AN ABSTRACT OF THE THESIS OF

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Title: Metabolic and Digestive Parameters of Cope's Gray Treefrog, *Hyla chrysoscelis.*

Abstract approved: Lynnette Sievert
(Thesis Advisor's Signature)

I conducted three experiments to compare metabolic and digestive parameters of Cope's Gray Treefrogs, *Hyla chrysoscelis*. I monitored oxygen consumption ($Vo_2$) rates of fasted and fed treefrogs (n = 8) in 4-h intervals over 24-h to determine if higher levels of resting $Vo_2$ occurred at night during the species' normal activity period. There were no significant differences among $Vo_2$ values at different times of day ($P = 0.33$), and no interaction between time of day and treatment group ($P = 0.50$). However, there was a highly significant difference ($P < 0.0001$) in $Vo_2$ between fasted and fed animals, with fed animals having $Vo_2$ rates 2.6 to 4.1 times higher than fasted animals. I measured the food passage time (FPT) of eight *H. chrysoscelis* at 24 C and six at 16 C to determine the effect of temperature on FPT. I fed adult male treefrogs a meal of a single marked cricket for four consecutive nights. I measured the amount of time between ingestion of marked food and defecation of each marker. All treefrogs ate marked crickets at 24 C, but only six treefrogs ate marked crickets at 16 C. Means ± SD of FPTs were 91.1 ± 29.2 h at 16 C and 38.2 ± 9.76 h at 24 C ($P = 0.006$). I fed 11 male *H. chrysoscelis* single crickets once daily at 2000 h for eight days to determine digestive efficiency. I dried fecal samples at 70 C and combusted with a Parr Instrument Company oxygen bomb calorimeter. The mean ± SD apparent digestibility coefficient for *H. chrysoscelis* was

81.1 ± 3.8%. Linear regression showed a positive linear relationship between calories consumed and calories in feces ($r^2 = 0.76$, $P = 0.0006$). Low resting metabolic rate, short FPT, and efficient digestion at 20 to 24 C correspond with the species' life history patterns.

# METABOLIC AND DIGESTIVE PARAMETERS OF COPE'S GRAY TREEFROG, *HYLA CHRYSOSCELIS*

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A Thesis

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by

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# PREFACE

The data and results from my thesis will be submitted to the journal Copeia for publication. I wrote my thesis in the style of that journal. The ESU Animal Care and Use Committee approved all procedures under permit number ESU-ACUC-99-002. All animals were collected under Kansas Department of Wildlife and Parks collection permit numbers SC-098-99, SC-113-2000, or SC-084-2002.

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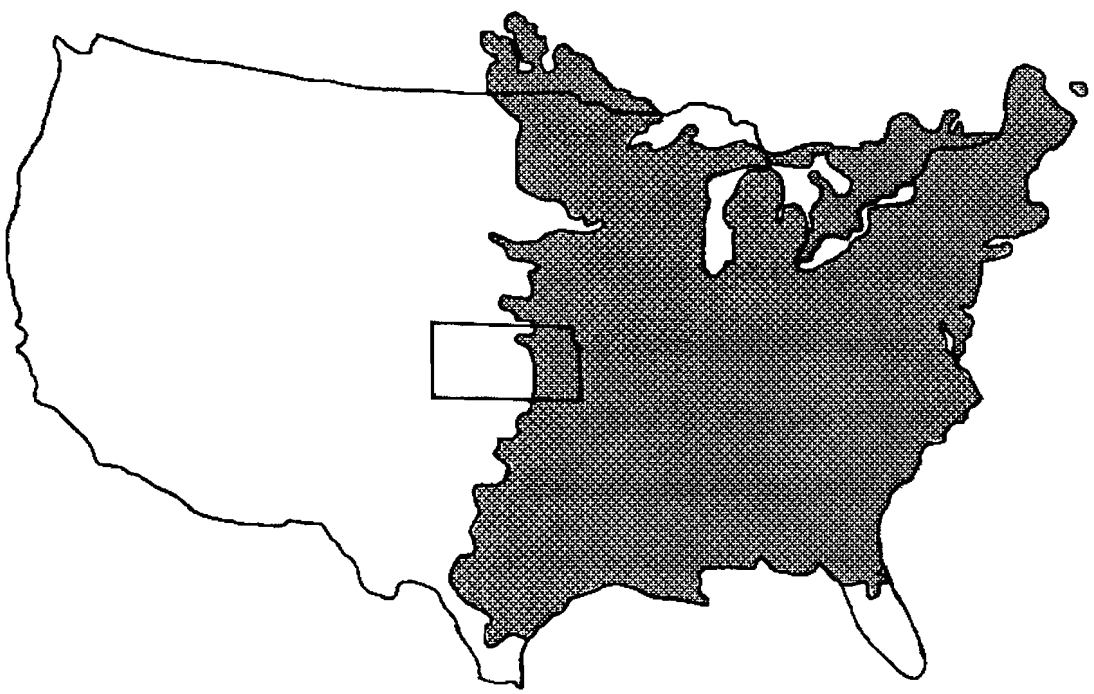
## INTRODUCTION

Cope's Gray Treefrog, *Hyla chrysoscelis*, is a nocturnal, arboreal frog that feeds on small invertebrates (Johnson, 1987). *H. chrysoscelis* is limited to the more mesic portions of North America (Fig. 1), and sometimes it shares habitat with a sibling species, *Hyla versicolor*, the Eastern Gray Treefrog (Hillis *et al.*, 1987). They live near fishless, woodland ponds (Collins, 1993) where they cling to tree bark or leaves with their sticky toe pads (Green, 1981).

*H. chrysoscelis* is an ectotherm with low energy requirements (Gatten *et al.*, 1992). This treefrog is nocturnal, and feeds, moves to and from water, and mates at night. Females mate and lay one clutch of eggs each spring (Blem *et al.* 1986). Male treefrogs call between temperatures of 16-32 C (Clarke, 1958) from 11 April to 26 June (Hillis *et al.*, 1987), and in Missouri, the treefrogs are active until October (Johnson, 1987). Gray Treefrogs are the only amphibians in Kansas capable of changing skin color to match their substrate (Collins, 1993), and thereby avoid detection by potential predators. Many of their characteristics, including their low daily energy requirement, mouth morphology, and cryptic coloration are correlated with a typical sit-and-wait forager (Pough *et al.*, 1998).

The metabolic rates of most ectotherms reflect their low energy requirements and low activity levels relative to endotherms (Pough *et al.*, 1998). Metabolic rates of ectotherms have been defined as: standard metabolic rate ($Vo_{2standard}$), resting metabolic rate ($Vo_{2rest}$) and maximum metabolic rate ($Vo_{2max}$). Pough *et al.* (1998) defined these as the metabolic rates of quiet, post-absorptive (fasted) animals during their normal resting period, during their normal activity period, and during maximal exercise. Cyclical

Figure 1. Distribution of *H. chrysoscelis* and *H. versicolor* in North America, adapted from Collins (1993), Conant and Collins (1998).



variations in $Vo_{2rest}$ over the course of a 24-h day defines the animal's diel cycle of metabolic rate. For example, if an animal's $Vo_{2rest}$ increases significantly at dawn and dusk, the animal would be crepuscular.

Within a species' normal active temperature range, higher temperatures tend to result in higher metabolic rates (Guimond and Hutchison, 1968; Brownlie and Loveridge, 1983; Beaupre *et al.*, 1993a) and greater digestive rates (Riddle, 1909; Skoczylas, 1970). Increases in body temperature ($T_b$) below the critical thermal maximum increase digestive rates, which allow the animal to empty the gut faster, thereby allowing for increased food consumption and growth potential (Freed, 1980; Christian, 1986).

Blem *et al.* (1986) found that in *H. chrysoscelis* digestive efficiency significantly increased over temperatures from 19 to 29 C. However, $Vo_2$ also increased significantly as ambient temperatures increased from 10 to 30 C. At high temperatures, an increase in calorie use beyond the increase in calorie uptake is disadvantageous, because it decreases the amount of surplus energy available to the animal for activities such as reproduction. Blem *et al.* (1986) concluded that *H. chrysoscelis* is not tolerant of high temperature, as demonstrated by its significant increase in metabolic rate in response to increases in temperature. Layne and Romano (1985) found that *H. chrysoscelis* demonstrated a tolerance of extreme low temperatures. The treefrog's capability to tolerate various temperatures may explain the northern distribution of the species, and lack of a population in much of the Florida Peninsula (Blem *et al.* 1986).

This thesis covers three areas of physiology in *H. chrysoscelis*. In Experiment 1, I measured daily patterns of resting metabolic rates in fasted and fed animals. In Experiment 2, I measured food passage time at two temperatures, and in Experiment 3 I assessed the digestive efficiency of Cope's Gray Treefrog when fed crickets (*Acheta*

*domestica*). Each of these physiological characteristics act in concert with one another. Increasing the metabolism associated with feeding, time of food passage, and efficiency of digestion all affect the fitness and growth capacity of ectotherms (Sibly, 1981; Hatch and Afik, 1999), and temperature affects all of these factors. Physiological tolerance to environmental changes, such as fluctuation in temperature, allows a species to take advantage of resources not available to less tolerant species (Pough, 1980). Understanding a species' physiology under controlled laboratory conditions offers valuable insight into the relationship between the physiology and natural history of the species.

*Experiment 1: Correlations of resting metabolism, feeding, and activity patterns*

The amount of time since feeding in ectotherms is of great importance to metabolic rate. An increase in metabolic rate following feeding typically occurs in animals (Gatten, 1980; Wang *et al.*, 1995; Secor and Diamond, 1997; Sievert and Bailey, 2000). This increase could obscure any possible diel cycle in metabolic rate if feeding in experimental animals is not reported. When animals are digesting and assimilating a meal, energy is required to move the food through the digestive tract, produce mRNA, and break down and absorb nutrients (Wang *et al.*, 1995; Secor and Diamond, 1997). The increase in post-feeding metabolic rates is termed Specific Dynamic Action (SDA) and represents an energetic cost to the animal.

I hypothesized that fasted Copes Gray Treefrogs would have lower metabolic rates than fed animals due to SDA, as seen in *Rana catesbeiana* (Secor and Diamond, 1996), *Bufo woodhousii* (Sievert and Bailey, 2000), and *Bufo marinus* (Wang *et al.*, 1995). The advantage of low metabolic rates in fasted animals is that it conserves energy

until more food is available. The cost of SDA is normally a small fraction of the energy supplied by the meal, but in some cases, the cost can be a substantial portion of the meal. Examples of this are *Python molurus* (Secor and Diamond, 1997), *Ceratophrys ornata,* and *Pyxicephalus adspersus* (Secor and Diamond, 1996), which eat so infrequently that the lining of the small intestine must essentially be re-built each time the animal eats. This is energetically expensive and results in SDA values that are 10 to 44 times higher than metabolic rates of fasted animals (Secor and Diamond, 1997).

Time of day has an impact on metabolic rate in many species. *Acris crepitans* is the only frog in the family Hylidae that has been monitored for a diel cycle. *Acris crepitans*, which is primarily diurnal, but active night and day during warm months (Johnson, 1987; Collins, 1993) does not exhibit a pronounced diel cycle of metabolic rate (Dunlap, 1969). The frogs had a tendency for peak $Vo_2$ at 1320, and lowest $Vo_2$ at 0320 h; however, no significant differences were found (Dunlap, 1969).

Some nocturnal anurans exhibit diel cycles of metabolic rates with the highest rates occurring at night, which is the normal active period. When *Rana pipiens* was maintained on photoperiods of LD 8:16 and 16:8, $Vo_2$ peaked soon after the beginning of scotophase and was lowest in the middle of photophase (Guimond and Hutchison, 1968). The nocturnal frog *Xenopus laevis* (Abel *et al.*, 1992) and nocturnal salamanders *Desmognathus ochrophaeus* (now *D. ocoee*) and *Plethodon websteri* (Sievert and Davis, 1995) all had higher $Vo_2$ during scotophase than photophase.

Many reptiles exhibit diel rhythms of oxygen consumption. The diurnal lizards *Sceloporus merriami* (Beaupre *et al.*, 1993a), *Lacerta sicula* (Cragg, 1978), and *Mabuya capensis* (Brownlie and Loveridge, 1983) had strong diel rhythms with $Vo_2$ highest during the day. However, in three burrowing species the diel cycles in $Vo_2$ were either

minimal in the diurnal *Proscelotes arnoldi* or absent in the crepuscular *Typhlosaurus cregoi* and the diurnal *Acontias meleagris* (Brownlie and Loveridge, 1983). Brownlie and Loveridge (1983) concluded that the fossorial life history of these animals might have diminished their diurnal rhythms. Cragg (1978) and Brownlie and Loveridge (1983) maintained the animals in constant darkness, which demonstrated that diel cycles of $Vo_2$ were endogenous.

Feder and Feder (1981) tested the correlation between daily activity and $Vo_2$ rates using three nocturnal gekkonid lizards. All three species had different patterns of $Vo_2$. *Cosymbotus platyurus* did not have a distinct high or low $Vo_2$ over a 24 h period. *Lepidodactylus lugubris* and *Hemidactylus frenatus* both had peak $Vo_2$ during scotophase, but the elevated $Vo_2$ of *L. lugubris* was transient whereas $Vo_2$ of *H. frenatus* remained high throughout scotophase. *Hemidactylus frenatus* competitively excludes *L. lugubris* due to its larger size and more efficient foraging tactics (Petren and Case, 1996), which suggests that sustained metabolic rates lend an adaptive advantage to *H. frenatus* over *L. lugubris*.

The purpose of Experiment 1 was two-fold: 1) to determine if Cope's Gray Treefrog exhibits a diel cycle of resting metabolism, and 2) to establish the effect of feeding on metabolic rate. I examined the daily resting metabolic rate of recently fed as well as fasted *H. chrysoscelis*.

*Experiment 2: Influence of temperature on feeding performance and food passage time*

Physiological tolerance to changes in the thermal environment and efficient feeding and digestion over a broad range of body temperatures allow an ectotherm to take advantage of available resources and increase energy intake (Blem *et al.*, 1986). The

motivation to feed and feeding performance are variable among species, dependent upon life history and environmental conditions.

Experiment 2 was an investigation of the amount of time required for food to pass through the digestive tract of *H. chrysoscelis* at two temperatures. According to Larsen (1992), food passage time in ectotherms is dependent upon the species, size, sex, nutritional condition, health, stress, season, temperature, and meal size and type. The purpose of this study was to investigate how the thermal environment affects the food passage time (FPT) of *H. chrysoscelis*.

Cold acclimation temperatures reduces feeding motivation and performance in *Bufo americanus* (Stevens, 1988a; 1988b; 1988c). When tested at acclimation temperatures between 5 C and 35 C, no American Toads ate at 5 C, and feeding performance was greatest at 20 C (Stevens, 1988b). When American Toads were gradually cooled from 20 to 10 C, and feeding performance was tested at 20 C, performance significantly decreased after four days (Stevens, 1988c). When *B. americanus* was rapidly cooled from 20 to 12 C, motivation to feed was significantly reduced after 7 h (Stevens, 1988a). Feeding rates increased at 30 C relative to 15 C in *Bufo bufo* (Larsen, 1992), and food intake was positively correlated with temperature in *Bufo boreas* at 14, 20, and 27 C (Lillywhite *et al.*, 1973). The lizard, *Platysaurus intermedius wilhelmi,* ceased feeding below 12 C (Alexander *et al.*, 2001). FPT was significantly shorter at 22 C than at 18 and 16 C, but did not decrease significantly from 22 to 34 C (Alexander *et al.*, 2001).

Digestive rates in *H. cinerea* increase as body temperatures increase due to basking (Freed, 1980). Animals that were allowed to bask showed an increase in food intake and growth (Freed, 1980). Riddle (1909) observed an increase in digestive rates of

*Rana virescens* (now *pipiens*) when ambient temperature was increased from 25 C to 30 C. Digestive processes in *Natrix natrix* were maximal at 25 C, slowed at 35 C, and ceased at 5 C (Skoczylas, 1970).

Because ambient temperature ($T_a$) greatly determines the $T_b$ of amphibians, some amphibians have developed thermoregulatory behaviors that maximize growth rates (Freed, 1980, Lillywhite *et al.*, 1973). To control $T_b$ and to accelerate digestive processes ectotherms can thermoregulate by moving to heat sources (e.g., warm rocks, rotting litter, or sunshine) or into the shade to avoid the heat source (Moll and Legler, 1971). With a few exceptions, many amphibians are poor thermoregulators due to high cutaneous evaporative water loss, and water conservation often takes precedence over thermoregulation (Brattstrom, 1979).

Post-feeding thermophily allows amphibians to more quickly and sometimes more efficiently digest food, maximizing caloric intake (Lillywhite *et al.*, 1973). Montane *Bufo bufo boreas* prefers higher temperatures after feeding as opposed to during a fast; however, *Bufo bufo halophilus* does not increase $T_b$ after feeding (Carey, 1978). Witters and Sievert (2001) measured the $T_b$ of fasted and fed *B. woodhousii* in a thermal gradient. Fed toads exhibited a preference for warmer temperatures during late afternoon and evening, which would increase digestive rate. Post-feeding thermophily has also been documented in some reptiles such as *Nerodia sipedon* (Sievert and Andreadis, 1999)

Food passage time of anurans has been noted in previous studies (Smith and Bragg, 1949; Larsen, 1984), but has rarely been quantified with consideration of temperature. The two studies that have quantified the effect of temperature on FPT in anurans used widely varying temperatures for comparison. Jiang and Claussen (1993) measured the rate of FPT in the newt, *Notophthalmus viridescens*, at 25 C and 5 C.

*Notophthalmus viridescens* passed waste at much slower rates at 5 C than at 25 C, with a mean minimum FPT at 5 C of 220 h versus 33 h at 25 C. These authors reported that the newts ingested and digested food at winter temperatures of 5 C. Gossling *et al.* (1980) measured FPT in hibernating and active *R. pipiens* at 4 C and 21 C, respectively. Hibernating *R. pipiens* maintained at 4 C passed chromic oxide markers at 5% the rate of active Leopard Frogs, which took 12 to 24 h to pass markers at 21 C.

The two temperatures chosen for this study, 16 C and 24 C, are temperatures at which male treefrogs are normally active (Clarke, 1958; Johnson, 1987; Collins, 1993). Because metabolic rates increase at higher temperatures in *H. chrysoscelis* (Blem *et al.*, 1986), I predicted that the FPT at 16 C would be significantly longer than at 24 C.

*Experiment 3: Apparent digestibility coefficient – a function of diet and temperature*

Johnson and Lillywhite (1979) defined digested energy as the calories absorbed through the animal's gut, or the number of calories consumed by the animal minus the calories of the feces. This is comparable to the assimilated energy, which is the number of useable calories retained by the animal, and takes into consideration the caloric content of the urine as well as the feces. In some animals, the nitrogenous wastes are incorporated in the fecal pellet, and measuring assimilation efficiency is more feasible. However, in many cases the amount of energy contained in the urine is negligible (Withers, 1992), and cannot be directly associated with a particular meal (Harwood, 1979).

Digestive efficiency (DE) and assimilation efficiency (AE) represent the percentage of consumed energy that is not defecated as waste. For my data, I have chosen to use the term apparent digestibility coefficient (ADC) (Beaupre *et al.*, 1993b;

Beaupre and Dunham, 1995; Raubenheimer, 1995) rather than digestive efficiency (DE), because I believe it more accurately describes the process I studied. The percent ADC represents the percent of energy that is digestible. The term DE suggests that all materials contained in the diet are a potential energy source and that undigested materials result from inefficient digestion. However, materials such as chitin and cellulose are indigestible and are excreted in the feces.

There is little information regarding digestive or assimilation efficiencies of anurans (Smith, 1976; Blem *et al.*, 1986). Smith (1976) compared the assimilation efficiencies of two snakes, *Elaphe guttata* (fed *Mus musculus*) and *Heterodon platyrhinos* (fed *Elaphe guttata*) and one toad, *Bufo terrestris* (fed *A. domestica*) at 25 C. He found that the snakes had significantly higher assimilation efficiencies than the toad. The lower assimilation efficiency of *B. terrestris* reflected the high chitin content of its food.

Fitzpatrick (1973) found that the assimilation efficiency of the salamander, *Eurycea bislineata*, significantly increased with an increase in temperature. *Eurycea bislineata* is relatively aquatic, which may explain its inability to adapt to sudden changes in temperature (Fitzpatrick, 1973). The assimilation efficiency of the salamander *Plethodon cinereus* is also temperature-dependent, and significantly decreases at temperatures exceeding 10 C (Merchant, 1970; Bobka *et al.*, 1981). Bobka *et al.* (1981) noted that the higher assimilation efficiencies do not correspond with the preferred temperature of *P. cinereus* as determined by Spotila (1972), and suggested that factors other than assimilation may play a role in temperature selection.

The size and type of food eaten is also a controlling factor in ADC (Skoczylas, 1970). Larger meals reduce the amount of surface area per volume of the meal that is exposed to digestive enzymes, and different food types contain a variety of indigestible

materials such as chitin (Kitchell and Windell, 1972) and cellulose (Johnson and Lillywhite, 1979; Bjorndal, 1987). The effects of food type and size on ADC have not been studied in amphibians.

The goal of Experiment 3 was to provide baseline data regarding the ADC of *H. chrysoscelis* fed crickets (*A. domestica*) at 21 C. Blem *et al.* (1986) compared the effects of temperature on the energy utilization in two treefrogs, *Hyla cinerea* and *H. chrysoscelis*, fed mealworms (*Tenebrio molitor*). Because temperature and methods were similar, the Blem *et al.* study offers comparison for the effect of food type on ADC.

*Hypotheses*

Experiment 1 had two hypotheses: 1) fasted frogs would have lower metabolic rates than fed animals, due to SDA in the fed animals, and 2) post-absorptive animals would exhibit a diel cycle of $Vo_2$, with higher levels at night, reflecting the nocturnal activity patterns of *H. chrysoscelis*. In Experiment 2, I hypothesized that FPT would be significantly shorter at 24 C than at 16 C. In Experiment 3, I estimated that the ADC of *H. chrysoscelis* would be between 75 and 90% based on literature values such as those described by Blem *et al.* (1986), with consideration of the differences in materials and methods, such as temperature and food type.

## MATERIALS AND METHODS

*Experiment 1: Diel metabolic rates of fasted and fed animals*

I collected 16 adult Cope's Gray Treefrogs in Lyon Co., KS, during June 1999. All experimentation and acclimation occurred at Emporia State University. I acclimated the treefrogs to 20 ± 1 C for one month in glass, 3.8-L containers with mosquito netting secured to the top of the container for ventilation. I placed basking lamps with 25 watt white incandescent light bulbs no closer than 30 cm above the jars. Basking lamps and the fluorescent room lights were on a 12:12 L:D photoperiod. Each container housed two treefrogs, which remained paired for the entire experiment. During acclimation, water was available *ad libitum*, and live adult crickets (*A. domestica*) or mealworm larvae (*T. molitor*) were fed to the animals three times per week.

I measured $Vo_2$ in two treefrogs at the same time due to the low rate of oxygen consumption in a single fasted individual. A sample size of eight was most feasible, because a larger sample size would have greatly extended the timeline of the experiment causing possible seasonal variation. The mean mass (±SD) of the pairs of treefrogs over the course of the study was 18.64 g ± 0.98 g. I reduced possible stress on the treefrogs caused by pairing by keeping them paired during acclimatization and habituation. I habituated paired treefrogs in a 235-ml experimental chamber for at least 30 minutes prior to measuring $Vo_2$. Habituation and data collection proceeded equally for fasted and fed animals. I considered the treefrogs to be fasted if they had not consumed food for four days prior to measuring $Vo_2$. I considered treefrogs fed if they had consumed at least one cricket between 24 and 48 h prior to experimentation.

A Columbus Instruments Model 180C open-flow gas sensor (Columbus, OH) measured the oxygen concentration of the air surrounding the animals within the

experimental chamber. I maintained the flow rate of the sample pump between 40 and 50 ml/min. The mean (± SD) flow rate was 41.1 ± 2.3 ml/min during the experiment. I calibrated the sensor to an ambient oxygen concentration of 20.95%. The gas sensor monitored the percent of oxygen in the chamber for one of every 11 minutes for 33 minutes. During the one-minute sample period, the gas sensor took continuous measurements and averaged them.

I collected data on all eight treefrog pairs between 20 July and 6 September 1999 at 0300, 0700, 1100, 1500, 1900, and 2300 ± 1 h. I monitored the $Vo_2$ of the treefrog pairs in 2 to 6-h time blocks starting at varying times to avoid an effect of sample order on $Vo_2$ levels (Appendix A). For each treefrog pair there were three repeated measures at each sampling period. At the end of each sample period, I coaxed each treefrog to urinate by gently handling the treefrog in a dry paper towel and then measured the total mass of the pair.

I recorded the percent oxygen in the chamber, and calculated the $Vo_2$ of the organisms in the chamber (Appendix B). I corrected the final $Vo_2$ to standard temperature and pressure and reported as mass specific. I analyzed the results with a two-way repeated measures analysis of variance (ANOVA) to determine if there were significant differences in $Vo_2$ of fasted versus fed animals over time.

*Experiment 2: Influence of temperature on food passage time*

I collected eight adult male *Hyla chrysoscelis* in Lyon Co., KS from May to June 2000. The treefrogs had a mean mass (± SD) of 7.6 ± 1 g at the beginning of the

experiment, and 8.5 ± 1 g at the end of the experiment. I acclimated the treefrogs for at least one month in 38-L aquaria at 24 ± 1 C on a 12:12 L:D photoperiod at Emporia State University. All acclimation, habituation, and testing were conducted under the same temperature and lighting regime. During acclimation, I provided the treefrogs with *A. domestica* three times a week and water *ad libitum*. Damp paper towels and radially cut PVC pipes provided moisture and shelter. The acclimation period lasted from time of capture to the beginning of the experiment in August, 2000.

The experimental protocol began by first placing all eight treefrogs in separate 1800-ml clear, plastic chambers for 24 h. I provided the treefrogs damp paper towels, and the chambers were well ventilated. At 2100 h, time zero of the experimental period, the treefrogs were fed a single, unmarked cricket. I fed each treefrog one marked cricket at 2100 h for the next four nights. The crickets were marked with a uniquely colored, 1.5 mm diameter glass bead, secured to each cricket's dorsum with Elmer's glue (Borden, Inc., Bainbridge, NY). I handled the treefrogs and their chambers minimally to avoid disturbance. For the duration of the experiment, I checked fecal pellets for beads at 2 h intervals each day between 0700 and 0100 h after feeding. To prevent the treefrogs from retaining material in the gut I offered unmarked crickets at 2100 h on succeeding nights until I collected all markers. I repeated the experiment at 16 ± 1 C following the same procedure. The animals were acclimated to 16 C for 24 h before the experiment started.

I calculated food passage time at each temperature by monitoring the amount of time between ingestion and defecation of each marked pellet. The mean time for all marked pellets expelled by each animal was then calculated. These means were averaged, with n = 8 at 24 C and n = 6 at 16 C. I performed an unpaired Satterthwaite

(1946) t-test to compare mean passage times at 24 C and 16 C using PSI Plot software (Polysoftware International, Salt Lake City, UT).

*Experiment 3: Apparent digestibility coefficient*

I collected 11 adult male *H. chrysoscelis* in Lyon Co., KS in May 2002. The mean mass of the animals ± SD was 7.07 ± 0.98 g, and mass ranged from 5.61 g to 8.45 g. During a one-month acclimation period, I fed treefrogs crickets (*A. domestica*) to satiety three times per week. Water was available *ad libitum*. The room was maintained at 21 ± 1 C and the lights were set for a 16:8 L:D cycle. I housed the animals in 1800-ml, ventilated, clear plastic containers and provided them with damp paper towels for shelter and moisture.

During Experiment 3, I fed the animals a single cricket once daily at 2000 h for eight days. I measured the wet mass of each live cricket prior to feeding it to the treefrog. I recorded the number of consumed crickets the following morning. I checked for fresh fecal material several times each day and placed any fecal samples in a drying oven at 70 C until all moisture was removed from the pellets. A fecal pellet was determined to be dry when its mass ceased to decrease over 24 h.

A bomb calorimeter (Model 1351, Parr Instrument Company, Inc., Moline, IL) was used to combust all samples. Prior to combustion, I placed each sample inside a gelatin capsule. I estimated the mean caloric content of the capsules by bombing six individual capsules. I estimated the mean number of calories in the crickets by bombing 12 representative *A. domestica*. Past studies have used Waldschmidt *et al.*'s (1986) value of 25.6 KJ/g dry mass of crickets. However, I felt it important to determine the caloric value of the specific size (mean ± SD: 0.26 ± 0.03 g) of crickets I used. I measured live

wet mass of crickets, then froze the crickets to euthanize them. As with fecal samples, I dried crickets at 70 C until all moisture was removed. I recorded dry mass of each cricket, and combusted them in the calorimeter.

Wet mass of the crickets consumed by the treefrogs was multiplied by the Kcal per gram of the 12 representative crickets to estimate calories consumed. I estimated the number of calories in the capsules that were bombed with the samples using the following equation: $Kcal_{cap} = Kcal/g_{cap}$ x mass of capsule used, where $Kcal_{cap}$ is the estimated number of calories in a capsule that was bombed with a fecal pellet or cricket, and $Kcal/g_{cap}$ is the mean number of calories/g in six representative capsules. The number of calories in the fecal pellets was determined by the following equations: $Kcal_{total} = F + Kcal_{cap} = 10,158\ (T)\ J \times 1\ cal/4.184\ J \times 1\ Kcal/1000\ cal = F + Kcal_{cap}$, where $Kcal_{total}$ is the total number of calories in the sample, F is the estimated number of calories in the fecal pellet, 10,158 is the correction value for the bomb calorimeter used, and T is the total change in water temperature caused by combusting the sample. Therefore, $F = Kcal_{total} - Kcal_{cap}$.

Percent ADC was calculated using the following equation: ADC = (C - F) / C x 100, where C is the Kcal consumed and F is the number of Kcal in the feces. I used linear regression to determine the relationship between C and F.

## RESULTS

*Experiment 1: Diel metabolic rates of fasted and fed animals*

No diel patterns were observed in the metabolic rate of *H. chrysoscelis* in either the fasted or fed groups (Figure 2). Fed animals had significantly higher mean metabolic rates than fasted animals ($P < 0.0001$, $F = 95.48$, $df_{1,15}$) (Appendix C). There was no treatment group by time interaction ($P = 0.50$, $F = 0.88$, $df_{5,80}$). There was no significant difference in $Vo_2$ over time when the two treatment groups were combined ($P = 0.33$, $F = 1.18$, $df_{5,80}$). Mean (± SD) $Vo_2$ for fasted and fed animals was 33.65 ± 4.74 µl/g·h and 117.43 ± 17.81 µl/g·h, respectively. Mean $Vo_2$ for fed animals ranged from 2.6 times to 4.1 times higher than the metabolic rates of fasted animals.

*Experiment 2: Influence of temperature on feeding performance and food passage time*

At 24 C, eight treefrogs ate four marked crickets each over four nights followed by eight unmarked crickets the fifth night. The treefrogs ate every cricket that was offered during the five days of feeding. Therefore, the ingestion at 24 C was 100%. At 24 C, the beads were defecated in the same sequence that they were fed to the treefrogs. The mean ± SD FPT at 24 C was 38.2 ± 9.76 h (Table 1). The minimum FPT at 24 C was 20 h.

After 24 h of acclimation to 16 C, six of eight treefrogs ate 15 marked crickets over four nights (Table 1) followed by seven unmarked crickets over the next two nights. Two of the treefrogs would not consume food at 16 C. Over the first five days following acclimation to 16 C, I offered 32 marked crickets to the treefrogs. The treefrogs consumed 15 marked crickets, giving an ingestion rate of 47%. I removed one of the

Figure 2. Oxygen consumption over time in fasted and fed *H. chrysoscelis* (n = 8). Solid lines represent fasted animals and dashed lines represent fed animals. Error bars represent one standard error above and below the mean.

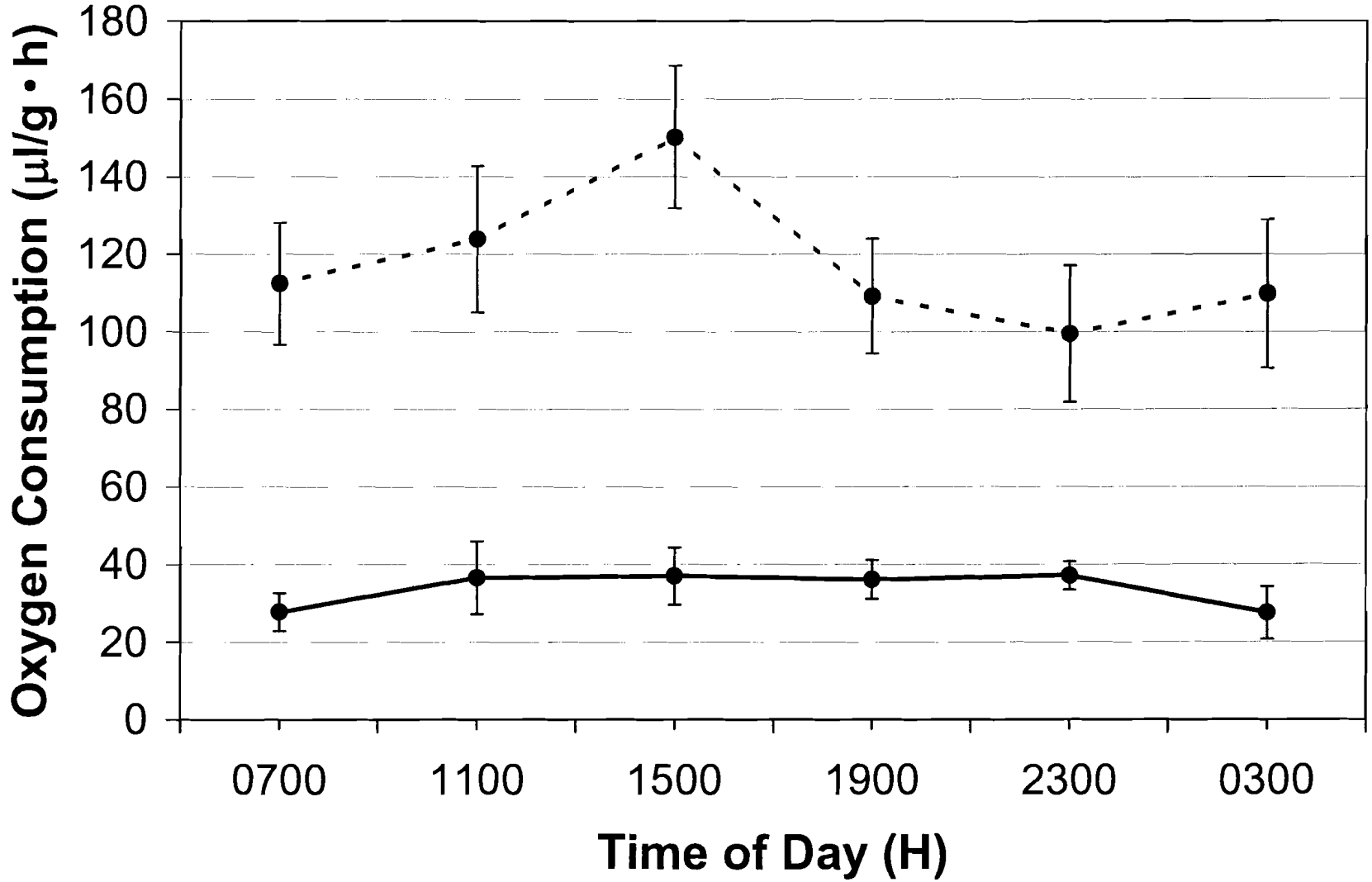

Oxygen Consumption (µl/g • h)
180
160
140
120
100
80
60
40
20
0
0700
1100
1500
1900
2300
0300
Time of Day (H)

Table 1. Passage time of individual beads in *H. chrysoscelis* at 24 and 16 C. Dashes represent uneaten beads.

| Passage Time of Bead at 24 C (h) | | | | | | |
|---|---|---|---|---|---|---|
| | Treefrog | Bead 1 | Bead 2 | Bead 3 | Bead 4 | Mean FPT (h) | SD |
| | 1 | 46 | 68 | 44 | 47 | 51.25 | 11.24 |
| | 2 | 24 | 32 | 24 | 33 | 28.25 | 4.92 |
| | 3 | 40 | 34 | 48 | 23 | 36.25 | 10.53 |
| | 4 | 40 | 48 | 24 | 21 | 33.25 | 12.89 |
| | 5 | 20 | 22 | 22 | 45 | 27.25 | 11.87 |
| | 6 | 32 | 48 | 24 | 69 | 43.25 | 19.86 |
| | 7 | 34 | 40 | 80 | 56 | 52.50 | 20.55 |
| | 8 | 22 | 46 | 22 | 45 | 33.75 | 13.57 |
| Mean | | | | | | 38.22 | |
| SD | | | | | | 9.76 | |
| Passage Time of Bead at 16 C (h) | | | | | | |
| | Treefrog | Bead 1 | Bead 2 | Bead 3 | Bead 4 | Mean FPT (h) | SD |
| | 1 | 106 | -- | 82 | -- | 94.00 | 16.97 |
| | 2 | 120 | 96 | -- | -- | 108.00 | 16.97 |
| | 3 | 60 | 82 | 94 | -- | 78.67 | 17.24 |
| | 4 | 44 | 46 | 74 | -- | 54.67 | 16.77 |
| | 5 | 138 | -- | -- | -- | 138.00 | |
| | 6 | 58 | 34 | 106 | 96 | 73.50 | 33.48 |
| | 7 | -- | -- | -- | -- | | |
| | 8 | -- | -- | -- | -- | | |
| Mean | | | | | | 91.14 | |
| SD | | | | | | 29.28 | |

eight treefrogs from the experiment after the fifth day due to lack of appetite and lethargy, from which it recovered fully at 24 C. The mean ± SD FPT at 16 C was 91.1 ± 29.2 h. At 16 C, one of the beads was not defecated in the same sequence that it was fed to the treefrog. The minimum FPT at 16 C was 34 h. A *t*-test demonstrated that mean FPT was significantly shorter at 24 than 16 C ($P = 0.006$, t = -4.25, df = 5.84). I collected data in 2-h intervals, and summarized it in 12-h intervals (Figure 3).

*Experiment 3: Apparent digestibility coefficient*

The mean caloric value/g ± SD of the 12 representative crickets was 1.42 ± 0.18 Kcal/g wet mass and 5.37 ± 0.53 Kcal/g dry mass (equivalent to 22.48 KJ/g dry mass). The mean caloric value of the meals over the eight days of the experiment was 0.218 ± 0.071 Kcal/day. Treefrog mass did not change significantly over the course of the experiment ($P = 0.167$, t = -1.49, df = 10). The mean ± SD wet mass of meals fed to the treefrogs per day was 3.7 ± 0.5% of the mean treefrog mass.

The estimated mean dry mass of meals fed to the treefrogs per day was 0.9 ± 0.3% of the mean treefrog mass. The mean ± SD ADC for *H. chrysoscelis* was 81.1 ± 3.8%. At an ADC of 81.1%, the animals were assimilating an average of 0.74 KJ/day. Regression analysis showed a linear relationship ($y = 0.2068x - 0.0036$, $r^2 = 0.76$, $P = 0.0006$) between consumed calories and calories in the feces (Microsoft Excel 2000) (Figure 4).

Figure 3. Food passage time in *H. chrysoscelis* at 24 (n = 32) and 16 C (n = 15). Solid bars represent marked fecal pellets defecated at 24 C and crosshatched bars represent pellets at 16 C.

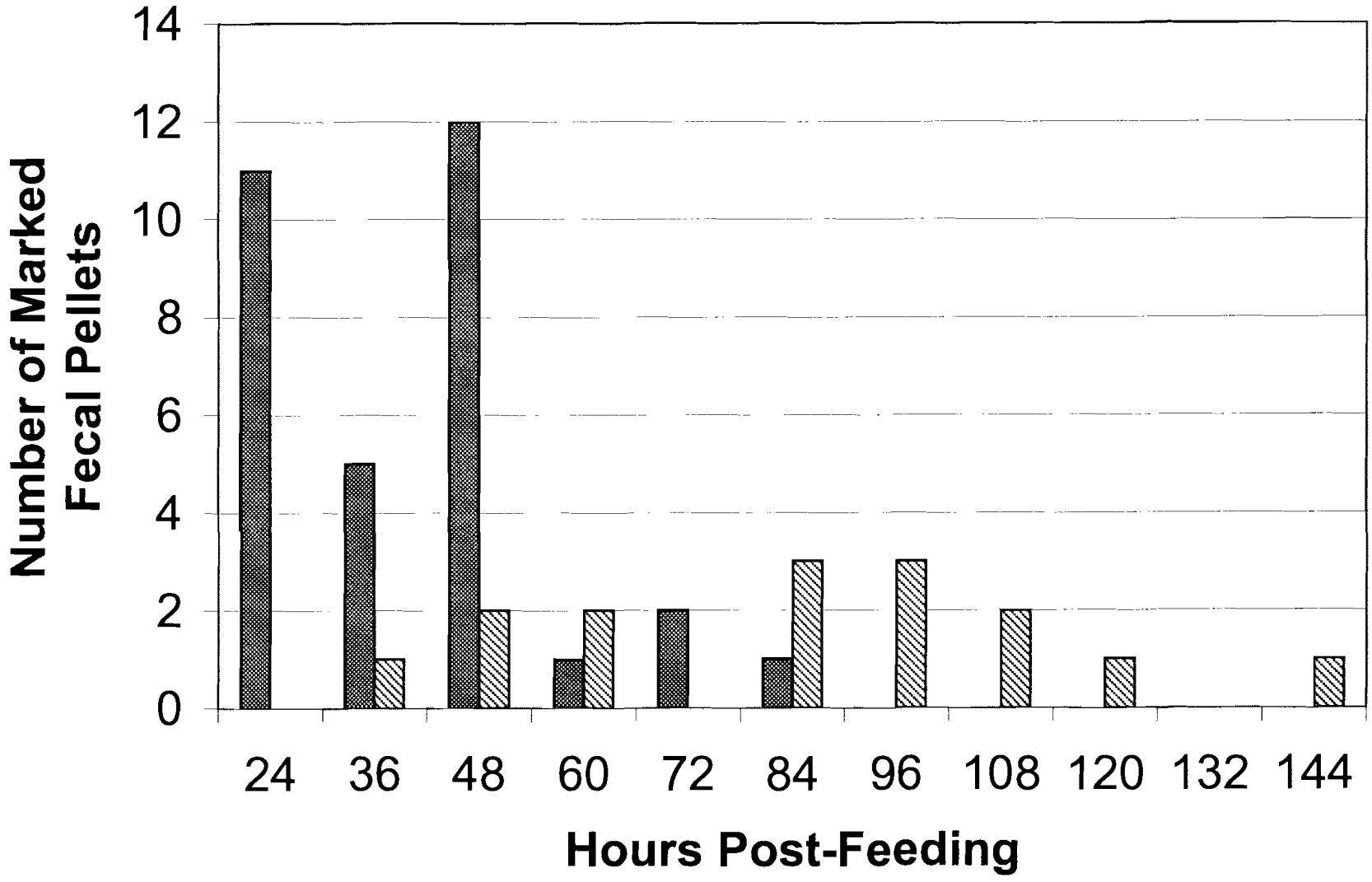
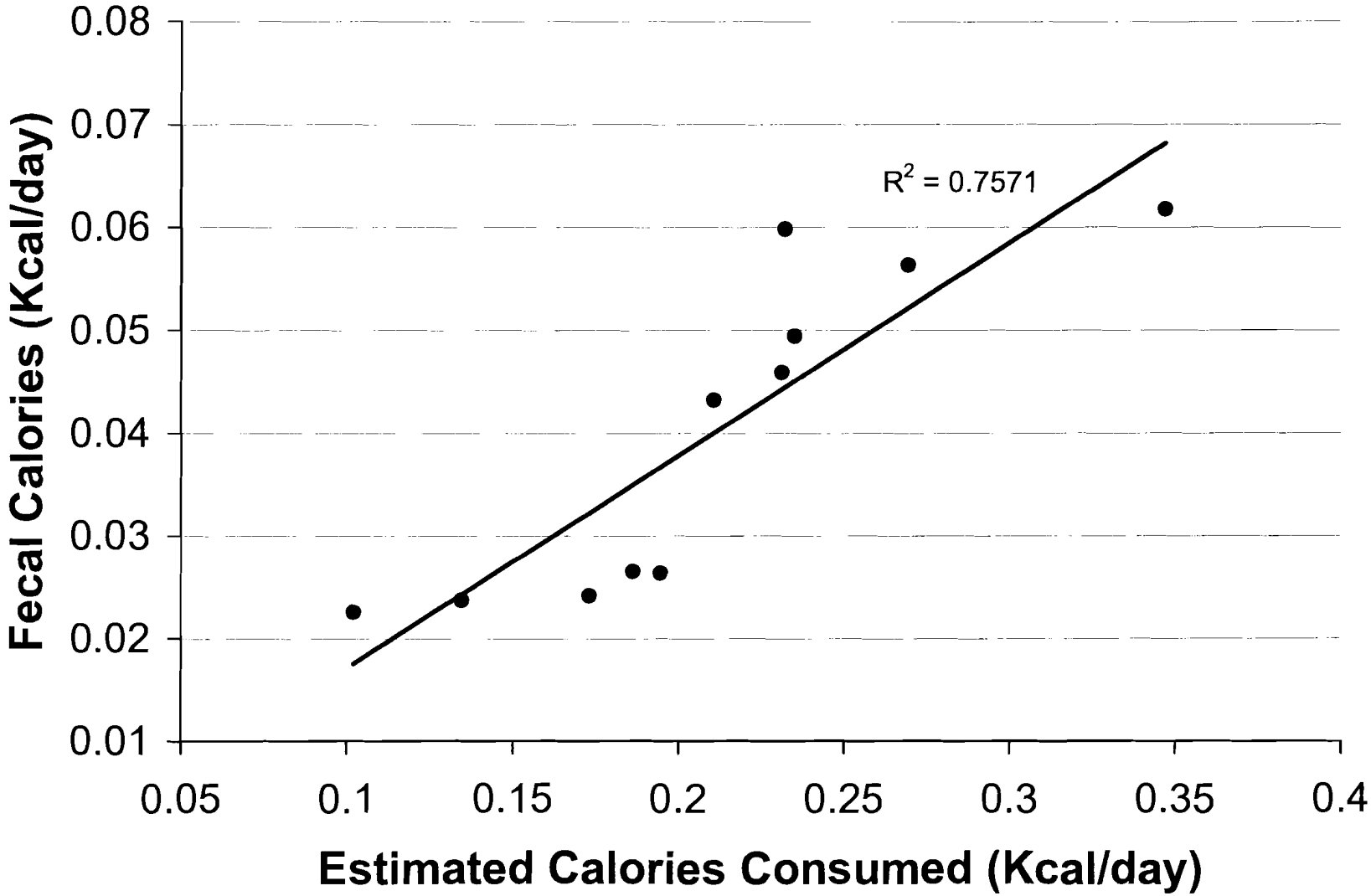

Number of Marked Fecal Pellets
0
2
4
6
8
10
12
14
24
36
48
60
72
84
96
108
120
132
144
Hours Post-Feeding

Figure 4. Linear regression of the number of calories consumed per day and the caloric content of feces per day in treefrogs fed crickets at 21C (n = 11, $P = 0.0006$).


0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
Fecal Calories (Kcal/day)
R2 = 0.7571
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
Estimated Calories Consumed (Kcal/day)

## DISCUSSION

*Diel metabolic rates of fasted and fed animals*

As expected, the metabolic rate of the fed animals was higher than that of fasted animals, indicating an increase in SDA of 2.6 to 4.1- fold in *H. chrysoscelis.* There is a paucity of information regarding SDA of frogs, but my results were similar to SDA studies in other anurans. In *Bufo woodhousii*, $Vo_2$ increased 1.7-fold 3 h after feeding (Sievert and Bailey, 2000). After peptone injection into the stomach, the metabolic rates of *Bufo marinus* increased two-fold (Wang *et al.*, 1995) within 5 to 6 h. In my study, the standard error in the fed animals was much higher than in the fasted animals, and although not statistically significant, there was greater variation in the mean $Vo_2$ rates over time in the fed animals. Because the animals did not eat at the same time prior to data collection and cricket size varied, the animals were at different stages of the SDA curve.

If the SDA in *H. chrysoscelis* approaches its metabolic scope, much of the animal's post-feeding activities would be limited to digestive processes. My data suggest that during SDA the treefrogs did not approach their aerobic capacity, which is generally 5 to 10 x resting rates (Withers, 1992). For many frequently feeding ectotherms, the energetic cost of SDA is generally a small fraction of the energy gained from the meal (Sievert and Bailey, 2000). One activity, calling in male frogs, has been shown to require the most aerobic metabolism known in ectothermic vertebrates, and exceeds the $Vo_2$ of forced exercise (Taigen and Wells, 1985). *Hyla versicolor* maintained a $Vo_{2rest}$ of 0.08 ml/g h, yet the $Vo_2$ during calling was 1.7 ml/g h (Pough *et al.*, 1992). Calling metabolic rates of such magnitude probably limit digestive functions. Because calling frogs are able to maintain such high levels of $Vo_2$, it is likely that the SDA in *H. chrysoscelis* is below

its metabolic capacity, and the cost of SDA does not prohibit the animals from activities that are moderately aerobic, such as hydroregulation or predator avoidance behaviors. However, the cost of calling during the breeding season may preclude feeding.

My data do not support my hypothesis that *H. chrysoscelis* would have a higher metabolic rate at night due to its nocturnal activity patterns. The metabolic rate of *H. chrysoscelis* is dependent upon the amount of time since food consumption rather than time of day. As with *H. chrysoscelis*, no significant effect of time of day was seen in resting metabolic rates of two frogs, *Colostethus nubicola* and *Eleutherodactylus coqui* (Taigen and Pough, 1983), nor in the hylid frog *Acris crepitans* (Dunlap, 1969). The life history traits of *E. coqui* are similar to those of *H. chrysoscelis*, in that the frogs remain in shelters during the day and become active during humid nights (Taigen and Pough, 1985).

Adult *H. chrysoscelis*, like *E. coqui*, are sit-and-wait predators with very few aerobic needs – beyond what is required for maintenance – while awake and resting. The $Vo_{2rest}$ determined for *H. chrysoscelis* in this study was lower than that of *H. chrysoscelis* at 20 C (Blem *et al.*, 1986) or for most hylids at similar temperatures (Gatten *et al.* 1992). Energy is expended primarily during brief periods of locomotion and in digestion of food. This is also seen in the genus *Colostethus*, which satisfies its energy requirements by consuming large, infrequent meals (Pough *et al.*, 1998). This is in contrast to amphibians that maintain higher oxygen consumption during the activity cycle (*R. pipiens*; Guimond and Hutchison, 1968), or where the metabolic rates correspond with photoperiod (*B. marinus*; Hutchison and Kohl, 1971; *X. laevis*; Abel *et al.*, 1992). The nocturnal marine toad, *B. marinus*, is a voracious active predator (Alexander, 1964). However, *X. laevis* is

relatively inactive, but exhibits voracious sit-and-wait feeding habits (Avila and Frye, 1978).

Taigen *et al.* (1982) found a correlation between anuran predatory behavior, locomotion type, and a dependence upon oxidative metabolism, termed the Aerobic Dependence Index (ADI). The animals that were sit-and-wait predators and jumpers, such as *E. coqui*, had lower ADIs with respect to active foragers and non-jumpers such as *B. americanus*. *H. chrysoscelis*, a passive forager that leaps long distances, fits well into Taigen *et al.*'s (1982) description of an animal with a low ADI. Advantages of a low aerobic capacity include the ability to occupy unique ecological niches not occupied by animals with higher energy and oxygen demands, and the ability to generate energy rapidly while maintaining low $Vo_{2rest}$ (Pough, 1980).

Active foragers tend to require more food because of their higher energy output for locomotion and foraging, while passive foragers consume larger, more infrequent meals (Taigen and Pough, 1985). Metabolic rates of *H. chrysoscelis* were driven by the SDA associated with passive food intake rather than the daily energetic demands of active foraging. A more exhaustive comparison of the diel resting metabolic rates of active and passive foragers would provide valuable insight into the relationships between the physiology and natural history of anurans.

*Influence of temperature on food passage time*

At 16 C, *H. chrysoscelis* ate less frequently and passed food through the digestive system at a slower rate than at 24 C. There was also more variation in the mean FPT at 16 C than at 24 C. These findings are consistent with temperature-dependent feeding energetics in other ectotherms. As temperature increases within the thermal limits of the

animal, physiological processes such as metabolic rates (Guimond and Hutchison, 1968; Brownlie and Loveridge, 1983; Beaupre *et al.*, 1993a) and digestive rates increase (Riddle, 1909; Greenwald and Kanter, 1979). At cold temperatures (10 C), Blem *et al.* (1986) found that metabolic rates of *H. chrysoscelis* were significantly lower than at warmer temperatures (20-30 C) and were dependent upon acclimation time. The authors found that energy assimilation also increased with increases in temperature from 19 to 29. The reduction of metabolic processes at cold temperatures serves to benefit the frog by reducing energy costs, and increasing the amount of time that energy stores can maintain them (Packard, 1972).

When treefrogs emerge from hibernation in April, the cool spring temperatures result in reduced motivation to feed. In Kansas, male gray treefrogs are actively calling at temperatures as low as 16 C (Clarke, 1958). However, in the laboratory at 16 C, *H. chrysoscelis* only ate 47% of the food that I presented to them. Calling is an expensive activity (Bucher *et al.*, 1982; Prestwich *et al.*, 1989) and metabolic rates are up to 22 times higher in calling animals than resting animals (Wells and Taigen, 1986; Wells and Taigen, 1989; Pough *et al.*, 1992). There are no known records of calling *H. chrysoscelis* feeding at low temperatures, although some of the treefrogs that were collected for these studies were calling at night in the lab at 21 C, and would consume crickets if fed prior to calling. If the animals are not motivated to feed at low temperatures, how are they fulfilling the high-energy demands of calling? It is possible that males are relying, in part, on the fat stored in the previous summer and carried through hibernation for calling at low temperatures in spring. Blem *et al.* (1986) measured lipid reserves of *H. chrysoscelis* from May to July and found lipid reserves to be highest at the end of the summer. However, it is unknown whether the animals in the Blem *et al.* study were able

to maintain high-level lipid reserves throughout hibernation, because these authors did not measure the lipid levels of pre-mating animals. Pinder *et al.* (1992), who reviewed the general pattern of seasonal energy reserves in amphibians, found that fuel is accumulated prior to overwintering to support hibernation and spring breeding. Therefore, it is likely that on cool spring nights male gray treefrogs are not motivated to eat, and must support calling with lipid reserves accumulated during the previous year.

The decreased motivation of *H. chrysoscelis* to feed at 16 C was problematic in measuring FPT at that temperature. Since fewer animals ate regular meals, the sample size was lower at 16 C. Greater retention times may have also increased the probability that the beads were separated from the meal. I offered the treefrogs food every day to reduce the likelihood that feces would be retained. At 24 C, treefrogs consumed a single cricket each day at 2100 h. In their natural setting at 24 C, these sit-and-wait predators probably feed more sporadically and may be able to consume greater numbers of smaller prey than the crickets I provided. An increase or decrease in meal size or meal frequency may have affected the observed FPTs (Larsen, 1992). My results showed a significant difference in FPTs at two temperatures when offered a standardized meal size.

The methods used in this study were probably more variable than other studies in which the animals are sacrificed and the gut contents are examined to determine digestive rate. My FPT results do not consider the amount of time that fecal matter is retained in the gut prior to defecation without being actively digested. However, my results more accurately reflect the FPTs of treefrogs in nature because the animals were not force-fed, and I did not sacrifice any treefrogs.

The treefrogs exhibited an increased metabolic rate 24 to 48 h following a feeding at 21 C. Frequently feeding anurans sometimes show a peak in oxygen consumption

associated with SDA within a few hours after feeding (*Bufo marinus*: Wang *et al.*, 1995; *Bufo woodhousii*: Sievert and Bailey, 2000). Frequent feeders such as *R. catesbeiana* experience modest 4-fold increases in oxygen consumption in comparison to the 10-fold increase in oxygen consumption of infrequent feeders such as *C. ornata* and *P. adspersus* (Secor and Diamond, 1996).

At 24 C, the animals were voluntarily ingesting food every 24 h and passing the food as waste within 38.2 ± 9.76 h. The data suggest that at these temperatures, the treefrogs are able to process a meal in less than 48 h. A combination of field observations of the feeding patterns of *H. chrysoscelis*, an investigation of the stomach evacuation time, and a precise determination of the magnitude and duration of the SDA would greatly enhance our knowledge of the feeding energetics of the species.

*Apparent digestibility coefficient*

The ADC of *H. chrysoscelis* in this study is complementary to the results of Blem *et al.* (1986), who examined the effect of temperature on ADC in *H. chrysoscelis* when animals were fed mealworm (*T. molitor*) larvae. Blem *et al.* found that the ADC ± SE at 19 C, 24 C, and 29 C was 89.1 ± 1.7%, 91.1 ± 1.4%, and 94.6 ± 1.0%, respectively. This demonstrated that increases in ambient temperature above 29 C significantly increased the ADC of *H. chrysoscelis*. Statistically insignificant changes in physiological factors, such as increases in metabolic rate, peristalsis, and ADC due to increases in temperature (Blem *et al.* 1986), may work in concert to decrease food passage time, as seen in Experiment 2. Conversely, a lower metabolic rate and lower absorption rates at low temperatures may increase the amount of time needed to extract nutrients from a meal,

causing the treefrog to retain the meal for a longer period. However, if material remains in the digestive tract at low temperatures, the animal is susceptible to the material rotting.

Meal type may account for the difference between the ADC in this study and that of Blem *et al.* (1986). My study animals were acclimated to 21 ± 1 C and had lower relative ADC (81.1 ± 3.8%) when fed crickets. The omnivorous lizard *Klauberina riversiana* is an example of how meal type can affect ADC. ADC of *K. riversiana* fed mealworms was 93% compared to 89% in animals fed apples. In general, herbivores have relatively low digestive efficiencies compared to insectivores. The gopher tortoise, *Gopherus polyphemus*, digested only 68% of the organic matter in legume leaves (Bjorndal, 1987). Vertebrates lack special enzymes that break down cellulose cells walls that protect the easily digested nutrients in plants (Sibly, 1981).

Similarly, the chitinous exoskeletons of crickets are indigestible by frogs and toads (Smith, 1976), due to a lack of chitinase (Kitchell and Windell, 1972), and are excreted in the feces. Licht and Jones (1967) tested the ADC of the lizard *Anolis carolinensis* when given three different food types. When given mealworm larvae, mealworm adults, and crickets, the ADC differed significantly between food types (88.9%, 54.4% and 69.5% respectively). Similarly, *Crotophytus collaris* had significantly lower ADC when fed adult *A. domestica* than when fed either *T. molitor* larvae or neonatal mice (Kearney, 2002). My data fit this pattern; the ADC of treefrogs fed mealworms (Blem *et al.*, 1986) was higher than that of treefrogs fed crickets. The ADC represents the percent of calories that are digestible, and underestimates the efficiency of digestion due to energy contained in indigestible materials such as chitin.

Blem *et al.* (1986) did not discuss meal size, which may be a factor in the ADC of the animals. In my study, I avoided variation in meal size by offering the treefrogs one

cricket within one size class per feeding; however, the treefrogs were not force-fed and therefore were able to control whether they ate or not.

At 21 C, I estimated the treefrogs consumed 0.22 Kcal/day, equivalent to 0.91 KJ/day. At an ADC of 81.1%, the animals were assimilating 0.74 KJ/day. Blem *et al.* (1986) found that *H. chrysoscelis* metabolized 0.07 KJ/day at 19 C, 0.31 KJ/day at 24 C, and 0.57 KJ/day at 29 C. The authors did not specify the mass of the food given to the treefrogs. Since the mean mass of my treefrogs remained constant, the amount of food given to them was appropriate. The amount of food that I supplied to the treefrogs was greater than the estimated intake requirement for the animals in the Blem *et al.* study.

The animals used in the Blem *et al.* study were collected in Virginia, which has a different climate from Kansas. Some ectotherms' physiologies vary with their geography. The lizard *Sceloporus undulatus* had variation in energy assimilation depending on the geography of the sample population. *Sceloporus undulatus* from New Jersey had lower energy intake than lizards from South Carolina when placed in the same laboratory conditions (Angilletta, 2001). Given the considerations of food type, meal size, and possible interspecific geographic variation in *H. chrysoscelis*, my results are similar to those of Blem *et al.* (1986).

## SUMMARY

At 20 to 24 C, normal temperatures at which the animal is active in nature, *H. chrysoscelis* had a low resting metabolic rate, short food passage time, and efficient digestion. As temperature decreases, and prey items become less active and less available, the ADC decreases along with a decrease in metabolic rate (Blem *et al.*, 1986), decreased motivation to feed, and a longer food passage time. Longer food passage times may be a compensation for the decrease in digestive efficiencies at low temperatures. Increased ADC at higher temperatures fuels higher metabolic rates and allows the animal to retain food for shorter time, clearing the stomach for a future meal.

At 20 C, the metabolic rate of *H. chrysoscelis* increased significantly, 2.6 to 4.1 times, within 24 to 48 h following a meal due to SDA, which is similar to the SDA of *Bufo woodhousii* (Sievert and Bailey, 2000) and *Bufo marinus* (Wang *et al.*, 1995). Food passage times at 21 C reflected an increased metabolic rate, as the animals were defecating approximately 38 h post-feeding. I estimate that at temperatures between 20 and 24 C, the animals are capable of processing a meal within 48 h, and feed once a day to every other day if feeding on meals similar in size and type to those provided in this study. Taigen and Pough (1985) stated that passive foragers must rely on large, infrequent prey items as opposed to active foragers, which consume large amounts of small-bodied prey. In their natural setting, *H. chrysoscelis* feeds on a wider variety of meal sizes and types than is generally supplied in laboratory studies.

The ADC of the treefrogs is dependent upon type of food, as seen with other ectotherms (Licht and Jones, 1967; Johnson and Lillywhite, 1979). I fed my treefrogs crickets, which resulted in slightly lower digestive efficiencies than treefrogs fed

mealworm larvae (Blem *et al.*, 1986). *Anolis carolinensis* showed a similar pattern when ADC was compared using crickets and mealworm larvae (Licht and Jones, 1967).

Because *H. chrysoscelis* is a sit-and-wait predator, it exhibits a low, non-cyclical resting metabolism similar to *E. coqui* (Taigen and Pough, 1983) and *A. crepitans* (Dunlap, 1969). The advantage of maintaining a low resting metabolic rate is that energy is conserved until more food is available. This is in contrast to active foragers such as *H. frenatus* (Petren and Case, 1996), which maintained higher metabolic rates during the activity cycle, and *B. marinus* (Hutchison and Kohl, 1971), in which the metabolic rate corresponded with photoperiod.

I estimate the cost of SDA to be lower than the aerobic scope of the animal considering the extremely high metabolic rates reported for calling frogs (Bucher *et al.*, 1982; Wells and Taigen, 1989). However, it is unknown whether *H. chrysoscelis* is capable of feeding during calling at low temperatures. Further research into lipid reserves of *H. chrysoscelis* during hibernation and breeding would greatly increase our knowledge of this animal's adaptive strategies for supporting high energy demands of reproduction.

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# APPENDICES

Appendix A. Timetable for diel $Vo_2$ measurements of fasted and fed *H. chrysoscelis* pairs.

| Treefrog Pair | Time of Day (H) | | | | | |
|---|---|---|---|---|---|---|
| | 0300 | 0700 | 1100 | 1500 | 1900 | 2300 |
| A-fasted | 23 Aug. 1999 | 23 Aug. 1999 | 20 July 1999 | 20 July 1999 | 20 July 1999 | 06 Sept. 1999 |
| B-fasted | | | | | | |
| C-fasted | | | | | | |
| D-fasted | | | | | | |
| E-fasted | 03 Sept. 1999 | 03 Sept. 1999 | 03 Sept. 1999 | 29 Aug. 1999 | 29 Aug. 1999 | 29 Aug. 1999 |
| F-fasted | | | | | | |
| G-fasted | | | | | | |
| H-fasted | | | | | | |
| A-fed | 10 Aug. 1999 | 11 Aug. 1999 | 13 Aug. 1999 | 13 Aug. 1999 | 13 Aug. 1999 | 10 Aug. 1999 |
| B-fed | | | | | | |
| C-fed | | | | | | |
| D-fed | | | | | | |
| E-fed | 19 Aug. 1999 | 19 Aug. 1999 | 21 July 1999 | 21 July 1999 | 20 Aug. 1999 | 18 Aug. 1999 |
| F-fed | | | | | | |
| G-fed | | | | | | |
| H-fed | | | | | | |

Appendix B. The following equation was used to calculate $Vo_2$ for *H. chrysoscelis*.

$$Vo_2 = Vi*Xi - Vo*Xo$$

where Vi is the airflow rate into the chamber, Vo is the airflow rate out of the chamber into the oxygen analyzer, Xi is the oxygen concentration of the room, and Xo is the oxygen concentration of the air flowing out of the chamber. The flow rate into the chamber was determined using the following equation:

$$Vo = Vi\ (Ni/No)$$

where Ni is the nitrogen concentration of the room (1 - Xi), and No is the nitrogen concentration of the chamber (1 - Xo). The final $Vo_2$ was determined by correcting for standard temperature and pressure:

$$Vo_2\ final = (Vo_2 * P * 273) / (273 + T) * 760$$

where P is the barometric pressure, and T is temperature. To correct for differences in mass among the animals, $Vo_2$ final was divided by total mass of the animals in the chamber and then multiplied by 60 min/hr to yield the total ml/g · h. This number was multiplied by 1000 to yield µl/g · h.

Appendix C. Repeated measures analysis of variance for fasted and fed groups (G) over time (T).

| Source | DF | SS(U) | MSS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 15 | 193230.73 | | | |
| G (Group) | 1 | 168521.80 | 168521.80 | 95.48 | 0.0000 |
| Error 1 | 14 | 24708.93 | 1764.92 | | |
| | | | | | |
| Within Subjects | 80 | 106134.14 | | | |
| T | 5 | 7782.09 | 1556.42 | 1.177 | 0.3291 |
| GT | 5 | 5810.85 | 1162.17 | 0.879 | 0.4998 |
| Error 2 | 70 | 92541.21 | 1322.02 | | |

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Angela M Babbit
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7-24-03
Date

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Signature of Graduate Office Staff

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