Correlation Analysis of Body Condition and Advertisement Call Morphology in Male *Hyla versicolor* and the Effect of Female Choice

BIOS 35502-01: Practicum in Field Biology

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Abstract:

Mating dynamics in *Hyla versicolor* are mainly determined by female choice of male advertisement calls; females choose mates for higher fitness and genetic quality. Body condition, the relative energy reserves of an individual, is related to fitness. Males with a higher body condition were hypothesized to have call morphology traits more preferred by females: longer call duration, more notes per call, and (based on Jorgensen and Gerhardt's 1991 study) a carrier frequency of 1.1+2.2 kHz. We measured the snout vent length (SVL) and mass of 24 male *H. versicolor* and recorded each of their advertisement calls. A residual index was used to determine body condition, and correlation analyses were done between the call morphology variables and body condition, SVL, and mass. Body condition surprisingly showed no correlation with the tested call morphology variables, but SVL and mass were significantly correlated to many variables. Larger males tended to have longer calls and lower carrier frequencies, traits that are more attractive to females; this implies that they have greater reproductive success. Their larger size influences their carrier frequency differently than smaller males, and it is possible that their greater absolute energy reserves allows them to create higher quality calls. This research shows evidence that a future study comparing relative versus absolute energy reserves in reference to call morphology and reproductive success could be useful in further understanding the trade-off between energy use for advertisement calls and reproductive success in *H. versicolor* males.

Introduction:

Many anurans have acoustic mating systems where the males produce advertisement calls to attract females. Producing higher quality advertisement calls has an energy cost to all organisms with this mating system; the benefit of the energy cost is having a greater chance to reproduce (Ryan 1988). Eastern gray tree frogs (*Hyla versicolor*) produce one of the most energetically expensive advertisement calls, recorded as more strenuous than regular physical movement (Taigen and Wells 1985).

Male *H. versicolor* aggregate in vegetation surrounding ponds at night and produce advertisement calls to attract conspecific females. In Northern Michigan, they often mate from mid-May to late June or when the night conditions are ideal, approximately above 15°C (Harding 1997). This time frame and temperature range varies across different years and locations due to shifts in weather and rainfall.

Body condition is an indicator of relative energy reserves, so males with a higher body condition should be able to produce higher quality calls. It is also assumed that body condition is related to relative fitness and quality of an individual (Peig and Green 2010). Females use the advertisement calls of male tree frogs to assess fitness and genetic quality, and thus males with a higher body condition are likely the ones to be producing female-preferred calls. For this reason, mating dynamics in *H. versicolor* are mainly determined by female choice. Females tend to prefer males with longer call duration, as it indicates higher genetic quality of offspring (Doty 2001). A study by Jorgensen and Gerhardt (1991) suggests that males with carrier frequencies of 1.0 kHz or 1.1 + 2.2 kHz are more successful in mating, while 1.4 kHz carrier frequencies are less successful. Additionally, temperature affects both mating preferences in female frogs and call morphology in male frogs (Brenowitz et al. 1985).

I hypothesize that *H. versicolor* males with a higher body condition will have a longer call duration, more notes per call, a low peak carrier frequency at about 1.0-1.1 kHz, and a high peak carrier frequency at about 2.2 kHz.

Methods:

Study Setting: 24 male *H. versicolor* were recorded and caught at three vernal ponds on the University of Notre Dame Environmental Research Center's (UNDERC) property; Wood Duck Pond, Pond U, and Pond V. The Boyd Lab assisted in this field work due to cooler night temperatures ending the mating season earlier than anticipated. All frog calls were recorded around 9:00 pm to midnight between May 23rd and May 30th in the year of 2018.

Recording and Catching: At each outing, all three ponds were visited and if there were sufficient *H. versicolor* advertisement calls, we would measure water and air temperatures, attempt to record the calling tree frogs in the field, and catch them. The recorder used was Sony Corporation's 2016 Stereo Digital Voice Recorder (ICD-SX2000). Male tree frogs were brought back to the lab to measure their mass and snout vent length (SVL). These two variables were used to create a residual index to determine body condition; the residual index is a recommended method in determining the body condition of amphibians as it has a normal distribution and does not compare absolute energy reserves (Băncilă 2010).

Advertisement Call Analysis: I used Cornell University's 2014 Raven Pro 1.5 software to analyze the recordings. Ten successive calls in each recording were identified, starting with the second call to avoid instances where the first call was cut off at the beginning of the recording. The duration from the first note of the second call to the last note of the eleventh call was recorded and later divided by ten to achieve the average time per call. Each of the ten calls were measured for seven variables: call and intercall duration, call duty cycle (a sum of the previous two variables), total number of notes, high and low carrier frequency peaks, and note duty cycle. Note duty cycle was calculated by identifying the first four peak notes of a call and averaging the duration of the four. The measurements were then averaged across all ten calls to create a single data point for each variable in one recording.

Statistical Methods: A Pearson's product-moment correlation test was used to find significant correlation between each call morphology variable and body condition, mass, and SVL. I used the R statistical programming language to compute my statistics and the R package ggplot2 to generate my figures.

Results:

The average SVL of recorded male tree frogs was 43.8 mm, and the average mass was 9.137 grams.

No call morphology variables were significantly correlated with the body condition index. However, several variables were correlated to mass and SVL: call duration, call duty cycle (significantly correlated to only mass, not SVL), number of notes per call and both low and high carrier frequency (all p-values listed in Table 1; degrees of freedom = 21, $p > 0.1$). Several other variables were approaching significance ($0.1 < p < 0.2$) in mass and SVL: average time per call, intercall duration, and call duty cycle (approaching in SVL, significant in mass).

Carrier frequencies tended to decrease as mass and SVL increased, while all other significant call morphology variables tended to increase as mass and SVL increased.

Temperature was not corrected for as the water temperature variation across the sampling nights was very minimal, with a range of 19.5°C - 24.5°C and an average of 22.06°C.

Discussion:

Tree frogs with higher body conditions were expected to produce calls that are more preferred by females due to a potential ability to use energy more effectively, but this hypothesis was disproved. Mass and SVL may not be good indicators of *H. versicolor* body condition on their own. Other body condition indexes could be compared with relevance to this study to examine other potential relationships. Additionally, other physiological factors should be studied to fully understand the energy trade-off of *H. versicolor* in creating advertisement calls such as oxygen consumption, metabolic levels, hormone levels, etc.

This experiment had fewer individuals in the extreme ranges of body condition than near the average body condition. A follow-up experiment could look at categorical differences between males with average, positive, and negative body condition and include more individuals in the negative and positive condition categories for a more even spread of data. This follow-up study could potentially find non-linear relationships between body condition and call morphology.

While body condition alone was not shown to be significantly correlated to any of the call morphology variables tested, mass and SVL were correlated to almost all tested variables. Average time per call and intercall duty cycle were not significantly correlated in this study, but they seemed to approach significance. With a larger sample size, the approaching variables may be found to be significantly correlated. Note duty cycle carried no significance throughout body condition, mass, and SVL; it is likely not an indicator of fitness nor size.

Taigen and Wells found that call duration in *H. versicolor* is highly positively correlated to oxygen consumption and therefore related to the metabolic energy available (1985). It is possible that because the larger males have higher absolute energy reserves and larger respiratory cavities, they were able to create longer calls with more notes per call, traits that are preferred by females (Doty 2001). This would not have been detected by the residual body condition index as it does not compare absolute energy reserves nor size.

Size also has a physical impact of what carrier frequencies can be produced. Smaller organisms are restrained by their physical acoustics to produce higher carrier frequencies, while larger organisms generally produce lower carrier frequencies. Bennet-Clark found supporting evidence for this relationship and discovered it to be related to energy efficiency in crickets. The most energy efficient carrier frequency a cricket produced depended on its size, a pattern in acoustic theory that has been further supported in many other anurans and insects (Bennet-Clark 1989; Prestwich 1994). However, McLister (2000) conducted a study of energy efficiency in *H. versicolor* and found that there was no energy trade-off between carrier frequency differences related to size; their study suggests that a smaller frog could produce lower frequency calls without taking a cost to their metabolic energy efficiency. Additionally, the environment acts as a selection force favoring lower frequency calls, as higher frequency calls experience more habitat degradation. However, there are other factors that may cancel out or lessen the effects of this environmental selection such as female choice, lack of need to transmit advertisement calls over longer distances, and physiological constraints (Kime, et al. 2000).

Size selective mating in *H. versicolor* was observed in Gatz's 1981 study; Gatz observed and measured males already in amplexus with females and compared them with single males. Since female choice in *H.versicolor* is almost solely based on advertisement calls, I deduce that the large, successful males in Gatz's study likely had high quality advertisement calls to attract the females. Gatz also found evidence that implied many dominant, actively calling males were significantly larger than subordinate satellite males. This study along with mine supports the idea that certain call morphology variables may indicate to females the size of the male and their potential ability to be dominant during the mating season, a trait that a female would want to pass onto her offspring so that her genes are carried into future gene pools.

The multiple advantages of low frequency calls and the lack of an energy trade-off in relation to size begs the question as to why smaller males were not seen to produce lower frequency calls. If their smaller vocal muscles don't allow for the lower frequencies that the larger males can achieve, then it would be assumed that there would be selection for larger males. If this size selection isn't occurring, it suggests that directional selection favoring lower frequencies in *H. versicolor* is canceled out or lessened by other ecological factors.

A brief glance at Figures 6.2, 6.3, 7.2, and 7.3 shows that males in this study with a carrier frequency of 1.1+2.2 kHz (a trait reported by Jorgensen and Gerhardt in 1991 to be preferred by *H. versicolor* females) had a mass of approximately 8 grams and an SVL of 42 mm. Interestingly enough, this combination appears to have either an average or slightly negative body condition and is slightly smaller than the average SVL and mass of my recorded frogs (43.8

mm and 9.137 grams). Considering it is highly supported that larger males have a greater reproductive advantage, it is possible that the results Jorgensen and Gerhardt found in 1991 are no longer as accurate in 2018, and/or the difference in location of our study areas affects female preferences. An up-to-date study on female preference on UNDERC property would help to analyze my data more specifically in reference to the relationship between male call morphology and female preference.

The body condition index used in this study did not show any effect on the variables measured, but this only implies more about how relative energy reserves and size affect a female tree frog's mate choices. It is supported that, in general, larger males (particularly those with lower frequency calls) have more attractive calls and are more reproductively successful, although the question is raised as to why smaller frogs do not produce lower frequency calls despite the multiple advantages. Future studies, some of which I have suggested in this paper, could help us further understand the relationship between energy trade-offs and reproductive success in *H. versicolor* and how extensively female preference plays a role.

Tables:

Call Morphology Variables	Body Condition Index		Mass		SVL	
	p-value	r^2	p-value	r^2	p-value	r^2
Average Time per Call	0.4453	0.02677	0.1199	0.10673	0.1616	0.087319
Call Duration	0.2237	0.06669	0.002951	0.339373	0.006471	0.293494
Intercall Duration	0.3865	0.034335	0.1235	0.10476	0.1827	0.079455
Call Duty Cycle	0.3525	0.039476	0.08852	0.126643	0.1381	0.097493
Number of Notes per Call	0.5273	0.018437	0.01429	0.244916	0.01314	0.250151
Low Carrier Frequency	0.4707	0.023944	0.01261	0.252699	0.01316	0.250054
High Carrier Frequency	0.3109	0.046722	0.0001145	0.503864	0.0001268	0.499326
Note Duty Cycle	0.3165	0.045664	0.3501	0.039859	0.5528	0.016273

Table 1: r^2 and p-values from correlation tests between call morphology variables and body measurements. Cells in yellow are significantly correlated; $p < 0.1$. Cells in green are approaching significance; $0.1 < p < 0.2$.

Figures:

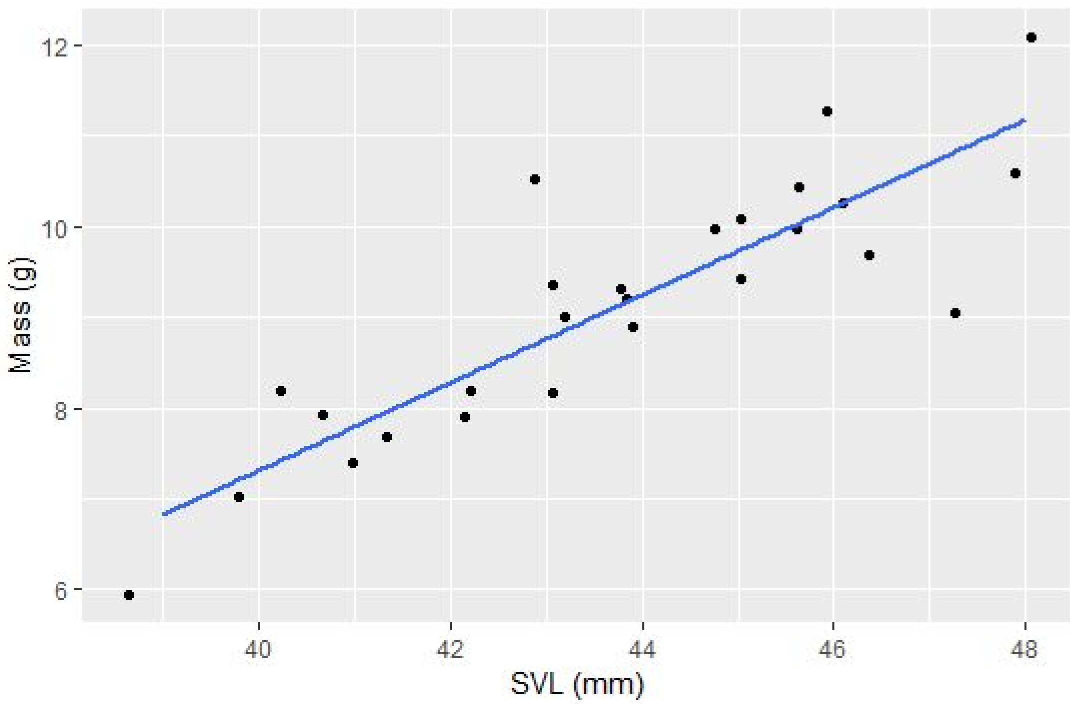

Figure 1: Body condition based off a residual index. Each point represents one frog; those above the trendline have a positive body condition, those below have a negative body condition.

<u>Call Duration Correlation Figures:</u>

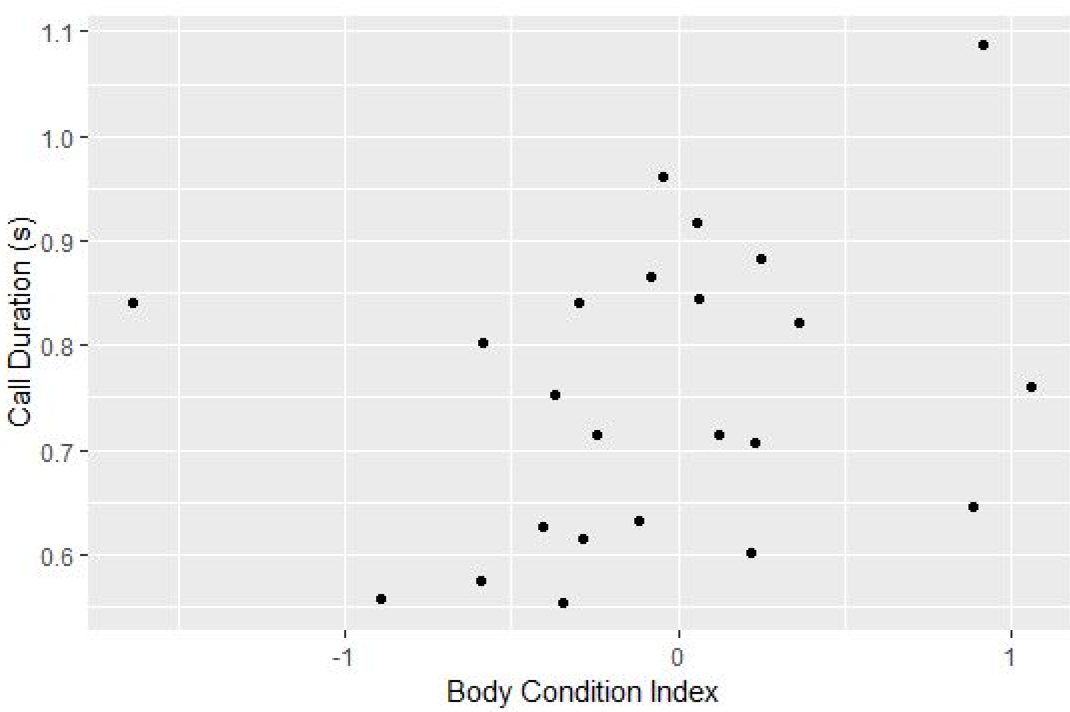

Figure 2.1: Body condition plotted against call duration. It is not significantly correlated; p = 0.2237.

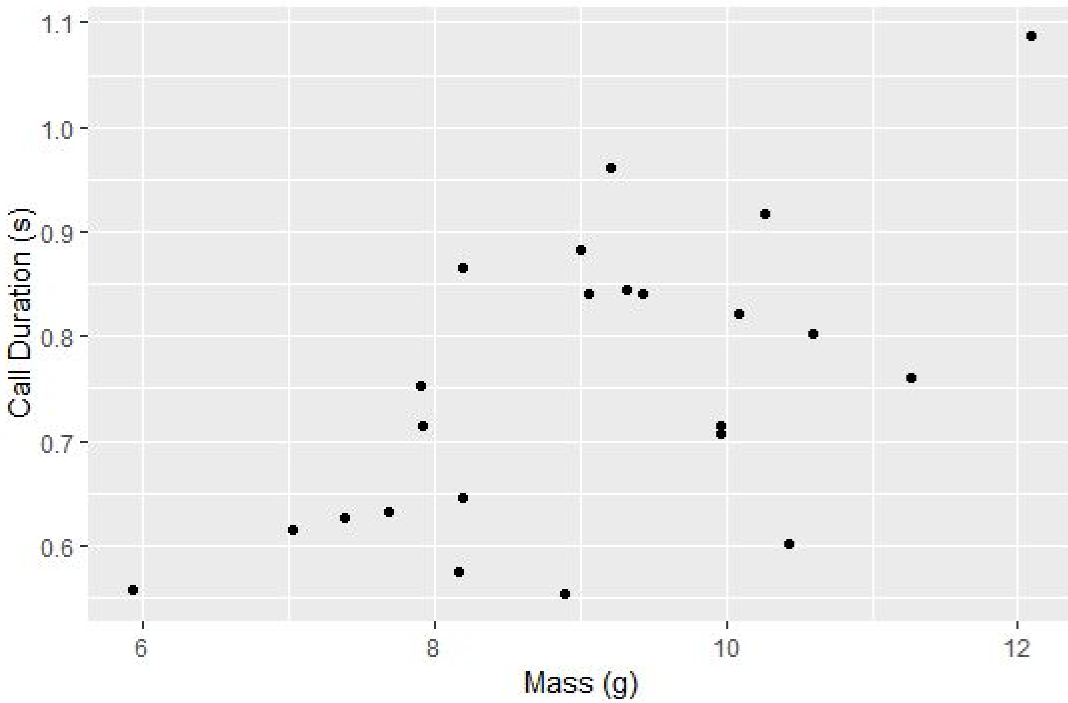

Figure 2.2: Mass plotted against call duration. It is significantly correlated; $p = 0.002951$, $r^2 = 0.339373$, 95% CI = 0.8069567.

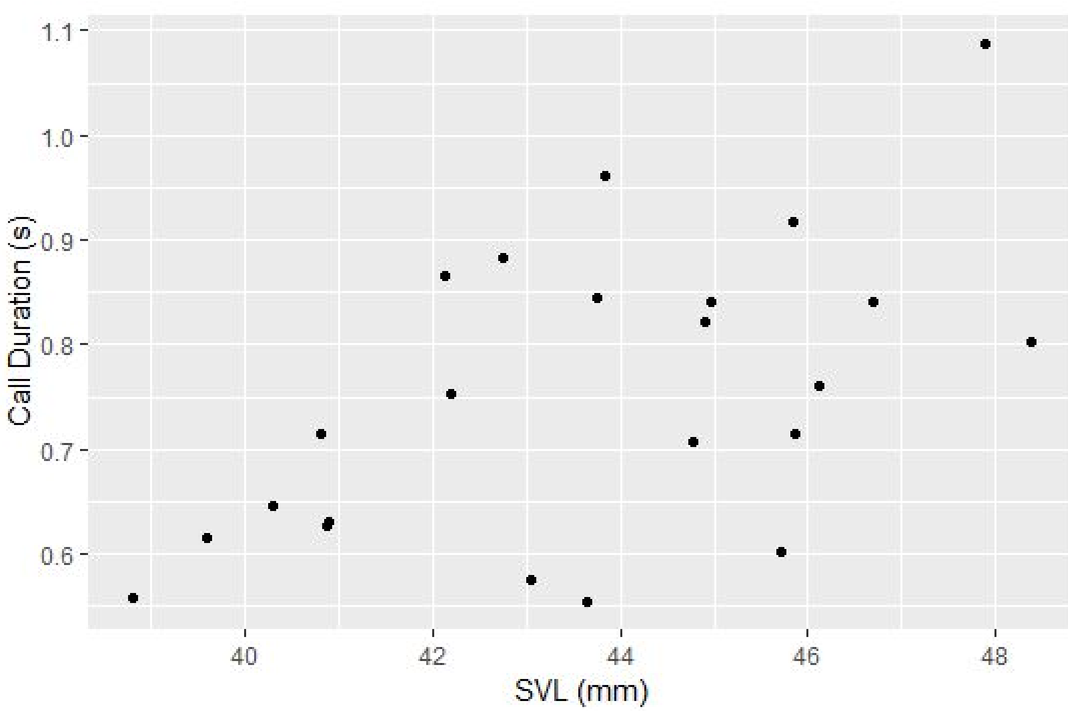

Figure 2.3: SVL plotted against call duration. It is significantly correlated; $p = 0.006471$, $r^2 = 0.293494$, 95% CI = 0.7847386.

Intercall Duration Correlation Figures:

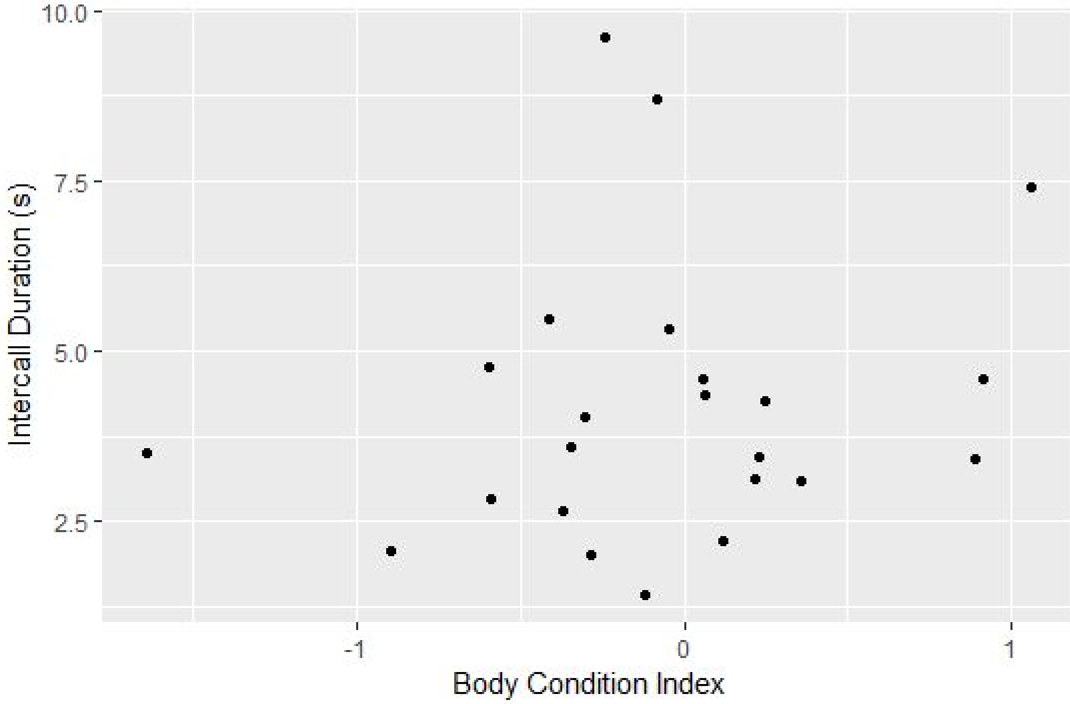

Figure 3.1: Body condition plotted against intercall duration. It is not significantly correlated; p = 0.3865.

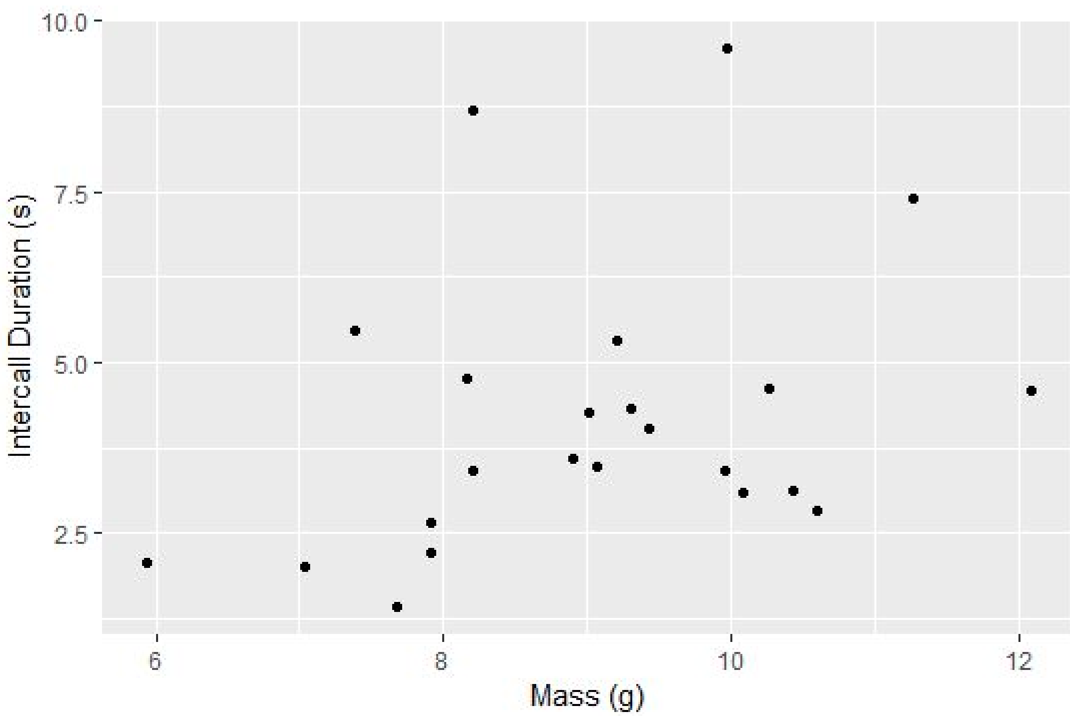

Figure 3.2: Mass plotted against intercall duration. It is not significantly correlated; p = 0.1235.

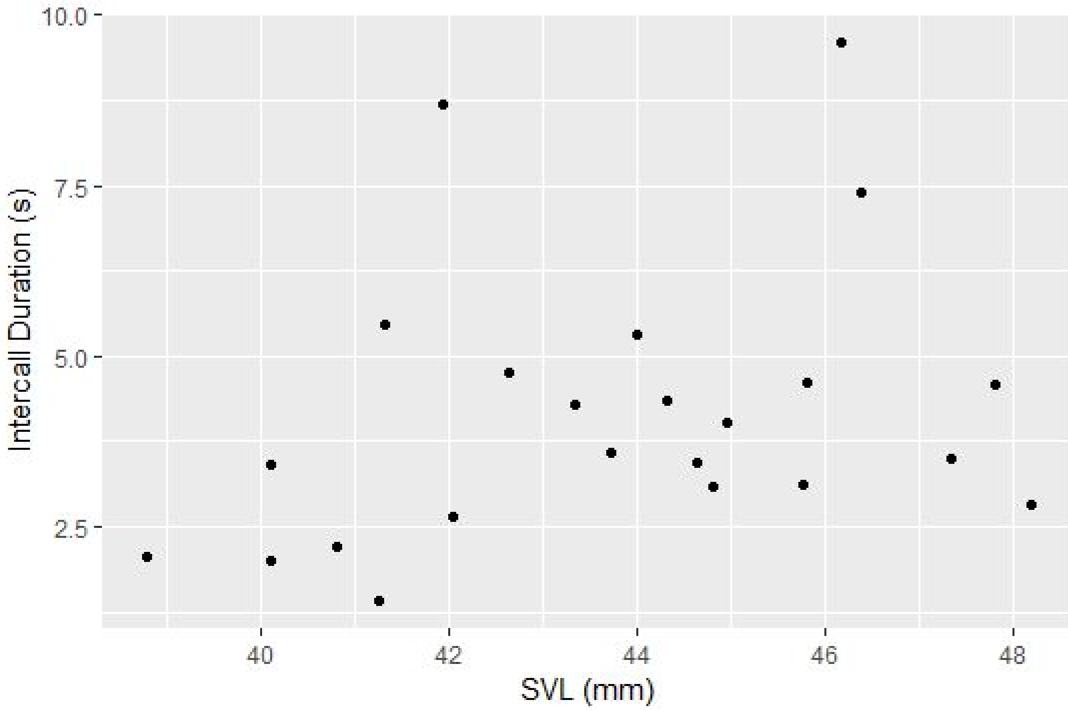

Figure 3.3: SVL plotted against intercall duration. It is not significantly correlated; p = 0.1827.

<u>Call Duty Cycle Correlation Figures:</u>

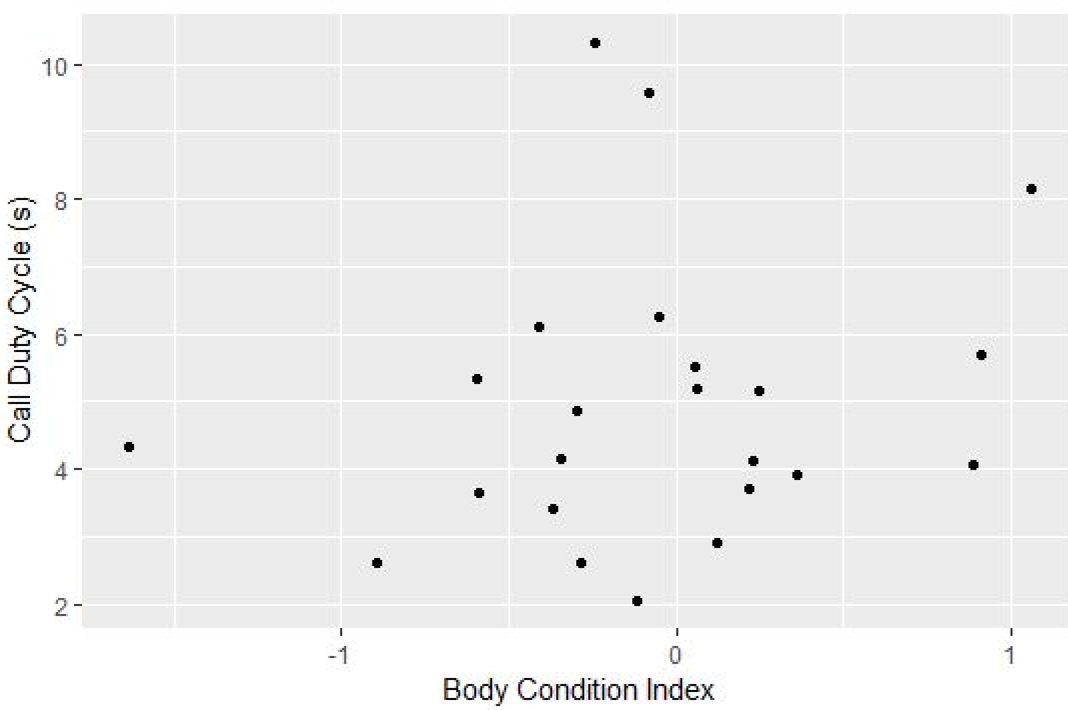

Figure 4.1: Body condition plotted against call duty cycle. It is not significantly correlated; p = 0.3525.

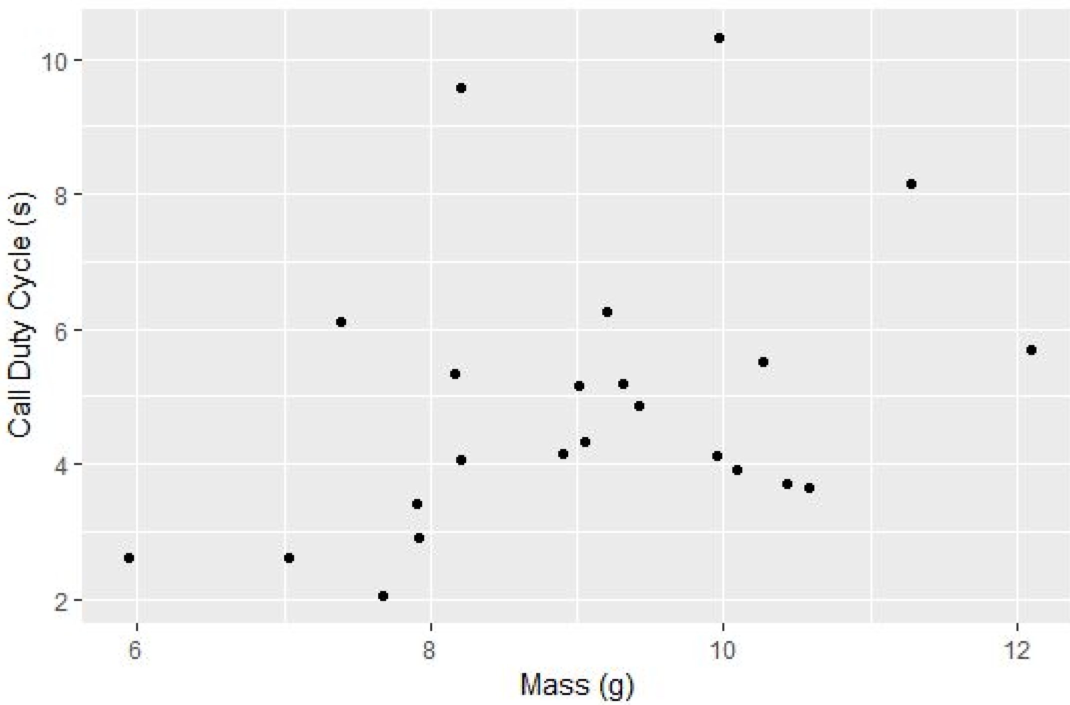

Figure 4.2: Mass plotted against call duty cycle. It is significantly correlated; $p = 0.08852$, $r^2 = 0.126643$, 95% CI = 0.67440569.

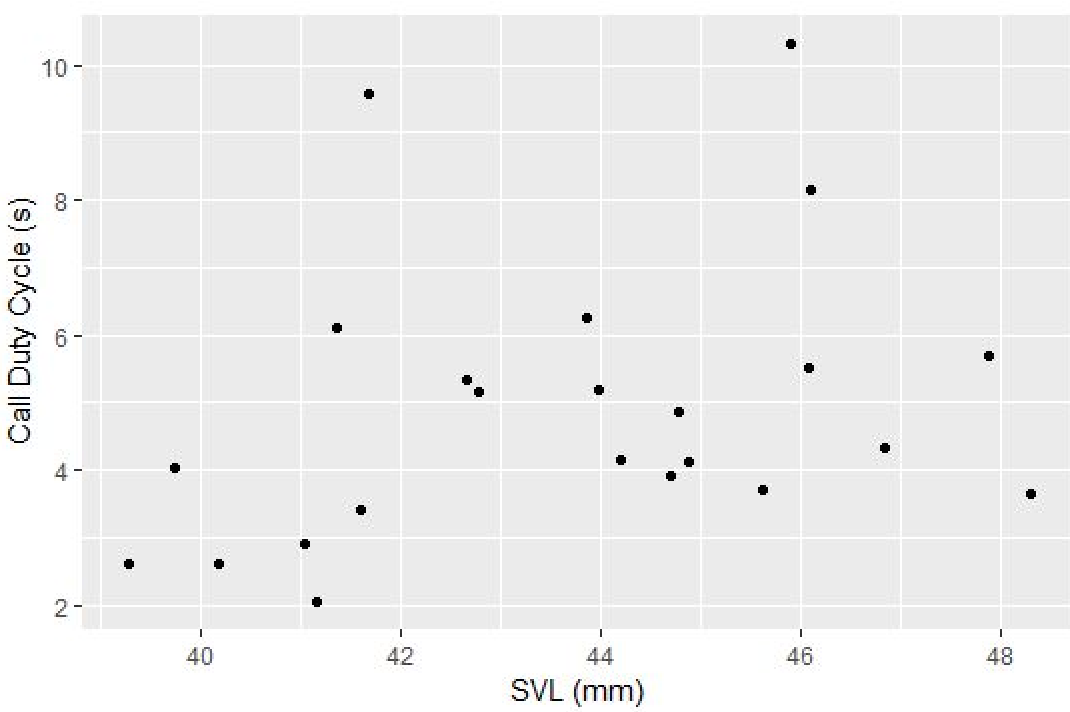

Figure 4.3: SVL plotted against call duty cycle. It is not significantly correlated; $p = 0.1381$.

Number of Notes per Call Correlation Figures:

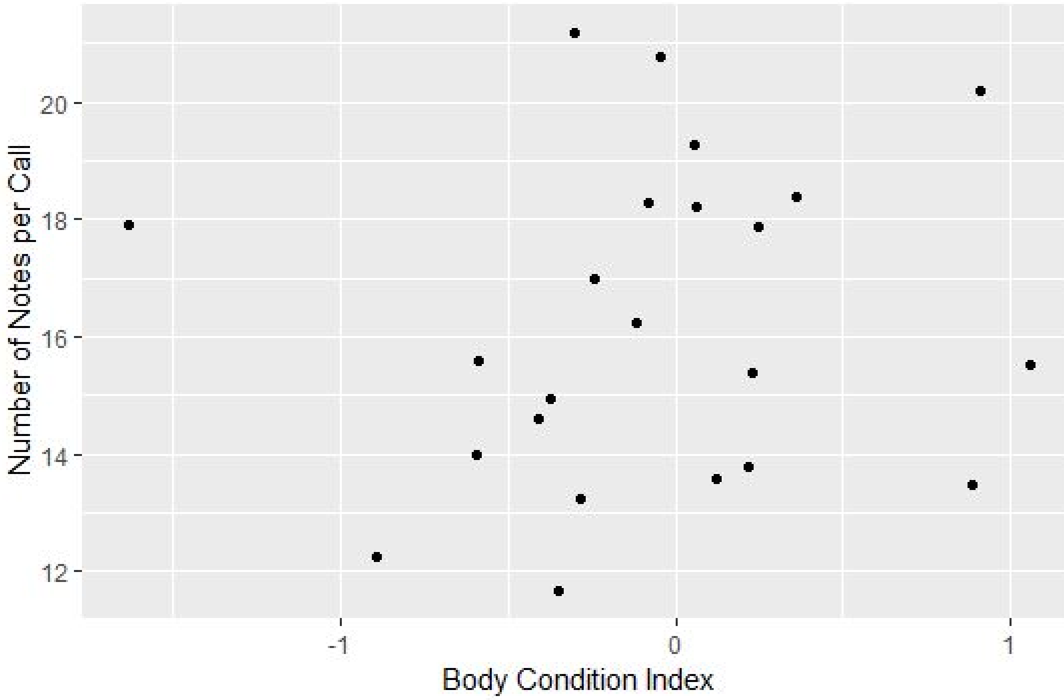

Figure 5.1: Body condition plotted against number of notes per call. It is not significantly correlated; p = 0.5273.

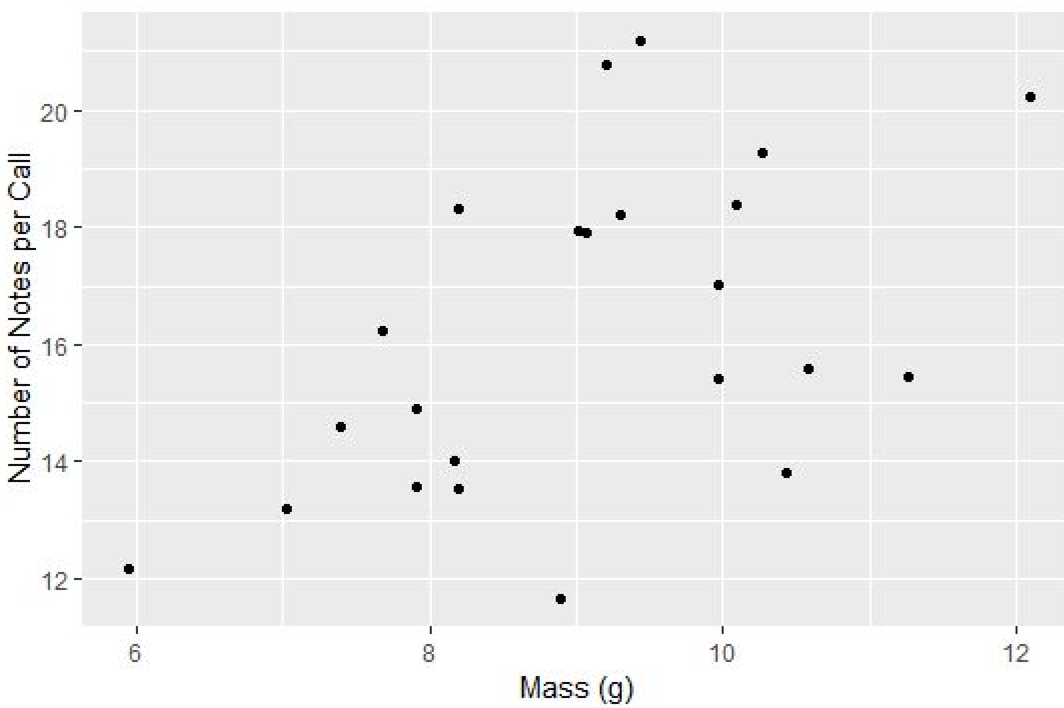

Figure 5.2: Mass plotted against number of notes per call. It is significantly correlated; p = 0.01429, r^2 = 0.244916, 95% CI = 0.7583785.

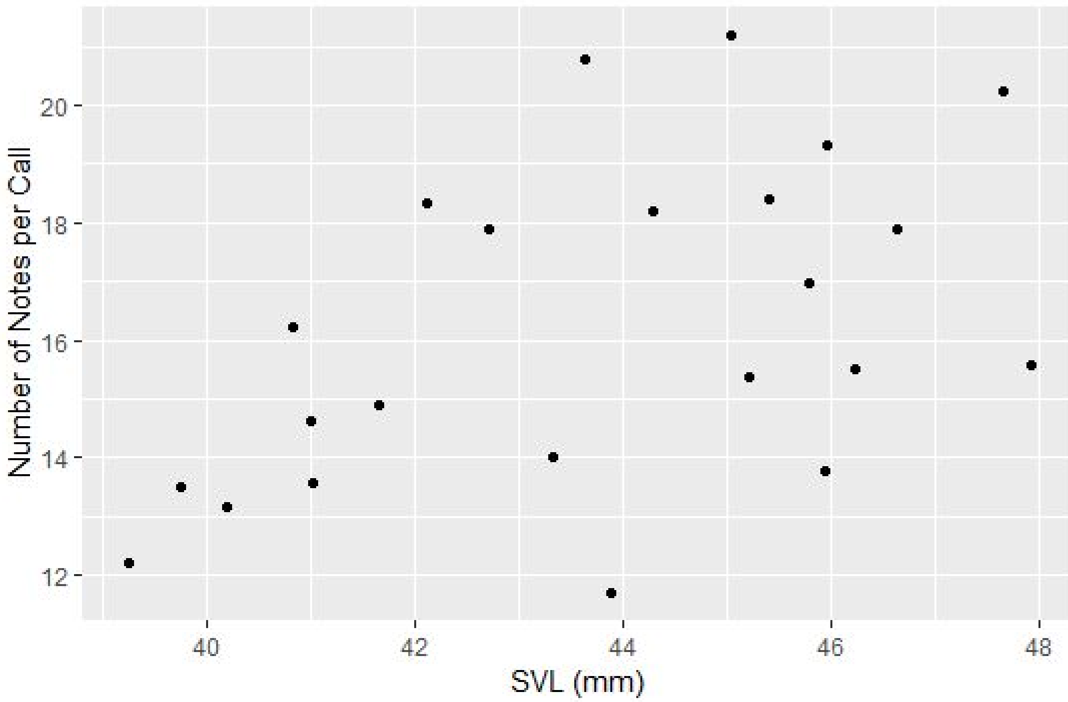

Figure 5.3: SVL plotted against number of notes per call. It is significantly correlated; p = 0.01314, $r^2 = 0.250151$, 95% CI = 0.7613877.

Low Peak Carrier Frequency Correlation Figures:

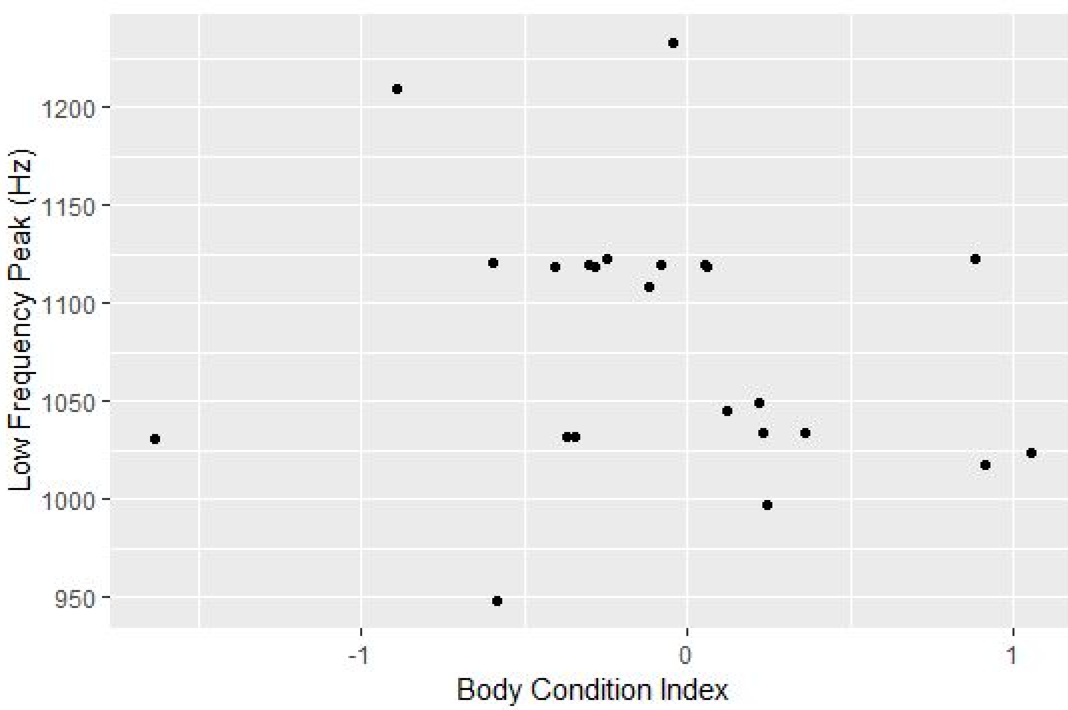

Figure 6.1: Body condition plotted against low peak carrier frequency. It is not significantly correlated; p = 0.4707.

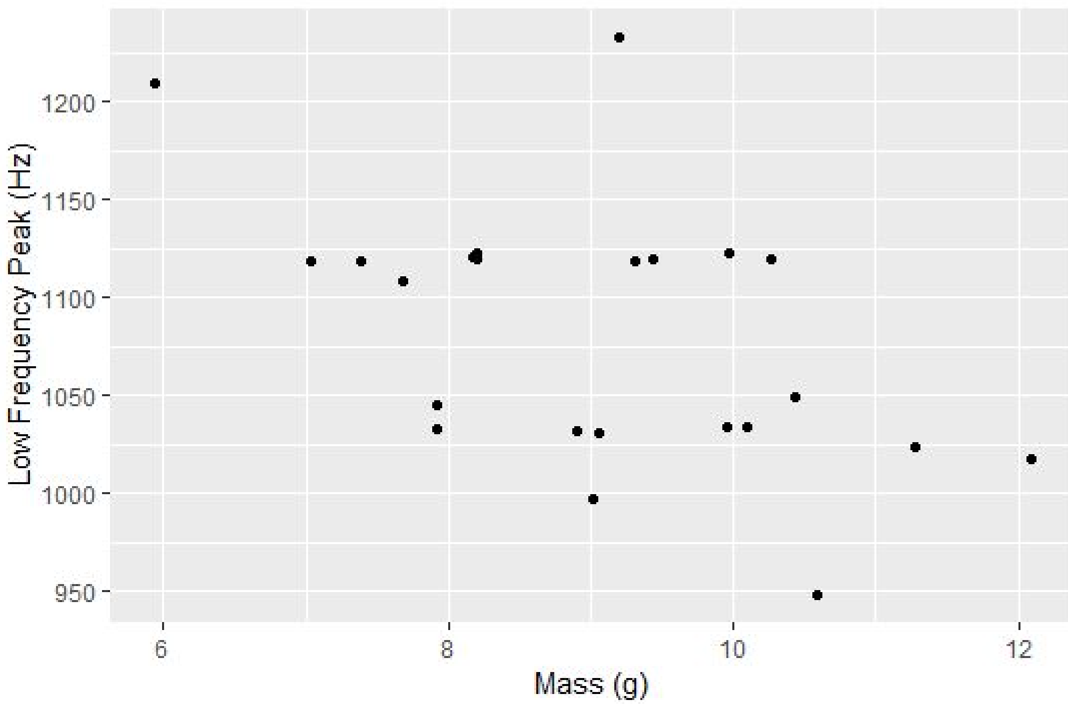

Figure 6.2: Mass plotted against low peak carrier frequency. It is significantly correlated; p = 0.01261, r^2 = 0.252699, 95% CI = -0.1257719.

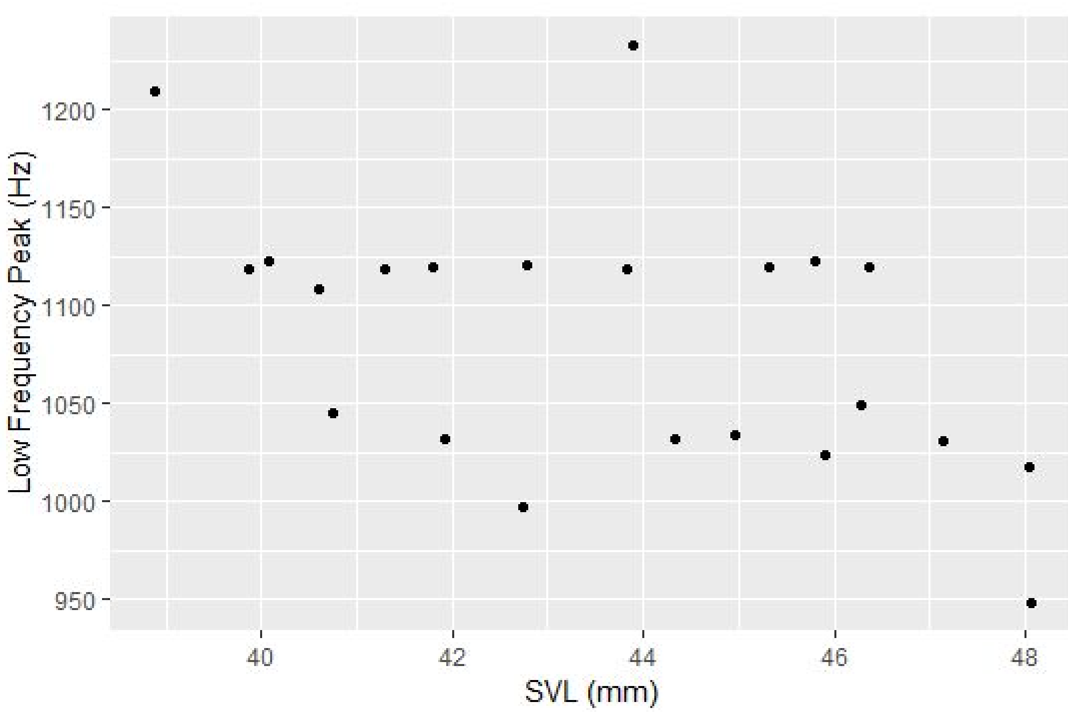

Figure 6.3: SVL plotted against low peak carrier frequency. It is significantly correlated; p = 0.01316, r^2 = 0.250054, 95% CI = -0.1222277.

High Peak Carrier Frequency Correlation Figures:

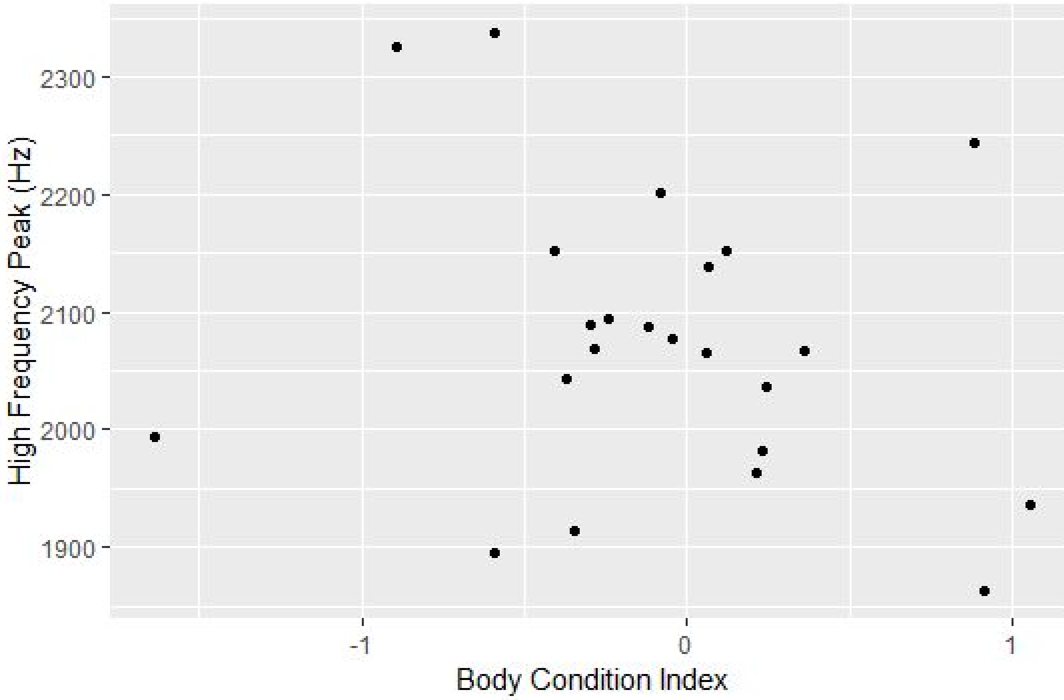

Figure 7.1: Body condition plotted against high peak carrier frequency. It is not significantly correlated; $p = 0.3109$.

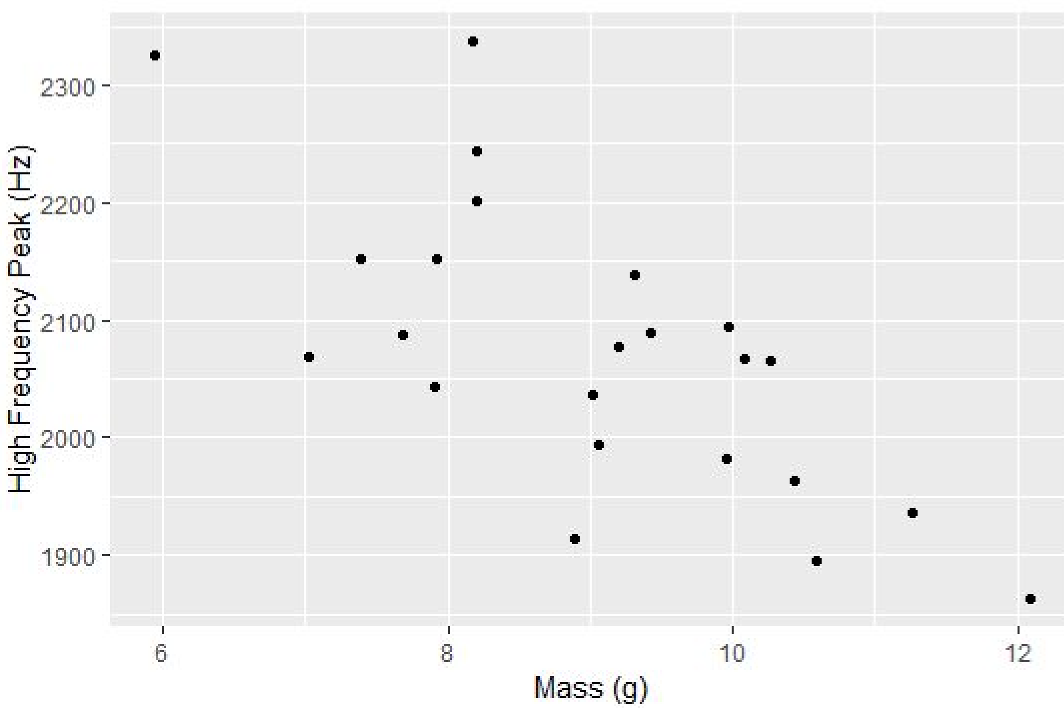

Figure 7.2: Mass plotted against high peak carrier frequency. It is significantly correlated; $p = 0.0001145$, $r^2 = 0.503864$, 95% CI = -0.4343075.

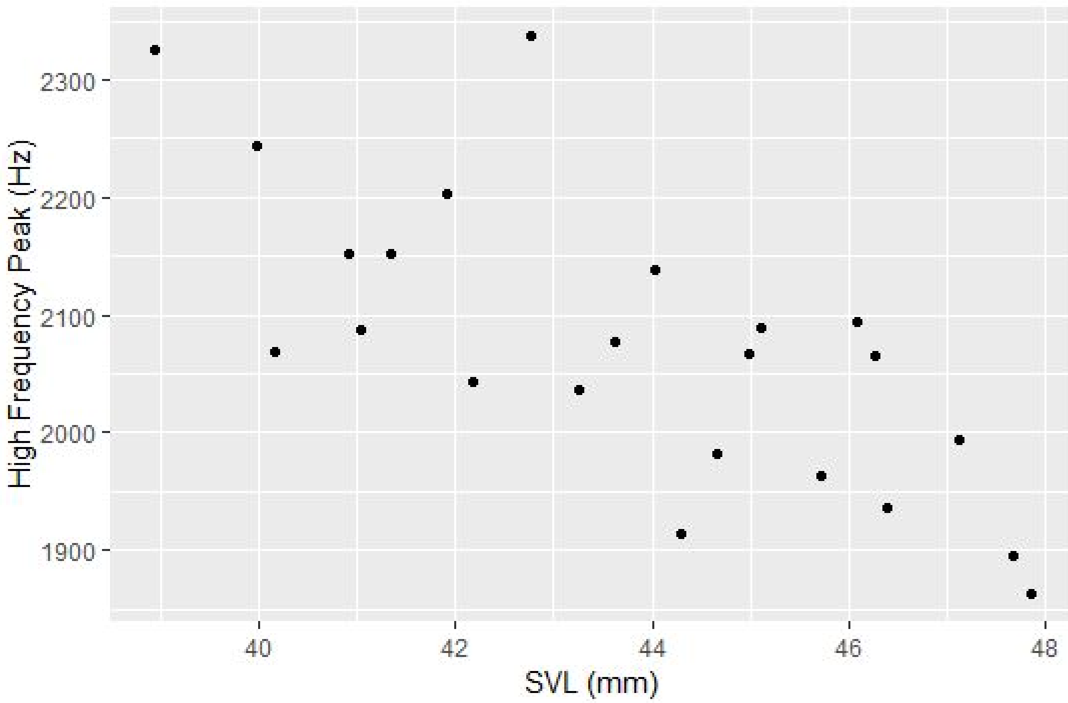

Figure 7.3: SVL plotted against high peak carrier frequency. It is significantly correlated; $p = 0.0001268$, $r^2 = 0.499326$, 95% CI = -0.4290120.

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