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Acknowledgements

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Abstract: Specific dynamic action (SDA) is the energy expended during ingestion, digestion, absorption, and assimilation of a meal, and is influenced by meal (type, size, composition, and temperature) and environmental temperature. Understanding the effect of meal type and environmental temperature on SDA in turtles is important in describing how *T. s. elegans* may acclimate with changing environmental temperatures. In this study, we conducted feeding trials in which we fed juvenile *T. s. elegans* duckweed and mealworm diets at 25°C and 30°C. We measured the rate of oxygen consumption as a proxy for metabolic rate after feeding for four 30 minute consecutive intervals. There was a strong effect of diet on SDA, but only at 30°C. Peak metabolic rate was significantly higher for turtles fed mealworms than those fed duckweed at 30°C. At 25°C, there was no significant difference between metabolic rates for turtles fed mealworms and turtles fed duckweed. Both turtles that ate mealworms and turtles that ate duckweed had higher metabolic rates overall at 30°C than at 25°C. Our findings suggest that mealworms are not more costly to digest than duckweed for juvenile *T. s. elegans* at 25°C, and indicate that temperature increases the cost of digestion and SDA in both mealworm and duckweed diets. Our findings should provide supporting information for diet selection of juvenile *T. s. elegans,* and for determining how *T. s. elegans* might acclimate to changing thermal landscapes.

Keywords: Specific dynamic action, red eared slider, ontogenetic diet shift, reptile, temperature

Introduction

The ability to obtain and process food into usable energy for maintenance, growth, reproduction, and storage is fundamental in determining an animal's ability to survive and reproduce (Avery et al. 1993). Differences in physiological responses may be ecologically important if they are considered within the framework of differences in foraging behavior. Foraging behavior is ultimately shaped by foraging efficiency: the ratio of metabolizable energy gained from food divided by the energy expended to acquire it. Multiple factors such as diet type, size, digestibility and habitat structure can influence foraging efficiency. Foraging efficiency can describe why animals eat what they eat. In addition to foraging efficiency, the costs and benefits of different diets can be important to describing diet selection (Robira et al. 2021).

Diet types can include carnivorous and herbivorous diets. Carnivorous diets require the animal to spend energy to catch moving prey that is easily digested and can provide substantial amounts of energy because of their protein content (Secor 2002). Herbivorous diets require the animal to forage on plants that have limited protein and do not provide as much energy. Animals that actively forage spend more energy in the process to pursue and consume prey than animals that passively forage. If the amount of energy gained from food does not exceed the amount of energy spent to obtain it, the meal is not valuable to the animal. Thus, animals that actively forage for their food will eat diets that contain larger amounts of energy than animals that passively forage.

Turtles provide a very interesting model for helping us understand the costs and benefits of different diets. Ontogenetic diet shifts are widespread in several turtle families, including red eared sliders (*Trachemys scripta elegans*); as turtles grow, they shift from a more carnivorous diet to a more herbivorous diet (Bouchard and Bjorndal 2006). *Trachemys scripta elegans* has an exceptionally broad diet in the wild, including vascular plants and invertebrates. Juveniles tend to eat items such as

worms, small insects, and small fish, and adults tend to forage for items such as duckweed, and water lettuce (Bouchard and Bjorndal 2006). As turtles develop, they begin to face maneuverability constraints and changes in their digestion ability. Juvenile turtles are small, and can easily maneuver through complex environments that require the animal to move around large obstacles such as aquatic plants. Larger turtles, however, have greater costs of locomotion than smaller turtles because of their size. Larger turtles experience an increased foraging effort as their rigid bodies constrain the ability to maneuver throughout littoral zone plants in search of prey (Hart 1983). In addition, as turtles grow in size, they begin to spend more energy pursuing and consuming invertebrates than juveniles because of the increased foraging effort. The net gain from each invertebrate pursued and consumed becomes progressively smaller with increasing turtle size (Parmenter and Avery 1990).

The ontogenetic diet shift is best understood by understanding the costs and benefits of the plant and animal diets. Animal diets are harder to catch, but are protein rich. Animal matter also requires the animal to use its own enzymes and nutrient transporters in order to digest. Juveniles are extremely efficient on high protein diets and are able to digest proteins to a greater extent than adults (Bouchard and Bjorndal 2005). Bouchard and Bjorndal (2005) found that shrimp fed juveniles grew 3.2 times faster and consumed 4.2 times more energy and 9.1 times more nitrogen than juveniles fed duckweed. Differences in digestibility of animal material between juveniles and adults may be attributed to ontogenetic shifts in enzyme production or in the densities and types of nutrient transporters (Buddington 1992). Juveniles benefit from a carnivorous diet because of their ability to retain more energy and nutrients from animal matter (Bouchard and Bjorndal 2006). With greater energy assimilation, animals are able to achieve faster growth rates, which is thought to be connected with lower juvenile mortality rates by minimizing time spent vulnerable to predators. (Wilbur 1975).

Juveniles tend to have higher rates of metabolism than older animals, which may partially be explained by the energy demands accompanying rapid growth and tissue synthesis.

Plant diets have less protein than animal diets, but are much more easily attainable. Plants are digested by bacteria present in the gut, rather than by enzymes. Juveniles can digest duckweed as well as adults, but juvenile intake is constrained on duckweed diets and juveniles have difficulty meeting their growth potential (Bouchard and Bjorndal 2005). Bouchard and Bjorndal (2005) found that juveniles fed duckweed only gained 161.1 mg of dry matter compared to 770.0 mg for those fed shrimp. Juveniles also assimilated substantially less energy and nitrogen than those fed shrimp. Thus, despite duckweed being a preferred food item of adults, juvenile *T. s. elegans* do not fare nearly as well on duckweed diets as on protein diets (Bouchard and Bjorndal 2005).

Ontogenetic diet shifts are often accompanied by habitat shifts from shallow to deeper water which consequently influences diet choices (Hart 1983; Congdon et al. 1992). Juveniles feed in shallow, warmer, more productive aquatic areas compared to adults, since adults cannot inhabit shallow areas due to maneuverability constraints (Parmenter and Avery 1990). These shallow aquatic areas often have a lot of invertebrate prey and vegetation that acts as cover to hide juveniles from predators. By foraging in warmer, more productive microhabitats, juveniles are able to maximize consumption and digestion rates and consequently maximize the net energy available for growth (Avery et al. 1993). Individuals with limited foraging opportunities and energy assimilation will have a slower rate of growth (Avery et al. 1993). In addition, as turtles get bigger their rigid shell makes it harder to maneuver around plants in shallow areas. Larger turtles also have fewer predators, and it becomes less risky to move out of these shallow aquatic areas. Thus, as turtles grow, they will move out of these shallow, warmer, and productive aquatic areas and shift to a more herbivorous diet to maximize their maneuverability and net energy.

Juvenile turtles require a large amount of energy to sustain their high growth rates. In turtles, growth rate may influence body size and consequently, clutch size, egg size, clutch frequency, and/or survivorship (Avery et al 1993). Male turtles mature on reaching a certain size, whereas females tend to mature at a certain age regardless of size (Bouchard and Bjorndal 2005). Faster juvenile growth would decrease age at maturity for males and increase maturity size for females (Bouchard and Bjorndal 2005). Differences in diet quality may account for differences in growth in populations, with higher protein diets resulting in increased growth rates (Parmenter 1980; Gibbons 1967, 1970). In addition, the type of food consumed by turtles should impose an important physiological constraint on food processing capacity (Bennett and Dawson 1976; Porter and Tracy 1983; Bjorndal 1985). Studies have compared the energy and protein contents of food consumed by juvenile *T. s. elegans* in these populations and found that dietary protein is an important factor affecting the growth of turtles (Parmenter 1980, Vogt and Guzman 1988).

Another cost of feeding is the costs of processing a meal, or specific dynamic action (SDA): the accumulated energy expended from the ingestion, digestion, absorption, and assimilation of a meal (Secor 2009). SDA response is measured by the increase in the rate of oxygen consumption (V_{O2}) relative to the accumulated energy expended to process a meal. The meal type, size, composition, and meal temperature, as well as environmental temperature can drastically impact the magnitude and duration of the metabolic response (Gienger et al. 2017; Secor 2009). Several studies have found SDA to contribute heavily to an animal's energy budget (Secor and Nagy 1994; Peterson et al. 1998; Secor 2002), and may be adaptively linked to feeding ecologies and digestive physiology. In animals with variable diets, food type can have a significant effect on SDA (Hailey 1998). A universal phenomena is that larger meals, either as absolute mass or relative to body mass, generate greater magnitudes of post-feeding responses. Zaidan and Beaupre (2003) found that larger meals

incurred a greater SDA in timber rattlesnakes, *Crotalus horridus*, because of the cost of processing more biomass. Secor (2002) found that marinus toad, *Bufo marinus*, had a steady increase in peak O_2 consumption, scope and SDA with each increase in meal size. Secor also found that meals entering the stomach intact and/or possessing a strong structure (e.g., chitinous exoskeleton) would require more enzymes, acids and mechanical churning than fragmented and/or soft-bodied meals (e.g. earthworms, rodents). In addition, meal composition (relative content of protein, carbohydrates, and fats) has well-established effects on the SDA response, with digestion of proteins from intact animal meals being more costly in digestion than leaves or fungi (Hailey 1998; Secor 2002). Several studies have shown that increasing protein content of a meal invokes a greater peak V_{O2}, longer duration of the metabolic response and an elevated SDA (Ross et al. 1992; Secor 2002). McCue (2006) found that animal tissue containing complete protein or complete mixtures of amino acids produces high SDA, but simpler proteins or incomplete amino acid mixtures produce a smaller or immeasurable SDA.

While there may be a correlation between high protein diets and high SDA, temperature may also have a critical role due to the complexities of the physiological and behavioral interactions involved. Metabolic rates of ectotherms can vary as a function of environmental temperature (Secor 2009). The shape of the SDA response appears to be affected by temperature in crustaceans (Whiteley et al. 2001), juvenile cod, *Gadus morhua*, (Soofiani and Hawkins 1982), burmese pythons, *Python molurus*, (Wang et al. 2003) and more. Generally, as temperature increases, standard metabolic rate (SMR) and peak metabolism also increases while the duration of metabolic response decreases. However, for crustaceans and burmese pythons, temperature had no effect on SDA (Whiteley et. al 2001; Wang et al. 2003). Secor and Faulkner (2002) found in the marine toad, *B. marinus*, body temperature impacts the profile of the postprandial metabolic response, having its largest influence on

SMR, peak oxygen consumption, and duration. In addition, their findings indicate that digestion of a given meal requires a fixed amount of energy and at lower body temperatures, the same total amount of absorption and assimilation seem to occur, but at a slower pace.

Higher environmental temperatures within habitats will likely cause increased food consumption rates, faster digestion rates, and higher digestive efficiencies in turtles, and other reptiles (Kepenis and McManus 1974; Parmenter 1980; Parmenter 1981; Davenport 1997). These same rates and efficiencies may increase the need for food abundance required for growth. With increased need, diets with significant amounts of animal protein may be essential to sustain high juvenile growth rates (Avery et al. 1993). Habitats without substantial amounts of protein would be unable to sustain the high juvenile growth rates. Examining the effect of these changes on turtles and other reptiles could provide information on how species may be impacted by habitat temperature variation.

In this study, we conducted feeding trials in which we fed juvenile *T. s. elegans* duckweed and mealworm diets at differing environmental temperatures. These foods differ in protein content and digestibility, allowing the importance of protein in SDA to be assessed. Understanding how differing meal types and environmental temperatures can impact the cost of digestion will be important in describing how this species may acclimate with current threats such as climate change. We predicted that (1) at both 25°C and 30°C, turtles fed mealworms would have a significantly higher peak oxygen consumption than turtles fed duckweed, (2) both diet treatments would have significantly higher peak oxygen consumption at 30°C than at 25°C and (3) both diet treatments would have significantly higher SMR at 30°C than at 25°C.

Methodology

Collection and Animal Husbandry. - This study was conducted at Otterbein University in Westerville Ohio for eight weeks during October, November and December 2021. We ordered ten hatchling red eared sliders from Concordia Turtle Farm LLC, and shipped to Otterbein University on September 2, 2021. We housed turtles in two groups of three individuals and one group of four individuals in plastic tubs (37cm x 17cm x 29cm). Each tub received heat and light from a 50 Watt Turtle Tuff splash proof halogen bulb and heat from a 13 Watt Reptisun 5.0 UVB mini compact fluorescent lamp on a 12hr cycle. We housed turtles with water ad libitum and basking sites (32cm x 17.5cm x 10cm) with anti-slip mats and resting/diving platforms that were suctioned underneath the light fixture before the trials (Figure 1). Before trials, turtles were fed Reptomin floating food sticks with 42.5% crude protein ad libitum for one hour every day. Turtles were randomly assigned a number between 1 and 10. Turtles were marked by notching marginal scutes of the carapace corresponding with their assigned number (Figure 2).

During trials, turtles were housed individually in clear plastic tubs (37cm x 17cm x 29cm). Each tub received the same light as before the trials, with heat and light from a 50 Watt Turtle Tuff splash proof halogen bulb and heat from a 13 Watt Reptisun 5.0 UVB mini compact fluorescent lamp on a 12hr cycle. Basking platforms remained suctioned underneath the light fixture. Basking sites were maintained at 27°C during the day and 23°-25°C during the night. In the first set of trials, water temperatures were maintained at 25°C with the use of the deep dome lighting. In the second set of trials, we increased the water temperature to 30°C with the addition of BOEESPAT Small Aquarium Heater.

Measuring Metabolic Rates. – We determined metabolic rate by measuring the rate at which the turtle consumed oxygen in a closed respirometer chamber (182.03mL). We placed the

respirometer chamber inside a water bath to maintain the desired temperatures of the trial (25°C or 30°C) (Figure 3). Temperatures and oxygen consumption were monitored and recorded through a WITROX 1 instrument (Loligo Systems; https://www.loligosystems.com/witrox-1-oxygen-meter-for-mini-sensors-1-x-o-1-x-temp). The WITROX system temperature probe measured temperature in the water bath.

The WITROX dipping probe oxygen mini sensor was inserted into the chamber and measured oxygen consumption of a turtle in an airtight glass respirometer chamber (Figure 3). The dipping probe optode was inserted into the capillary tube probe port, and the tip was projected 2.54cm into the chamber. We sealed the port and the chamber lid with screws, and measured the partial pressure of oxygen every second by $mg \cdot L^{-1}$. We plotted the partial pressure of oxygen as a function of time and used the slope as our measure of oxygen consumption. We multiplied the slope by the volume of air space in the chamber. To calculate the volume of air space, we first weighed the chamber. We then filled it completely with water and reweighed it. The difference between these masses allowed us to calculate the air volume in the chamber. On day seven of the trial, we measured each turtle volume before feeding using a 250mL conical graduated cylinder. We filled the graduated cylinder to the 100mL line and measured the displacement of the turtle. After individual turtle volume was assessed, their volume was subtracted from the air volume in the chamber to calculate the amount of air present with the addition of each turtle. Slope was then multiplied by 60 seconds to convert it to minutes. All values for oxygen trace were converted to $mg\ O_2 \cdot min^{-1}$.

We used two different diets, one animal matter and one plant matter. For our animal matter, we used Fluker's Freeze- Dried Mealworms (*Tenebrio molitor*) with 46.64% crude protein. We grew duckweed (*Lemna minor*) in tubs outside the lab as our plant matter diet. We randomly assigned turtles to a meal treatment each week, either mealworm or duckweed. This study followed a repeated

measure design. Trials lasted one week, with four consecutive trials done at 25°C and four at 30°C. At each temperature, turtles were randomly assigned either duckweed or mealworms, and then assigned the opposite meal for the following trial. This was repeated so that we measured the metabolic rate of each turtle twice on each diet at each temperature.

Turtles were fed their designated meal treatment for three days to acclimate them to the diet. Duckweed was provided ab libitum to enable free grazing throughout the three days. Mealworms were fed ad libitum for one hour, and leftovers were collected every day to prevent water spoilage. We then fasted turtles for three days to make the turtle postabsorptive. On day seven, we measured standard metabolic rate (SMR) to establish a baseline for each turtle. We then fed the turtle their assigned meal treatment ad libitum for one hour. Following feeding, turtles were individually placed inside the chamber and V_{O2} was recorded for four 30 minute consecutive intervals, for a total of two hours. No more than three runs were done in a day. Peak O_2 consumption was determined as the fastest rate, occurring at the 60 minute interval.

Statistical Analyses. – We used a general linear model (GLM) to test for the effects of diet and temperature on SMR and peak O_2 consumption after controlling for the effect of body mass (covariate). Individual turtle measurements were averaged so that each turtle had one SMR and peak O_2 measurement per meal treatment at each temperature. Correlations between turtle mass, volume, and carapace length were assessed using linear regressions. Correlations were assessed using all measurements made for each turtle per trial, with each turtle being represented eight times. All tests were conducted using the Statistical Package for the Social Sciences (SPSS) with comparisons being considered statistically significant when $p < 0.05$.

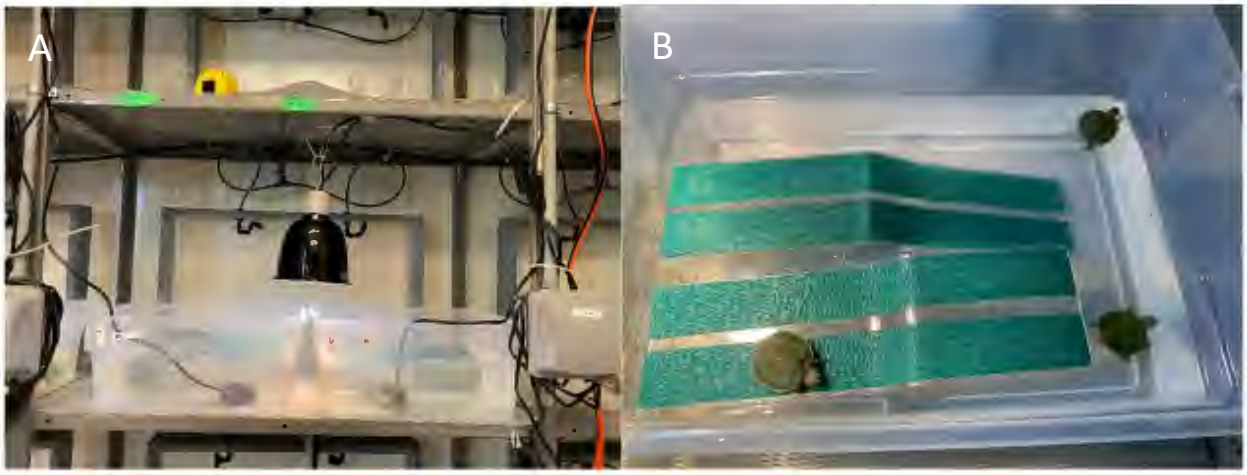

Figure 1 Images of turtle housing throughout the study. A) Side view of two turtle housing tubs filled with water located under UVB and heat light fixture, and B) above view of turtle housing tub with three turtles before trials begun.

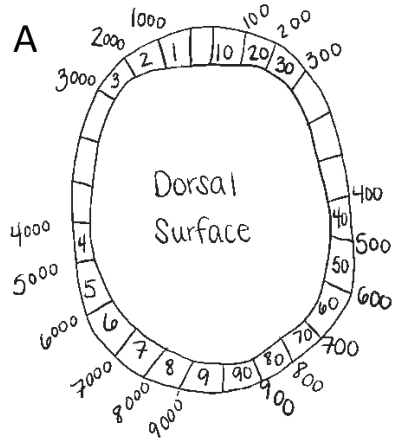

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Figure 2 Images of marginal scute numbering system for turtles. A) Marginal scute diagram used to number and identify individual turtles during study, and B) turtle with notched scute identifying turtle as number 2.

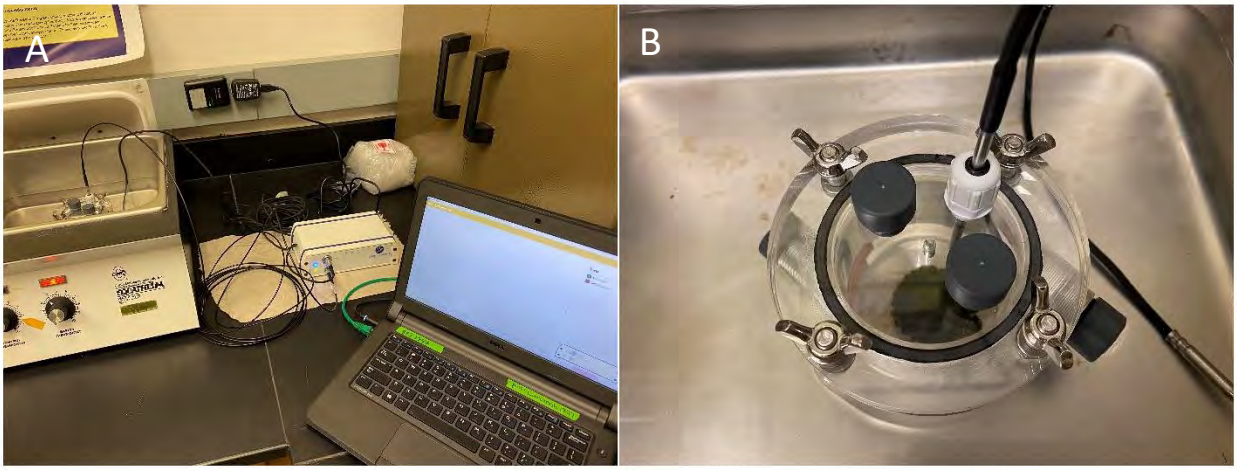

Figure 3 Images of WITROX respirometer system and set up. A) WITROX recording V_{O2} measurements of a turtle in an airtight glass respirometer chamber placed in water bath for temperature control, and B) Turtle inside chamber placed in the water bath while V_{O2} measurements are recorded.

Results

Turtle mass ranged from 7.02g to 18.8g (10.05 ± 0.006 g) throughout the period of the study with individuals growing an average of 2.4g ± 0.222 g. Turtle mass and turtle volume were highly correlated ($F_{1,380}$ = 4,220.76, $p < 0.001$, Figure 4), as were carapace length and turtle mass ($F_{1,380}$ = 1,649.50, $p < 0.001$), and turtle volume and carapace length ($F_{1,380}$ =1,142.39, $p < 0.001$).

We measured metabolism of ten hatchling red eared sliders at 25°C and 30°C. For each trial, metabolic rates started low at the average turtle SMR, and then gradually rose to the peak O_2 consumption at 60 min (Figure 5), followed by a decrease back to near average SMR at 120 min (Figure 5). Average consumption did not decrease to the original turtle SMR, suggesting that even more energy was required for processing than the peak suggests.

At 25°C, there was no significant difference between metabolic responses. SMR was not significantly higher for turtles fed mealworms than turtles fed duckweed ($F_{1,17}$ = 0.13, $p = 0.727$, Figure 6). Peak O_2 consumption was also not significantly higher for turtles fed mealworms than turtles fed duckweed at 25°C ($F_{1,17}$ = 0.03, $p = 0.868$, Figure 6). There was no significant effect of body mass on O_2 consumption for SMR ($F_{1,17}$ = 0.21, $p = 0.654$) or peak O_2 consumption ($F_{1,17}$ = 1.01, $p = 0.329$) at 25°C.

Whereas there was no significant difference between diets at 25°C, we did find a significant difference at 30°C. SMR was not significantly higher for turtles fed mealworms than turtles fed duckweed at 30°C which suggests that the diet is a contributing factor to the differing metabolic response ($F_{1,17}$ = 3.28, $p = 0.088$, Figure 6). Peak O_2 consumption was significantly higher for turtles fed mealworms than turtles fed duckweed at 30°C ($F_{1,17}$ = 6.86, $p = 0.018$, Figure 6). There was no

significant effect of body mass on SMR ($F_{1,17} = 0.25$, $p = 0.622$), but there was a significant effect of body mass on peak O_2 consumption ($F_{1,17} = 11.08$, $p = 0.004$), which suggests that body mass only plays a significant role when a meal is being processed at 30°C.

Both diet treatments had significantly higher peak O_2 consumption and SMR at 30°C than 25°C. SMR for turtles fed duckweed was significantly higher at 30°C then at 25°C ($F_{1,17} = 30.84$, $p < 0.001$, Figure 7). Peak O_2 consumption for turtles fed duckweed was significantly higher at 30°C then at 25°C ($F_{1,17} = 66.24$, $p < 0.001$, Figure 7). There was no significant effect of body mass on SMR ($F_{1,17} = 0.35$, $p = 0.560$), but there was a significant effect on peak O_2 consumption ($F_{1,17} = 18.05$, $p = 0.001$).

SMR for turtles fed mealworms was significantly higher at 30°C then at 25°C ($F_{1,17} = 199.58$, $p < 0.001$, Figure 8). Peak O_2 consumption for turtles fed mealworms was significantly higher at 30°C then at 25°C ($F_{1,17} = 87.16$, $p < 0.001$, Figure 8). There was no significant effect of body mass on SMR ($F_{1,17} = 0.04$, $p = 0.842$), but unlike turtles fed duckweed, there was no significant effect on peak O_2 consumption ($F_{1,17} = 1.27$, $p = 0.275$).

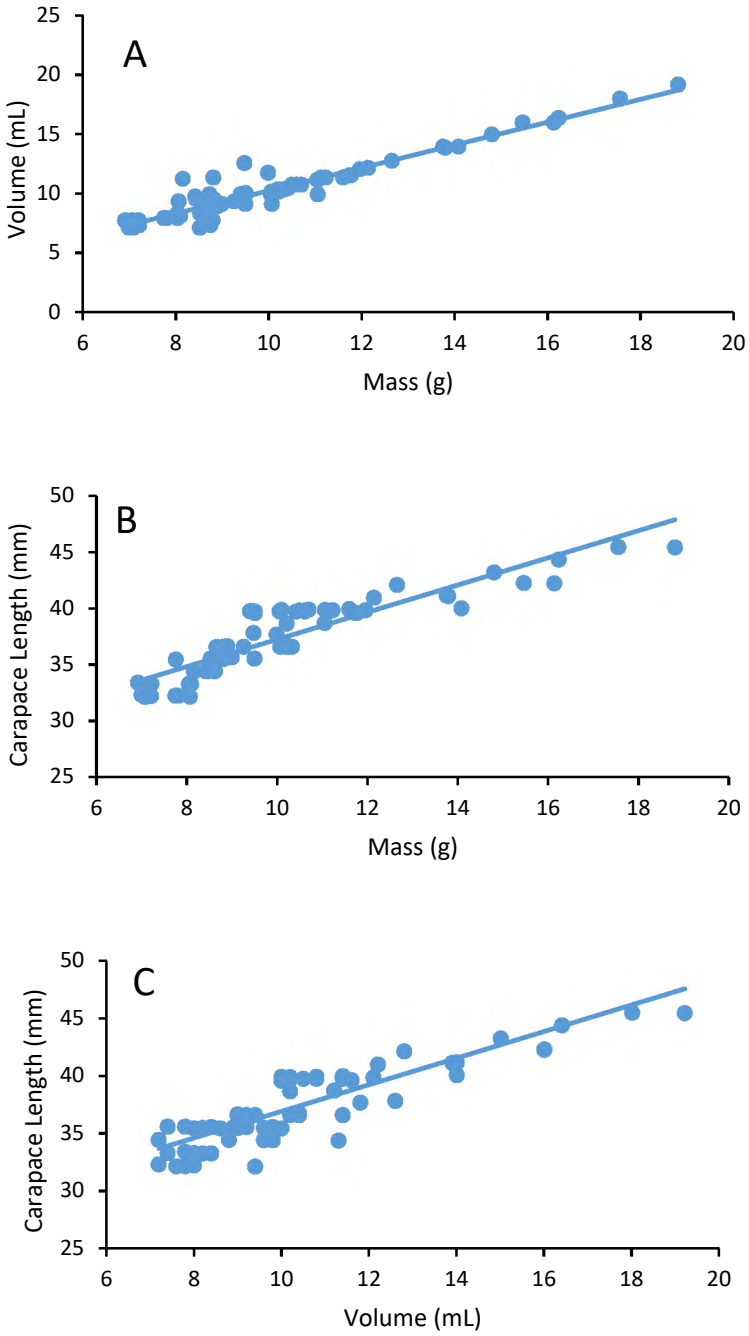

Figure 4 Correlations between (A) *T. s. elegans* volume (ml) and mass (g) (B) turtle carapace length (mm) and mass (g) and (C) turtle volume (ml) and carapace length (mm) from each turtle per trial, with each turtle being represented eight times.

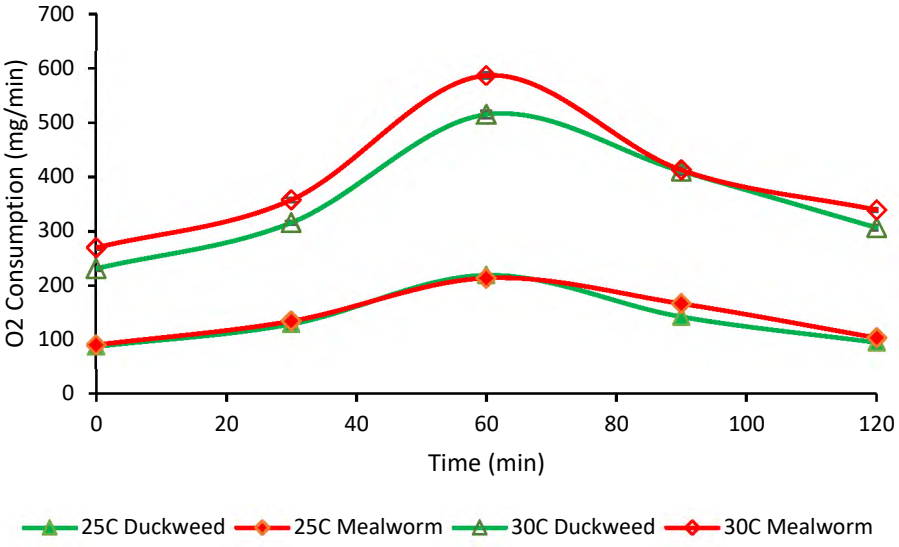

Figure 5 Oxygen consumption rates (mg/min) and time (min) at 0, 30, 60, 90, and 120 minutes for *T. s. elegans* fed duckweed (*Lemna minor*) and mealworm (*Tenebrio molitor*) diet treatments at 25°C and 30°C with standard error bars (some too small to see).

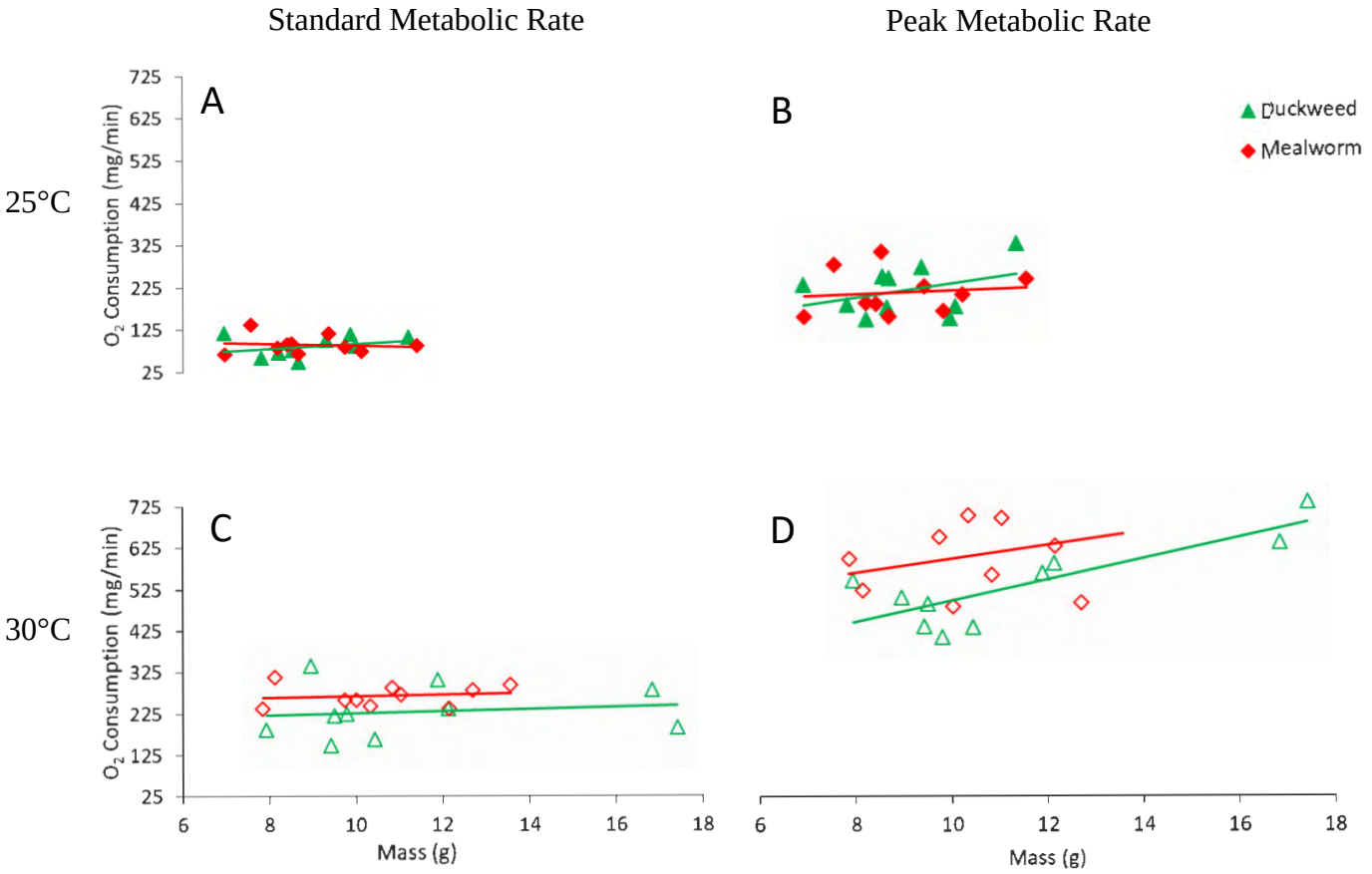

Figure 6 Average O_2 consumption (mg/min) and *T. s. elegans* (g) mass for turtles fed mealworm (*Tenebrio molitor*) and turtles fed duckweed (*Lemna minor*) for (A) standard metabolic rate at 25°C (B) peak metabolic rate at 25°C (C) standard metabolic rate at 30°C, and (D) peak metabolic rate at 30°C.

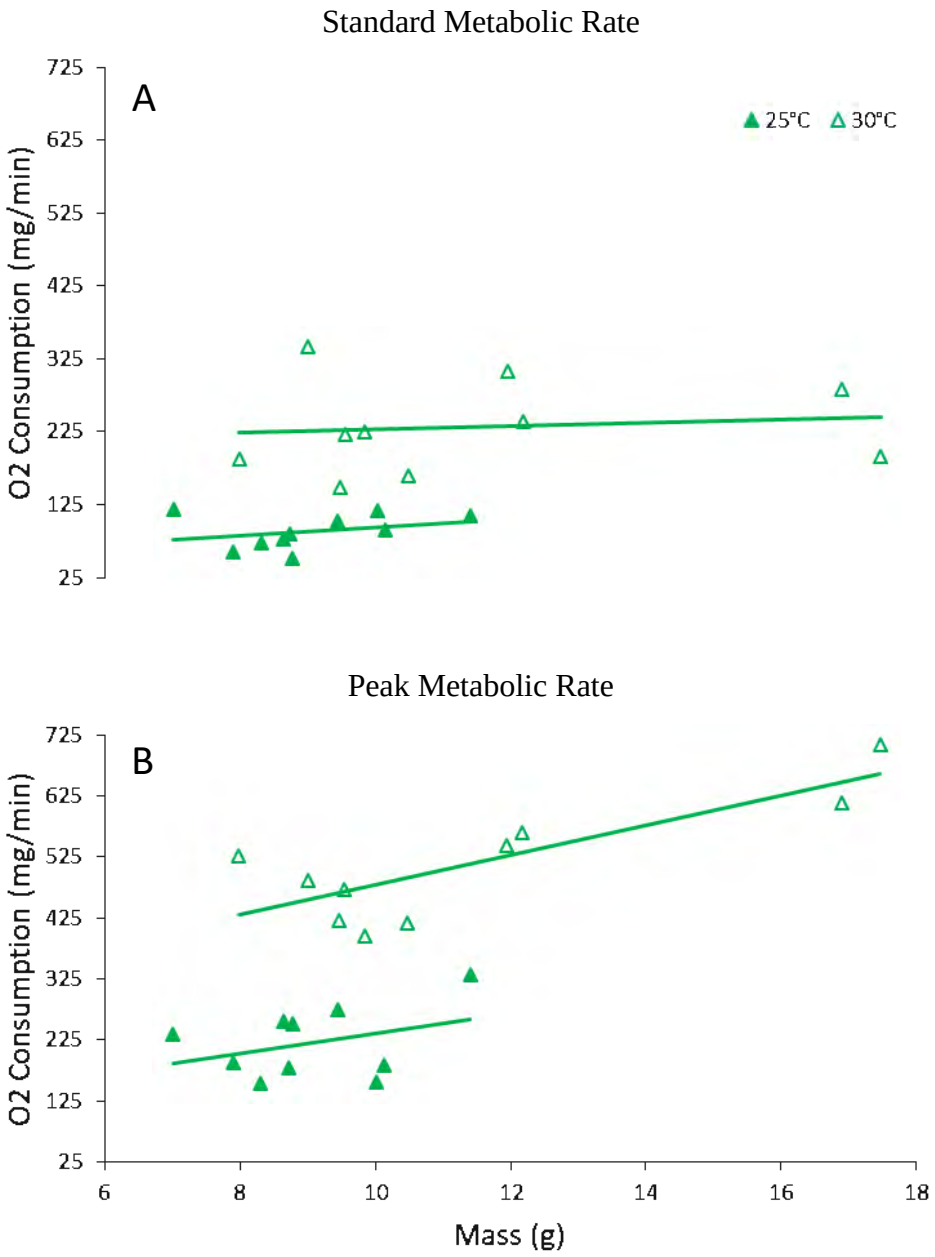

Figure 7 Average O_2 consumption (mg/min) and *T. s. elegans* mass (g) for turtles fed duckweed (*Lemna minor*) at 25°C and 30°C for (A) standard metabolic rate and (B) peak metabolic rate.

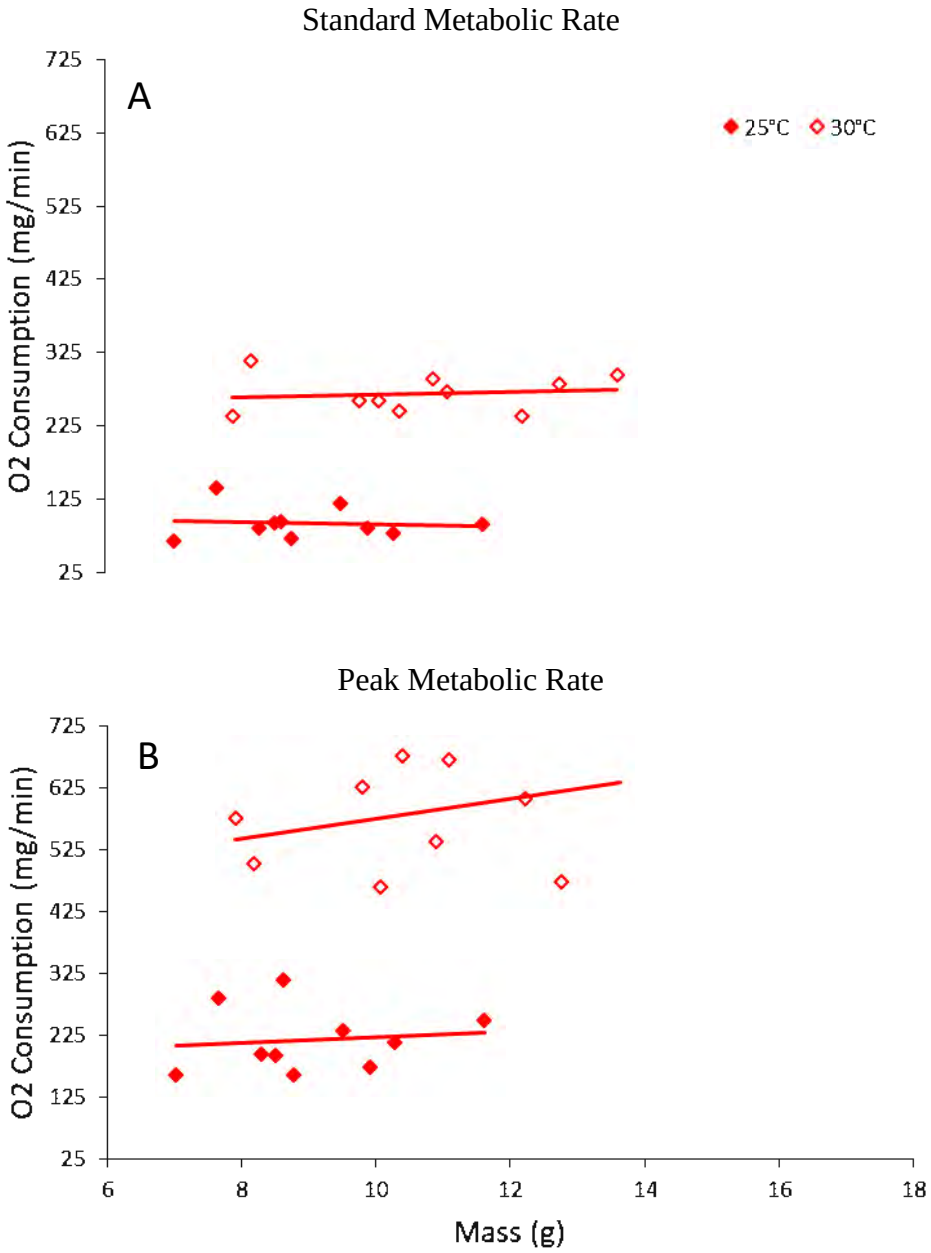

Figure 8 Average O_2 consumption (mg/min) *T. s. elegans* mass (g) for turtles fed mealworm (*Tenebrio molitor*) at 25°C and 30°C for (A) standard metabolic rate and (B) peak O_2 consumption.

Discussion

We predicted that both diet treatments would have a higher peak oxygen consumption and SMR at 30°C than at 25°C, and that turtles fed mealworms would have a significantly higher peak oxygen consumption than turtles fed duckweed at both 25°C and 30°C. We found that there was a strong effect of diet on SDA, but only at the warmer temperature. At 30°C, peak metabolic rate was higher for turtles that ate mealworms than those that ate duckweed, but there was no significant difference between diets at 25°C. Turtles overall had significantly higher metabolic rates with both meal treatments when environmental temperature was increased and did not return to baseline at the end of the 120 minutes, suggesting that even more energy was required for processing than the peak suggests.

Since turtles are ectotherms and ectotherm body temperature is driven by environmental temperature, it makes sense that SMR and SDA were significantly higher at 30°C than at 25°C. As temperature increases, the metabolic rate and the general maintenance cost of the animal increase. Thus, it is more expensive to operate at a warmer temperature. In the wild, the aquatic areas that juvenile *T. s. elegans* inhabit average at about 25°C. With current climate change models, water temperatures for juvenile aquatic areas are set to rise to about 30°C (Vliet et al. 2013). Ligon et al. (2012) examined the effects of thermal environment on pre- and post-hatching *T. s. elegans* and found that across a 4°C range of body temperature, thermal sensitivity was unaffected by long-term temperature exposure. This suggests that if *T. s. elegans* natural habitat experienced a long-term temperature increase (e.g. climate change), thermal sensitivity would persist over time. It is important to note that Ligon et al. (2012) found that turtles that were incubated at 30.5°C were relatively unaffected by the increased temperature, unlike those incubated at 26.5°C. In the wild it is likely that these temperatures would change very gradually, rather than abruptly. Thus, it is possible that these

turtles would be unaffected by the increased temperatures at least between pre- and post-hatchling life stages. More studies that measure the interactive effects on acclimation of adult turtles in changing thermal environments over time may be useful in describing how these effects might occur in a natural environment.

Digestive physiology plays an important role in the ontogenetic diet shift of *T. s. elegans*. We found no significant difference between metabolic rates when turtles were fed different mealworm and duckweed treatments at 25°C. Our findings suggest that at 25°C, there is no difference in cost of digestion between mealworms than duckweed diets. Previous studies have found that animal matter that is intact and/or possessing a strong structure (e.g., chitinous exoskeleton) are harder to digest than leaves and fungi (Hailey 1998; Secor 2002). Our findings suggest that mealworms, which have a chitinous exoskeleton, are not more costly to digest than duckweed for juvenile *T. s. elegans* at 25°C. We expected that animal matter would have a higher SDA and would be more costly to digest because of the energy needed to breakdown and assimilate protein. Animal matter requires the animal to produce more enzymes and nutrient transporters in order to digest and assimilate the meal. Plant matter, however, is digested through the use of existing bacteria in the gut. Metabolically, it costs more for the animal to digest animal matter than plant matter. Our findings could be explained by the differences found in the abilities of juveniles and adults to digest and assimilate animal material, including shifts in enzyme production or in the densities and types of nutrient transporters necessary for efficient animal matter digestion and assimilation.

While our data did not observe a difference between diets at 25°C, there was a significant difference at 30°C. Our findings suggest that temperature increases the cost of digestion and SDA in both mealworm and duckweed diets and that animal diets become more costly at higher temperatures than plant diets. This makes sense because it is more costly for turtles to operate at warmer

temperatures, and consequently digestion would also become more costly. An animal's metabolic compensation is produced through the process of acclimation and, whether "perfect" or "incomplete", allows the effects of temperature to be dampened or reduced (Ligon et al. 2012). Interestingly, Ligon (2012) found that hatchlings produced incomplete metabolic compensation between 26.5°C and 28.5°C, and near-perfect compensation between 28.5°C and 30.5°C. Organisms might fail to achieve "perfect" compensation if experiencing physiological constraints (e.g. enzymatic activity, membrane structure, organ function), or when energetic costs associated with it outweigh the benefits (Angilleta et al. 2006; Guderly and St. Pierre 1999). These previous studies suggest that turtles were more likely to achieve metabolic compensation at 30°C. While we didn't calculate the metabolic compensation in particular, we found that there is an increased cost of digestion for both mealworm and duckweed treatments at 30°C. To reach metabolic compensation our turtles would have to retain more energy from their meals than normal to outweigh the increased costs of digestion, so it is unlikely that metabolic compensation was "perfect" during our study at 30°C. In addition, we found that at 30°C turtles generally were more active and consumed more of their meal within the hour of feeding before metabolic rate was measured. Since we did not measure meal size before feeding, we could not quantify how much more turtles ate at 30°C. Generally, larger meals generate a larger SDA because of the cost associated with processing more biomass. It is possible that these differences in meal size could have also attributed to the differences in SDA seen between temperature and even diet types. Thus, our findings could ultimately be attributed to the interactions between temperature, diet type, and diet size. Future studies aiming to further measure the effects of meal size, diet type, and environmental temperature on SDA would be useful when determining *T. s. elegans* ability to acclimate metabolically to changing thermal environments.

An important factor not considered in this study is the capacity for *T. s. elegans* to acclimate metabolically to gradual changes in temperature. Here we changed the temperature of their environment from 25°C to 30°C. Slow changes in environmental thermal regimes, such as seasonal changes, or changes occurring over time with climate change, may also influence patterns of metabolism (Gienger et al. 2017). In other studies that have aimed to specifically address the effects of thermal acclimation, it is clear that turtles have the capacity to reach "perfect" metabolic compensation with changing environmental temperatures (Gatten 1978; Wood et al. 1978; Hochscheid et al. 2004). This capacity could have implications for the mechanisms by which *T. s. elegans* respond to seasonal and long term climate changes (Janzen, 1994). Behavioral thermoregulation via basking is well documented in turtles (Dreslik 2000), and it is probable that the precision with which animals thermoregulate would be unaffected during the majority of the active season. However, the duration of the active season and the proportion of individuals' activity budget dedicated to thermoregulation could be affected. Physiological adjustments to shifting temperatures could offset the need for behavioral adjustments. Finally, physiological compensation has the potential to provide much faster and more plastic responses to climate changes than do population-level genetic shifts. Given the fast rate at which global temperatures are predicted to rise (Kacholia and Reck 1997), combined with the protracted life-history characteristic of most turtle species, capacity for temperature compensation could play a crucial role in the ecology of these animals. The data here measured under acute temperature change should provide supporting information for determining how *T. s. elegans* might acclimate to changing thermal landscapes. In addition, this study may provide additional information on factors influencing diet selection in turtles with ontogenetic diet shifts.

While our study measured SDA after fasting turtles for three days, other studies such as Hailey et al. (1987) found substantial increases in SDA in *K. spekii* feeding on leaves when turtles were not fasted prior. Carter and Brafield (1992) found that the size of the SDA in continuously fed grass carp depends partly on food intake on the previous day. While the increased SDA in continuous feeding could be attributed to a simple additive effect of food remaining in the gut from the previous day, it would be important to consider these differences in SDA and their additives in the complexities of wild juvenile *T. s. elegans* foraging behavior with increased environmental temperature. In addition, studies measuring the effects of meal size on SDA with gradual changes in environmental temperature could be influential in describing how the importance of meal size might change.

Turtles varied in body mass from 7.02g to 18.8g throughout the period of the study, so our metabolic measurements were limited to small juveniles. We found that body mass had a significant effect on peak O_2 consumption at 30°C when comparing diets. Our findings were likely a result because the warmer temperature took place later in the study and turtles had more body mass variability. With more variability, we were able to detect the effect of body mass. Ligon et al. (2012) found a strong correlation between metabolic rate and mass among juveniles but not among hatchlings. This difference among age classes is due in large part to the low variability in mass among hatchlings, and probably also reflects differences in turtles' digestive state. Future studies that aim to further describe body mass effect with increased temperatures could provide a better understanding on the importance of body size in meal processing.

Conclusion

Ultimately, the impact of diet on metabolic response in juvenile *T. s. elegans* is best reflected by the changes in physiological and behavioral interactions due to increased temperature. We predicted that both diet treatments would have a higher peak oxygen consumption and SMR at 30°C than at 25°C, and that turtles fed mealworms would have a significantly higher peak oxygen consumption than turtles fed duckweed at both 25°C and 30°C. We found that there was a strong effect of diet on SDA, but only at the warmer temperature. Our findings make sense because it is metabolically more costly to operate at a warmer temperature for ectotherms such as turtles. Our findings suggest that at 25°C, there is no difference in cost of digestion between mealworms than duckweed diets, possibly being explained by juvenile *T. s. elegans* increased efficiency to digest and assimilate animal matter compared to adults. While we did find turtles fed mealworms had a significantly higher SDA than turtles fed duckweed at 30°C, this may be attributed to the increased amount of food consumed among turtles. Turtles overall had significantly higher metabolic rates with both meal treatments when environmental temperature was increased, suggesting that temperature and diet size may increase the cost of digestion and SDA in *T. s. elegans* for both mealworm and duckweed diets.

Research should be done to determine the capacity for *T. s. elegans* to acclimate metabolically to gradual changes in temperature. Slow changes in environmental thermal regimes, such as seasonal changes, or changes occurring over time with climate change, may also influence patterns of metabolism (Gienger et al. 2017). It is possible that these turtles would be unaffected by the increased temperatures between pre- and post-hatchling life stages, but research should be done to describe how adults may be affected considering their long life span. In addition, further studies that aim to

describe the effect of environmental temperature on digestive ability and metabolic compensation could be influential in describing the capability of these turtles to acclimate to changing environments.

Overall, our findings provide some information about how temperatures of juvenile *T. s. elegans* habitats can influence metabolism. Further research investigating how turtles may benefit from different diet types and sizes in varying temperatures can be useful wildlife facilities and zoos. This information will be valuable to wildlife rehabilitation facilities and zoos in preparing suitable habitats for juvenile *T. s. elegans*, and allows for greater consideration to be taken when considering what affects climate change will have on animal populations.

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April 26, 2021

Please consider this memo your formal approval of your research protocol by the Otterbein University Animal Care and Use Committee. Your reference number is: 20210402.

Thank you for your submission. Please do not hesitate to contact me with additional questions.

Best regards,

Sheri Birmingham, DVM
Chair, OUACUC