Summer Internship Project
Report

Effect Of Developmental Stage Diet On Adult Size And Weight

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Last but not least, I would like to acknowledge the collective support of my family, whose encouragement and understanding have been pivotal in ensuring the successful completion of this project.

Thank You
C L Srinivas

Contents

Abstract

Baffling is an alternative reproductive strategy used by male tree crickets *(Oecanthus henryi)* to enhance their chances of attracting females. Several factors including size is known to affect the baffling propensity of adult male crickets. Additionally, in other insect systems, development stage diet has been shown to have a pivotal role in determining morphological and physiological characteristics of the adult. In this project, I have tried to study the effect of developmental stage diet on adult size and weight in *O.henryi*. After being exposed to two distinct diet categories (rich and poor) during development, the adults showed significant difference in size, weight, rate of size gain and rate of weight gain. A better analysis of the data can be performed using a mixed model, taking into account the age of the nymphs at capture and/or the time spent under the dieting conditions. This would provide better insight into the effect of developmental stage diet on adult size and weight in *O.henryi*.

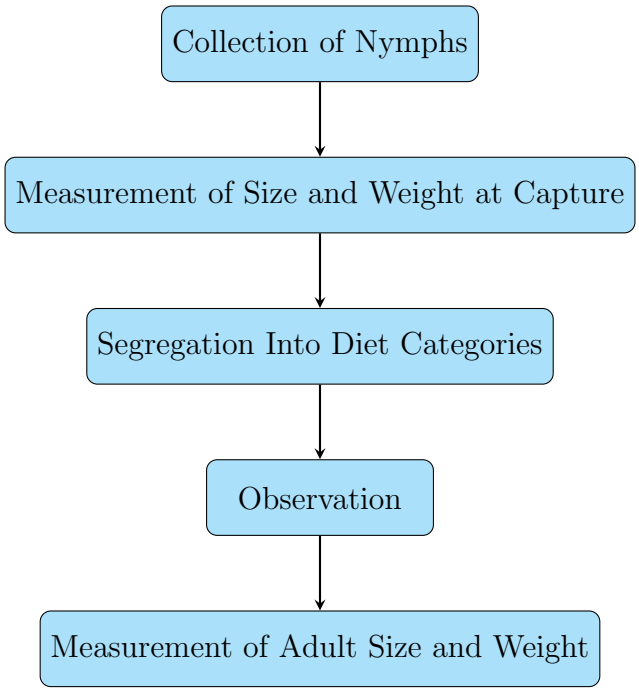
1 Introduction

Oecanthus henryi is a species of tree cricket found commonly around the campus. They live, feed and lay their eggs on their host plant Hyptis Suaveolens, bushes of which, are abundant here in NISER. Adults can be anywhere between 9mm to 15mm long and weigh around 18mg to 30mg. Nymphs (smaller in size) resemble the adults except for the absence of wings. Nymphs develop into adults through moulting. Each stage in the developmental sequence to become an adult is called an instar. The final moulting to emerge as the fully formed adult is called eclosion. Males and females can be distinguished by the difference in their wing structure (Figure 2a) and by the presence of ovipositor on the latter. *O.henryi* males attract mates by producing mating calls. Males produce mating calls by stridulation. Some males (usually softer, smaller males) make baffles to circumvent a phenomenon called acoustic short circuiting (Figure 2b). It is not apparent whether this baffling behaviour is genetically conferred or manifested in accordance with the status of the male. This alternate reproductive tactic (ART) is employed largely by low status males (softer, smaller males)[1]. However, it has been observed that even loud and/or large males (who are edge callers in the wild), when provided with a large enough leaf, baffle under lab conditions[1]. This suggests that baffling might be a condition dependent strategy. Size and loudness (non-baffling SPL) seem to affect baffling propensity, hence, factors that affect size and loudness are also of our interest[2]. It has been shown that post eclosion diet has significant effect on baffling propensity[2]. In this project I have tried to analyze the influence of development stage diet on adult size and weight.

(a) (b)

Figure 1: (a) On the left, male and on the right, female *Oecanthus Henryi*, (b) A baffling male *O.Henryi*

2 Experimental Design



3 Methods

3.1 Collection of nymphs

O.henryi nymphs were collected from field sites (refer attached figures 2a to 2c) inside NISER campus for a duration of 45 days (9 June 2023 to 24 July 2023). Nymph collection from the field was conducted usually from 1930hrs to 2100hrs. Headlamps strapped to the forehead provided bright, targeted light which was crucial for spotting the tiny nymphs.

Specimen containers (100ml) with perforated lids (done before hand using a compass or a hot soldering iron) were used for capturing and subsequently housing the developing nymphs.

(a) FS I (b) FS II (c) FS III

Figure 2: (a) FS I, (b) FS II, (c) FS III

3.2 Distribution of nymphs in the field sites

It was interesting to note that even though Hyptis plants are abundant and spread out across the field site, most of them have very few to no nymphs. Majority of the nymphs are concentrated in a bush/thicket or a few closely arranged bushes. For convenience let me call these bushes **hotspots**. This observation is consistent with two (FS1&FS2) of the three field sites that I have visited. It is my suspicion that this skewed distribution of nymphs can be attributed at least in part to the fact that nymphs don't poses wings and hence are not extremely mobile. It would seem reasonable to assume that they would spend the entirety of their juvenile lives in the bush of their birth or at best in bushes within jumping distance from the bush of birth. This phenomenon is something that has caught my attention during the past 2 months, the significance and accuracy of this observation along with the multitude of possible factors behind this requires further investigation.

3.3 Spotting nymphs

As far as is apparent to me, there is no sure shot way to ascertain the presence of nymphs in an area or shrub without actually looking for them. However, I found that there are a few cues that indicate the presence of nymphs on a plant. The presence of an active population (calling males and females attracted to them) on a bush means there might be nymphs in the plant. Usually, the hotspots are also busy sites, populated by calling males and females that are attracted to them. Another reliable cue are the holes left on the leaves by adults and nymphs alike as a result of feeding. Plants with abundant chewed up leaves have better chance of hosting nymphs as opposed to those without. It's like the old adage, where there is smoke, there is fire.

3.4 Difficulties of nymph hunting

3.4.1 Inherent difficulties

Since nymphs are considerably small, well camouflaged and silent, it is difficult to spot them. It becomes near impossible when the bushes are thick and impenetrable. Rain significantly reduces nymph availability in its wake, the reasons however are not apparent

to me. A luck factor is definitely involved as the number of nymphs caught per field trip fluctuates dramatically.

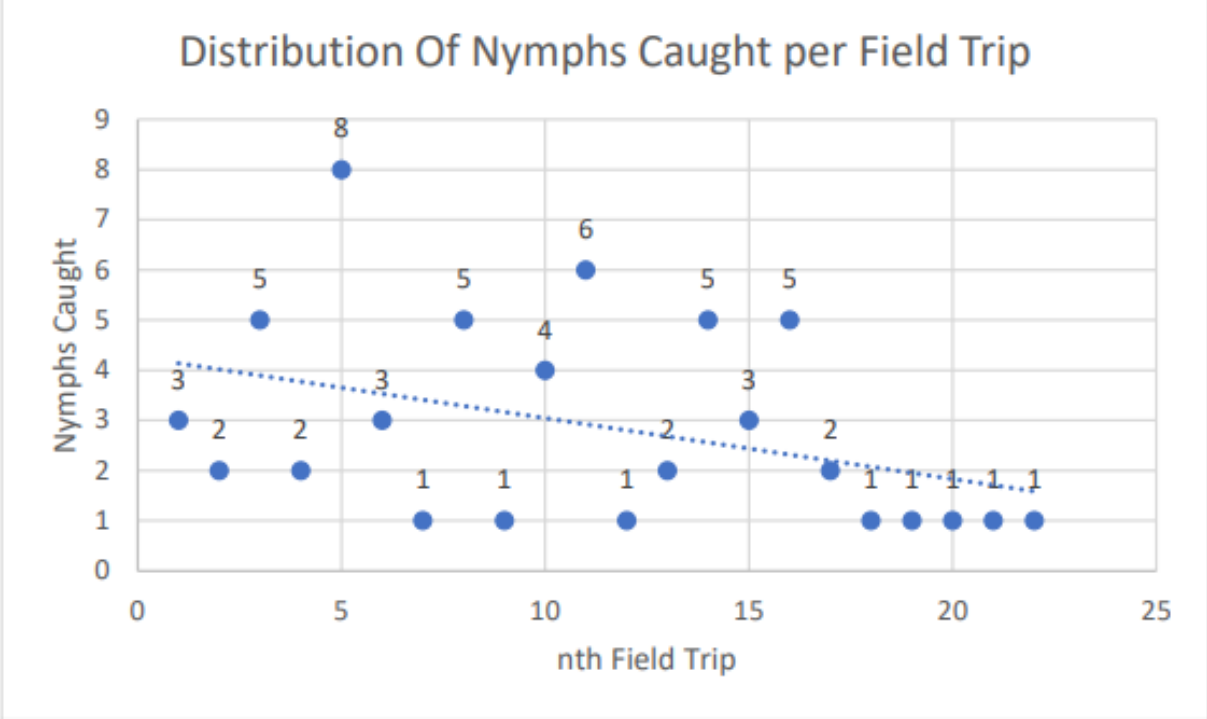


Figure 3: Distribution of number of nymphs caught per field trip

3.4.2 A canine concern

It is unfortunate to realize that our four-legged canine friends find the light from the headlamp extremely disturbing. Once spotted (by them), they are immediately alarmed and bark continuously. This often culminates in an entire pack of dogs moving in to check what is moving in the bushes. This unwanted attention has led to a few field trips being aborted.

3.5 Measurement of size and weight at capture

3.5.1 Measurement of size

The captured nymphs are labelled with the date of capture and a name. The name consists of an uppercase letter, either P or R followed by a number. The letter indicates the diet category to which the nymph has been assigned and the number is the unique identification mark for the nymph within its diet category. For example, the name "P1" indicates that it is the nymph with serial number "1" in the "Poor" diet category. The labelled individuals are kept in -20°C for a duration of time anywhere between 20 seconds to 2 minutes depending on the size of the individual. Very young nymphs are anesthetized relatively quickly at -20°C. Longer exposure can cause fatality. The anesthetized individuals are photographed using the microscope under a suitable magnification (Figure 6). Length is measure from the anterior tip of the head to the posterior end of the abdomen using Zeiss Labscope software. I try to orient the anesthetized nymph in such way that the dorsal

side lies flat against the stage of the microscope, exposing the ventral side to the lens. After measurement the individuals are transferred back to their respective containers.

Figure 4: Length measurement

3.5.2 Measurement of weight

An empty specimen container (100ml) was first weighed and tared on the weighing machine. The nymph was then transferred to the empty container and weighed along with the container. This process was repeated for all the nymphs during the initial phase of the experiment. Later, for more accurate readings, the nymphs were anesthetized with cold (similar to anesthetization during size measurement) and placed directly on the weighing machine.

3.6 Dieting and observation

3.6.1 Dieting

The nymphs in the poor diet category (labelled with capital letter "P") were provided fresh leaves, flowers (when available) * and damp cotton ball. The leaves and flowers serve as food source and the damp cotton ball provides a source of water. The leaves and flowers are replaced every day with freshly plucked ones. Care should be taken to ensure that other organisms like spiders, nymphs (very small) or worms are not present on the leaves that are introduced into the specimen containers as food.
***By the end of June there were some showers of rain which changed the morphology of the Hyptis plants. The new leaves were much bigger and the plants had no flowers.**

The nymphs in the rich diet category (labelled with capital "R") were provided with finely diced apple pieces, leaves* and dog food. Apple was diced and stored in a specimen container (100ml) at 4°C. One 100ml container of diced apple would last around 10 days before it needed to be replaced with fresh diced apple (if not sooner due to increased demand for apple pieces resulting from a large population). I found that storing apple pieces at -20°C is suboptimal for shelf life, and I found out, the hard way. A lot of the apple pieces got soggy on de-freezing and caused fungal growth. At 4°C, the shelf life improved drastically and fungal growth on the pieces was nil. Apple pieces and leaves were replaced every day with fresh ones. Dog food was replaced if it had fungus.
***Till July 3rd 2023, the nymphs were not given leaves.**
Their diet consisted only of dog food and apple.
Rich diet from June 14th to July 3rd – Apple + Dog Food
Rich diet post June 3rd – Apple + Dog Food + Leaves

3.7 Observation

The nymphs were regularly monitored every day, mostly between 1130hrs and 1230hrs.

3.7.1 Eating and excretion

There was a significant difference in the number of fecal pellets in the containers of the nymphs in rich diet as compared to those of the nymphs in poor diet. The nymphs that fed solely on leaves produced much greater number of fecal pellets as compared to those that fed on apple, dog food and leaves*. I suspect this could be due to the fiber content in the leaves which might be absent in apple and dog food. Hence even though the nymphs must have been eating/sucking juices from the apple pieces they did not produce much feces.
***From 14th June to 3rd July when the nymphs in rich diet category were given just apple pieces and dog food, the amount of feces produced was extremely less to none.**

3.7.2 Wing growth and moulting

Nymphs increase in size by moulting multiple times. Nymphs start to grown wing buds around the penultimate instar stage, after which there is no dramatic increase in size. After the final moult, the nymph emerges as fully formed adult with functional wings. It was observed that wing growth and moulting were adversely affected by fungus. All individuals who died from fungal infection developed severe deformities before death. Mostly, these nymphs moult into an adult with deformed wings, in some cases, normal adults, after fungal infection developed deformities in their wing structure. Some nymphs also died in the process of moulting, where some part of their deformed body remained attached to the old exoskeleton. It appears to me that at least one of many possible, existing ways in which fungal infection might manifest itself in these nymphs, is in the form of such wing deformities and miss-moultings. It was noted that during the period from 14th June to 3rd July, the incidence of deformity in developed adults was high in "Rich" category. This frequency decreased once leaf was provided along with dog food and apple. It might suggest that the fiber content in leaves and/or the leaf surface is an important factor that promotes normal wing development/adult eclosion. The dates of

moulting were very inconsistently observed because most of the time the nymphs would eat up the evidence before I could get to it. Hence, the instances of observed moultings are few. Of the few observed, all of them are of nymphs in later stages of development.

3.7.3 Death and its causes

The date and cause of death was recorded for every individual. During the initial phase of the experiment, few nymphs died during cold exposure and/or from after effects of cold exposure (for anesthetization). Initially there were incidents of death among the nymphs in "Rich" diet category due to fungus. Fungal growth was caused due to apple pieces becoming soggy upon de-freezing post storage at -20°C. The reminder of deaths recorded were due to unknown/natural causes(presumably).

3.7.4 Adult eclosion

Date of adult eclosion was recorded for each individual. Adults were measured on the day of eclosion. Adult females were released after measurement, and adult males were maintained in the lab. Date of release was recorded for the females.

3.8 Adult size and weight measurement

Adults received a special mark (tick) on their respective specimen containers as a mark of completion of development. This helped in easy identification of the adults also. Marked adults were then taken for size and weight measurements. Same procedure was followed as in measurement at capture.

4 Observation

A total of **41** individuals were observed from capture till adult eclosion. Among these, **19** individuals were from poor diet category **(Np = 19)** and 22 were from rich diet category **(Nr = 22)**. Out of the observables recorded, size at adulthood, weight at adulthood, development period, life expectancy (for those individuals that died after adult eclosion) were analysed. Further, size gain, weight gain, size gain per day, weight gain per day was computed and analysed for these individuals. From this dataset, individuals who had spent at least 10 days in either diet category were filtered and analysed separately. A total of **27** individuals satisfied this criterion. Out of this, 12 individuals were from poor diet category **(Np* = 12)** and **15** individuals were from rich diet category **(Nr* = 15)**. All statistical tests were performed using R version **4.3.1 (2023-06-16 ucrt) – "Beagle Scouts" Copyright (C) 2023 The R Foundation for Statistical Computing Platform**. All the datasets were tested for normality (using Shapiro Wilkins Test) and heteroscedasticity (F test). Data that were found to be normally distributed were analysed using Unpaired Two Sample T Test. All the other datasets were analysed using Wilcoxon Rank Sum Exact Test or alternatively for non-contiguous data, using Wilcoxon Rank Sum Test with continuity correction.

4.1 Raw Data

4.1.1 Size

Adult size was normally distributed among the poor diet category individuals (Shapiro-Wilk normality test, n = 18, W = 0.94331, p-value = 0.3298), whereas in the rich diet category it was not (Shapiro-Wilk normality test, n = 23, W = 0.87606, p-value = 0.008387). The data was found to be homoscedastic (F = 0.71821, num df = 17, denom df = 22, p-value = 0.4906). Median size of both categories was compared and showed that median size of the individuals in poor diet category was less than that of individuals in rich diet category (Wilcoxon rank sum exact test, Size(a)(P) and Size(a)(R), W = 123, p-value = 0.01356).

4.1.2 Weight

Adult size was normally distributed among the individuals in both diet categories (Poor, n = 19, Shapiro-Wilk normality test, W = 0.94632, p-value = 0.3415) (Rich, n = 23, Shapiro-Wilk normality test, W = 0.94199, p-value = 0.1983). The data was found to be homoscedastic (F- test, F = 0.43579, num df = 18, denom df = 22, p-value = 0.07814). Mean weight of individuals in both diet category was compared and found that mean weight of the individuals in poor diet category was less than that of the individuals in rich diet category (Welch Two Sample t – test, t = - 4.0774, df = 40, p-value = 0.0001052).

4.1.3 Size gain

Size gain was taken to be the difference between the weight at adult eclosion and the weight measured at capture. Size gain was normally distributed amongst individuals in the poor diet category (Shapiro-Wilk Test, n = 15, W = 0.96163, p-value = 0.7206) whereas it was not the case amongst the individuals in rich diet category (Shapiro-Wilk Test, n = 23, W = 0.9047, p-value = 0.03164). The data was found to be homoscedastic (F = 1.3431, num df = 14, denom df = 22, p-value = 0.5201). Median size gain of both diet categories did not show significant difference (Wilcoxon rank sum exact test, W = 178, p-value = 0.8828).

4.1.4 Weight gain

Weight gain was taken to be the difference between the weight at adult eclosion and the weight measured at capture. Weight gain was normally distributed amongst individuals in the rich diet category (Shapiro-Wilk Test, n = 23, W = 0.9519, p-value = 0.3203) whereas it was not the case amongst the individuals in poor diet category (Shapiro-Wilk Test, n = 18, W = 0.86905, p-value = 0.01717). The data was found to be homoscedastic (F = 1.5344, num df = 17, denom df = 22, p-value = 0.342). Median weight gain of both diet categories did not show significant difference (Wilcoxon rank sum exact test, W = 172, p-value = 0.3688).

4.1.5 Size gain per day (S.G/D.P)

Size gain per day was calculated as the size gain divided by development period for an individual. S.G/D.P was normally distributed amongst individuals in the poor diet

category (Shapiro-Wilk Test, n = 15, W = 0.97715, p-value = 0.9463) whereas it was not the case amongst the individuals in rich diet category (Shapiro-Wilk Test, n = 23, W = 0.69069, p-value = 1.063e-05). The data was found to be homoscedastic (F = 0.14777, num df = 14, denom df = 22, p-value = 0.0006198). Median S.G/D.P of both diet categories were compared and found that median S.G/D.P of individuals in poor diet category was less than that of individuals in rich diet category.(Wilcoxon rank sum test with continuity correction, W = 81, p-value = 0.003288).

4.1.6 Weight gain per day (W.G/D.P)

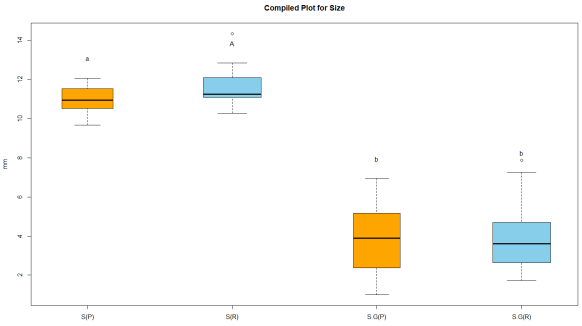
Weight gain per day was calculated as the weight gain divided by development period for an individual. W.G/D.P was normally distributed amongst individuals in the poor diet category (Shapiro-Wilk Test, n = 18, W = 0.92818, p-value = 0.1804) whereas it was not the case amongst the individuals in rich diet category (Shapiro-Wilk Test, n = 23, W = 0.81871, p-value = 0.0007756). The data was found to be homoscedastic (F = 0.50906, num df = 17, denom df = 22, p-value = 0.1595). Median W.G/D.P of both diet categories were compared and found that median W.G/D.P of individuals in poor diet category was less than that of individuals in rich diet category.(Wilcoxon rank sum test with continuity correction, W = 60, p-value = 5.94e-05).

4.1.7 Development period

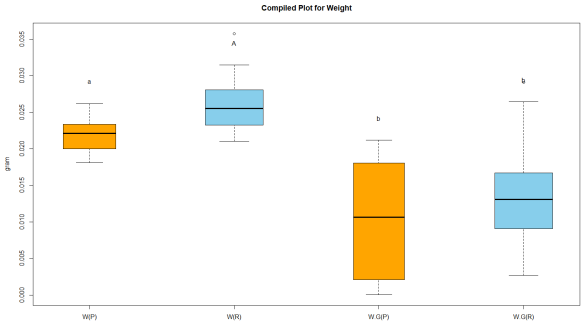
Development period was calculated as the duration (in days) between date of capture and date of adult eclosion. Development period was normally distributed amongst the individuals in poor diet category (Shapiro-Wilk test, n = 20, W = 0.93257, p-value = 0.173) but the same was not the case amongst the individuals in the rich diet category (Shapiro-Wilk test, n = 24, W = 0.88638, p-value = 0.0112). The data was found to be homoscedastic (F = 0.7355, num df = 19, denom df = 23, p-value = 0.5005). Median development period of individuals in both diet categories was not significantly different (Wilcoxon rank sum test with continuity correction, W = 261, p-value = 0.6282).

4.1.8 Life expectancy

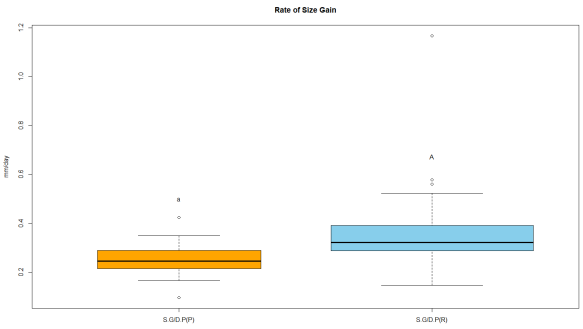
Life expectancy was calculated as the duration (in days) from date of capture to date of death (if recorded). Life expectancy was found to be normally distributed amongst individuals of both poor (Shapiro-Wilk test, n = 21, W = 0.91682, p-value = 0.07498) and rich (Shapiro-Wilk test, n = 15, W = 0.92369, p-value = 0.2192) diet categories. The data was found to be homoscedastic (F = 1.4696, num df = 20, denom df = 14, p-value = 0.4655). Mean life expectancy for individuals of both diet categories was not significantly different (Two Sample t-test, t = -0.75176, df = 34, p-value = 0.4574).



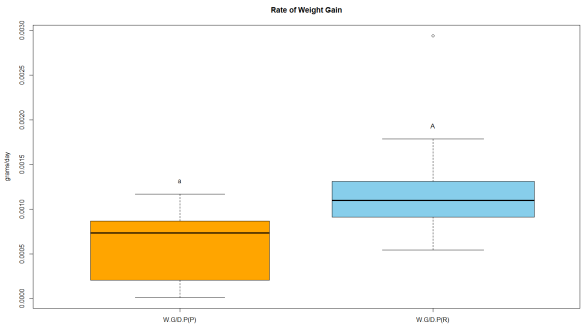
(a) Size and size gain (Rich vs Poor)



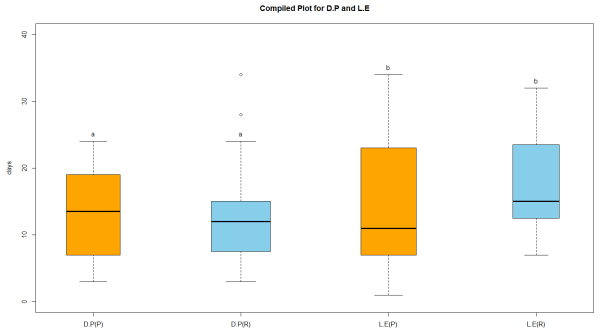
(b) Weight and weight gain (Rich vs Poor)



(c) Rate of size gain (Rich vs Poor)



(d) Rate of weight gain (Rich vs Poor)

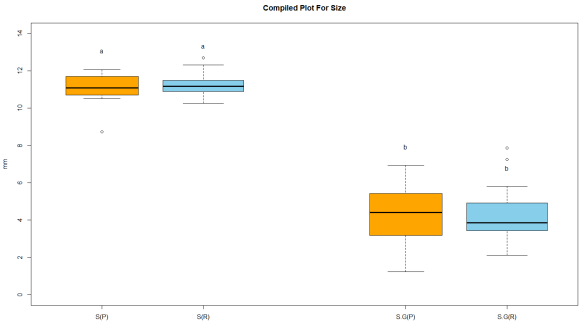


(e) Development Period and Life expectancy (Rich vs Poor)

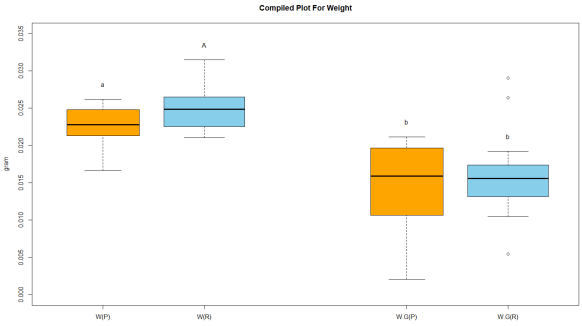
Figure 5: *S - Adult size, W - Adult weight, S.G - Size gain, W.G - Weight gain, D.P - Development period, L.E - Life expectancy.* ***Note boxes with letters in different cases are significantly different***

4.2 Clean Data

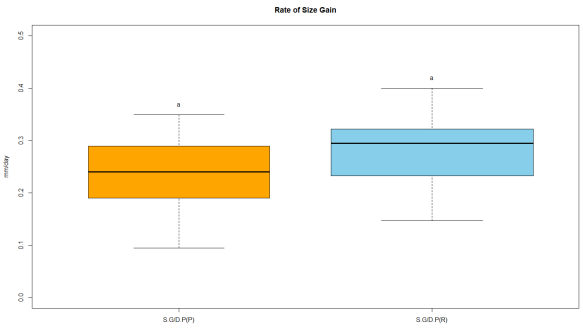
In this data set (Np = 12, Nr = 15), values for all the observables viz. Size, Weight, Size gain, Weight gain, S.G/D.P, W.G/D.P were found to be normally distributed. Out of these, mean Weight and mean W.G/D.P were found to be significantly different amongst the individuals in the two diet categories (Unpaired Two Sample t-test).



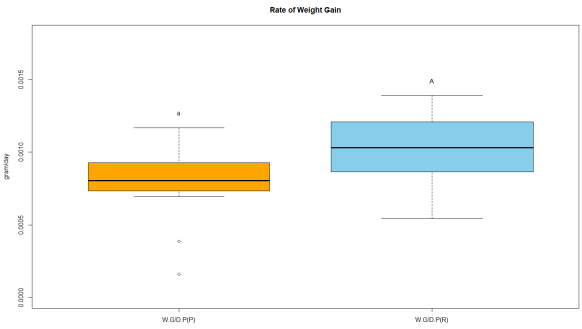
(a) Size and size gain (Rich vs Poor)



(b) Weight and weight gain (Rich vs Poor)



(c) Rate of size gain (Rich vs Poor)



(d) Rate of weight gain (Rich vs Poor)

Figure 6: *S - Adult size (Welch Two Sample t-test, t = -0.82318, df = 18.837, p-value = 0.4207), W - Adult weight (Welch Two Sample t-test, t = -2.1239, df = 24.837, p-value = 0.04382), S.G - Size gain (Welch Two Sample t-test, t = -0.20213, df = 22.681, p-value = 0.8416), W.G - Weight gain (Welch Two Sample t-test, t = -0.78687, df = 22.844, p-value = 0.4394), S.G/D.P - Welch Two Sample t-test, t = -1.5386, df = 23.906, p-value = 0.137, W.G/D.P - Welch Two Sample t-test, t = -2.3026, df = 22.462, p-value = 0.01546.* ***Note boxes with letters in different cases are significantly different***

5 Conclusion

Upon analysis of the **raw data**, four parameters viz. size, rate of size gain, weight and rate of weight gain showed significant difference between individuals of the two diet categories. Whereas, upon analysing the **cleaned data**, it was found that only weight and rate of weight gain showed significant difference between individuals of the two diet categories. A more comprehensive analysis using a mixed model which can account for the time spent under each diet category is required to provide a better insight into the effect of developmental stage diet on adult size and weight in *O.henryi* nymphs.

References

[1] Rittik Deb, Sambita Modak, and Rohini Balakrishnan. "Baffling: a condition-dependent alternative mate attraction strategy using self-made tools in tree crickets". In: *Proceedings of the Royal Society B: Biological Sciences* 287 (Dec. 2020), p. 20202229. DOI: 10.1098/rspb.2020.2229.

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