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Summer Weight Changes in a Gray Treefrog (*Hyla versicolor/chrysoscelis* Complex) Population in Maryville, Missouri

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Abstract: *Late spring and summer represent a critical ecological period for members of the Gray Treefrog and Cope's Gray Treefrog species complex. During this time several energy goals must be met to ensure reproduction takes place followed by a period of preparation for winter dormancy. We hypothesized that the effects of this period could be measured by changes in weight, where weight in both sexes would decline during the reproductive part of the season (late spring/early summer), followed by an increase in weight for both sexes during the late summer in preparation for winter. We tracked a total of 97 male and 63 female treefrogs from four ponds and two lake inlets over a period of seven summers (2015–2021). We regressed weights of both sexes against ordinal dates for the summer reproductive period (May–June, 123–182) and winter preparatory period (July–August, 183–261). For both sexes we found a significant overall weight loss in the summer reproductive period (males: $F_{1,129}$, $P = 0.0013$, females: $F_{1,220}$, $P = 0.0038$) followed by a weight gain in the winter preparatory period (males: $F_{1,317}$, $P < 0.0001$, females: $F_{1,209}$, $P < 0.0001$) over the seven-year period. However, on a yearly basis, these significant weight change patterns were only seen in some years (summer reproductive, males: 42%, females: 17%; winter preparatory, males: 42%, females: 42%). We conclude a general pattern of weight loss during the reproductive period of the season followed by a general pattern of weight gain, but that this effect may be masked in some years by other ecological factors.*

Key words: Hyla versicolor, Hyla chrysoscelis, *weight, breeding*

Introduction

Late spring and summer represent an important period for members of the Gray Treefrog and Cope's Gray Treefrog species complex (*Hyla versicolor*, LeConte/*chrysoscelis*, Cope; hereafter treefrog). Most breeding takes place during late spring through mid-summer, and this energy-expensive period is followed by preparations for overwinter dormancy, another energetically expensive time (Smith et al., 2003; Briggler and Johnson, 2021). As such, the summer energy budget is an important component of the annual fitness of individuals in treefrog populations. This study explores the importance of this energy budget in terms of weight change in relation to both sexes over the breeding and winter preparatory time periods.

In northwest Missouri, treefrog breeding typically reaches its peak in early May and lasts through the end of June or early July, with small amounts of breeding extending even to August (Briggler and Johnson, 2021). Males will establish choruses at shallow ponds, or similar sites where fish are unlikely to prey on eggs, allowing females to approach and select mates based on site and call characteristics (Fellers, 1979; Schwartz et al., 2001). This is particularly energetically expensive for males, who may produce over 5000 calls a night (Wells and Taigen, 1986). However, females incur energy costs as well, traveling nightly to visit choruses and using fat supplies to produce eggs (Johnson et al., 2007; Rastogi et al., 2010). It should be no surprise then, that both males and females lose weight during this time (Rastogi et al., 2010).

During winter, dormancy typically occurs in leaf litter above the frost-line, where temperatures are moderated, but still often below freezing (Layne, 1999; Johnson et al., 2008). Cryoprotectants are secreted by the liver and take the form of glucose (*Hyla chrysoscelis*) broken down from glycogen (*Hyla versicolor*, Costanzo et al., 1992; Irwin and Lee, 2003). Regardless of the energy expenses incurred during breeding, treefrogs should rebuild nutrient supplies as soon as possible in preparation for winter dormancy. Indeed, Kovacs et al. (2007), in a study of the diet of congener *Hyla arborea*, reported higher feeding intensity in the period prior to dormancy (August-September).

The selective pressure to maintain nutrient levels during the breeding season, and to rebuild nutrient supplies for winter dormancy should be detectable in the form of weight shifts in individuals during the summer months. If true, this would suggest that current monitoring studies for treefrogs should include weight measurements during the summer. We hypothesized a detectable decline in weight during the main part of the breeding period (May – June) for a population of treefrogs in Nodaway County, Missouri. We further hypothesized that this would be followed by a detectable weight gain in the subsequent period (July – August).

Methods

We conducted this study at Northwest Missouri State University and the Mozingo Outdoor Education and Recreation Area (MOERA, Lat-Lon: 40° 26' 26" N, 94° 46' 16" W) in Nodaway County, Missouri, between the months of May and August, 2015–2021. Using PVC pipe traps (Boughton et al., 2000), we sampled four ponds (three at MOERA and one on campus) and two inlets of Mozingo lake, which borders the MOERA site. The campus pond (7875 m^2) 9.6 km to the west of MOERA was sampled only in 2015 and 2016. Ponds and inlets at the MOERA site were located an average (± SD) of 456.4 ± 214.66 m apart. The smallest pond (300m^2) often dried at least once each August, while the next smallest (975m^2) dried once in August 2018. Ponds were not known to be stocked with fish nor were they ever inundated by the neighboring lake. The average size of the ponds was (mean ± SD) 2775m^2 ± 3467m^2, and both inlets and ponds were shallow (~ 1 – 1.5 m deep). The total area of the inlets were not covered by our sampling protocol, but the area that was covered (as measured by the outermost PVC pipes) was (mean ± SD) 1243m^2 ± 416m^2.

At each pond or inlet, within 50m of the water's edge, we placed 4 vertical standing PVC pipes in line with a randomly selected tree. This was done such that the pipes formed a line from the tree to the water's edge. The line of 4 pipes included a 1-m standing pipe placed respectively 2m and 5m from the tree closest to the water, and a 1-m standing pipe placed 2m from the tree in the direction away from the water. A fourth, shorter pipe (0.6m) was attached to the tree at about 1.5m high (Boughton et al., 2000). This array of four pipes was repeated 3 more times for a total of 16 pipes at each pond or inlet. All PVC pipes had a 3.8 cm diameter (Boughton et al., 2000; Pittman et al., 2008).

We checked pipes for treefrogs twice per week on consecutive days. If treefrogs were found in the PVC pipes, they were carefully moved to a plastic sandwich bag, sexed by noting throat coloration (males have a darker throat from the effect of calling) measured from cloaca to tip of snout and weighed to the nearest 0.05 grams using a 30g Pesola scale©. Treefrogs were then removed from the bags, returned to their pipe, and empty bags were weighed to subtract the weight of the bag and any debris left in the bag. Individuals were marked using visible implant alphanumeric tags (VIA tags, Northwest Marine Technology, Inc, Shaw Island, Washington, USA) just under the skin in the right tibiofibular section of the leg (Clemas et al., 2009). Each treefrog was examined for a tag when captured. If the individual was tagged the information gathered was cataloged under the specimen's unique identification number. If the individual was not tagged, a tag and identification number would be issued. We weighed an individual treefrog no more than once per week. We used new bags for every treefrog or group of treefrogs found in a single pipe.

We used linear regression to determine the degree to which individual treefrogs on average lost weight during the early part of the reproductive period (May-June) and gained weight during the winter preparatory period (July-August). Independent regressions were first conducted for each sex, combining data across bodies of water (inlet or pond) and years to offset the effects of low sample sizes. All treefrogs were included in the analysis regardless of the number of times they were captured over the summer. We then tested for yearly effects for males and females by conducting regressions by sex and year. Treefrog weights were regressed against the independent variable of ordinal date. For this study's field seasons, these numbers ranged between 123–182 for May through June; and 183–261 for July through August. Leap years occurred in 2016 and 2020 with a total of 366 days as opposed to 365, but this was ignored as it would have minimal effect on the overall pattern being assessed. For each regression analysis, $\alpha = 0.05$, but Bonferroni adjustments were made to account for the effect of multiple regressions (1 per year by sex). Residuals were analyzed graphically to assess departures from homoscedasticity (Zar, 1999).

Results

We captured and tracked the weights of 97 male and 63 female treefrogs from 2015 – 2021. Average male weight for May-June (± SD) was 4.89 ± 1.98g, while for females it was 5.31 ± 2.31g. Average male weight for July-August (± SD) was 5.59 ± 1.70g, while for females it was 5.71 ± 2.06g. Overall, these weights did not differ between sexes or by time period (May-June, July-August, $F_{3,23}$, $P = 0.6432$). The average proportion of males recaptured at least once in May-June (± SD) was 0.64 ± 0.25, and 0.74 ± 0.15 in July-August. These males were recaptured on average (± SD) 2.33 ± 0.66 times (May-June), and 3.26 ± 0.59 times (July-August). The average proportion of females recaptured at least once in May-

June (± SD) was 0.56 ± 0.19, and 0.69 ± 0.18 in July-August. These females were recaptured on average (± SD) 2.17 ± 0.64 times (May-June), and 2.91 ± 0.92 times (July-August). Males that were captured once did not differ in average weight from males captured more than once in May-June (t = 1.36, df = 90, $P = 0.1782$) or July-August (t = 0.12, df = 23, $P = 0.9073$). Females that were captured once did not differ in average weight from females captured more than once in May-June (t = 0.69, df = 54, $P = 0.4900$) or July-August (t = 1.57, df = 22, $P = 0.1308$).

To assess overall patterns of weight change for both sexes during both time periods we conducted four overall regressions, combining data collected across all seven summers for all bodies of water sampled (male reproductive period, male winter preparatory period, female reproductive period, and female winter preparatory period). We found a significant overall weight loss in the summer reproductive period in both sexes (males: $F_{1,129}$, $P = 0.0013$, females: $F_{1,220}$, $P = 0.0038$) followed by a significant weight gain in the winter preparatory period in both sexes (males: $F_{1,317}$, $P < 0.0001$, females: $F_{1,209}$, $P < 0.0001$).

We then conducted regressions for each summer of the study to determine if this pattern was consistent each year. For the summer reproductive period, males had a significant negative slope in three out of seven years (Bonferroni adjustment of $\alpha \leq 0.0071$, Table 1). During years of significant weight loss, males lost an average (± SD) of 0.028 ± 0.002 grams per day, or 1.652 grams over the period of May-June. We caught no females in May or June of 2015, so performed six independent regressions for the years 2016 – 2021 and found a significant negative slope in only one out of six years (Bonferroni adjustment of $\alpha \leq 0.0083$, Table 1). During this single year of significant weight loss, females lost 0.145 grams per day, or 8.555 grams over the period of May-June (some females this particular summer weighed as high as 13 grams at the beginning of the season). Years showing a significant decline in weight for males during the reproductive period were 2015, 2020 and 2021; with a significant decline in weight for females in 2020 only.

For the winter preparatory period, males had a significant positive slope in three out of seven years (Table 2). During years of significant weight gains, males gained an average (± SD) of 0.038 ± 0.010 grams per day, or 2.964 grams over the period of July-August. Similarly, females had a significant positive slope occurring in three out of seven years (Table 2). During years of significant weight gains, females gained an average (± SD) of 0.033 ± 0.007 grams per day, or 2.574 grams over the period of July-August. Years showing a significant increase in weight in winter preparatory period in males were 2015, 2016, and 2020; with a significant increase in weight for females in 2016, 2019, and 2020.

Table 1. Expected male and female weight loss in May and June for treefrogs in four ponds and two inlets of Mozingo Lake, Nodaway County, Missouri for the years 2015-2021. The slope ($\beta 1$) and standard deviation (SD) of the slope are provided with P-values marked with an asterisk when significant, $\alpha = 0.05$ and with a Bonferroni adjustment to 0.0071 for individual years. The coefficient of determination (r^2) represents the proportion of data explained by ordinal date. The sample size (n) represents the number of treefrogs that were tracked for a given summer. Females were not captured in this time period for 2015.

	Male				Female			
Year	$\beta 1$ (SD)	*P*-value	r^2	n	$\beta 1$ (SD)	*P*-value	r^2	n
2015	−0.075 (0.013)	<0.0001*	0.713	8	–	–	–	–
2016	0.002 (0.011)	0.8516	0.001	18	0.020 (0.013)	0.1374	0.086	14
2017	−0.049 (0.034)	0.1641	0.105	8	−0.012 (0.046)	0.7926	0.004	7
2018	0.017 (0.021)	0.4179	0.016	14	−0.004 (0.022)	0.8460	0.001	10
2019	−0.037 (0.014)	0.0103	0.170	12	0.036 (0.030)	0.2456	0.120	6
2020	−0.030 (0.008)	0.0007*	0.307	23	−0.145 (0.016)	<0.0001*	0.761	15
2021	−0.070 (0.020)	<0.0001*	0.632	14	−0.037 (0.023)	0.2742	0.085	11
Combined	−0.020 (0.010)	0.0013*	0.046	97	−0.037 (0.014)	0.0038*	0.064	63

Table 2. Expected male and female weight loss in July and August for treefrogs in four ponds and two inlets of Mozingo Lake, Nodaway County, Missouri for the years 2015-2021. The slope ($\beta 1$) and standard deviation (SD) of the slope are provided with P-values marked with an asterisk when significant, $\alpha = 0.05$ and with a Bonferroni adjustment to 0.0071 for individual years. The coefficient of determination (r^2) represents the proportion of data explained by ordinal date. The sample size (n) represents the number of treefrogs that were tracked for a given summer.

	Male				Female			
Year	$\beta 1$ (SD)	*P*-value	r^2	n	$\beta 1$ (SD)	*P*-value	r^2	n
2015	0.048 (0.005)	<0.0001*	0.594	16	0.027 (0.065)	0.693	0.028	4
2016	0.067 (0.006)	<0.0001*	0.494	32	0.066 (0.009)	<0.0001*	0.392	18
2017	0.074 (0.043)	0.1065	0.146	8	0.075 (0.067)	0.2967	0.135	5
2018	0.036 (0.022)	0.1094	0.083	12	0.016 (0.019)	0.3998	0.019	12
2019	0.014 (0.021)	0.5033	0.013	10	0.102 (0.023)	0.0068*	0.797	2
2020	0.051 (0.006)	<0.0001*	0.651	10	0.055 (0.010)	<0.0001*	0.410	14
2021	0.034 (0.062)	0.6131	0.070	3	0.017 (0.049)	0.7387	0.006	10
Combined	0.045 (0.004)	<0.0001*	0.268	65	0.035 (0.007)	<0.0001*	0.112	65

Discussion

Overall patterns of weight change matched our hypotheses for both sexes. We documented weight declines in May-June, followed by weight increases in July-August. Nevertheless, our results varied highly by year and sex. Significant weight loss during May-June appeared to be more commonly true for males than females. This difference between the two sexes likely reflects the heightened energy requirements males undergo by calling (Taigen and Wells, 1985). However, even for males, in some years significant weight loss was not detected. It is possible that male energy loss over this time is usually offset by foraging bouts during the day or during absences from chorusing (Murphy, 1994; Wells et al., 1995; Johnson et al., 2007). As such, measuring weights on a weekly basis would not be precise enough to detect energy losses in most years. Instead, we would be detecting only unusually poor periods where energy losses in the form of weight loss would last for a longer period. This would be true for females as well, but our results suggest that significant weight loss occurs in fewer years during this period than for males. Females during this period may travel to assess multiple breeding ponds and tend to locate at the periphery of a population's distribution possibly for this purpose or to avoid foraging competition with males who are in higher densities around breeding ponds (Johnson et al. 2007). Despite the greater degree of movement and consequent energy cost this may require, it is likely only for a short period of time, perhaps as short as 48 hours in some populations (Johnson et al. 2007), and so was not detectable by this study (sampling two consecutive days per week).

Later in the summer (July-August), both sexes show significant weight gains in three of seven years, with overlap in years 2016 and 2020. During this period prior to cooler weather, one would expect increased foraging to offset any costs associated with breeding, but also to prepare for winter dormancy by increasing glycogen stores (Irwin and Lee, 2003; Costanzo et al., 1992). This would presumably result in weight gain as late summer progressed. Our inability to detect these weight gains in most years is again likely due to the coarseness of our sampling strategy, such that we were only able to detect major shifts in weight. Large scale changes in weight may only occur in some years, potentially high-quality years in terms of prey availability, offsetting competitive pressures.

One caveat to our analysis is that individual frogs were tracked at varying rates over the summer. Some were captured multiple times, some were captured just once. Those treefrogs that were reweighed more consistently over the course of the summer contributed more data to the analysis. If treefrogs that were likely to use PVC pipes for longer periods (allowing multiple measurements) differed from the larger population, it would bias our results. However, we were unable to find a significant difference between treefrogs captured only once and treefrogs captured multiple times, suggesting that this was not the case. A second caveat is that the conditions of our study meant that treefrogs could join or contribute a weight measurement at any time. This would be problematic if younger, and presumably lighter treefrogs joined later than older and heavier treefrogs because it would falsely imply a weight loss. We did on occasion see older females who had survived the winter start using pipes early, be measured a couple of times, then not be captured again, but this was rare and would be unlikely to bias results heavily. Other bias could include environmental factors such as general weather conditions and a short sampling period that excluded the months of March, April, September, and October.

Our results suggest that there are detectable weight shifts in some years during the summer, presumably reflecting a reproductive season (May – June) and winter preparatory season (July–August). This indicates that the summer period overall (May–August) in northwest Missouri is an ecologically important period for treefrogs, requiring the balancing of multiple goals, such as reproducing and preparing for winter. In addition, there is an inherent variability in annual conditions affecting treefrogs' ability to meet these goals. We suggest monitoring protocols for populations should include weight measurements, especially during summer.

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