

Investigating the relationship between body condition and phonotaxis of male *Hyla versicolor*
and *Rana clamitans* towards a conspecific chorus

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Abstract

Males of both *Hyla versicolor* and *Rana clamitans* vocalize during their breeding seasons. However, these calls serve different functions: for *R. clamitans*, the call primarily serves to maintain a territory and ward off other males, with females choosing mates primarily based on breeding site rather than on call morphology as in *H. versicolor*. I investigated the relationship between body condition, as calculated by the residual index method, and phonotaxis of male *H. versicolor* and *R. clamitans* to a conspecific chorus of fellow males in a laboratory setting. We found that *R. clamitans* moved significantly farther from the speaker during trials in which a conspecific chorus was playing, suggesting that their overall tendency is to move away from a chorus of fellow males. We also found that in this laboratory setting, patterns in phonotaxis relative to body condition did not emerge until the second day, after which in both species as body condition increased, time spent moving decreased, as did distance from start in *R. clamitans*. Collectively, this suggests that, for both species, frogs with a lower body condition exhibit decreased phonotaxis towards a conspecific chorus, though further research is needed on the effect of elements of the laboratory setting, such as artificial light, that may be manipulating natural relationships between body condition and phonotaxis.

Introduction

Both male *Hyla versicolor*, commonly known as the gray tree frog, and male *Rana clamitans*, the green frog, produce loud vocalizations during their breeding seasons (Christie et al. 2010, Moreno-Gómez et al. 2015). Female *H. versicolor* are known to exhibit positive and selective phonotaxis (referring to movement in relation to sound) towards these signals from distances as far as 100 meters (Bee 2007, Christie et al. 2010). In more territorial species such as *Rana clamitans*, these calls, beyond attracting females, mediate male-male competition through maintaining spacing between competitors (Moreno-Gómez et al. 2015). For *R. clamitans*, the primary function of these calls is to maintain a territory, with females choosing mates primarily based on breeding site rather than on call morphology (Owen and Perrill 1998).

Yet some species of frogs have been shown to exploit these acoustic signals for reasons beyond chorus spacing and mediating male-male aggression. The sound of the conspecific (referring to same species) chorus serves as an acoustic beacon for the males of certain species to gather in large breeding aggregations (Bee 2006). One potential explanation for this selective

male phonotaxis is that such behavior may increase the chances of unpaired males to find and clasp a female partner (Bee 2006).

Furthermore, not all males are consistent callers; numerous anuran species have also been reported adopting non-calling "satellite" tactics and behaviors, with these "satellite males" lingering in intimate proximity to conspecific callers to intercept mates attracted to these "advertising males" (Leary et al. 2004). These actively calling males were found to have a significantly higher body condition than the satellites, potentially because the production of calls is so highly energy intensive (Leary et al. 2004). As such, satellite behavior may be caused by physiological restraints that inhibit sustained calling activity, resulting in subsequent "foraging" mating behavior. It has also been suggested that such a classification of "satellite" or "advertising" male is not static but rather condition-dependent. In other words, a male will cease calling when the energy required to vocalize is more than it can afford (Leary et al. 2004). This type of satellite behavior has been observed in *H. versicolor* (Wells 1977b).

My study builds off this research by further investigating the relationship between body condition, as calculated by the residual index method (Bancila et al. 2010), and phonotaxis of male *H. versicolor* and *R. clamitans* to a conspecific chorus of fellow males in a laboratory setting. The laboratory setting stripped away other cues available to the frog in the field, such as olfactory, visual, and magnetic stimuli, and solely looked at the influence of auditory sound on movement towards or away from a breeding aggregation (Christie et al. 2010).

Because these species' exhibit different breeding patterns and strategies, I predicted there to be different, converse relationships between body condition and phonotaxis. My hypothesis was that male *H. versicolor* with a lower body condition would exhibit greater phonotaxis

towards a conspecific chorus of fellow males, while male *R. clamitans* with a lower body condition would exhibit weaker phonotaxis towards a conspecific chorus of fellow males.

Methods

Sampling and Data collection

I opportunistically sampled 15 male green frogs from two vernal ponds, Roach Lake, and Bay Lake on the property of the University of Notre Dame Environmental Research Center. Due to a short breeding season for *H. versicolor*, a sample size of only 9 male gray tree frogs was obtained from the vernal ponds on property. Each frog was kept in a separate, labeled container for no longer than 2 days.

Before each trial, I measured the frogs' SVL (snout-vent-length, mm) and mass (g) to assess their body condition using the residual index method (Figure 1). A frog with a greater mass than expected for its SVL had a higher body condition, and vice versa.

Each frog underwent two days of trials, both including a conspecific chorus trial and a silent trial. The order of the trials was randomized, as was the order in which the frogs were trialed, and the direction of the arena to account for extraneous, confounding noise and light. I placed each frog in the center of a long hallway arena (dubbed the “centipede”) 300 centimeters in length, 6 inches in height, and 6 inches in width, that had a working speaker at one end set to 88 decibels and a mute replica speaker at the other to act as a visual control. The frog was placed under a mesh cup that allowed for them to hear the presence or absence of the conspecific chorus. After a one-minute acclimation period, I then lifted the cup and allowed the frog to freely move in either direction for a subsequent 5 minutes. The top of the hallway arena was covered with plastic wrap to prevent escape out of the top; however, the frog was allowed to escape out

whichever end it chose. During those 5 minutes, I recorded the frogs' actions (hop (H), walk (X), turn (O), or still (-)) and location every 15 seconds until escape or the end of the trial period. At the end of the 5-minute trial, I then recorded final distance to both speaker and start.

Statistical analysis

I used a paired t-test to assess whether there was a significant change in body condition between the first and second day for either species. To assess phonotactic response to a conspecific chorus, I used the nonparametric Kruskal Wallis test to analyze differences between silent and conspecific chorus trials in final distance from speaker, final distance from start, time spent moving (proportional to total time in arena), and latency (time until initial movement) for each species, testing day 1 and day 2 separately. Using data from the conspecific trials, I also ran the Spearman rank correlation to test for significant relationships between body condition and final distance from speaker, final distance from speaker, time spent moving, and latency, again testing day 1 and day 2 separately. As the arcsin square root transformed data for time spent moving in *H. versicolor* was normally distributed on the second day, I was able to use a regression to analyze this relationship to body condition. All statistical tests were performed using R (R Core Team 2017).

Results

There was no significant change in body condition between day 1 and day 2 in *R. clamitans* ($t_{\text{obs}} = -0.35315$, $df = 14$, $p=0.7292$, Figure 2) or *H. versicolor* ($t = 2.0793$, $df = 4$, $p\text{-value} = 0.1061$, Figure 3).

R. clamitans moved significantly farther from the speaker during trials in which a conspecific chorus was playing than during silent trials (Kruskal-Wallis chi-squared = 5.141 p-value = 0.02337, Figure 4). On the second day, as body condition increased, distance from start decreased ($S=873.65$, $p=0.0299$, Figure 5), and time spent moving generally decreased, though this relationship was not statistically significant ($S=784.45$, $p=0.1387$, Figure 6).

For *H. versicolor*, as body condition increased, time spent moving decreased ($y = -0.54485x + 1.02572$, Adjusted R-squared: 0.944, p-value=0.0001292, Figure 7).

Discussion

Overall, male *R. clamitans*, as expected, appeared to experience greater phonotaxis away from a conspecific chorus as body condition decreased, moving farther from the start and spending overall more time moving proportional to total time in the arena. This is consistent with the known breeding behavior and intersexual selection of green frogs as a relatively territorial species, using calls to keep competing males at a distance (Moreno-Gomez et al. 2015, Wells 1977a).

However, for gray tree frogs, rather than finding converse relationships to *R. clamitans* between body condition and phonotactic behaviors, I found instead one similar pattern in time spent moving. During conspecific trials, *H. versicolor* increased their time spent moving as body condition decreased, but this increased movement was not in any significant direction towards or away from a conspecific chorus, nor was there any significant difference in time moving between chorus and silent trials. Lack of statistically significant relationships may be largely due to the small sample size of *H. versicolor*, though this initial pattern suggests that the males of these two species may respond in a similar manner to a conspecific chorus. This would be

contrary to my expectations because the call of *H. versicolor* does not serve the same directly territorial function as *R. clamitans* (Owen and Perrill 1998).

However, for both species, patterns of significance did not emerge until the second day of trials. My study was set up in a laboratory setting that was notably artificial in comparison with both frogs' natural breeding habitat, and as amphibians are highly sensitive to environmental quality and habitat changes, this may provide some explanation for patterns being overshadowed on the first day of trials (Bancila et al. 2010).

The most distinct difference between the laboratory setting and the frogs' natural habitat was the presence of artificial light in my experimental design, particularly potent because such presence of artificial light is one factor that has been shown to affect male green frog behavior (Baker and Richardson 2006). A study on male *R. clamitans* found in a rural setting free from artificial light exposure, similar to the conditions of my own study site, found that exposure to light caused disorientation and more frequent movement in the presence of artificial light (Baker and Richardson 2006). However, this study was performed with a habituation period to artificial light of only 5 minutes and stated the need for further research on whether populations can adjust to more prolonged exposure.

Additionally, certain frog species are more robust to exposure to artificial light than others, as is the case with *H. versicolor*. Artificial light did not significantly affect mate choice behavior in female *H. versicolor*, with no difference in mate preference and ranking, choosiness, or approach behavior (Underhill and Höbel 2018). There was also no difference in response rate, suggesting that artificial lighting did not frighten the frogs to the extent that they were more inclined to refuse to move or flee (Underhill and Höbel 2018). These results combatted predictions of relaxed preferences, decreased choosiness, and stealthier phonotactic approach

under artificially lit conditions, suggesting that *H. versicolor* are a species more robust to artificial light exposure. However, I cannot rule out that artificial light may have affected the phonotactic behavior of the *male H. versicolor* trialed in my experiment.

By the second day of trials, all frogs had been exposed to the artificial laboratory settings for upwards of a 24-hour acclimation period. Though not consistently exposed to artificial light during the entirety of the first day, as lights were turned off in the holding room at night, this is something that could be controlled for in future experiments to more accurately account for and test differences in habituation to artificial light and the subsequent effect upon the relationship between body condition and phonotaxis.

In the execution of my design, all trials for both species were held under artificial lighting, with the second days' trials having a longer acclimation period to artificial light than the first day. There was no difference in procedure between the first and second day, suggesting that different habituation time to artificial light could be a potential factor that lead to patterns emerging on the *second* day but not the first, particularly for *R. clamitans* (Baker and Richardson 2006).

It would be beneficial to repeat this experiment running trials in ambient as well as artificial light, in order to see if similar patterns emerge in phonotaxis relative to body condition, and whether or not these patterns appear on the first or second day. Additionally, running trials for a third day to allow for a yet greater acclimation time to artificial lab conditions may tease out further patterns that were yet insignificant by the second day, notably including latency (time until initial movement) as it relates to body condition.

Overall, the patterns found in my experiment suggest that male *R. clamitans* of lower body condition move away from a conspecific chorus, while those of a higher body condition are

more likely to stand their ground when faced with the territorial calls of other males. In both species, frogs of a higher body condition spent proportionally less time moving in response to a conspecific chorus, suggesting that both species may have a similar relationship between body condition and phonotaxis. A larger sample size may have further revealed these patterns. However, as these patterns emerged only on the second day, my study prompts the need to further research the effect of elements of the laboratory setting, such as artificial light, that may be initially manipulating natural relationships between body condition and phonotaxis in male *H. versicolor* and *R. clamitans*.

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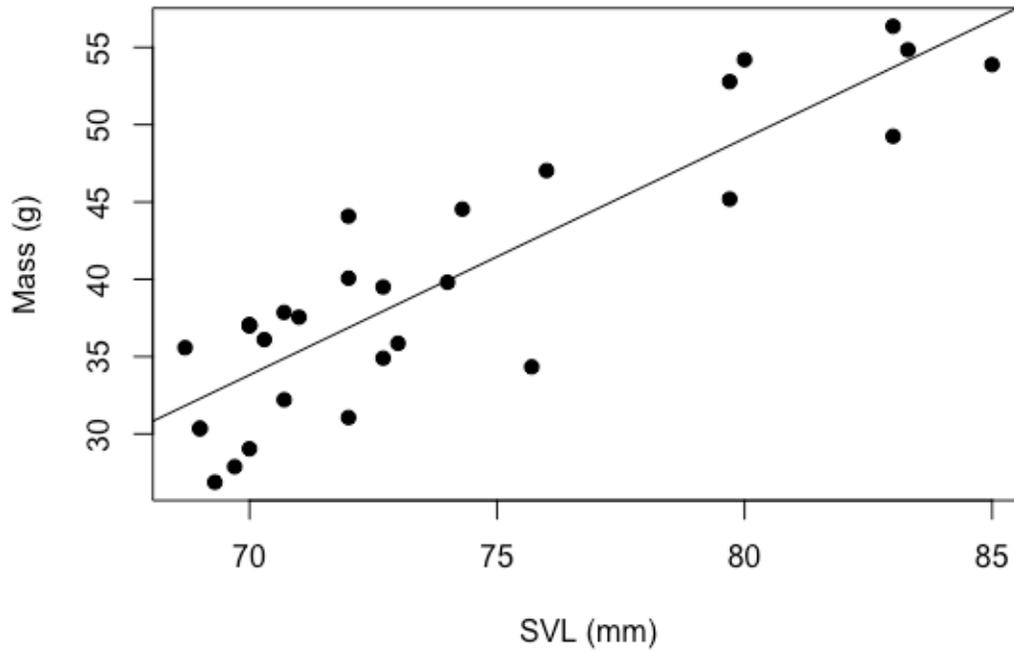
Figures

Figure 1. Residual index method of measuring body condition in *R. clamitans* (conspecific trials, days 1 and 2). Plotting SVL (snout-vent-length) and mass, I used the regression line ($y=1.5301x - 73.2953$) to calculate the difference between observed and expected mass as a measure of body condition. Above is the plot for only green frogs ($n=30$).

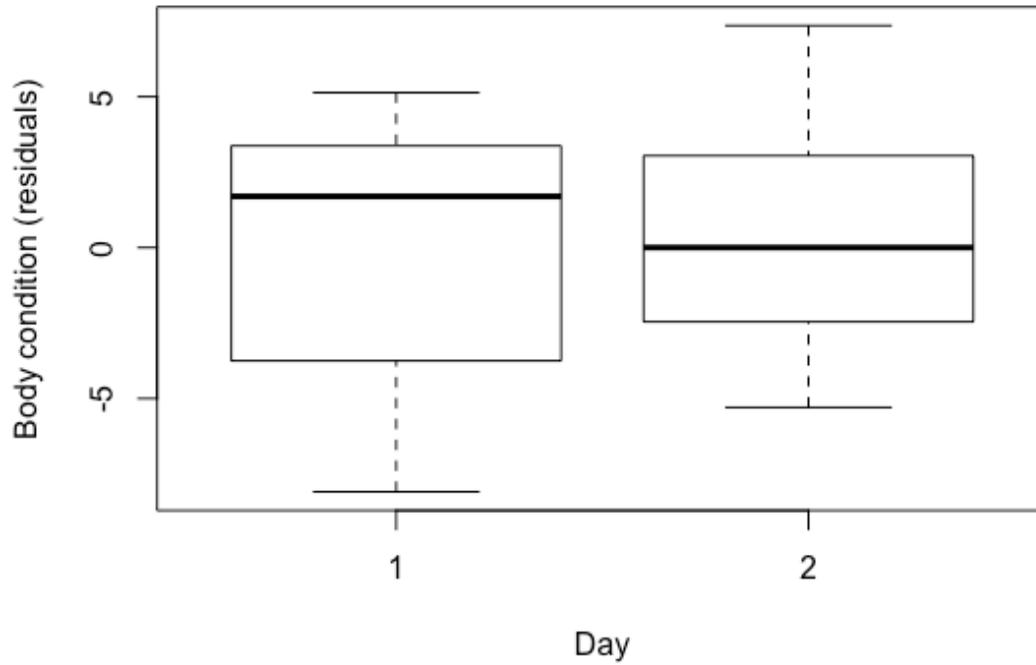


Figure 2. Change in body condition by day in *R. clamitans*. No significant change in body condition between days ($t_{\text{obs}} = -0.35315$, $df = 14$, $p=0.7292$). $n=15$.

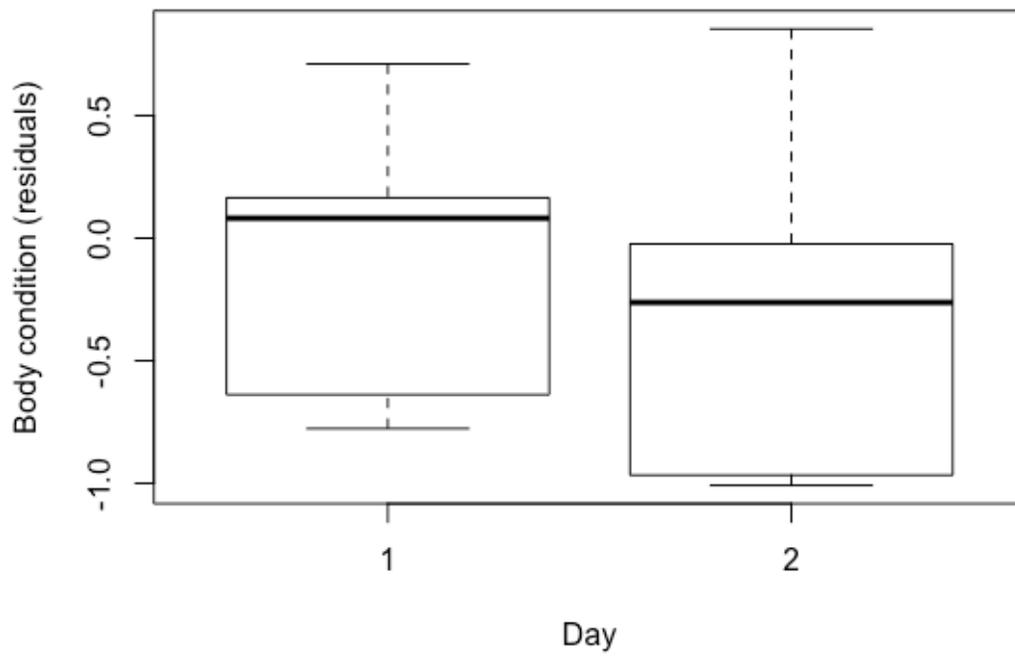


Figure 3. Change in body condition by day in *H. versicolor*. No significant change in body condition between days ($t = 2.0793$, $df = 4$, $p\text{-value} = 0.1061$). $n=5$.

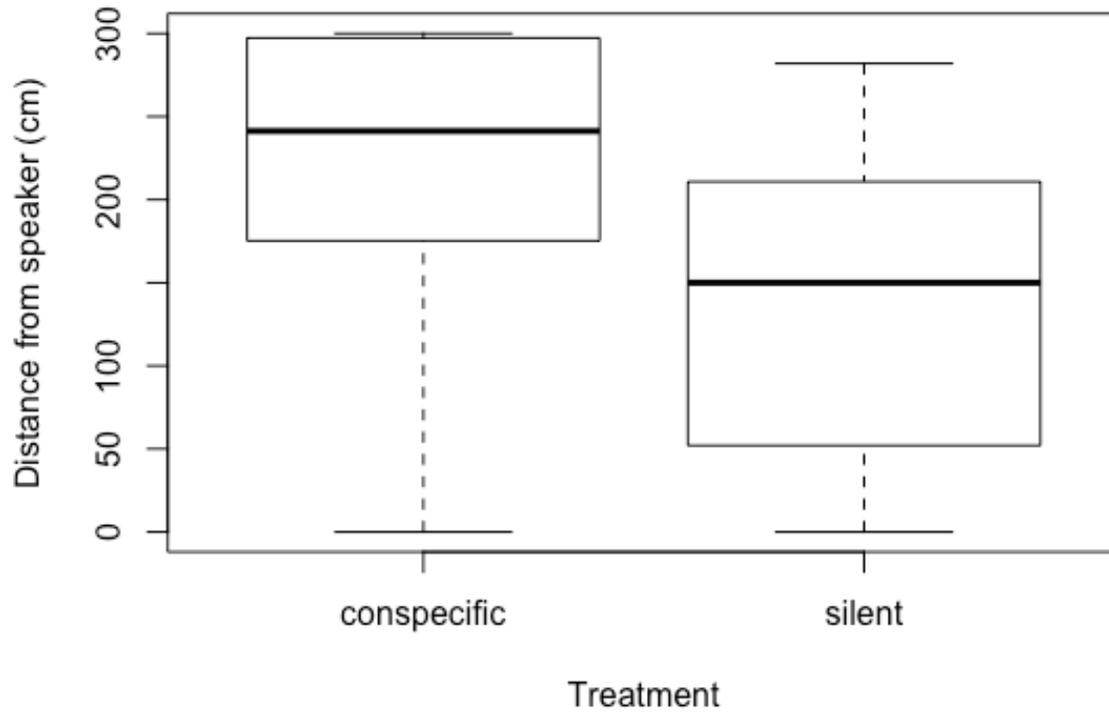


Figure 4. Distance from speaker by trial treatment in *R. clamitans* (day 2). Green frogs moved significantly farther from the speaker during conspecific chorus trials (Kruskal-Wallis chi-squared = 5.141 p-value = 0.02337). n=15.

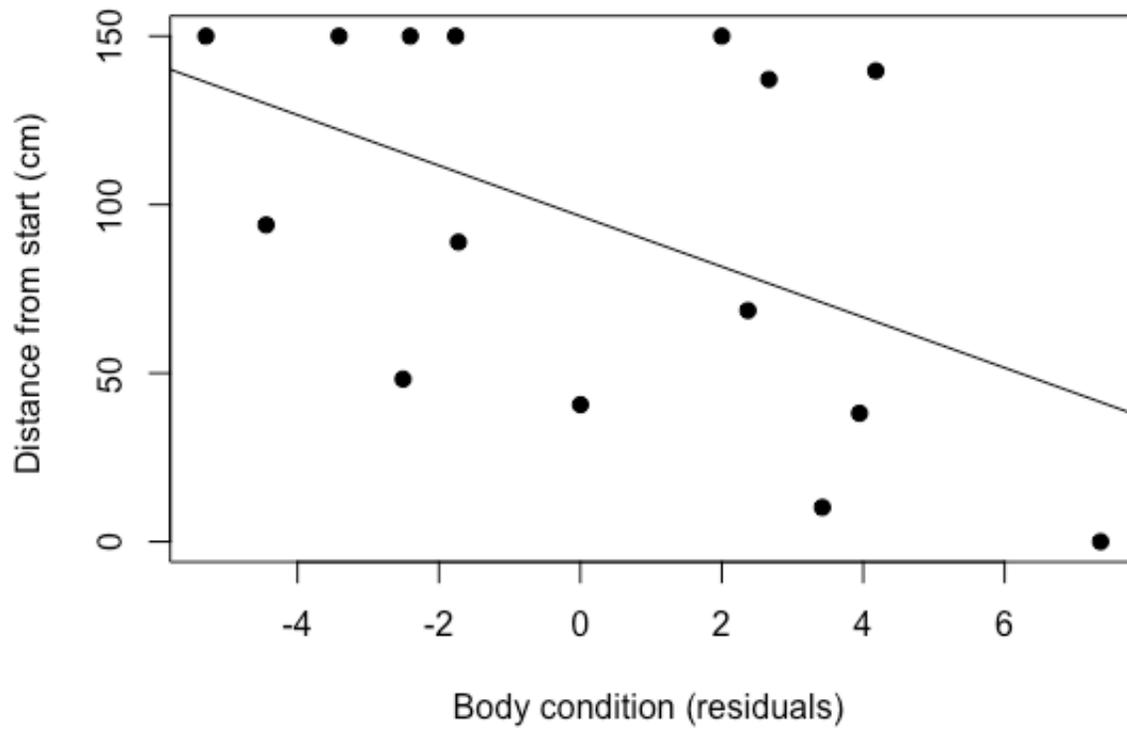


Figure 5. Distance from start related to body condition in *R. clamitans* (day 2). As body condition increased, distance from start decreased ($y = -7.507x + 96.565$, $S = 873.65$, $p = 0.0299$). $n = 15$.

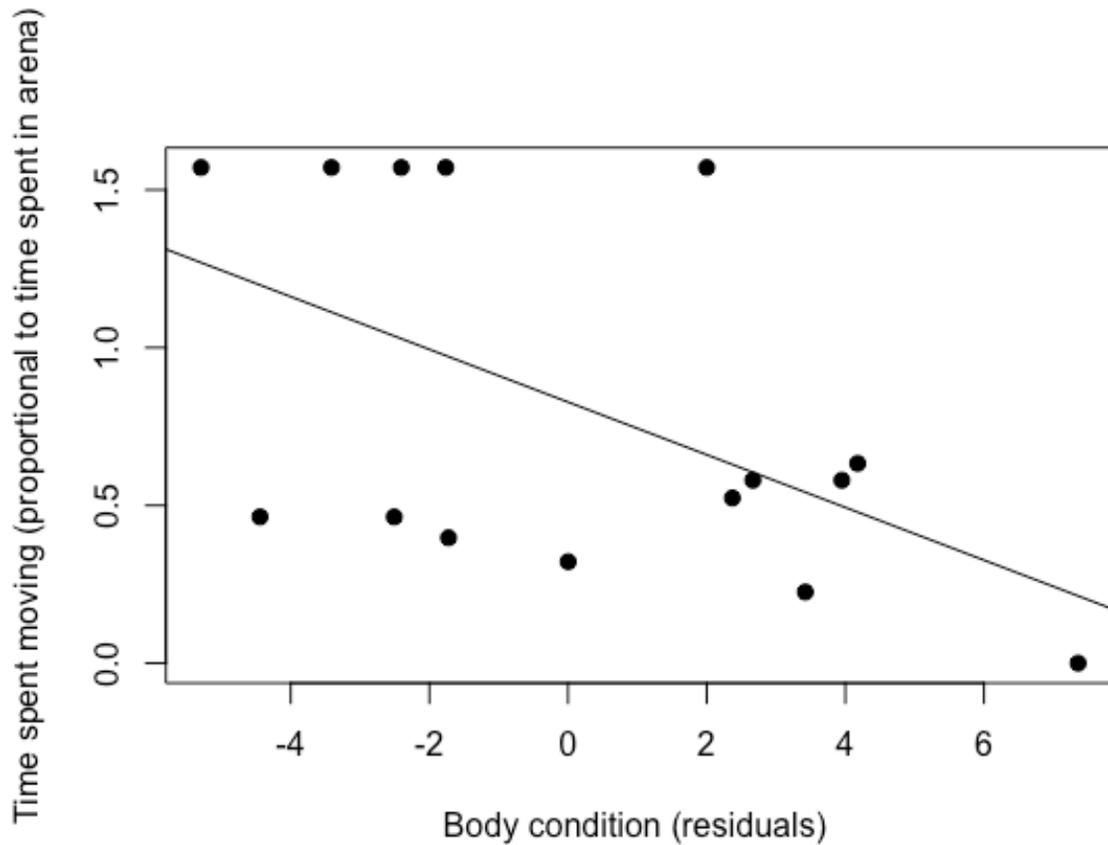


Figure 6. Time spent moving related to body condition in *R. clamitans* (day 2). Time moving was calculated as a proportion to total time spent in the arena before escape or the end of the trial. Data was transformed using the arcsin square root transformation. As body condition increased, time spent moving decreased ($y = -0.05386x + 0.47578$, $S = 784.45$, $p = 0.1387$). $n = 15$.

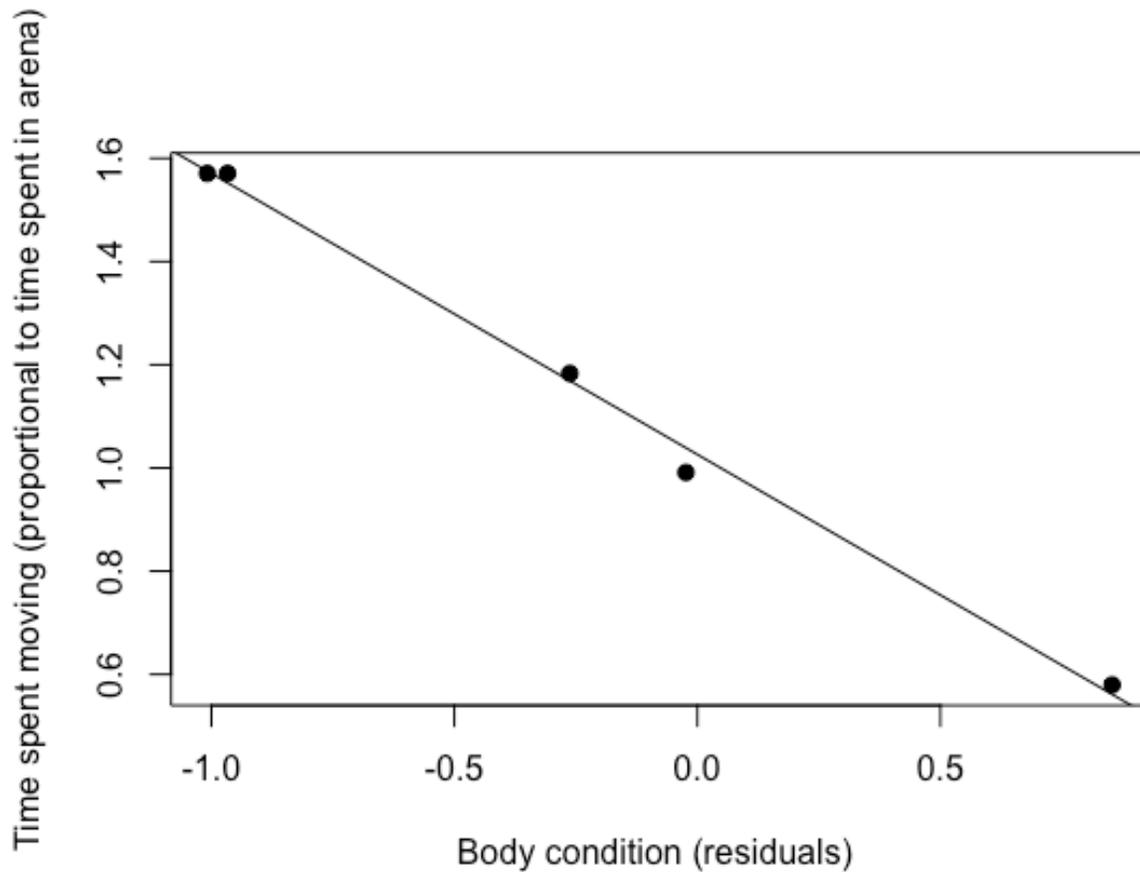


Figure 7. Time spent moving related to body condition in *H. versicolor* (day 2). Time moving was calculated as a proportion to total time spent in the arena before escape or the end of the trial. Data was transformed using the arcsin square root transformation. As body condition increased, time spent moving decreased ($y = -0.54485x + 1.02572$, Adjusted R-squared: 0.944, p -value=0.0001292). $n=5$.