bacteria (reviewed in Blatteis, 1998b). The decreased viability of iron-dependent bacteria such as *Salmonella typhimurium* and *Escherichia coli* in the face of an increase in ambient temperature is dependent on the rate of biosynthesis of compounds involved in iron transport such as siderophores and enterochelins (Garibaldi, 1972; Kochan, 1977).

As bacterial cell temperatures increase, production of these iron transport compounds decreases, which decreases the ability of the bacteria to reproduce and gain a foothold in the febrile host's body (Garibaldi, 1972; Kochan, 1977). The numerous studies that show fever or an increase in body temperature after exposure to disease to decrease mortality rates across all groups of vertebrates indicate that fever is beneficial and therefore an adaptive response to disease. Such findings, along with studies on other important physiological responses, led Williams and Nesse to coin the term "Darwinian Medicine" (reviewed in Kluger, 1998). Data suggest that the adaptive roles of fever in fighting disease fall into three basic categories: fever as a highly regulated response, the evolutionary history of fever, and the role of fever in decreasing morbidity and mortality rates (Kluger, 1998). These findings have led physicians away from the standard Western medicine approach to fighting disease, which often had them treating adaptive responses to disease rather than the disease itself, towards a more modern approach which involves treating the harmful effects of an infection or disease without interfering with the body's adaptive response that is fever (Styrt and Sugarman, 1990; Milton, 1998; Ryan and Levy, 2003**)**.

The first body of evidence suggesting that fever is an adaptive mechanism for coping with disease deals with the very highly regulated processes involved in increasing the thermoregulatory set-point of the body and maintaining higher body temperatures to match this set-point while insuring that body temperature does not increase to dangerous levels. When an organism is faced with an exogenous pyrogen such as LPS from a bacterial cell wall, the host organism releases many types of endogenous pyrogens to increase body temperature and many types of endogenous cryogens to modulate the rise

in body temperature and insure that body temperature does not increase to damaging levels (Kluger, 1991; Kluger, 1998). Increasing body temperature to a degree high enough to be beneficial but low enough to be safe involves precise management of numerous cytokines, hormones, and effector responses via complex feedback loops. The complexity of these temperature-regulating processes argues for the evolution of fever as a host defense mechanism rather than as a simple symptom of disease (Kluger, 1998).

The second body of evidence suggesting fever to be an adaptive mechanism for coping with disease deals with the ancient phylogenetic history of fever (Kluger, 1998). Almost all endothermic vertebrates, ectothermic vertebrates, and many invertebrates exhibit fevers in the face of endotoxin (Kluger, 1998). In addition, organisms as simple as a single-celled paramecium show higher temperature preferences after exposure to pyrogens (Kluger, 1998). Invertebrate ectotherms that display a behavioral fever in response to a supposed pyrogen include *Nephelopsis obscura* (leech), *Limulus polyphemus* (horshoe crab), *Cambarus bartoni* (crayfish), *Penaeus duorarum* (shrimp), *Homarus americanus* (lobster), *Buthus occitanus* (scorpion), *Androctonus australis* (scorpion), *Acheta domesticus* (cricket), *Onymacris plana* (beetle), *Gromphadorhina portentosa* (cockroach), *Gryllus bimaculatus* (cricket), *Cammula pelucida* (grasshopper), and *Melanoplus sanguinipes* (grasshopper) (Casterlin and Reynolds, 1977b; Casterlin and Reynolds, 1979; Cabanac and Guelte, 1980; Casterlin and Reynolds, 1980; Bronstein and Conner, 1984; Louis et al., 1986; Boorstein and Ewald, 1987; McClain et al., 1988; Cabanac, 1989; Carruthers et al., 1992; Adamo, 1998). Reptiles that display a behavioral fever in response to a supposed pyrogen include *Crotaphytus collaris* (collared lizard), *Dipsosaurus dorsalis* (desert iguana), *Oplurus cyclurus* (Madagascan swift),

Gerrhosaurus major (Sudan plated lizard), *Varanus exanthematicus* (savannah monitor), *Iguana iguana* (green iguana), *Sceloporus orcutti* (granite spiny lizard), *Sauromalus obesus* (chuckwalla), *Callopistes maculatus* (monitor tegu), *Agama agama* (common agama), *Pituophis melanoleucus* (gopher snake), *Arizona elegans* (glossy snake), *Thamnophis sirtalis* (common garter snake), *Alligator mississippiensis* (alligator), *Chrysemys picta* (painted turtle), *Clemmys insculpta* (wood turtle), and *Terrapene carolina* (box turtle) (Vaughn et al., 1974; Bernheim and Kluger, 1976a; Kluger, 1978; Kluger, 1979a; Firth et al., 1980; Monagas and Gatten Jr., 1983; Muchlinski, 1985; Lang, 1987; Muchlinski et al., 1989; Hallman et al., 1990; Ortega et al., 1991; Cabanac and Gosselin, 1993; Don et al., 1994; Muchlinski et al., 1995; Burns et al., 1996; Muchlinski et al., 1998; Cabanac and Bernieri, 2000; Deen and Hutchison, 2001). Amphibians that show a behavioral fever in response to a supposed pyrogen include *Necturus maculosus* (mudpuppy), *Rana catesbeiana* (bullfrog tadpoles), *Rana pipiens* (leopard frog tadpoles), *Rana esculenta* (edible frog), *Hyla cinerea* (green treefrog), and *Bufo marinus* (tropical toad) (Casterlin and Reynolds, 1977a; Kluger, 1977; Myhre et al., 1977; Hutchison and Erksine, 1981; Muchlinski, 1985; Sherman et al., 1991; Lefcort and Eiger, 1993). Fish that display a behavioral fever in response to a supposed pyrogen include *Carrassius auratus* (goldfish), *Lepomis macrochirus* (bluegill), and *Micropterus salmoides* (largemouth bass) (Reynolds, 1977; Reynolds et al. 1978a; Reynolds et al., 1978b; Muchlinski, 1985; Cabanac and LaBerge, 1998).

Some vertebrates do not develop a fever in response to a supposed pyrogen, but negative results in the field of thermal biology do not necessarily indicate the absence of the phenomenon in question. Negative results for the development of a fever may

indicate that an inappropriate stimulus such as the incorrect pyrogen or incorrect dose was used for the animal at hand. For example, rats require doses of LPS three orders of magnitude higher than do rabbits to produce a fever, and mice require doses of LPS three orders of magnitude higher than do rats to produce a fever (Kozak et al., 1994; Tocco-Bradley et al., 1985). Application of an inappropriate high dose of LPS could result in endotoxic shock which would trigger a decrease in body temperature that would then mask the phenomenon of fever in a given study, whereas application of an inappropriate low dose of LPS simply may not trigger a change in thermoregulatory set-point in some animals (Kluger, 1998). Other reasons for the apparent lack of fever in some organisms in some studies include the elevated levels of glucocorticoids in stressed animals that inhibit the production of prostaglandins (Hong and Levine, 1976; Lewis and Piper, 1975) or inhibit the release of endogenous pyrogens (Gander et al., 1980; Snyder and Unanue, 1982). Even though the question of the evolution of fever cannot be addressed directly through the study of present day organisms, the widespread occurrence of this complex phenomenon and the similarity of the mechanisms of this phenomenon from species to species suggest that the febrile response evolved hundreds of million years ago (Kluger, 1998). The evolutionary conservation of this energetically expensive response also suggests that its value must outweigh its cost and therefore that fever is indeed an adaptive response to disease.

The third body of evidence suggesting fever is an adaptive mechanism for coping with disease involves the effects of fever on morbidity and mortality. Studies on the correlation between body temperature and survival rates in organisms that have been exposed to bacterial pathogens are often difficult to interpret because not only are the

organisms in correlational studies often exposed to different doses of a pathogen, but the pattern of benefit of fever is not a linear correlation with an increase in body temperature. For a given species, an increase in body temperature a few degrees above "normal" correlates positively with an increase in survival rate; however, further increases in body temperature correlate with a decrease in survival rate. Body temperature is positively correlated with survival rate up to a certain body temperature in humans with a bacterial infection (Bryant et al., 1971; Weinstein et al., 1978; Hoefs et al., 1980; Mackowiak et al., 1980). However, one study which showed no correlation between fever and survival rate did show a positive correlation between hypothermia and mortality rates in both newborns and adults (Dupont and Spink, 1969). In addition, the spontaneous regression of certain types of cancer in humans has been linked correlationally to the fevers associated with bacterial or viral infections (Hobohm, 2005). New England white rabbits exposed to *Pasteurella multilocida* that developed a fever up to 2.25˚C above normal body temperature showed a positive correlation between survival rate and body temperature (Kluger and Vaughn, 1978). Animals that increased their body temperature above this 2.25˚C range had a lower survival rate. Toms et al. (1977) showed a statistically significant negative correlation between the amount of live virus found in the nasal passages of ferrets infected with different strains of influenza virus and the body temperature of these animals at four hour intervals after inoculation. *In vitro* studies involving the same set of viruses indicate that elevation of ambient temperature decreases the replication rate of the viruses (Toms et al., 1977). In humans with sepsis, fever has been associated with improved survival and shorter duration of the disease (Hasday and Garrison, 2000). Goldfish infected with *Aeromonas hydrophila* and allowed to

thermoregulate behaviorally chose febrile temperatures and survived (Covert and Reynolds, 1977). These correlational studies indicate that moderate fevers are beneficial in fighting disease, but extremely high fevers are maladaptive.

Conversely, a number of studies have shown an increase in mortality or morbidity in response to the application of an antipyretic substance in animals infected with a bacteria or virus. In goats inoculated with *Trypanosoma vivax,* treatment with flurbiprofen, an antipyretic drug, induced one hundred percent mortality (Van Miert et al., 1978). Rabbits who were infected with *P. multocida* and then had their preoptic anterior hypothalamus infused with an antipyretic drug exhibited lower body temperatures and higher mortality rates than rabbits infected with the bacteria and infused with a control solution (Vaughn et al., 1980). Ferrets infected with various influenza viruses and then treated with sodium salicylate exhibited an attenuation of fever, increased concentrations of live viruses in nasal washes, and an increase in the duration of the illness compared to control animals not receiving the antipyrogenic drug (Husseini et al., 1982). Rabbits infected with rinderpest virus (RPV) and treated with mefanamic or acetylsalicyclic acid (antipyrogens) exhibited various degrees of antipyresis, increased mortality, and longer duration of illness than rabbits infected with RPV and not given an antipyrogen (Kurosawa et al., 1978). Seven of 12 *D. dorsalis* injected with live *A. hydrophila* and sodium salicylate failed to select febrile temperatures in a thermal gradient and subsequently died (Bernheim and Kluger, 1976b). All lizards that chose febrile temperatures survived the bacterial infection (Bernheim and Kluger, 1976b). An increase in mortality and morbidity rates in response to the suppression of fever by

antipyretic drugs bolsters the idea that fever is a beneficial adaptation against disease rather than a maladaptive symptom of disease.

Additional studies on hyperthermia and hypothermia in both endotherms and ectotherms support the findings of these correlational and antipyretic studies that fever can decrease mortality and morbidity rate, while antipyresis can increase mortality and morbidity rates. Pigeons and rabbits infected with pneumococcal bacteria and artificially maintained at hypothermic temperatures exhibited increased mortality rates (Strouse, 1909; Muschenheim et al., 1943). Although reduction of body temperature through antipyretic drugs increased mortality rates in rabbits, physical cooling of rabbits infected with *P. multocida* decreased mortality rates (Vaughn et al., 1987). Because body temperature during physical cooling is below the set-point temperature and because antipyretic drugs, during antipyretic cooling, actually change the set-point temperature to a lower level, these studies indicate that activation of heat production and conservation responses in a cold-defense response during physical cooling that are not activated during antipyretic cooling may enhance survival in some organisms (Vaughn et al., 1987). Banet (1981) reported similar results for rats infected with *Salmonella enteritidis*. In addition, newborn endotherms do not have the metabolic machinery or behavioral ability to thermoregulate precisely so they have a limited ability for a febrile response. However, hyperthermia in humans, rabbits, mice, and dogs reduces mortality rates (Pembrey, 1895; Carmichael et al., 1969; Teisner and Haahr, 1974; Haahr and Mogensen, 1977). Mice kept at a high ambient temperature after infection with rabies virus had lower mortality rates (Bell and Moore, 1974). *D. dorsalis* housed at higher temperatures (hyperthermic) after inoculation with *A. hydrophila* had higher survival rates than those

housed at lower temperatures (hypothermic) (Kluger et al., 1975). Goldfish infected with *A. hydrophila* and held at hyperthermic, normothermic, and hypothermic temperatures showed similar results, with higher temperatures correlating with increased survival rates (Covert and Reynolds, 1977). Crickets held at high ambient temperatures after infection with the intracellular parasite *Rickettsiella grylli* survived the infection whereas those held at lower ambient temperatures died (Louis et al., 1986). Boorstein and Ewald (1987) found that grasshoppers infected with the protozoan *Nosema acridophagus* and held at febrile temperatures had both higher survival and higher growth rates than those held at afebrile temperatures. In addition, humans allowed to go hypothermic during and after colorectal surgery had more infections and longer hospital stays than those who were held at normothermic temperatures (Kurz et al., 1996). The variety of organisms that exhibit enhanced survival rates in response to fever or elevated body temperatures supports the hypothesis that fever is a beneficial adaptation for fighting disease.

Comparisons of Thermoregulation Among Organisms

Despite differences in methods of controlling body temperature, both endotherms and ectotherms display a high degree of similarity in response to pyrogens and cryogens or antipyrogens (Kluger, 1979a). In many vertebrates including fishes, amphibians, reptiles, birds, and mammals, thermoregulation is mediated by the hypothalamus (Bligh, 1973; Kluger, 1979b). The phylogenetic conservation of this process allows researchers to apply behavioral and physiological patterns seen in response to pyrogens and antipyrogens across all vertebrate species (Kluger, 1979a). The difficulty of altering mammalian body temperature for any length of time without seriously interrupting other

life-supporting mechanisms introduces many hazards to the interpretation of data from studies on mammalian fever. A benefit of the shared system of thermoregulatory mediation and the comparative ease of manipulating ectotherm body temperature in a laboratory setting is that anything learned about the febrile response in ectotherms may be applied to endotherms. This characteristic of the febrile response, along with the ability of the animal to thrive in a laboratory setting, makes *D. dorsalis* a perfect model organism for studying many aspects of thermoregulation. Indeed, *D. dorsalis* is the ectothermic vertebrate historically used to display the benefits of the fever response to disease for both endotherms and ectotherms (Vaughn et al., 1974).

Justification for this Study

In response to higher levels of exogenous pyrogen, the lizard's white blood cells should phagocytose more pyrogen. In turn, this may increase the amount of endogenous pyrogen released which could increase the change in set-point temperature employed by the animal. Exposure to an exogenous pyrogen during the animal's peak activity hours may cause a greater effect than exposure at trough activity hours. The animal's higher metabolic rate during peak activity may allow for a stronger immune response and, therefore, a greater amount of pyrogen phagocytosed by the host's white blood cells. This may lead to an enhanced release of endogenous pyrogen and a greater fever response. In a related way, an animal with low energy reserves may not be able to support the higher metabolic rate associated with the fever response and may employ other mechanisms to depress the set-point temperature or to become hypothermic (Deen and Hutchison, 2001).

Response of body temperature to an antipyrogen is dose-dependent in *D. dorsalis* (Bernheim and Kluger, 1976b), but variation in activity levels and metabolic rate correlated with time of day of injection may counteract or amplify the influence of dose. Dose of antipyrogenic substances may induce an absolute change in the firing rate of warm-sensitive neurons rather than a percent change in firing rate. Because warm-sensitive neurons are firing at different rates when the lizard is at different body temperatures, the predicted absolute change in firing rate will be a bigger percent change at the lower body temperatures associated with an injection at trough activity times than the percent change recorded at the higher body temperatures associated with the peak activity times. This phenomenon may result in different magnitudes of change in body temperature in response to the same dose of antipyrogen and/or pyrogen depending on the starting body temperature of the lizard.

Previous studies addressing the initiation of a febrile response by exogenous and endogenous pyrogens have focused primarily on whether a particular supposed pyrogen would actually cause a fever in a particular species and the survival value of this mechanism (Kluger, 1978). Few studies have focused on the intrinsic attributes of the vertebrate febrile response itself. Because *D. dorsalis* exhibits the typical ectothermic vertebrate thermoregulatory response to fever-inducing agents, examination of the response in this animal should shed light on fever across vertebrate ectotherms. My studies were thus designed to determine:

1) Effects of Time of Injection, Dose of Pyrogen, Antipyrogen, and Sex on Body Temperature

2) Effects of Body Mass and Body Condition on Body Temperature

3) Effects of Time of Injection, Dose of Pyrogen, and Dose of Antipyrogen on Thermoregulatory Precision

4) Characteristics of the Basic Febrile Response including latency period, rate of temperature rise, maximum temperature, duration of the febrile response, and rate of return to normal temperature

These studies are unique in that they concentrate on the fundamental characteristics of the febrile response in lizards rather than simply on the existence of the phenomenon, attempt to elucidate the chronopharmocological aspects of exogenous pyrogens and antipyrogens, explore interactions between time of day and dose of exogenous pyrogens and antipyrogens on the basic fever event, and begin to determine the importance of body energy reserves in thermoregulatory responses. The information gained from these studies will increase our understanding of the vertebrate response to fever-causing agents and the manner in which environmental conditions and the physiological condition of a particular lizard at a particular time interact to create a singular thermoregulatory response.

MATERIALS AND METHODS

Animals, captive care, acclimatization

I collected 156 adult desert iguanas (*Dipsosaurus dorsalis*) of similar size (mean snout-vent length = 12.0 ±1.1 cm, mean mass 55.3 ±15.5 g) from La Paz, Maricopa, Pima, Pinal, Mohave, and Yuma Counties, Arizona during May 2000 and May 2001. Animals were housed in groups of 10-30 individuals in 2.5 m X 0.75 m X 1.0 m cages with a 12-cm deep sandy substrate. Water was provided *ad libitum* in reptile waterers obtained from Farnum Pet Products® (Phoenix, AZ). Lizards were fed daily on a diet of soaked guinea pig chow sprinkled with Reptivite® (a reptile vitamin and mineral supplement) and a salad consisting of assorted chopped vegetables and fruit (spinach, collard, mustard, turnip, Romaine lettuce, carrots, apples, yellow squash, zucchini, and tomatoes). Lizards were maintained at room temperature (23.0-24.5°C) and were provided basking lamps between 0600 and 1800 h CST daily to provide a LD 12:12 photoperiod.

Lizards were placed in environmental chambers for 4 to 5 days at 30°C and a photoperiod of LD 12:12 (0600 h-1800 h) for acclimatization prior to testing. Animals were fed daily and offered water *ad libitum* as described above. Food was removed 36-48 hours prior to experimentation to avoid potential digestive influences on thermoregulation. Trials were conducted between August 2000 and January 2002.

Agents and dosages

I obtained purified (lyophilized powder prepared by phenol extraction) lipopolysaccharide (LPS, L-2630) from the cell wall of the bacteria, *E. coli* (serotype

0111-B4), and acetylsalicylic acid (aspirin, $C_9H_8O_4$, A-5376) from the Sigma Chemical Company (St. Louis, MO). I dissolved LPS powder over low heat in reptile Ringer's to form solutions of three different concentrations: 0.2125 g l^{-1}, 2.125 g l^{-1}, 21.25 g l^{-1}. I dissolved acetylsalicylic acid over low heat with a drop of ethanol in reptile Ringer's to form a solution of 14.167 g l^{-1} concentration. For LPS only injections, lizard body mass was divided by 85 g to calculate injection volume of either the 0.2125 g l^{-1} (for the "low" dose, 2.5 mg LPS per kg lizard body mass) or 2.125 g l^{-1} (for the "high" dose, 25 mg LPS per kg lizard body mass) solutions to maintain a constant injection volume per each gram of body mass and to maintain the appropriate dose for each animal. To insure that no animal received more than a 1 ml injection volume, I based my calculations on my largest lizard whose mass = 85 g. Larger injection volumes could interfere with total body water and affect thermoregulatory choices. For the combination LPS + acetylsalicylic acid injections, lizard body mass was multiplied by 0.9 and then divided by 85 g to determine the injection volume of acetylsalicylic acid and lizard body mass was multiplied by 0.1 and then divided by 85 g to determine the injection volume of the 2.125 g l^{-1} solution (for the low dose, 2.5 mg LPS per kg lizard body mass) and 21.25 g l^{-1} (for the high dose, 25 mg LPS per kg lizard body mass). These calculations insured that all lizards would receive the same volume of solution per gram of body mass with all doses and combinations of LPS and acetylsalicylic acid. Control treatments consisted of no injection. Pilot studies showed no significant difference (paired t-tests on mean Tb of 14 lizards in each group every hour over a 3 day period, df = 71, t = -0.26 p = 0.80) in Tb between lizards receiving no injection and lizards receiving an injection of saline solution consistent in volume with experimental treatments (1 ml per 85 g lizard body mass).

Measurements of body temperatures and data acquisition

To allow lizards to thermoregulate through choice of substrate temperature, I used linear thigmothermal gradients (Sievert and Hutchison, 1988; Tu and Hutchison, 1995) measuring 210 X 22 X 23 cm with a temperature range between 3.60 ± 0.19 and 75.91 ± 1.14°C. The cold end of the gradient was maintained by the air temperature of the cold room that contained the gradients and the hot end was maintained by a series of hot pads attached to the underneath side of the gradient. Two wide-spectrum fluorescent lights suspended 40 cm above each gradient and attached to an automatic timer maintained a photoperiod of LD 12:12 (centered at noon CST) (Sievert and Hutchison, 1989). Gradients were cleaned with 70% ethanol at least 12 hours before each run to insure olfactory neutrality for each trial.

Body temperature was measured by 22-gauge copper-constantan thermocouples dipped in Epoxy® and inserted approximately 1 cm into the lizard's cloaca. Two pieces of tape wrapped around the tail immediately below the vent and approximately 1 cm below the vent held the wires in place. To allow for habituation, I inserted the thermocouples and placed the lizards at the midpoint of each gradient 3 to 4 h prior to the start of body temperature recording. At 1200 h or 2400 h, I injected the animals with the appropriate dose and combination of LPS and/or acetylsalicylic acid and returned them to the same point on the gradients from which I had removed them. Any control animals in the gradients were handled at the same time the experimental animals were handled and returned to the same point on the gradient from which I had removed them. Animals were injected only once with LPS and/or acetylsalicylic acid. Body temperature was recorded every 15 min for three days (72 h) starting at 1200 h through the cloacal

thermocouple connected to an Omega 50® data logger (Omega Engineering, Stamford, CT). Trials were conducted in four thigmothermal gradients simultaneously throughout the course of experimentation. Animals were returned to original care conditions post trial and data were downloaded to a computer for analysis. All data were collected between the months of August and March to avoid the influence of breeding activities on thermoregulation.

Experimental design

Trials were conducted with injections at both 1200 h and 2400 h for both doses of LPS (2.5 mg kg^{-1} and 25 mg kg^{-1}) and for both doses of LPS (2.5 mg kg^{-1} and 25 mg kg^{-1}) + acetylsalicylic acid (150 mg kg^{-1}), and with the control (no injection) for a total of nine treatment groups of 10-13 lizards (including both males and females) each. Animal run order was determined by rank based on body condition as defined by mass (g) divided by SVL (cm). Animals were placed in each treatment category from the rankings of body condition in ascending (highest mass to SVL ratio) and then descending (lowest mass to SVL ratio) order alternately as that treatment was run which resulted in an even distribution of body conditions within each treatment. Treatments were run in order (2.5 $mgkg^{-1}$ LPS at noon, 2.5 $mgkg^{-1}$ LPS at midnight, 2.5 $mgkg^{-1}$ LPS + antipyrogen at midnight, 25 $mgkg^{-1}$ LPS at noon, controls, 2.5 $mgkg^{-1}$ LPS + antipyrogen at noon, 25 $mgkg^{-1}$ LPS + antipyrogen at noon, 25 $mgkg^{-1}$ LPS at midnight, 25 $mgkg^{-1}$ LPS + antipyrogen at midnight) as determined by random number table. Four animals were run at a time (each with a different treatment as determined by the order of treatments), which resulted in an even temporal distribution of treatments throughout the experimental

period. I compared body condition among treatment blocks before ($p = 0.90$) and after ($p = 0.74$) runs with 2 one-way ANOVAs to insure an even distribution of body conditions among treatment blocks.

Data analysis

Body-temperature data were averaged for each individual lizard over each 1 h period to produce a time series of 72 points describing lizard body temperature over the 72 hour trial period. Because body temperatures for females within each treatment block were significantly different from body temperatures for males and the sample size of females was low (1-3 per treatment group), only the data from male lizards were analyzed (7-10 per treatment). Temperature (Appendix 1) and morphometric (Appendix 2) data for females is shown in the appendices. To control for amount of time spent in the dark and amount of time spent in the light, only the first 48 hours after injection were compared among treatment groups.

To determine the effects of time of injection and dose on lizard body temperature, I divided the data into two blocks of treatment groups, those that included an injection of only the pyrogen and those that included an injection of pyrogen + antipyrogen, and ran 3 separate two-way ANOVAs and 3 separate Holm-Sidak Multiple Comparison Procedures on mean body temperatures for the total 48 hours after injection, day 1, and day 2 for each block of treatment groups for a total of 6 ANOVAs and 6 Holm-Sidak Multiple Comparison Procedures. Data points for the ANOVAs were obtained by averaging Tb over the total 48 hours of the trial run, over the first 24 hours after injection (day 1), and over the second 24 hours after injection (day 2) for each animal individually. To avoid

pseudo-replication of control data points, I used data from both the non-injected controls from the pilot studies and from the experimental studies. The control data from both the experimental studies and from the pilot studies were split in half and distributed to the noon and midnight control data sets based on the even distribution of body conditions. Control data points for analysis were obtained from the first 48 hours of the run for the noon comparisons, and from the first 48 hours after midnight for the midnight comparisons.

I then calculated the means of the variances in Tb from all lizards for each hourly time period for each treatment block for a total of 72 data points describing variance in Tb for all lizards within a treatment block. To compare thermoregulatory precision among treatment blocks, I averaged all hourly variances for each lizard individually to obtain a single point describing variance for that animal for the total 48-hour time period after injection. I also calculated mean variance for each animal individually for the first 24 hours after injection (day 1) and for the second 24 hours after injection (day 2). Control data points were obtained in the same manner as the control data points for body temperature comparisons. I then ran 6 two-way ANOVAs and 6 Holm-Sidak Multiple Comparison Procedures on mean variance of lizard body temperature for the total run period (48 hours), day 1, and day 2 for factors of time of injection and dose in the presence of only pyrogen and in the presence of pyrogen + antipyrogen to determine the effects of these parameters on lizard thermoregulatory precision.

Pearson's Product-Moment Correlation tests were run for body condition pre-run, body condition post-run, mass pre-run, mass post-run, SVL, and mass change against mean body temperature for each of the two days and for the first 48 hours after injection

for each of the treatment blocks to determine whether body temperature varies with morphometric parameters in *D. dorsalis*.

Because the data showed no clear endpoints for latency period, rate of temperature rise or fall, duration of response, or rate of return of body temperature to normal, I ran two-way ANOVAs with factors of time of injection and dose and Holm-Sidak Multiple Comparison Procedures on the skewness and kurtosis of the body temperature curves of each individual lizard for the first 24 hours after injection to compare the shapes of the curves between treatments. Comparisons of mean skewness values between treatment blocks that were significantly different allowed a qualitative determination of relative latency periods by showing whether the body temperature curves of individuals within a treatment block were shifted to the right or to the left compared to the curves of individuals within other treatment blocks. Treatments that induced body temperature curves farther to the left (more positive skewness) had shorter latency periods from the time of injection to the onset of a response (change in Tb) than those treatments that induced body temperature curves farther to the right (more negative skewness). Comparisons of mean kurtosis values among treatment groups that were significantly different allowed a qualitative determination of relative duration of response by showing whether the body temperature curves of individuals within a treatment block tended to be more peaked or flat compared to the curves of individuals within other treatment blocks. Treatments that induced more peaked curves (higher kurtosis) had a shorter duration of response than those treatments that induced flatter body temperature curves (lower kurtosis).

Statistical tests were performed with SigmaStat® software (Version 3.0, SPSS Inc., Chicago, IL). This experimental protocol was approved by the University of Oklahoma Animal Care and Use Committee, Assurance Number 73-R-100. Animals were collected under scientific collecting permit SP626421from the Arizona Game and Fish Department.

RESULTS

Effects of Time of Injection and Dose on Tb in the Presence of Only Pyrogen for the Total Run Period, Day 1, and Day 2

The pattern of Tb varied across groups receiving only pyrogen (Figure 1). Analysis of the primary factors for the total run period indicated that time of injection and dose independently affected body temperature response to an injection of pyrogen, but the amplitude of overall lizard body temperature response was not regulated by the interaction effect of time of injection and dose (Table 1). Average mean Tb during day 1 was not significantly affected by either time of injection or dose independently, nor the interaction between time of injection and dose (Table 2); but on day 2 there was a significant effect of time of injection and dose independently (Table 3). However, the low P-value for the interaction effect on day 2 ($P = 0.072$) may indicate a biological effect of that parameter on lizard Tb at that time.

A two-way ANOVA for mean Tb over the total run period (48 hrs) for lizards that received only the pyrogen revealed a significant main effect of time of injection ($P = 0.023$) and dose ($P = 0.018$) (Figures 2-4, Table 1). A Holm-Sidak Multiple Comparison Procedure revealed significant differences ($P < 0.05$) between these pairs: for dose: high vs. low; and for time of injection: noon vs. midnight. Analysis of dose effects showed that lizards receiving the high dose (33.73 ± 1.68 ˚C) exhibited higher mean Tbs than lizards receiving the low dose (27.40 ± 1.54 ˚C), but lizards receiving the low dose (27.40 ± 1.54 ˚C) or the high dose (33.73 ± 1.68 ˚C) had the same mean Tb as the controls (32.65 ± 1.81 ˚C) (Figure 3). Analysis of the time of injection effects indicated lizards

injected at noon (33.56 ± 1.43 ˚C) had higher mean Tbs for the total run period than did lizards injected at midnight (28.96 ± 1.32 ˚C) (Figure 4).

Further analysis of the timing of effects revealed that the overall differences in Tb across treatments were present mainly on day 2. The results of the two-way ANOVA for mean Tb over day 1 in lizards receiving only pyrogen showed no significant effects of time of injection, dose, or the interaction between the two (Figures 5-7, Table 2). The two-way ANOVA for mean Tb over day 2 in lizards receiving only pyrogen revealed a similar pattern of significance and non-significance as the two-way ANOVA for the total run period (Figures 8-10). The results of a two-way ANOVA for mean Tb for day 2 in lizards that received only the pyrogen showed significant main effects of both time of injection ($P = 0.013$) and dose ($P=0.016$) (Figures 9-10, Table 3). A Holm-Sidak Multiple Comparison Procedure revealed significant differences ($P<0.05$) between these pairs: for dose: high vs. low, and controls vs. low; and for time of injection: noon vs. midnight. Analysis of dose effects indicated lizards that received the high dose (32.58 ± 2.06 ˚C) or the control treatment (32.15 ± 2.22 ˚C) exhibited higher mean body temperatures than lizards that received the low dose (25.05 ± 1.89 ˚C), but lizards that received the high dose (32.58 ± 2.06 ˚C) had the same mean Tb as the controls (32.15 ± 2.22 ˚C) (Figure 9). Analysis of the time of injection effects indicated that lizards injected at noon (33.04 ± 1.75 ˚C) had higher mean Tbs for the total run period than did lizards injected at midnight (26.82 ± 1.61 ˚C) (Figure 10).

Effects of Time of Injection and Dose on Tb in the Presence of Pyrogen + Antipyrogen for the Total Run Period, Day 1, and Day 2

Analyses of the factors of time of injection and dose on mean Tb for the total run period and day 1 for lizards that received the pyrogen + antipyrogen indicated that neither of these factors independently nor interactively affected Tb in the presence of antipyrogen (Tables 4-5). Two-way ANOVAs on mean Tb during the total run period and day 1 for lizards that received pyrogen + antipyrogen revealed no statistically significant differences (Figures 11-17, Tables 4-5). However, dose on day 2 had a significant overall effect (P = 0.040) in the presence of antipyrogen (Figure 19, Table 6). Analysis of dose effects on day 2 with a Holm-Sidak Multiple Comparison Procedure revealed no significant pairwise comparisons.

Effects of Time of Injection and Dose on Variance in Tb in the Presence of Only Pyrogen for the Total Run Period, Day 1, and Day 2

The pattern of lizard variance in Tb and therefore, thermoregulatory precision, varied across groups receiving pyrogen only (Figure 21). A two-way ANOVA on variance of lizard body temperature for factors of time of injection and dose of pyrogen showed no significant main effects or interaction effects for the total run period or day 2 (Tables 7, 9). However, there was a significant main effect of dose on day 1 (P = 0.020), and a possible biologically significant interaction effect of time of injection and dose (P = 0.056) (Table 8). A Holm-Sidak Multiple Comparison Procedure revealed significant differences ($P < 0.05$) between this pair: for dose: high vs. controls. Analysis of main

effects on day 1 indicated that lizards injected with the high dose of LPS (12.18 ± 2.13 ˚C) had higher variance in Tb than the control lizards (3.36 ± 2.13 ˚C) (Table 8).

Effects of Time of Injection and Dose on Variance in Tb in the Presence of Pyrogen + Antipyrogen for the Total Run Period, Day 1, and Day 2

The pattern of lizard variance in Tb and therefore, thermoregulatory precision, varied across groups receiving pyrogen + antipyrogen (Figure 22). A two-way ANOVA on variance of lizard body temperature for factors of time of injection and dose of pyrogen for animals that received pyrogen + antipyrogen showed no significant main effects or interaction effects for the total run period or day 2 (Tables 10, 12). However, day 1 showed both a significant main effect of dose (P = 0.020) and time of injection (P = 0.039) (Table 11). A Holm-Sidak Multiple Comparison Procedure revealed significant differences ($P<0.05$) between these pairs: for dose: high vs. controls; for time of injection: midnight vs. noon. Analysis of main effects on day 1 indicates that lizards injected with the high dose + antipyrogen (7.96 ± 1.20 ˚C) had higher variance in Tb than the control lizards (3.36 ± 1.15 ˚C), and lizards injected at midnight had lower variance (4.65 ± 0.92 ˚C) than lizards injected at noon (7.42 ± 0.91 ˚C) (Table 11).

Effects of Body Condition and Body Mass on Body Temperature

Pearson's Product Moment Correlation tests for individual body condition pre-run, body condition post-run, mass pre-run, mass post-run, SVL, and mass change against mean body temperature for each of the two days, and the total time period of the run for each of the nine treatment blocks showed that Tb varies with morphometric parameters

under some circumstances (Table 13). Significant correlations appeared in the two treatment blocks that received pyrogen + antipyrogen at midnight, in the treatment block that received the high dose of pyrogen at midnight, and in the controls with higher mean individual Tbs occurring with higher morphometric values. For animals that received the low dose of LPS + antipyrogen at midnight, mass pre-run was significantly positively correlated with mean individual Tbs on day 2 ($P = 0.027$, $r = 0.69$), and total run period ($P = 0.011$, $r = 0.76$); mass pre-run was nearly significantly positively correlated with mean individual Tbs on day 1 ($P = 0.061$, $r = 0.61$); mass post-run was significantly positively correlated with mean individual Tbs on day 2 ($P = 0.019$, $r = 0.72$), and total run period ($P = 0.007$, $r = 0.78$); mass post-run was nearly significantly positively correlated with mean individual Tbs on day 1 ($P = 0.057$, $r = 0.62$); SVL was nearly significantly positively correlated with mean individual Tbs for day 2 ($P = 0.078$, $r = 0.58$); body condition pre-run was significantly positively correlated with mean individual Tbs on day 1 ($P = 0.028$, $r = 0.69$), day 2 ($P = 0.037$, $r = 0.66$), and total run period ($P = 0.007$, $r = 0.78$); body condition post-run was significantly positively correlated with mean individual Tbs on day 1 ($P = 0.024$, $r = 0.70$), day 2 ($P = 0.025$, $r = 0.70$), and total run period ($P = 0.004$, $r = 0.82$). For animals that received the high dose of LPS + antipyrogen at midnight, SVL was nearly significantly positively correlated with mean individual Tbs for day 1 ($P = 0.100$, $r = 0.57$) and the total run period ($P = 0.098$, $r = 0.59$). For animals that received the high dose of LPS at midnight, mass change was significantly positively correlated with mean individual Tbs for day 2 ($P = 0.027$, $r = 0.76$), and for the total run period ($P = 0.044$, $r = 0.72$). For animals that received the control treatment, mass pre-run was nearly significantly positively correlated

with mean individual Tbs for day 2 (P = 0.077, r = 0.51), and for the total run period (P = 0.084, r = 0.50); body condition pre-run was nearly significantly positively correlated with mean individual Tbs for day 1 (P = 0.066, r = 0.52), and significantly positively correlates with mean individual Tbs for day 2 (P = 0.048, r = 0.56), and for the total run period (P = 0.049, r = 0.56); body condition post-run was nearly significantly positively correlated with mean individual Tbs for day 1 (P = 0.095, r = 0.48), for day 2 (P = 0.073, r = 0.51), and for the total run period (P = 0.074, r = 0.51). No other correlations were significant ($P \leq 0.05$) or nearly significant ($0.05 < P < 0.10$).

Effects of Time of Injection and Dose on Latency Period of Tb Change

A two-way ANOVA on skewness of individual body temperature curves for the first 24 hours after injection with factors of time of injection and dose showed no statistically significant main effect of time of injection nor any statistically significant interaction effects (Table 14). The main effect of dose was significant (P = 0.016) with lizards injected with the high dose of LPS having body temperature curves with the highest skewness values (0.43 ± 0.34), followed by high dose + antipyrogen (0.39 ± 0.34), low dose of LPS (-0.22 ± 0.34), and low dose of LPS + antipyrogen (-0.81 ± 0.34).

Effects of Time of Injection and Dose on Duration of Response

A two-way ANOVA on kurtosis of individual body temperature curves for the first 24 hours after injection with factors of time of injection and dose showed a significant main effect of time of injection (P = 0.007) (Table 15). A Holm-Sidak Multiple Comparison Procedure revealed a significant difference between treatment

groups of noon and midnight ($P < 0.05$) within time of injection. Analysis of the time of injection effect indicates that lizards injected at noon had body temperature curves with higher kurtosis values (3.01 ± 0.69, shorter duration of response) than lizards injected at midnight (kurtosis = 0.23 ± 0.72).

DISCUSSION

Effects of Time of Injection, Dose, and Antipyrogen on Body Temperature

Both time of injection and dose affected the magnitude of mean Tb for animals that received only pyrogen for the total run period and day 2. In all comparisons, lizards that received the high dose of LPS had statistically higher Tbs than the lizards that received a low dose of LPS. However, in all comparisons, lizards receiving the high dose of LPS and lizards receiving the low dose of LPS had Tbs statistically similar to that of the controls. One interpretation of the similarity in Tb for animals that received the control treatment and animals that received the high or the low dose of LPS may be that stress played a role in determining Tb. Glucorticoids released during stress have a suppressive effect on the production (Lewis and Piper, 1975; Hong and Levine, 1976) or release (Gander et al., 1980; Snyder and Unanue, 1982) of prostaglandins which are essential to the elevated Tb of a fever response. In addition, handling stress and novel environments may induce stress hyperthermia in some circumstances (Kluger, 1991), which may further confound the interpretation of results in cases where a fever response occurs at a lower magnitude than the response to the handling stress. Even though all efforts were made to insure equal handling of all treatment groups, handling stress may have affected the thermoregulatory choices of the animals in this study.

Romanovsky and Szekely (1998) compared studies on Tb in various animals at different stages of disease and under the influence of various doses of pyrogen to conclude that a pathogen may induce opposite thermoregulatory responses depending on the quantity of the agent and the health of the host. My data, which show that lizards receiving the low dose of pyrogen exhibited Tbs opposite to the Tbs of the lizards that

received the high dose for the total run period and day 2, are consistent with these conclusions. Do Amaral et al. (2002) obtained the same pattern of results when they compared Tbs in *Terrapene carolina* that received a high dose and a low dose of LPS; turtles that received a high dose exhibited a fever and turtles that received a low dose exhibited hypothermia. The mechanism responsible for lowering Tb in response to some doses of LPS may be the same that is responsible for endotoxic shock in which the Tb thresholds for the activation of heat-defense mechanisms and the activation of cold-defense mechanisms become dissociated and the thermoeffector responses become less sensitive to changes in Tb (Romanovsky and Szekely, 1998). Because the application of an exogenous pyrogen such as LPS triggers both pyrogenic and cryogenic activities in the thermal control pathways of the body, the induction of hypothermia by LPS is a logical result of the dissociation of the thresholds for Tb maintenance in some circumstances (Romanovsky and Szekely, 1998). The proposed triggers for activating dissociation between these two thresholds are stress hormones such as adrenocorticotropin, whose levels rise under unfavorable conditions such as poor nutrition or physical restraint and, subsequently, result in hypothermia (Szekely and Szelenyi, 1982; Shido et al., 1989; Long et al., 1991; Romanovsky and Szekely, 1998). Because all animals in my studies were attached to a thermocouple wire and placed within the confines of a thigmothermal gradient, my results, which indicate that high doses of LPS induce increased Tbs and that low doses of LPS induce decreased Tbs are, in hindsight, not surprising.

In all comparisons, lizards exposed to only pyrogen at noon showed higher mean Tb for the total run period and for day 2 than did those injected at midnight. The lower temperatures available in the desert at night may predispose *D. dorsalis* to choosing

lower Tbs at night (or in the dark if in a thermal gradient) regardless of other physiological influences because light may act as a "token stimulus" for heat in these heliothermic animals (Fraenkel and Gunn, 1940; Cowles, 1962). Alternately, the circadian rhythms in Tb of these animals may not be completely overridden by exposure to an environmental stimulus such as a pathogen which would predict that organisms would show higher Tb during the day independent of other influences (Gelderloos, 1976). My data show some evidence of a basic diel cycle of Tb in these lizards independent of dose or time of injection. The Tbs chosen by animals in response to an injection at noon which were higher than the Tbs chosen in response to an injection at midnight may reflect their circadian rhythms.

Animals injected with pyrogen + antipyrogen showed no significant differences in mean Tbs across any groups for the total run period or day 1. This lack of difference in mean Tb among treatment groups suggests that the antipyrogen attenuated the effects of dose and time of injection on mean Tb for the total run period and day 1. Because all groups had Tbs similar to the controls for the total run period and day 1, I conclude that the presence of antipyrogen counteracts the effects of the pyrogen in certain circumstances. Bernheim and Kluger (1976a) demonstrated similar results with their study on fever and antipyresis in *D. dorsalis*. A low dose of antipyrogen (1.5 mg/lizard) attenuated the effects of a fever slightly, a medium dose (7.5 mg/lizard) returned Tb to the level of the controls, and a high dose (15 mg/lizard) lowered Tb below the level of the controls and killed the animals. My average dose of antipyrogen of 8.2 mg/lizard (based on the mean lizard mass of 55.3 g) is similar to Bernheim and Kluger's medium dose that returned the lizard's Tb to the level of the controls, so my results for lizards that received

antipyrogen agree with these previous studies. On day 2, dose had an overall significant effect on Tb in the presence of a pyrogen with control animals having the highest Tbs which seems to contradict Bernheim and Kluger's data. However, because Bernheim and Kluger did not take measurements on day 2, I conclude that the effects of the antipyrogen may have lasted longer than the effects of the pyrogen which would result in a depressed Tb by the end of the run period.

Effects of Body Condition on Body Temperature

In the presence of a pyrogen, time of injection and dose of the pyrogen become important factors in thermoregulatory decisions for *D. dorsalis*. Lizards treated with the low dose of LPS + antipyrogen at midnight exhibited mean Tbs for total run period, day 1, and day 2 that significantly or nearly significantly ($0.05 < P < 0.10$) correlate positively with body condition (mass/SVL) pre-run, body condition post-run, mass prior to the trial run, and mass post run. This may mean that body condition is one of the primary factors determining how a lizard thermoregulates under stressful conditions such as the presence of a low dose of pyrogen + antipyrogen. However, if the presence of a low dose of pyrogen + antipyrogen in conjunction with morphological characteristics were the only stressors to regulate Tb, then all groups exposed to this combination should have shown these correlations between Tb and morphological characteristics. Because they did not, I conclude that the time of injection (midnight) must have an overriding influence on thermoregulatory decisions that are based on energy reserves. Because animals injected at midnight had longer durations of response than animals injected at noon, animals exposed to the low dose of pyrogen + antipyrogen at midnight react by

choosing temperatures correlated with body condition. This likely allows the lizards to conserve energy in accordance with how much is available to the animal in the form of body reserves. If the infection is likely to have a longer duration, then body stores may become more important in determining the lizard's possible thermoregulatory reaction. If body reserves as indicated by body condition are low, then the lizard may demonstrate a lower Tb appropriate for conserving energy.

A similar pattern of thermoregulation is shown across the entire three day run period. As body mass decreases, body temperature tends to decrease across time in all treatment groups. In a previous study, I showed that the drop in energy reserves available to the lizard in the form of body mass may trigger this decrease in Tb to conserve energy (Deen and Hutchison, 2001).

An additional explanation for the positive correlation between Tb and body condition pre-run, body condition post-run, mass pre-run, and mass post-run in lizards that received the low dose of pyrogen + antipyrogen at midnight is that lizards my physiologically simply regard this treatment as no different from the control treatment. Lizards that received the control treatment exhibited Tbs that were significantly or nearly significantly positively correlated with body condition pre-run, body condition post-run, and mass pre-run. These data indicate that under no bacterial stressor, body energy reserves as indicated by body mass and body condition may be the most important determinant of Tb. The addition of antipyrogen to the system of lizards that received the low dose of pyrogen at midnight may return the firing rate of hypothalamic neurons to "normal" which would result in a Tb pattern similar to that seen in the control animals with body energy reserves being the most important factor in determining Tb.

For the total run period and day 1, lizards subjected to the high dose of pyrogen + antipyrogen at midnight, and for day 2, those lizards subjected to the low dose of pyrogen + antipyrogen at midnight, exhibited mean Tbs that nearly significantly correlated with SVL. This may mean that older animals exhibit higher Tbs under certain circumstances. Because reptiles continue to grow throughout their lives, older animals tend to have larger SVLs. Older animals may be less susceptible to various stressors because they are more experienced, so their production of glucocorticoids may be lower. This in turn could result in higher Tbs than younger lizards of smaller sizes under some circumstances.

Lizards subjected to the high dose of pyrogen at midnight exhibited mean Tbs that significantly positively correlated with mass change for the total run period and day 2. This positive correlation between Tb and mass change may be a result of an increase in immune function because lizards injected at midnight exhibited a longer duration of response than lizards injected at noon as shown by comparisons of kurtosis values, and lizards injected with the high dose of LPS had the highest body temperatures. These activities require the use of extra energy. Not only is energy output higher due to an increase in the metabolic rate, the immune system which also requires energy is similarly triggered by the high dose of pyrogen. The amount of immune activity possible will depend upon metabolic rate, so lizards with higher Tbs will have greater immune system activity and, therefore, would expend more energy over and above that needed to support body function at a higher Tb than lizards with lower Tbs. I hypothesize that the mass change is due to energy spent on the metabolic rate and on immune activity. Because the amount of energy necessary to simply keep an animal alive is similar among animals

within the relatively small size range used within this study, animals that picked higher Tbs and lost more mass may have had more immune activity. The longer duration of response in lizards exposed to a high dose of pyrogen at midnight may play a role in determining how an animal may thermoregulate in response to a pyrogen because other groups, including animals injected with the high dose at noon, did not show this correlation between mass change and Tb. Time of exposure to a pathogen may dictate the length of the illness. Therefore an exposure at midnight may provide information indicating that the duration of the illness may be long, so the lizard demonstrates Tbs appropriate to its energy reserves. Animals with lower mass select lower Tbs to conserve energy. Animals exposed to a pyrogen at noon may not be subjected to this limitation in the thermoregulatory decision-making process because their duration of illness is likely to be shorter with a lower probability of running out of energy reserves.

Because all significant correlations between Tb and morphometric parameters occurred in lizards exposed to the control treatment, to the pyrogen at midnight, or the combination of pyrogen + antipyrogen at midnight, I conclude that in the presence of a stressor, time of injection is the most important factor in determining whether or not energy reserves play a role in thermoregulatory decisions.

Effects of Time of Injection, Dose of Pyrogen, and Dose of Antipyrogen on Thermoregulatory Precision

Animals injected with only pyrogen showed no significant differences in variance among any groups for factors of time of injection and dose for all 48 hours or for day 2, which indicates that the animals subjected to the different doses of pyrogen at noon and

midnight for the entire time period and for day 2 had similar thermoregulatory precision. On day 1, controls had lower variance in Tb than lizards injected with the high dose of LPS. This indicates that the controls had higher thermoregulatory precision. Because lizards injected with the high dose had higher Tbs than those subjected to the control treatment, the higher thermoregulatory precision of those animals that received the control treatment is probably simply a result of their Tbs at noon already being close to the Tbs appropriate for their noon activity level. In the thigmothermal gradients, if the lizard had no need to change Tb, it had no need to move. On the other hand, the lizards that received the high dose of LPS needed to move more in the gradient to change Tb in response to the pyrogen. If the lizard did not move, thermoregulatory precision would be high. If the lizard did move, thermoregulatory precision would be low.

In addition, lizards had a faster response to the high dose of LPS than to the low dose as measured by skewness of the Tb curves. If lizards are reacting to the injection of the high dose of pyrogen sooner than to the low dose of pyrogen, then they would have lower thermoregulatory precision faster because they are moving more sooner. Because lizards injected with the high dose show the lowest thermoregulatory precision as measured by variance for only the first day after injection and not the second day, my results agree with this prediction.

Animals injected with the pyrogen + antipyrogen showed no significant differences in mean variances in Tbs for the factors of time of injection and dose between treatment groups for the total run period and for day 2. This lack of a difference in mean variance in Tb between treatment groups indicates that those lizards which received both pyrogen + antipyrogen may not physiologically “regard” these treatments as any different

from the control treatment and employ the same patterns of thermoregulation as they would under normal circumstances. This would result in similar thermoregulatory precision (as measured by variance) in all groups. Because my dose of antipyrogen should have returned my lizards to a Tb similar to that of the controls as shown by Bernheim and Kluger (1976a), the antipyrogen should perfectly counter the effects of the pyrogen on the warm-sensitive neurons in the hypothalamus with the result that the lizard perceives no change in set-point temperature. Therefore, thermoregulatory precision in all groups that received the antipyrogen should be similar to thermoregulatory precision in groups that received the control treatment. The lower thermoregulatory precision of lizards that received the high dose of pyrogen + antipyrogen on day 1 may be an indication that my dose of antipyrogen was not quite high enough to totally counter the effects of the high dose of pyrogen on the warm-sensitive neurons in the hypothalamus during the initial reaction to a high dose of pyrogen.

Characteristics of the Basic Febrile Response

The characteristics of the basic fever response are difficult to analyze because the data showed no clear endpoints for latency period, rate of temperature rise or fall, duration of response, or rate of return of body temperature to normal because individual variation was large. However, analysis of the chronopharmacology of the response to pyrogen and antipyrogen dose give some insight into how environmental factors such as time, dose of the pyrogen, and presence of an antipyrogen affect the basic fever response in *D. dorsalis*. Evaluation of the skewness and kurtosis of individual curves within and among each treatment group allowed qualitative comparisons of latency period and

duration of response. Time of injection affected the duration of the response as measured by the kurtosis of the Tb curves to an antipyrogen and/or a pyrogen, but neither dose nor the interaction effect between time of injection and dose affected duration of the response. Midnight injections induced longer responses than noon injections. At midnight in the desert, lizards would be subjected to a lower and smaller range of thermoregulatory possibilities than at noon. Animals exposed to only pyrogen at midnight exhibited lower mean Tbs for the total run period and day 2 than those exposed to pyrogen at noon regardless of dose which may reflect the constricted range of possible thermoregulatory choices in nature.

Lower metabolic rates associated with these lower Tbs at night would inhibit the activity of the animal's immune system, giving the invading bacteria a chance to multiply and get a strong foothold in the animal's body before more thermoregulatory choices become available and the animal could increase body temperature, metabolic rate, and immune system activity (Kluger, 1991). As a result, the lizard may require a longer time period (longer duration of response) to cope with the larger bacterial infection. By choosing Tbs appropriate to higher levels of bacterial infection for a longer period of time, the lizards may be compensating for limits in physiological response to the initial infection and demonstrating coadaption between behavioral and physiological thermoregulatory processes (Huey and Bennett, 1987; Garland et al., 1991). The higher variance in Tb, indicating a lower degree of thermoregulatory precision, shown by the animals subjected to the high dose of pyrogen than those subjected to the control treatment and the statistical similarity between thermoregulatory precision of control animals and those subjected to the low dose of pyrogen may simply be a reflection of the

degree to which a lizard must change Tb. Because fever is dose-dependent (Bernheim and Kluger, 1976a) higher concentrations of pyrogen may cause lower thermoregulatory precision when the animals need to move more in a thigmothermal gradient to change Tb. My data are consistent with this explanation of thermoregulatory precision because the control animals and the animals injected with the low dose of pyrogen had similar thermoregulatory precision, and the control animals exhibited higher thermoregulatory precision than animals injected with the high dose.

Comparisons of skewness curves suggest that dose has a significant effect on latency period of the response to an antipyrogen and/or a pyrogen in *D. dorsalis*. The high dose of LPS with or without an antipyrogen resulted in Tb curves with a shorter latency period from the time of injection to the time of response than the Tb curves of the animals that received the low dose of LPS with or without the antipyrogen. These results indicate that higher concentrations of bacteria may result in a more immediate thermoregulatory response whether or not that response is attenuated by any environmental factor that may induce a reduction in Tb such as the antipyrogen did in this study. The higher concentrations of exogenous pyrogen resulting from a higher concentration of bacteria may result in the formation of more circulating endogenous pyrogen more quickly than lower concentrations of bacteria, which may result in a faster response by the lizard. As thermoregulatory behavior and thermoregulatory physiology may be coadapted (Huey and Bennett, 1987; Garland et al., 1991), this possible link between speed of physiological response and speed of thermoregulatory choice should be independently tested.

Conclusions

Overall, dose of a pyrogen and antipyrogen, time of injection, and morphological parameters affect thermoregulatory behavior in *D. dorsalis* but not necessarily in an intuitively predictable fashion. Pyrogens and antipyrogens may act independently or interact physiologically on the thermoregulatory neurons in the brain to change the set-point temperature and trigger changes in behavior that alter lizard body temperature. My data agree with similar studies that show that a high dose of LPS may trigger fever whereas a low dose may trigger hypothermia in some cases (Romanovsky and Szekely, 1998; do Amaral et al., 2002), but a medium dose of antipyrogen will bring Tb back to the level of the controls (Bernheim and Kluger, 1976a). Some aspects of thermoregulatory behavior are affected not only by the magnitude of the stressor, but also by the timing of the exposure to the stressor. Duration of a response is affected by the timing of the stressor (midnight versus noon injections), but how quickly a lizard reacts to a stressful condition may only be affected by the amplitude of the stressor (dose). My results indicate that midnight exposures to a pathogen result in a longer duration of response, and high doses of a pyrogen result in a shorter latency period before the onset of temperature change in response to a pyrogen.

In addition to dose and time of injection, the energy reserves of a lizard may affect how it responds to a pyrogen under certain conditions. Except for the controls, all groups exhibiting a positive correlation between Tb and aspects of animal morphology that indicate something about the energy reserves of the animal (such as body condition, mass, or mass change) were injected at midnight. From this dependence on timing of exposure, I concluded that time of injection may be the most important factor in

determining whether or not energy reserves are important in determining Tb choice in lizards that are not in extremely poor body condition. Because midnight injections resulted in longer durations of response as measured by kurtosis of Tb curves, the correlation between energy reserves and Tb in lizards injected at midnight may be an adaptation for conserving energy when an illness is likely to be protracted.

Thermoregulatory precision in these studies was affected by both time of injection and dose of the pyrogen. Midnight injections produced higher thermoregulatory precision than noon injections on day 1 which may be a reflection on the timing of the active period of *D. dorsalis.* Because these are diurnal organisms, activity including movement in the thigmothermal gradient to find a different Tb may be suppressed, which would result in a lower variance and a higher precision of Tb. The controls and the lizards injected with the low dose of LPS had similar thermoregulatory precision, and the controls exhibited higher thermoregulatory precision than those injected with the high dose. Because the high dose induced higher Tbs than the controls, this lower precision exhibited by animals exposed to the high dose may occur because they must move in a thigmothermal gradient to obtain Tbs appropriate for their dose of pyrogen.

I conclude that time of exposure, dose, the interaction of the two, presence or absence of an antipyrogen, and energy reserves all affect thermoregulatory decisions in *D. dorsalis* whether it be in the form of what temperature to select or when to select it. In a complex environment with many stimuli, organisms are constantly weighing the relevance of both internal and external information and reacting in ways which will affect not only their survival but their reproductive success. Hopefully, future studies will

determine the degree to which each of these factors contributes to the overall thermoregulatory response in ectotherms.

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Table 1: Two-way ANOVA results for differences in mean body temperatures of *D. dorsalis* for the first 48 hours after injection between factors of time of injection and dose for lizards that received pyrogen only (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	237.86	237.86	5.61	0.023
Dose	2	376.96	188.48	4.45	0.018
Time of injection X Dose	2	134.94	67.47	1.59	0.216
Residual	40	1694.81	42.37	----	----
Total	45	2437.47	54.17	----	----

Table 2: Two-way ANOVA results for differences in mean body temperatures of *D. dorsalis* for day 1 between factors of time of injection and dose for lizards that received pyrogen only (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	99.87	99.87	2.52	0.120
Dose	2	222.87	111.43	2.81	0.072
Time of injection X Dose	2	18.52	9.26	0.23	0.793
Residual	49	1583.77	39.59	----	----
Total	54	1924.88	42.78	----	----

Table 3: Two-way ANOVA results for differences in mean body temperatures of *D. dorsalis* for day 2 between factors of time of injection and dose for lizards that received pyrogen only (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	433.42	433.42	6.81	0.013
Dose	2	584.25	292.12	4.59	0.016
Time of injection X Dose	2	356.83	178.41	2.81	0.072
Residual	40	2544.39	63.61	----	----
Total	45	3905.32	86.79	----	----

Table 4: Two-way ANOVA results for differences in mean body temperatures of *D. dorsalis* for the first 48 hours after time of injection between factors of time of injection and dose for lizards that received pyrogen + antipyrogen (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	140.33	140.331	2.14	0.151
Dose	2	343.00	171.50	2.62	0.085
Time of injection X Dose	2	12.94	6.47	0.10	0.906
Residual	41	2688.31	65.57	----	----
Total	46	3188.05	69.31	----	----

Table 5: Two-way ANOVA results for differences in mean body temperatures of *D. dorsalis* for day 1 between factors of time of injection and dose for lizards that received pyrogen + antipyrogen (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	241.05	241.05	3.37	0.074
Dose	2	120.41	60.20	0.84	0.438
Time of injection X Dose	2	33.04	16.52	0.23	0.795
Residual	41	2930.99	71.49	----	----
Total	46	3344.00	72.70	----	----

Table 6: Two-way ANOVA results for differences in mean body temperatures of *D. dorsalis* for day 2 between factors of time of injection and dose for lizards that received pyrogen + antipyrogen (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	91.32	91.32	0.97	0.331
Dose	2	658.64	329.32	3.49	0.040
Time of injection X Dose	2	12.21	6.11	0.06	0.937
Residual	41	3871.29	94.42	----	----
Total	46	4626.01	100.57	----	----

Table 7: Two-way ANOVA results for differences in mean variance in body temperatures of *D. dorsalis* for the first 48 hours after injection between factors of time of injection and dose for lizards that received pyrogen only (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	10.05	10.05	0.11	0.738
Dose	2	129.32	64.66	0.73	0.488
Time of injection X Dose	2	330.20	165.10	1.86	0.168
Residual	42	3721.64	88.61	----	----
Total	47	4176.43	88.86	----	----

Table 8: Two-way ANOVA results for differences in mean variance in body temperatures of *D. dorsalis* for day 1 between factors of time of injection and dose for lizards that received pyrogen only (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	110.61	110.61	1.64	0.207
Dose	2	581.99	290.99	4.31	0.020
Time of injection X Dose	2	415.82	207.91	3.08	0.056
Residual	42	2834.66	67.49	----	----
Total	47	3887.39	82.71	----	----

Table 9: Two-way ANOVA results for differences in mean variance in body temperatures of *D. dorsalis* for day 2 between factors of time of injection and dose for lizards that received pyrogen only (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	16.91	16.91	0.12	0.728
Dose	2	22.07	11.03	0.08	0.923
Time of injection X Dose	2	279.29	139.65	1.01	0.372
Residual	42	5795.68	137.99	----	----
Total	47	6136.78	130.57	----	----

Table 10: Two-way ANOVA results for differences in mean variance in body temperatures of *D. dorsalis* for the first 48 hours after time of injection between factors of time of injection and dose for lizards that received pyrogen + antipyrogen (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	9.64	9.64	0.61	0.440
Dose	2	8.60	4.30	0.27	0.764
Time of injection X Dose	2	14.46	7.23	0.46	0.638
Residual	42	667.52	15.89	----	----
Total	47	700.28	14.90	----	----

Table 11: Two-way ANOVA results for differences in mean variance in body temperatures of *D. dorsalis* for day 1 between factors of time of injection and dose for lizards that received pyrogen + antipyrogen (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	89.32	89.32	4.56	0.039
Dose	2	169.03	84.52	4.32	0.020
Time of injection X Dose	2	41.43	20.71	1.06	0.356
Residual	42	822.08	19.57	----	----
Total	47	1137.25	24.20	----	----

Table 12: Two-way ANOVA results for differences in mean variance in body temperatures of *D. dorsalis* for day 2 between factors of time of injection and dose for lizards that received pyrogen + antipyrogen (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	0.02	0.02	0.0004	0.985
Dose	2	22.36	11.8	0.26	0.770
Time of injection X Dose	2	83.50	41.75	0.98	0.383
Residual	42	1787.27	42.55	----	----
Total	47	1895.38	40.33	----	----

Table 13: Significant ($P < 0.05$) and nearly significant ($0.05 < P < 0.10$) Pearson's Product-Moment Correlations between mean body temperature and body condition pre-run (mass pre-run/snout-vent length), body condition post-run (mass post-run/snout-vent length), mass pre-run, mass post-run, snout-vent length, mass change for day 1, day 2, and the total run period in *D. dorsalis.* Significant results are in bold. (*r* = Pearson's correlation coefficient, *P* = probability, md = midnight injection, LPS + A = pyrogen + antipyrogen)

	Day 1	Day 2	Total Run Period (2 Days)
Body Condition, Pre-run	**2.5 mgkg^{-1} LPS + A md (*P* = 0.028, *r* = 0.688)** Controls (*P* = 0.066, *r* = 0.524)	**2.5 mgkg^{-1} LPS + A md (*P* = 0.037, *r* = 0.662) Controls (*P* = 0.048, *r* = 0.558)**	**2.5 mgkg^{-1} LPS + A md (*P* = 0.007, *r* = 0.784) Controls (*P* = 0.049, *r* = 0.556)**
Body Condition, Post-run	**2.5 mgkg^{-1} LPS + A md (*P* = 0.024, *r* = 0.702)** Controls (*P* = 0.095, *r* = 0.482)	**2.5 mgkg^{-1} LPS + A md (*P* = 0.025, *r* = 0.698)** Controls (*P* = 0.073, *r* = 0.513)	**2.5 mgkg^{-1} LPS + A md (*P* = 0.004, *r* = 0.818)** Controls (*P* = 0.074, *r* = 0.512)
Mass Pre-run (g)	2.5 mgkg^{-1} LPS + A md (*P* = 0.061, *r* = 0.611)	**2.5 mgkg^{-1} LPS + A md (*P* = 0.027, *r* = 0.692)** Controls (*P* = 0.077, *r* = 0.508)	**2.5 mgkg^{-1} LPS + A md (*P* = 0.011, *r* = 0.759)** Controls (*P* = 0.084, *r* = 0.497)
Mass Post-Run (g)	2.5 mgkg^{-1} LPS + A md (*P* = 0.057, *r* = 0.618)	**2.5 mgkg^{-1} LPS + A md (*P* = 0.002, *r* = 0.720)**	**2.5 mgkg^{-1} LPS + A md (*P* = 0.007, *r* = 0.783)**
SVL (cm)	25 mgkg^{-1} LPS + A md (*P* = 0.100, *r* = 0.574)	2.5 mgkg^{-1} LPS + A md (*P* = 0.078, *r* = 0.581)	25 mgkg^{-1} LPS + A md (*P* = 0.098, *r* = 0.586)
Mass Change (g)		**25 mgkg^{-1} LPS md (*P* = 0.027, *r* = 0.764)**	**25 mgkg^{-1} LPS md (*P* = 0.044, *r* = 0.720)**

Table 14: Two-way ANOVA results for skewness in body temperature curves between factors of time of injection and dose for *D. dorsalis* (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	4.38	4.38	2.25	0.137
Dose	3	21.31	7.10	3.66	0.016
Time of injection X Dose	3	5.29	1.76	0.91	0.442
Residual	76	147.68	1.94	----	----
Total	83	179.57	2.16	----	----

Table 15: Two-way ANOVA results for kurtosis in body temperature curves between factors of time of injection and dose for *D. dorsalis* (DF = degrees of freedom, SS = sum of squares, MS = mean squares, P = probability).

Source of variation	DF	SS	MS	F	P
Time of injection	1	162.25	162.25	7.74	0.007
Dose	3	32.75	10.92	0.52	0.669
Time of injection X Dose	3	14.35	4.78	0.23	0.877
Residual	76	1593.39	20.97	----	----
Total	83	1803.43	21.73	----	----

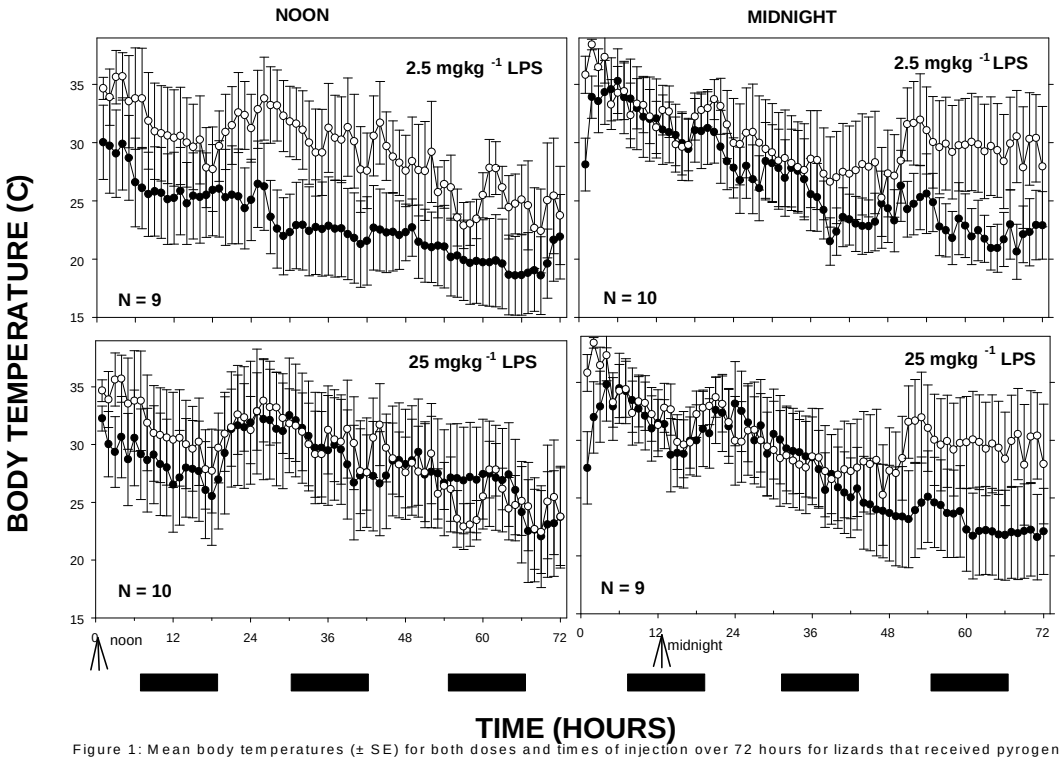

Figure 1: Mean body temperatures (± SE) for both doses and times of injection over 72 hours for lizards that received pyrogen only. White circles indicate control lizards and black circles indicate treatment lizards. Arrows indicate injection times. Black bars indicate scotophase. Statistical comparisons in the study included data from only the first 48 hours after injection. Control N = 8.

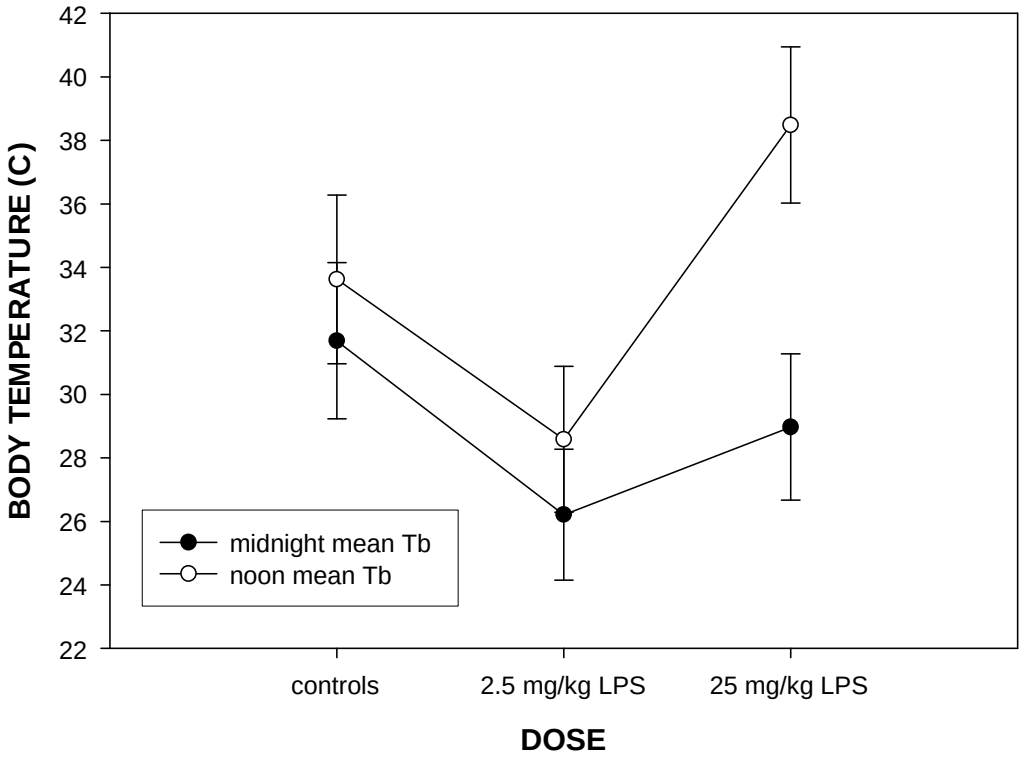

Figure 2: Mean body temperatures (± SE) of lizards injected with only pyrogen by time of injection and dose for all 48 hours. N = 8, 9, or 10.

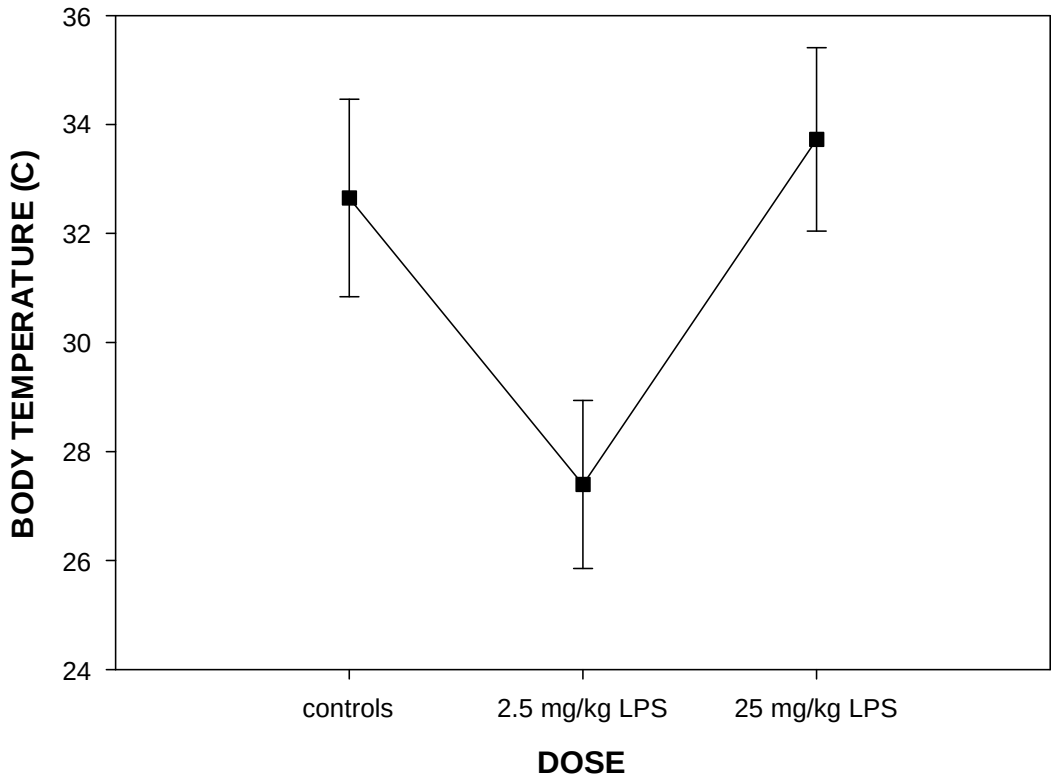

Figure 3: Mean body temperatures (± SE) of all lizards injected with only pyrogen by dose for all 48 hours. N = 16 or 19.

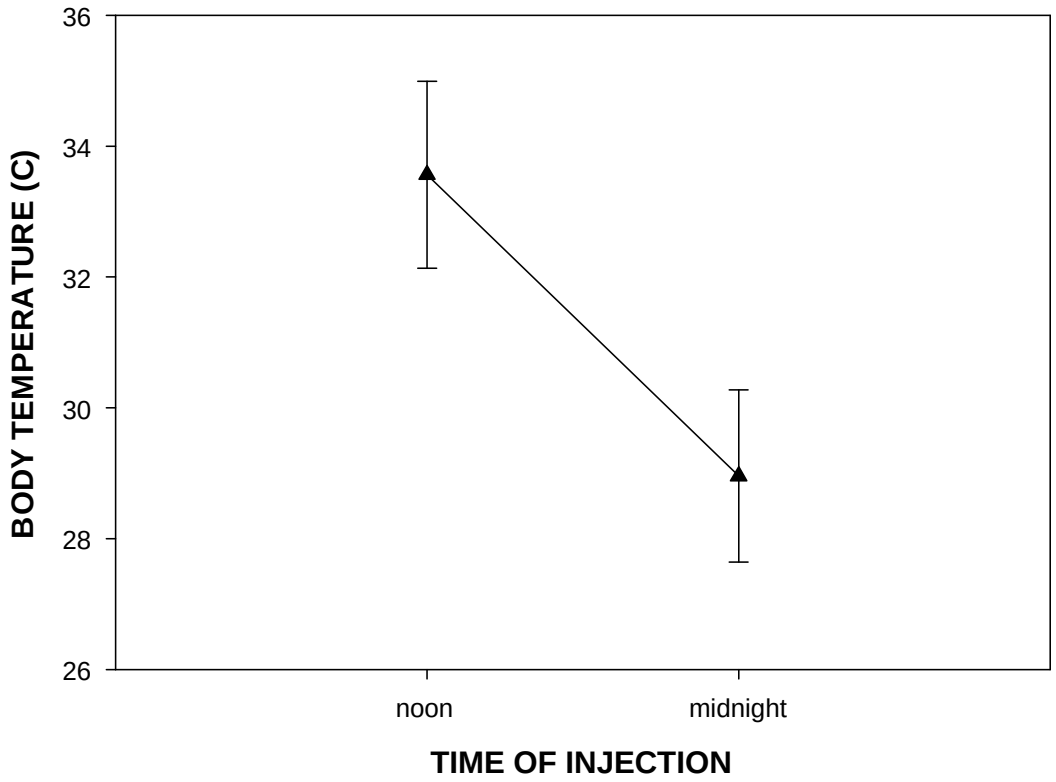

Figure 4: Mean body temperatures (± SE) of all lizards injected with only pyrogen by time of injection for all 48 hours. N = 27.

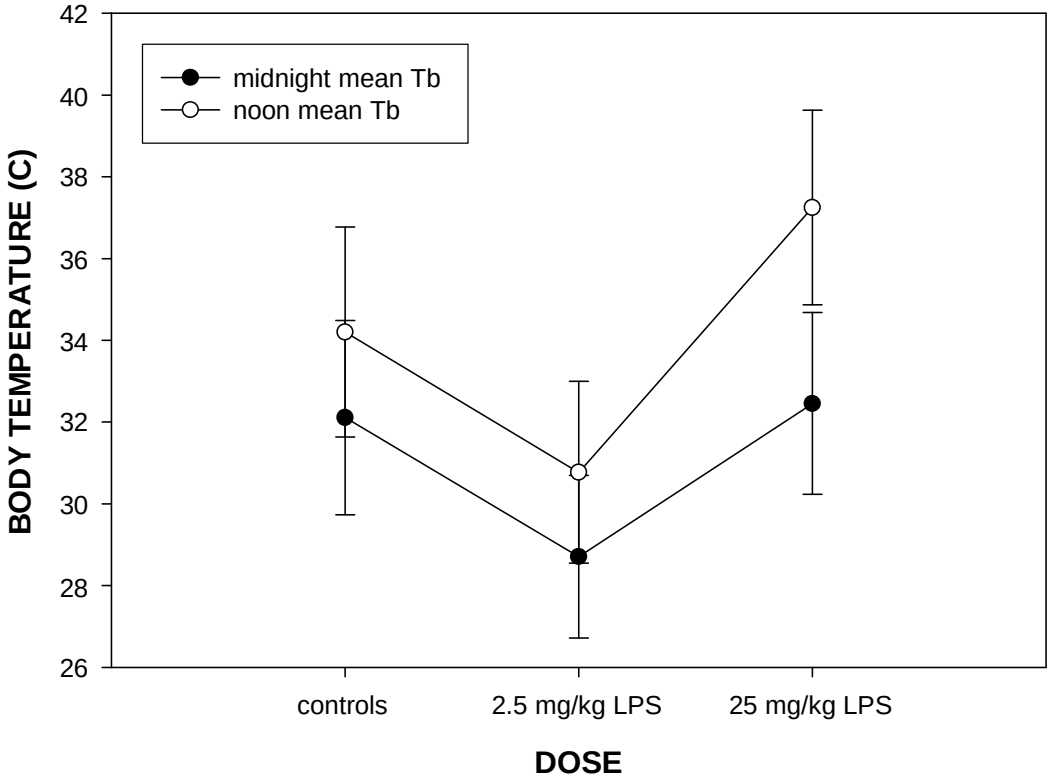

Figure 5: Mean body temperatures (± SE) of lizards injected with only pyrogen by time of injection and dose for day 1. N = 8, 9, or 10.

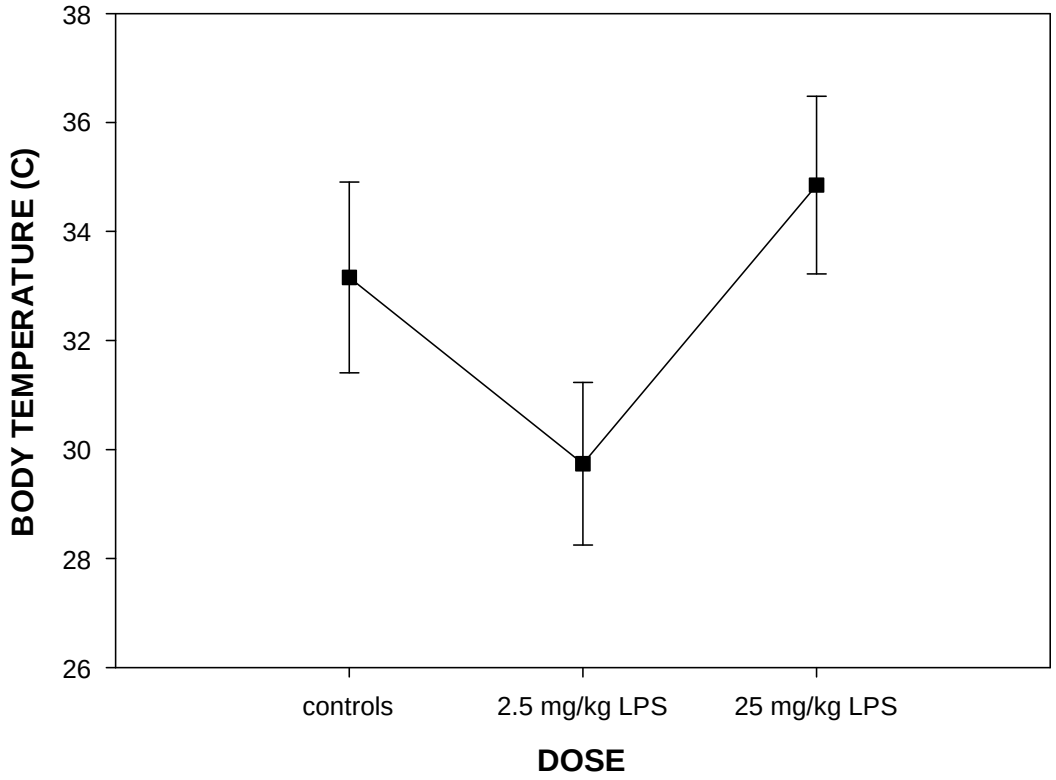

Figure 6: Mean body temperatures (± SE) of all lizards injected with only pyrogen by dose for day 1. N = 16 or 19.

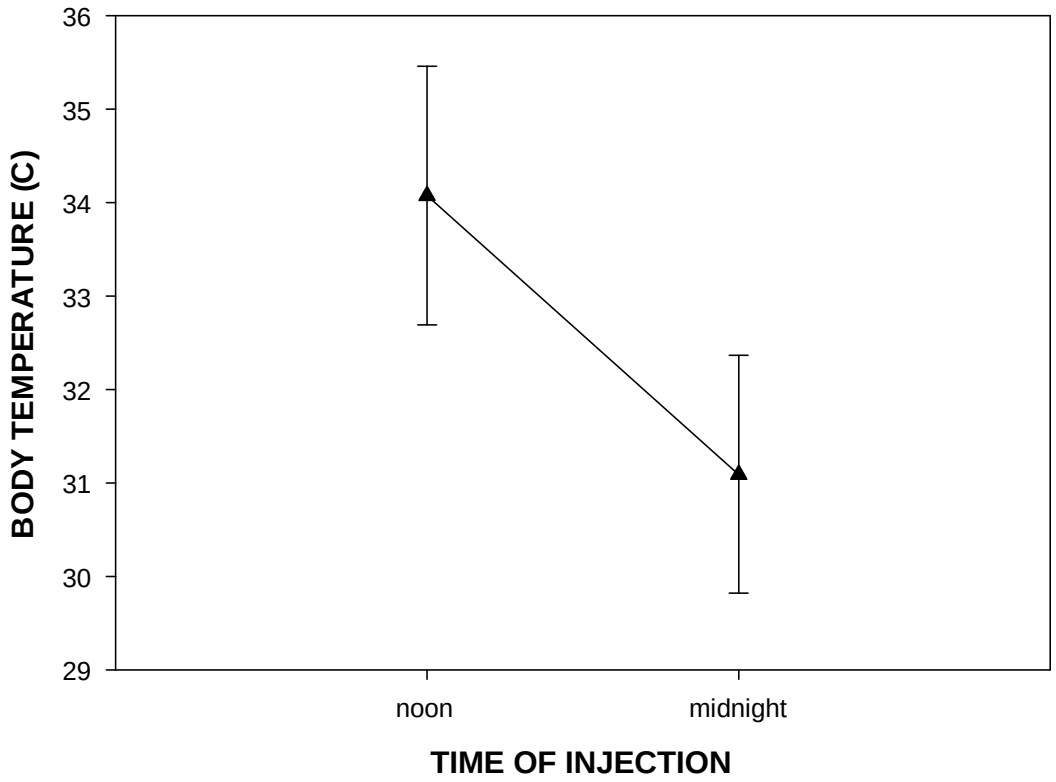

Figure 7: Mean body temperatures (± SE) of all lizards injected with only pyrogen by time of injection for day 1. N = 27.

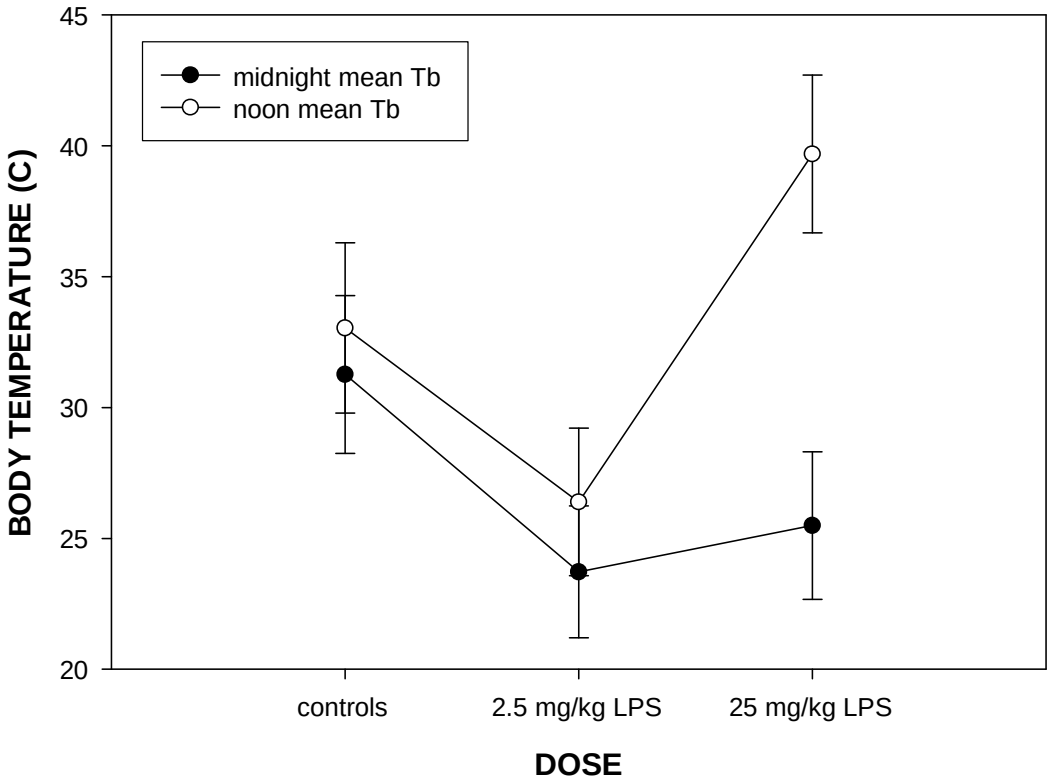

Figure 8: Mean body temperatures (± SE) of lizard injected with only pyrogen by time of injection and dose for day 2. N = 8, 9, or 10.

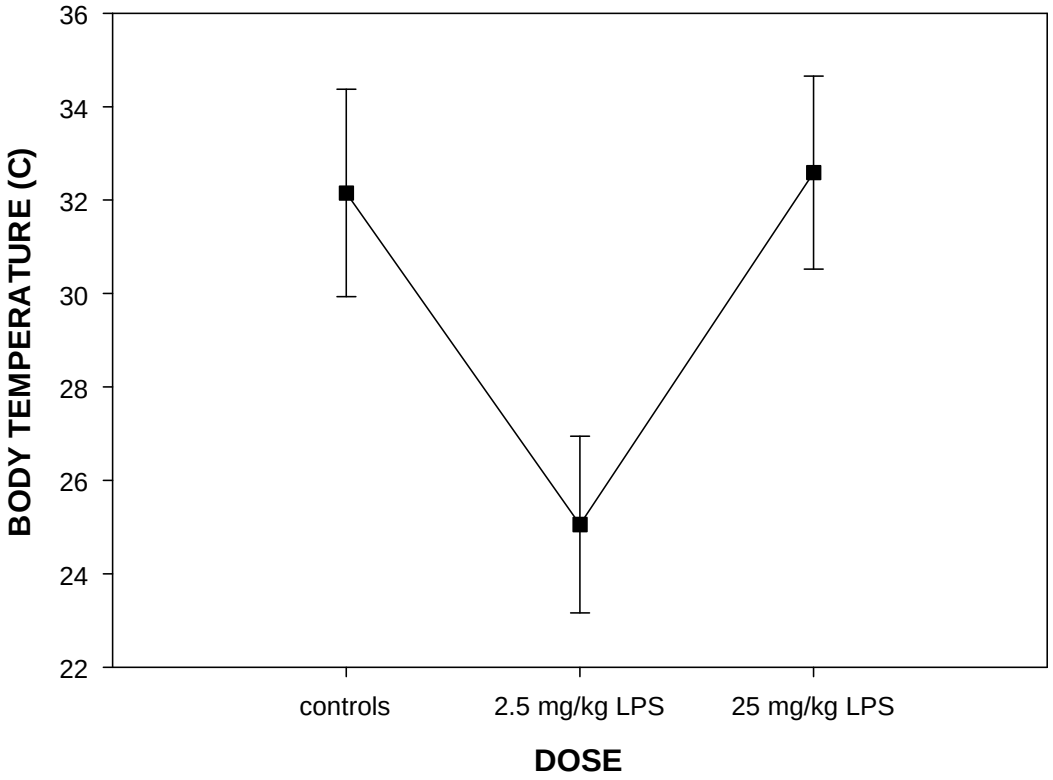

Figure 9: Mean body temperatures (± SE) of all lizards injected with only pyrogen by dose for day 2. N = 16 or 19.

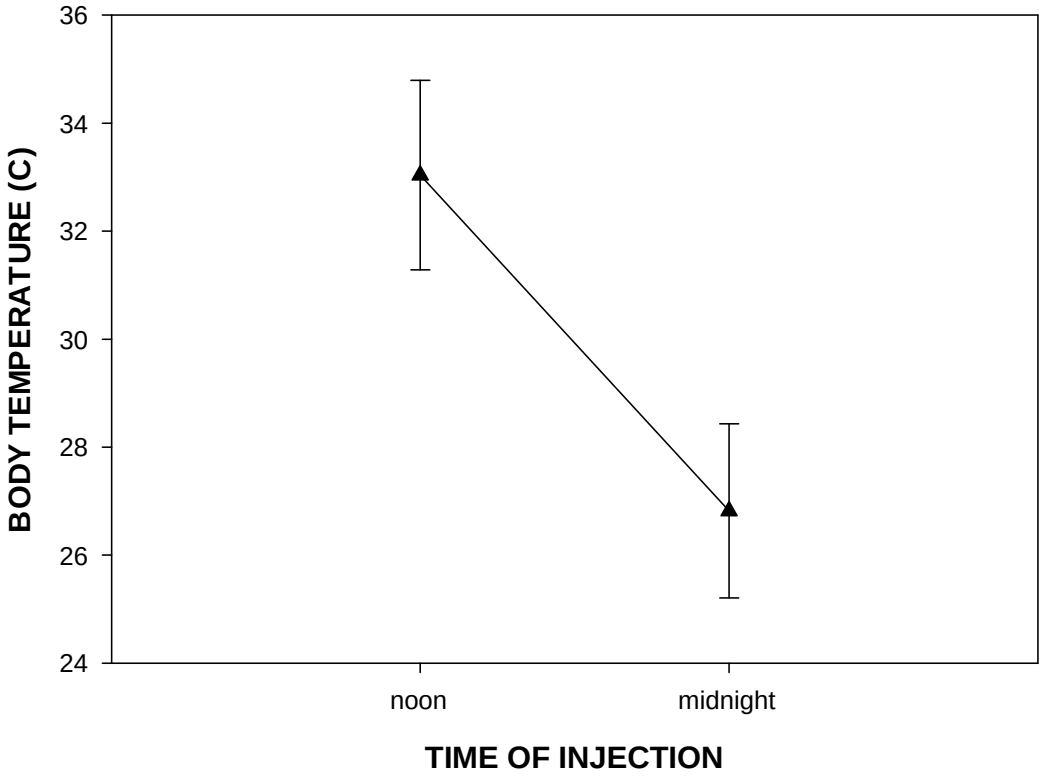

Figure 10: Mean body temperatures (± SE) of all lizards injected with only pyrogen by time of injection for day 2. N = 27.

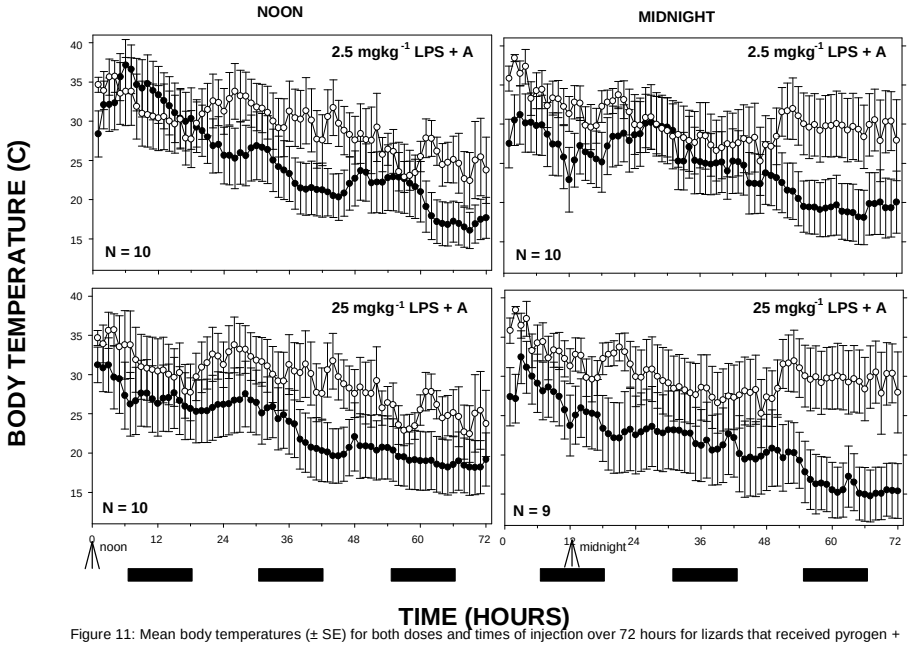

Figure 11: Mean body temperatures (± SE) for both doses and times of injection over 72 hours for lizards that received pyrogen + antipyrogen. White circles indicate control lizards and black circles indicate treatment lizards. Arrows indicate injection times. Black bars indicate scotophase. Statistical comparisons included data from only the first 48 hours after injection. Control N = 8.

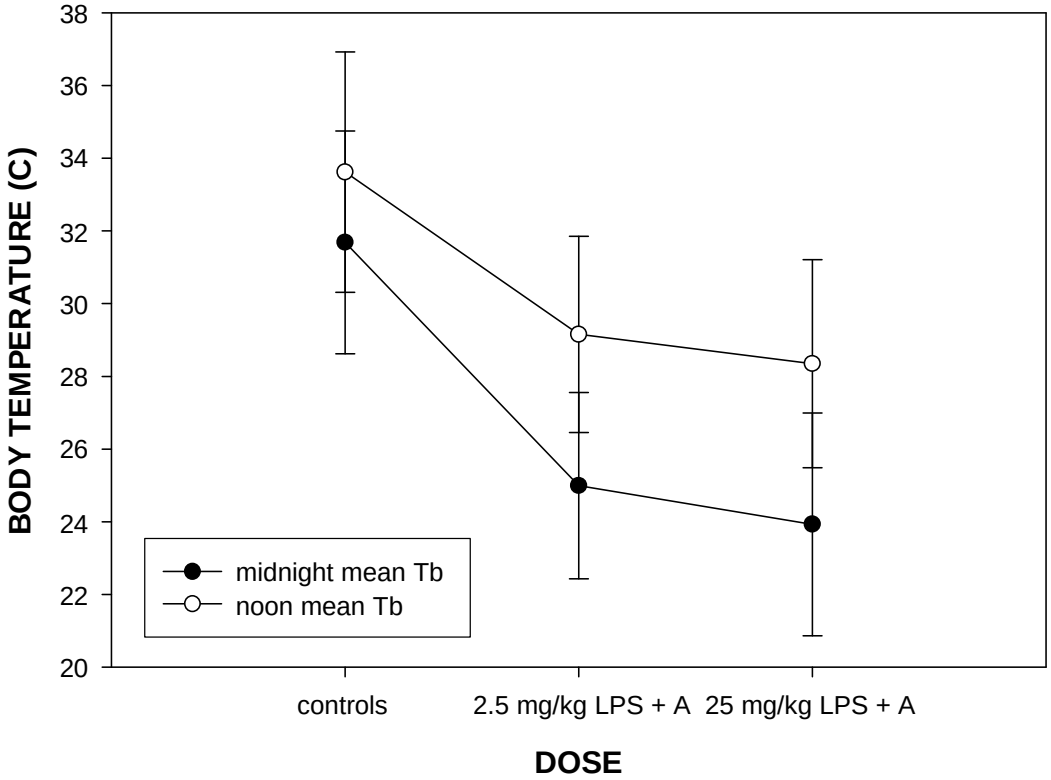

Figure 12: Mean body temperatures (± SE) of lizards injected with pyrogen + antipyrogen by time of injection and dose for all 48 hours. N = 8, 9, or 10.

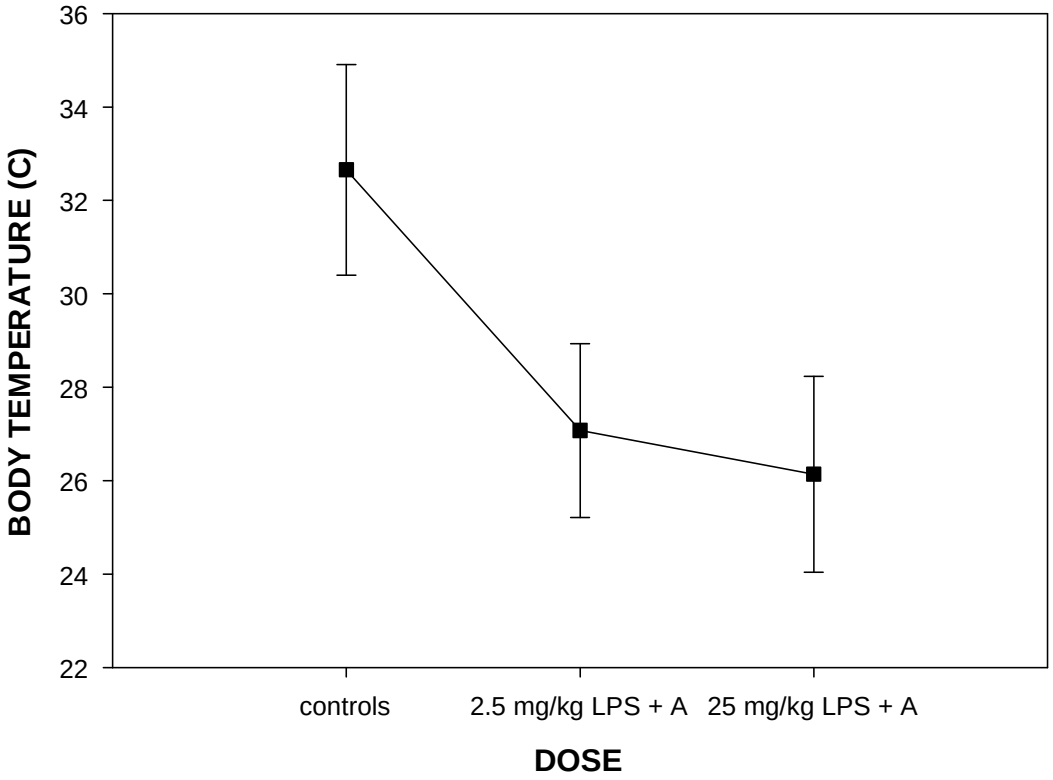

Figure 13: Mean body temperatures (± SE) of all lizards injected with pyrogen + antipyrogen by dose for all 48 hours. N = 16, 19, or 20.

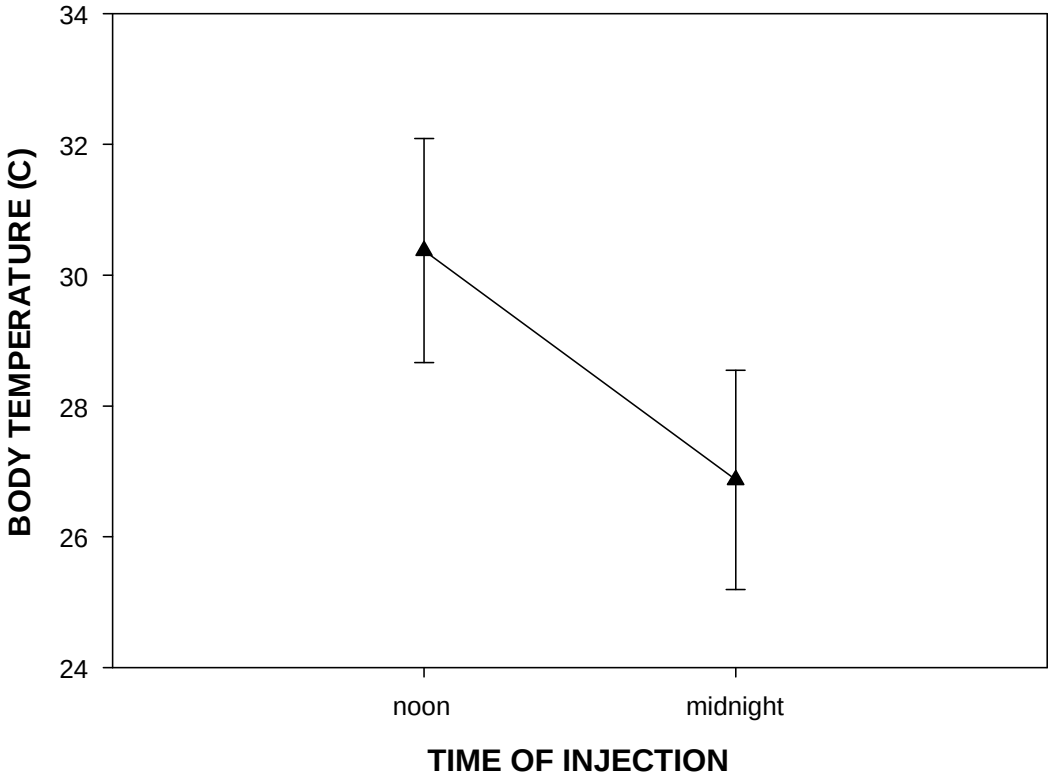

Figure 14: Mean body temperatures (± SE) of all lizards injected with pyrogen + antipyrogen by time of injection for all 48 hours. N = 27 or 28.

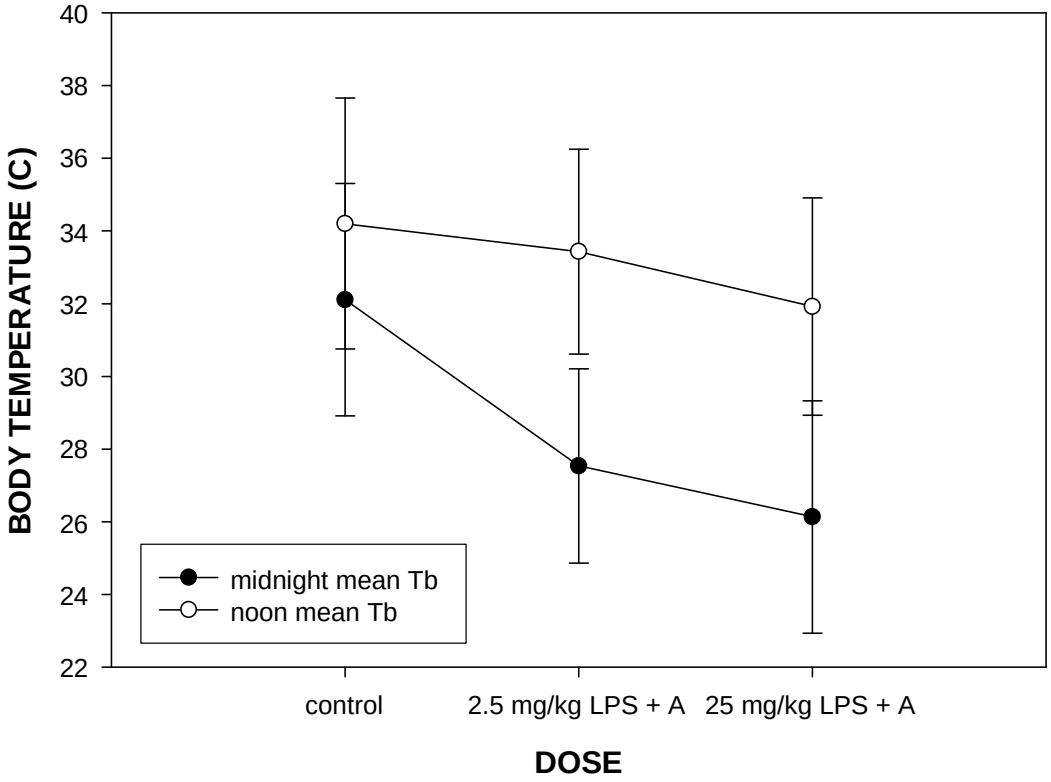

Figure 15: Mean body temperatures (± SE) of lizards injected with pyrogen + antipyrogen by time of injection and dose for day 1. N = 8, 9, or 10.

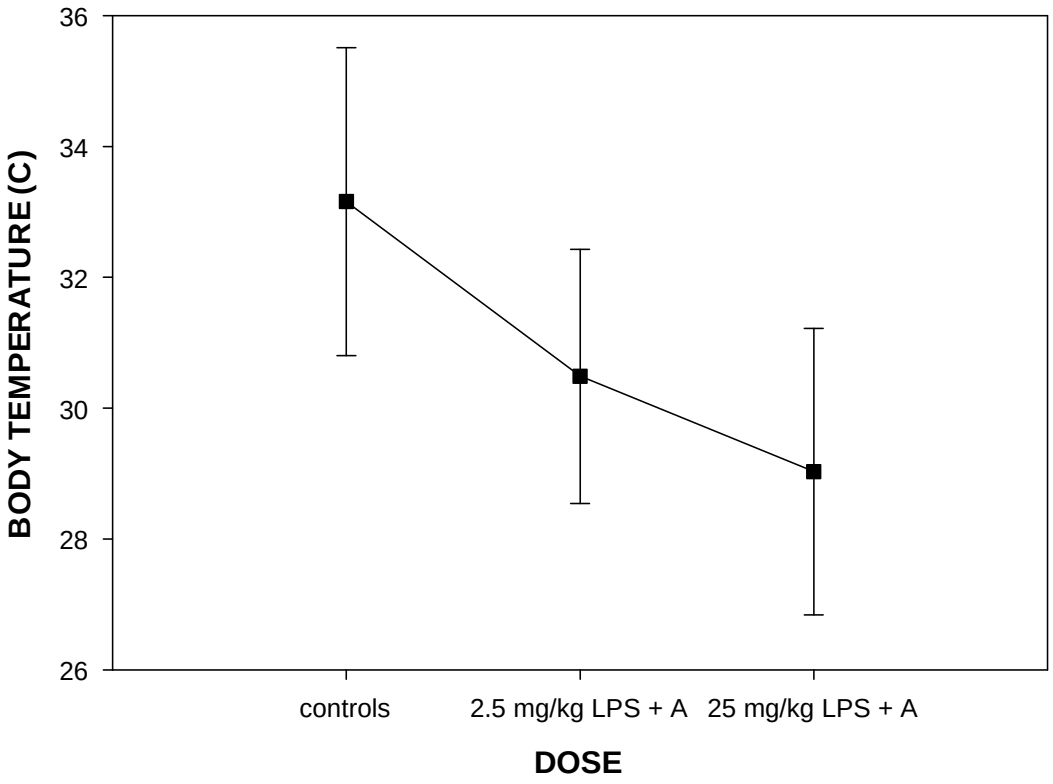

Figure 16: Mean body temperatures (± SE) of all lizards injected with pyrogen + antipyrogen by dose for day 1. N = 16, 19, or 20.

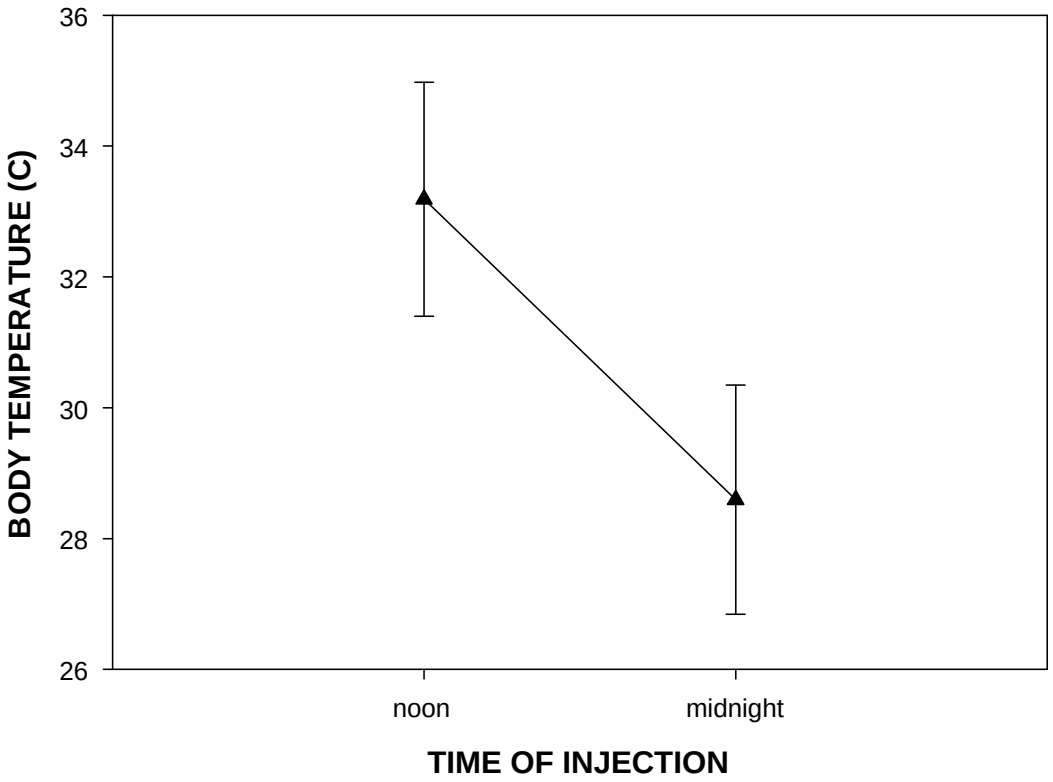

Figure 17: Mean body temperatures (± SE) of all lizards injected with pyrogen + antipyrogen by time of injection for day 1. N = 27 or 28.

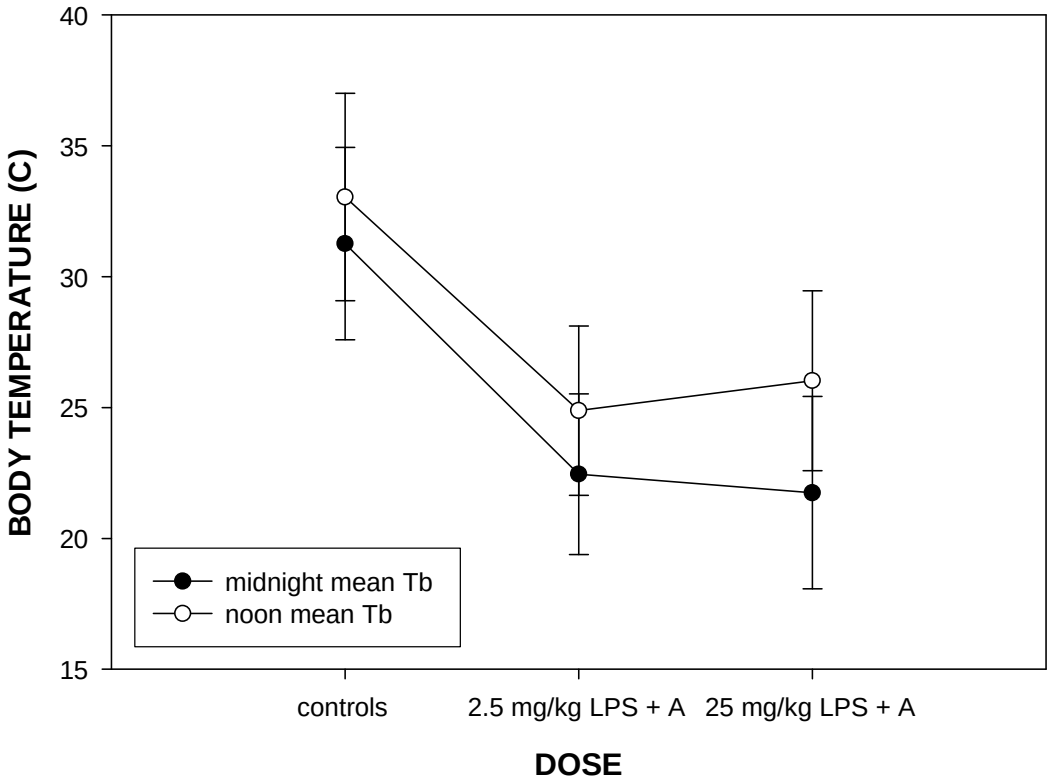

Figure 18: Mean body temperatures (± SE) of lizards injected with pyrogen + antipyrogen by time of injection and dose for day 2. N = 8, 9, or 10.

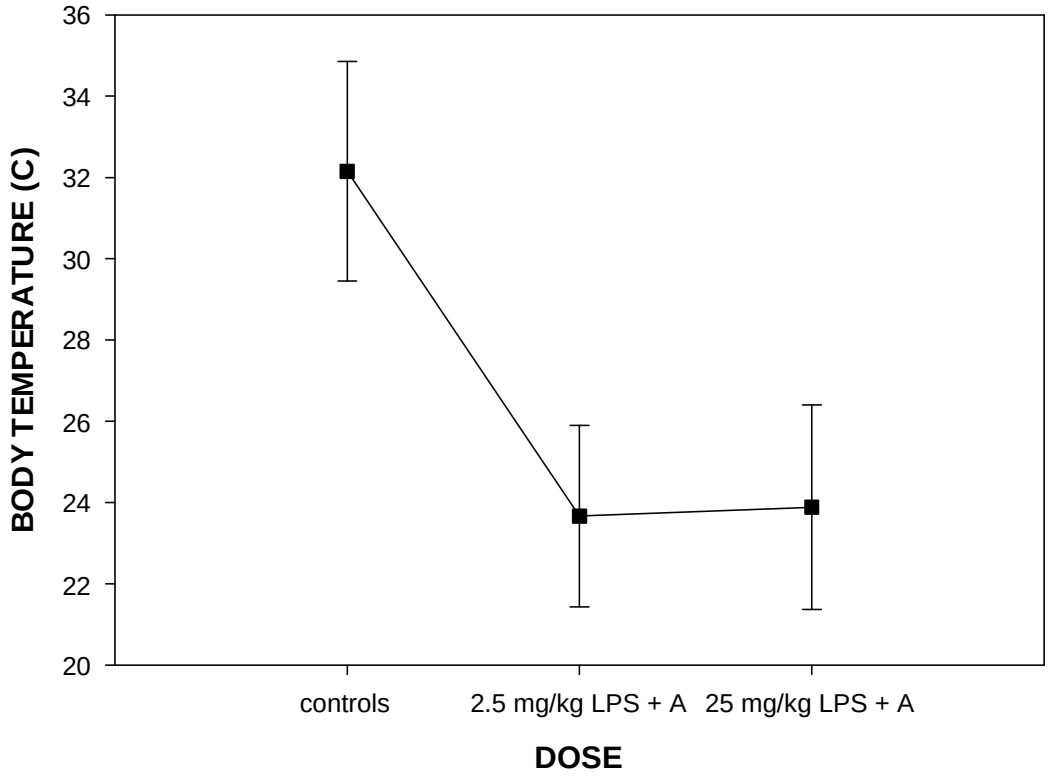

Figure 19: Mean body temperatures (± SE) of all lizards injected with pyrogen + antipyrogen by dose for day 2. N = 16, 19 or 20.

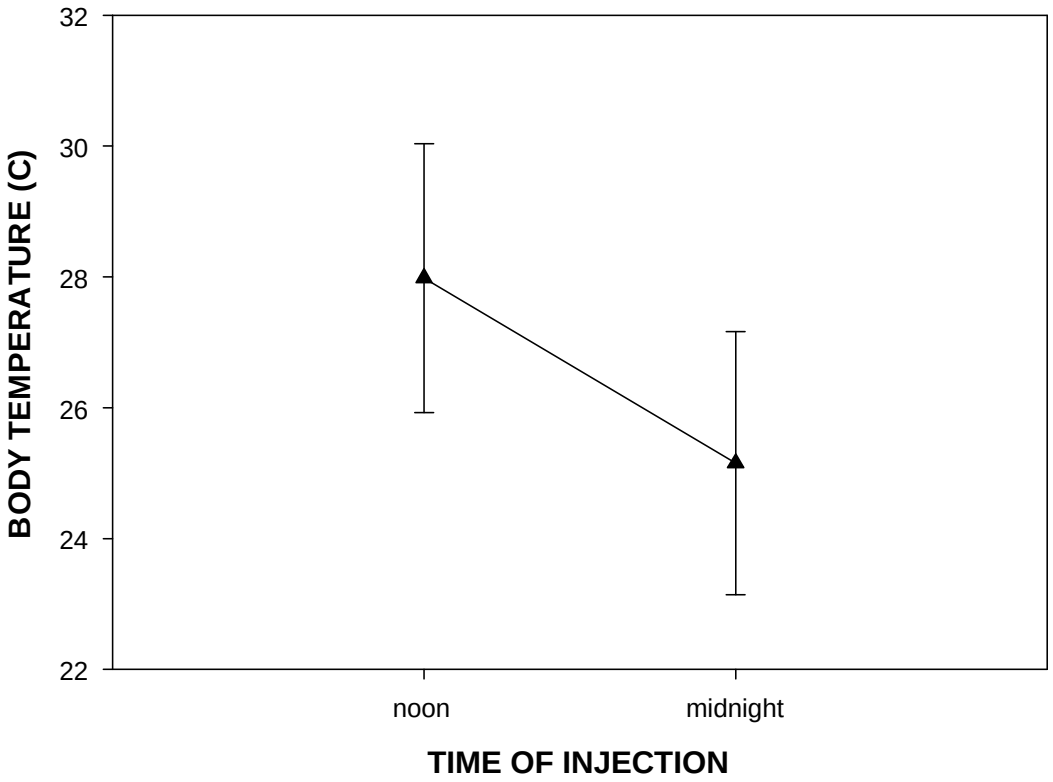

Figure 20: Mean body temperatures (± SE) of all lizards injected with pyrogen + antipyrogen by time of injection for day 2. N = 27 or 28.

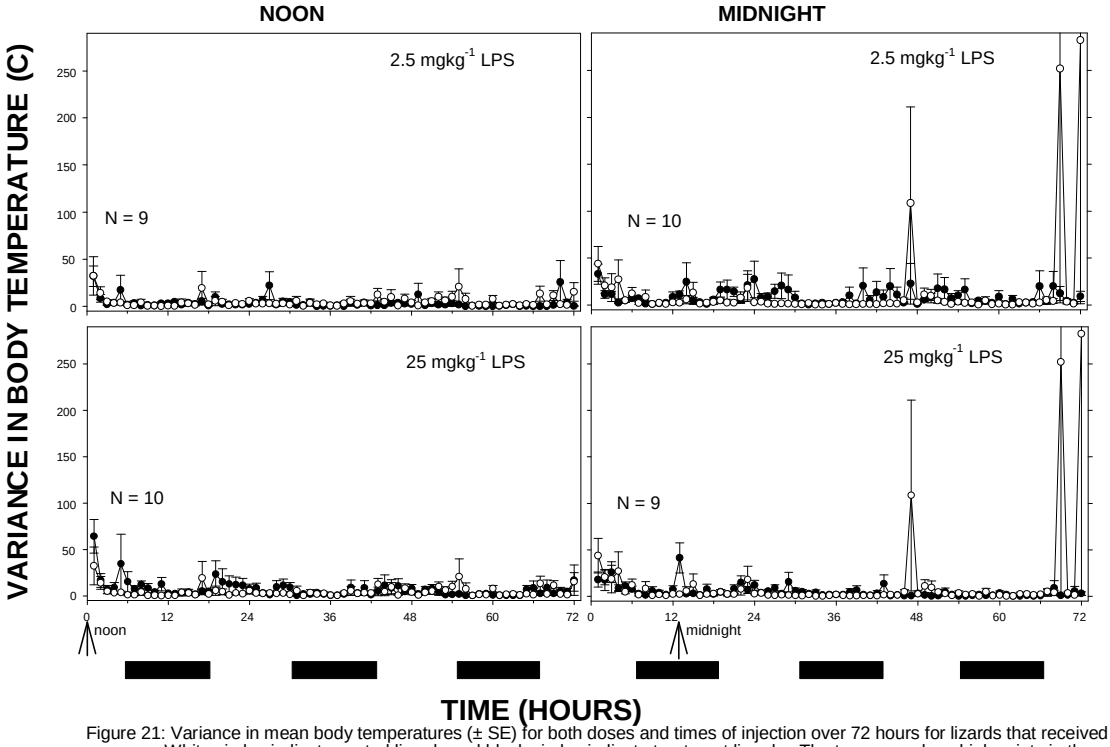

Figure 21: Variance in mean body temperatures (± SE) for both doses and times of injection over 72 hours for lizards that received only pyrogen. White circles indicate control lizards and black circles indicate treatment lizards. The two anomalous high points in the midnight control treatment line occur at hr 69 (252 ± 250) and hr 72 (282 ± 281). Arrows indicate times of injection. Black bars indicate scotophase. Statistical comparisons included data from only the first 48 hours after injection. Control N = 8.

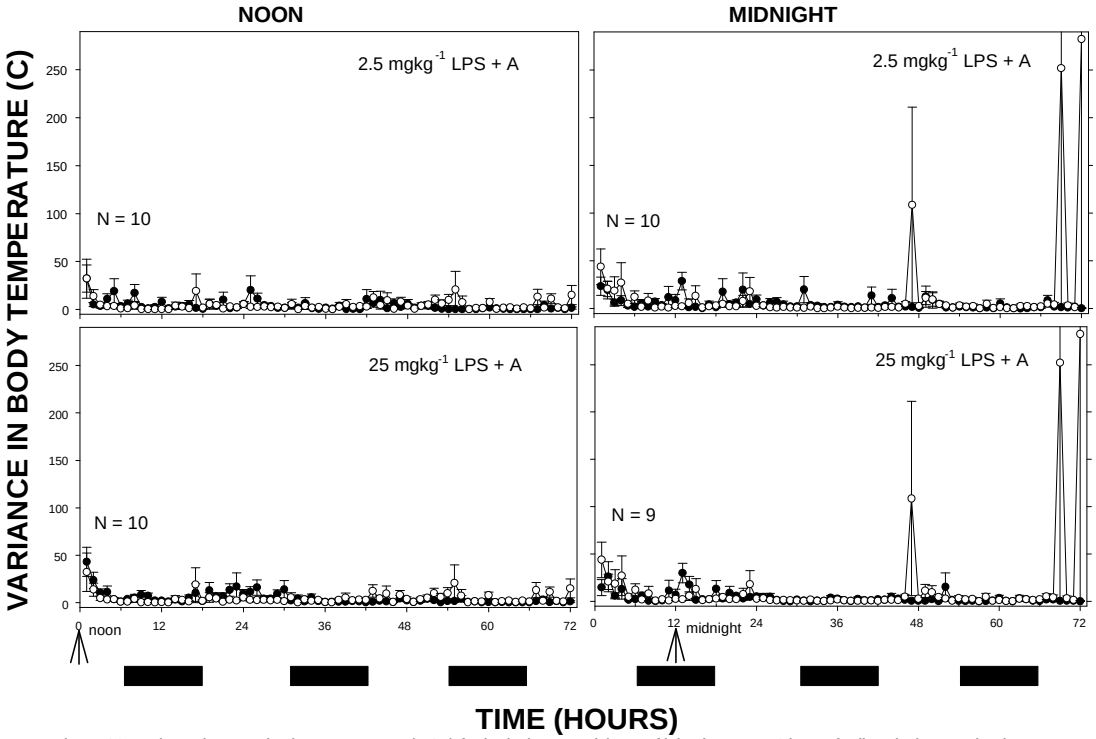

Figure 22: Variance in mean body temperatures (± SE) for both doses and times of injection over 72 hours for lizards that received pyrogen + antipyrogen. White circles indicate control lizards and black circles indicate treatment lizards. The two anomalous high points in the midnight control treatment line occur at hr 69 (252 ± 259) and at hr 72 (282 ± 281). Arrows indicate time of injection. Black bars indicate scotophase. Statistical comparisons included data from only the first 48 hours after injection. Control N = 8.

Appendix I

Mean body temperatures (C) for individual female *D. dorsalis* over the 72 hour trial period.

	non-injected			noon				midnight			
Time (Hrs)		controls		low	high	low + A	high + A	low	high	low + A	high + A
♀ ID	56	65	03	01	53	27	81	09	96	78	87
1	36.5	40.9	24.4	6.7	32.5	20.1	32.6	41.3	36.9	10.1	39.5
2	33.3	33.7	38.7	6.2	28.5	37.0	38.2	40.0	36.1	12.8	38.6
3	33.5	38.1	31.7	6.4	29.1	37.5	38.5	41.6	38.1	15.7	38.1
4	36.5	40.7	35.5	6.5	33.5	38.3	39.5	37.0	36.3	31.8	37.1
5	28.8	38.3	38.2	6.6	33.1	38.3	40.1	33.3	34.0	38.0	38.1
6	32.7	39.2	36.8	6.6	35.4	40.4	39.7	33.4	34.2	37.1	38.7
7	25.2	39.0	36.0	6.7	36.7	39.4	40.7	34.7	34.8	34.1	37.6
8	19.9	38.2	38.6	6.6	37.3	39.2	39.9	37.0	33.4	32.3	36.0
9	21.0	38.2	37.6	6.6	31.5	37.9	40.4	42.7	28.1	25.5	37.8
10	22.1	34.9	34.0	6.6	25.8	38.6	39.5	39.7	31.4	20.5	36.8
11	20.1	35.1	28.0	6.7	22.1	38.6	40.3	34.5	31.4	17.9	36.2
12	17.8	28.8	27.4	6.6	21.1	40.9	39.8	32.6	32.8	16.8	35.1
13	16.9	29.1	26.6	6.4	21.1	41.1	41.4	33.4	34.3	25.4	37.4
14	20.9	40.1	26.5	6.8	20.8	40.2	37.6	35.1	36.5	27.6	40.3
15	21.0	36.7	24.2	6.6	23.2	39.3	41.7	35.0	35.6	26.7	36.3
16	24.9	39.8	20.3	6.7	19.3	37.4	38.9	33.8	34.9	26.6	36.4
17	24.4	39.6	23.8	6.7	17.0	39.3	42.1	31.9	34.8	26.2	35.6
18	23.4	36.6	19.3	6.6	18.0	38.1	41.5	30.9	35.2	23.1	36.0
19	22.8	38.3	19.9	6.9	14.7	38.0	40.6	30.2	36.1	21.1	37.2
20	28.1	34.4	14.6	6.6	14.2	36.5	39.7	28.0	35.8	21.1	38.0
21	32.8	33.8	11.7	6.7	25.5	35.8	38.9	26.3	36.2	22.4	36.6
22	31.3	33.7	27.3	6.8	19.5	36.7	39.0	27.7	36.9	24.5	37.6
23	17.0	33.1	21.0	6.9	17.7	39.8	40.6	29.7	36.5	23.7	36.1
24	14.8	35.0	26.1	7.1	15.9	39.1	39.4	25.9	40.9	23.7	34.8
25	15.0	34.0	21.5	6.9	20.0	39.1	37.8	37.8	35.9	23.6	40.8
26	21.5	36.4	24.6	6.6	20.5	35.5	39.1	40.5	36.1	26.5	39.2
27	16.7	34.6	27.1	6.8	23.9	32.9	38.9	41.1	35.5	26.7	41.0
28	14.7	35.1	28.9	6.6	26.2	32.7	40.2	43.1	38.4	30.6	40.2
29	19.6	36.2	30.3	6.7	28.1	39.4	40.2	41.8	32.1	30.5	38.7
30	26.9	37.3	30.3	6.8	30.3	40.1	40.8	37.6	35.7	32.9	39.1
31	26.3	37.1	33.8	6.4	31.9	38.6	38.5	39.2	32.2	33.9	40.5
32	22.3	37.1	33.5	6.5	33.5	37.9	40.6	38.9	32.0	34.9	39.0
33	24.4	36.6	35.4	6.5	28.4	29.6	38.9	37.9	30.0	33.5	39.3
34	23.7	37.3	36.5	6.4	23.1	23.0	37.9	33.7	32.9	30.3	40.2
35	20.1	35.3	30.8	6.7	21.1	22.7	36.2	30.3	33.2	25.8	35.5
36	16.1	35.0	25.0	6.7	20.2	24.6	36.6	36.0	30.8	28.0	33.3
37	24.4	32.5	21.6	6.6	19.8	21.6	36.9	36.7	26.3	25.1	39.9
38	27.5	33.3	19.8	6.6	18.5	18.6	34.7	36.2	22.5	22.0	39.1
39	27.6	32.1	19.2	6.6	18.1	18.3	33.6	37.9	24.5	7.5	38.5
40	27.6	31.4	18.0	6.8	19.0	18.9	30.6	40.9	22.9	5.9	39.2
41	26.4	33.0	16.8	6.7	20.1	15.2	35.7	41.7	24.9	5.4	39.3
42	25.3	30.6	15.8	6.6	15.5	14.8	37.0	41.1	23.9	4.8	37.8
43	25.8	29.7	14.9	6.6	14.3	15.9	34.5	40.6	31.9	4.4	39.9
44	25.4	30.1	14.1	6.9	14.0	16.7	33.2	41.2	34.7	4.2	35.2
45	24.6	30.0	13.2	6.8	13.7	16.3	32.5	39.9	36.2	4.2	37.0

46	22.4	29.3	13.7	6.8	14.2	17.5	33.7	39.4	38.4	4.5	39.5
47	19.2	29.2	14.5	6.4	16.6	19.5	32.4	38.0	38.2	6.5	37.4
48	18.3	24.6	16.0	6.7	20.5	24.8	40.2	32.4	40.3	10.8	39.7
49	19.6	23.7	19.1	6.7	24.3	36.4	40.0	33.4	39.8	14.7	42.6
50	17.6	29.5	23.1	6.6	27.0	35.7	39.3	36.4	40.5	17.5	36.2
51	19.1	32.7	24.7	6.6	27.0	35.0	37.1	36.5	37.3	19.8	27.4
52	13.2	36.4	28.4	6.6	28.7	39.6	38.2	41.0	36.7	27.1	25.7
53	13.7	33.1	30.3	6.6	31.4	40.3	37.5	42.2	36.9	33.0	22.1
54	13.6	34.8	31.8	6.5	31.5	39.2	38.4	40.6	36.9	29.1	20.5
55	13.2	36.2	32.8	6.4	36.3	40.3	38.3	36.8	35.6	29.5	18.8
56	13.3	37.5	33.0	6.4	36.2	41.9	35.5	41.5	37.4	30.9	17.5
57	13.0	38.4	34.9	6.6	34.8	41.0	35.9	41.9	37.0	29.6	16.4
58	13.2	38.6	33.6	6.6	41.5	34.5	35.0	37.3	36.3	23.4	15.4
59	13.4	38.4	35.6	6.6	38.5	28.7	36.8	33.1	36.7	20.2	13.9
60	13.4	32.4	27.4	6.6	42.4	20.9	36.3	30.1	35.4	17.9	13.9
61	13.1	26.5	24.3	6.6	40.6	19.0	35.3	26.6	34.2	16.8	13.7
62	13.4	22.9	21.4	6.6	43.0	16.8	34.7	20.7	33.8	16.2	13.5
63	13.3	20.9	19.3	6.9	41.8	15.5	34.0	21.2	33.4	15.3	13.2
64	13.4	19.9	19.0	6.7	41.5	15.5	35.1	21.2	33.2	14.3	12.9
65	13.8	18.5	17.5	6.8	36.4	15.3	34.4	19.9	33.5	13.6	12.9
66	13.7	17.3	18.0	6.5	33.5	16.2	34.0	18.5	32.6	12.3	12.7
67	14.1	16.5	16.2	6.9	25.9	15.5	32.7	19.2	32.8	7.5	12.8
68	13.5	15.7	16.5	6.7	24.2	15.2	34.2	18.7	32.8	6.7	12.9
69	13.6	15.1	14.6	6.7	20.0	14.7	33.6	18.3	31.7	6.3	13.1
70	13.5	14.8	20.4	6.8	29.2	14.9	33.1	17.2	31.0	6.4	13.0
71	13.5	15.5	18.1	7.0	35.0	15.6	34.6	17.6	32.3	7.2	13.1
72	13.9	15.9	15.2	6.9	34.2	17.1	33.9	18.0	32.9	8.9	13.2

Appendix II

Female *D. dorsalis* morphometrics.

♀ ID	Snout-Vent Length (cm)	Mass Pre-Run (g)	Mass Post-Run (g)
56	10.5	30.2	28.0
65	9.9	31.5	28.7
03	11.1	38.3	37.0
01	11.1	32.1	29.2
53	11.7	31.3	23.6
27	11.0	40.4	35.9
81	10.7	39.1	37.1
09	11.0	47.2	44.3
96	11.4	34.2	32.6
78	10.3	29.5	28.6
87	11.0	39.3	37.0