The effects of dietary choice on lifespan in *drosophila melanogaster*

Data analyses was done in R v. 4.1.1. Various factors such as the effect of sex and mating status were tested using various statiscal models.

Males vs Females

Feeding behaviour

A linear model was used and the significance of day was tested using an ANOVA model. It was found that day was significant ($F_{8,903}$ = 0.941, P = 0.05). ANOVA analysis also showed that there was significance between the sexes and the interaction effect ($F_{8,903}$ = 35.36, P = <0.001), so this was kept in the model.

The significance of diets between sexes and within sexes was analysed.

There was a statistically significant difference found between a 1:2 diet and an 8:1 diet on the female assay on both day 1 and day 2 (Tukey test: t_{903} = -5.088, P < 0.001). On day 1, the there was a mean average of 1.43 +/- S.E. 0.154 flies per patch but on day 2 there was an average of 2.36 +/- S.E. 0.230 flies per patch on the 8:1 diet.

The significance of sex differences was analysed using a generalised linear model with quasipoisson to count for overdispersion. It was found that there was a significant difference in male and female flies choosing a diet higher in protein (a 2:1 diet). A tukey test was used to analyse this ($t_{903}8\sim$ = 13.048, P < 0.001). Day was not considered in this model.

Mated vs Virgin females
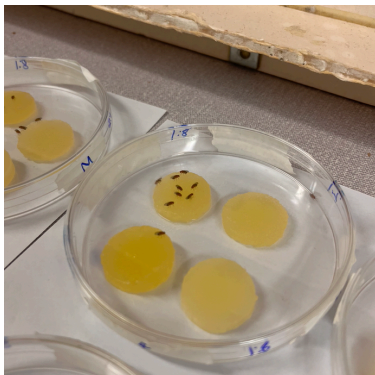
Feeding behaviour

A generalised linear model was used with quasipoisson to count for overdispersion. Day was dropped from the model as there was found to be no significant effect of including day in the model (ANOVA: $F_{1,1159}$ = 0.8,

P = 0.35). There was found to be a very strong statistically significant effect however on the interaction of diet and type (whether the fly was mated or virgin) (ANOVA: $F_{3,1159}$ = 0.8, P = < 0.0001).

Post hoc analysis showed there were statistically significant differences for the diets chosen between mated and virgin female *drosophila melanogaster*. Between the types of fly, there was a significant difference in choosing a diet high in protein (8:1). (Tukey test: z_{1151} = 5.301, P < 0.001). With a mean average of 2.74 +/- S.E. 0.138 mated females per patch and a mean average of 1.64 +/- S.E. 0.146 virgin females. This shows that mated females will prefer a diet which is high in protein, indicating that mated females are sensible enough to chose a diet which is good for pregnancy, contributing to the growth of their offspring.
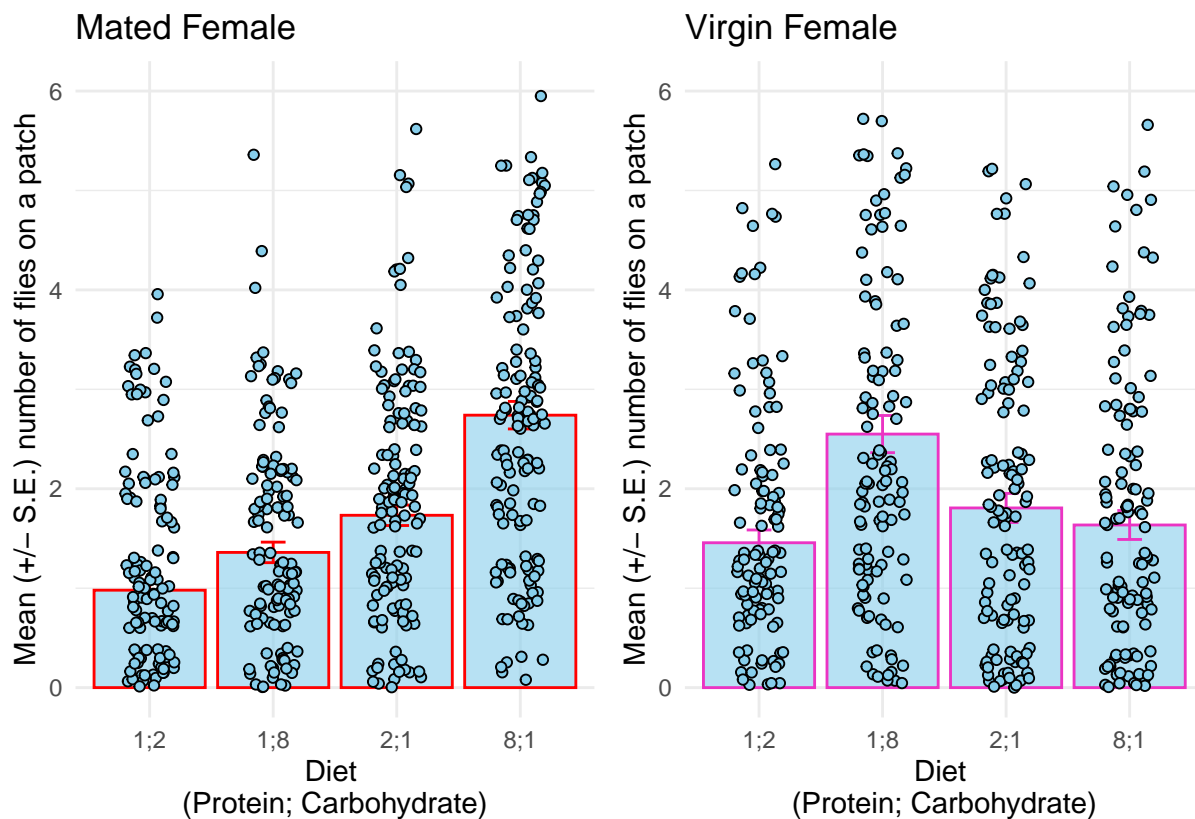

Figure 1: A boxplot comparing feeding behaviour of mated and virgin females. Figure shows a plot with the mean average +/- S.E. of where the mated females preferred to feed (left), compared with a mean average +/- S.E. of where the virgin females preferred to feed (right).

The significance of diet on mated and virgin *drosophila melanogaster* seperatley to look for the signifiance in choosing a particular diet. For mated females, it was found that there was a statistically significant difference in choosing a diet high in protein and low in carbohydrate (8:1) over a diet which was low in protein and high in carbohydrate (1:2). Post hoc analysis (Tukey test: z_{1151} = -9.065, P < 0.0001). There was a mean average of 2.74 +/- S.E. 0.138 flies per patch on an 8:1 diet, and only a mean average of 0.98 +/- S.E. 0.085 flies per patch on a 1:2 diet. Emphasising again the significance on a diet which is high in protein for a mated female.

For virgin females, they were a lot less 'choosy' about the diets they chose to feed and spend time on, although there was a significant difference found (Tukey test: z~~ = 4.443, P 0.0002) between diets 1:8 and 8:1. There was a mean average of 2.55 +/- S.E. 0.128 flies per patch on the 1:8 diet and only a mean average of 1.64 +/- S.E. 0.146 flies per patch on the 8:1 diet.

Oviposition behaviour

The significance of oviposition preference was tested on both mated and virgin female *drosophila melanogaster*

The first experiment investigated was looking into mated and virgin egg count. A generalised linear model was quasipoisson was used to count for overdispersion, it was found that there was not a significant difference in the interaction effect between where mated females laid their eggs and where virgin females laid their eggs (ANOVA: $F_{3,64}$ = 0.069, P = 0.9), so this factor was not tested in the model.

Looking at the significance of diet choice in mated and virgin female flies individually showed there was a significant difference in the diet in which a mated female chose to lay their eggs, investing into protein and carbohydrate differences, there was a mean average of 121 +/- S.E. 14.1 eggs laid on the 1:8 diet and only a mean average of 4.21 +/- S.E. 1.52 eggs laid on the 8:1 diet, showing a statistically significant difference (Tukey test: t~~ = 7.342, P = < 0.0001). Investigating the significance into where virgin females laid their eggs showed there was also a statistically significant difference in a 1:8 diet and a 8:1 diet (Tukey test: t~~ = 4.127, P = 0.0026). With an average of 74.4 +/- S.E. 17 eggs laid on the 1:8 diet and a mean average of 1.25 +/- 1.25 SE eggs laid on the 8:1 diet.
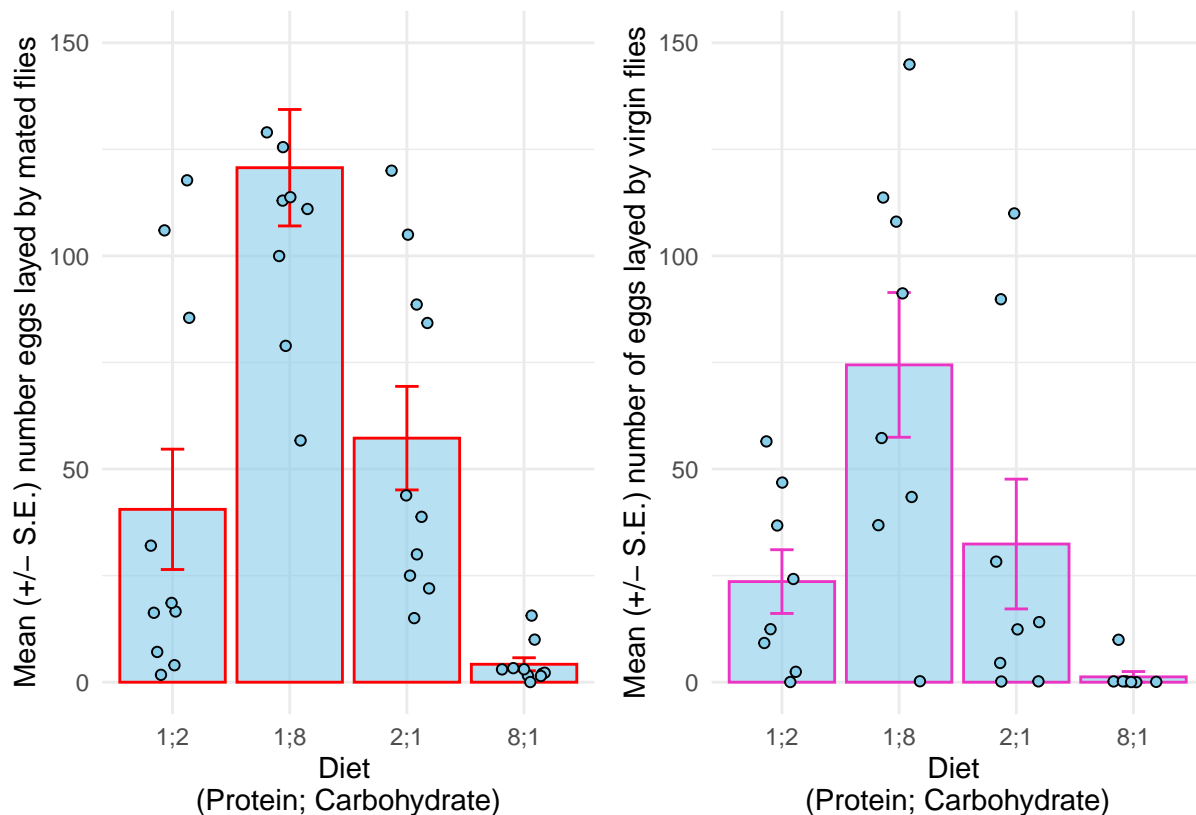

Figure 2: A boxplot showing the eggs counted from mated females and virgin females. Boxplot shows the mean average +/- S.E. eggs from the varying protein: carbohydrate food patches.

The same experiment was repeated, where the oviposition preference of mated females was investigated. To test for the significance in oviposition behaviour in the mated females, a linear model was used with.

It was found that although there was not a lot of differences, there were significantly more offspring emerging from diets 1:8 than diets 1:2 (t~~ = 2.379, P = 0.019). Overall there was a mean average of 114 +/- S.E. 22.5 offspring emerging from 1:8 diets and a mean average of 68.9 +/- 10.6 S.E. offspring emerging from the 1:2 diets (Figure 3). As these diets are both diets which are 'low in protein' it shows that although there

is not a lot of preference for any particular diets when mated females are laying their eggs. Data for virgin female egg laying was not collected for this experiment.
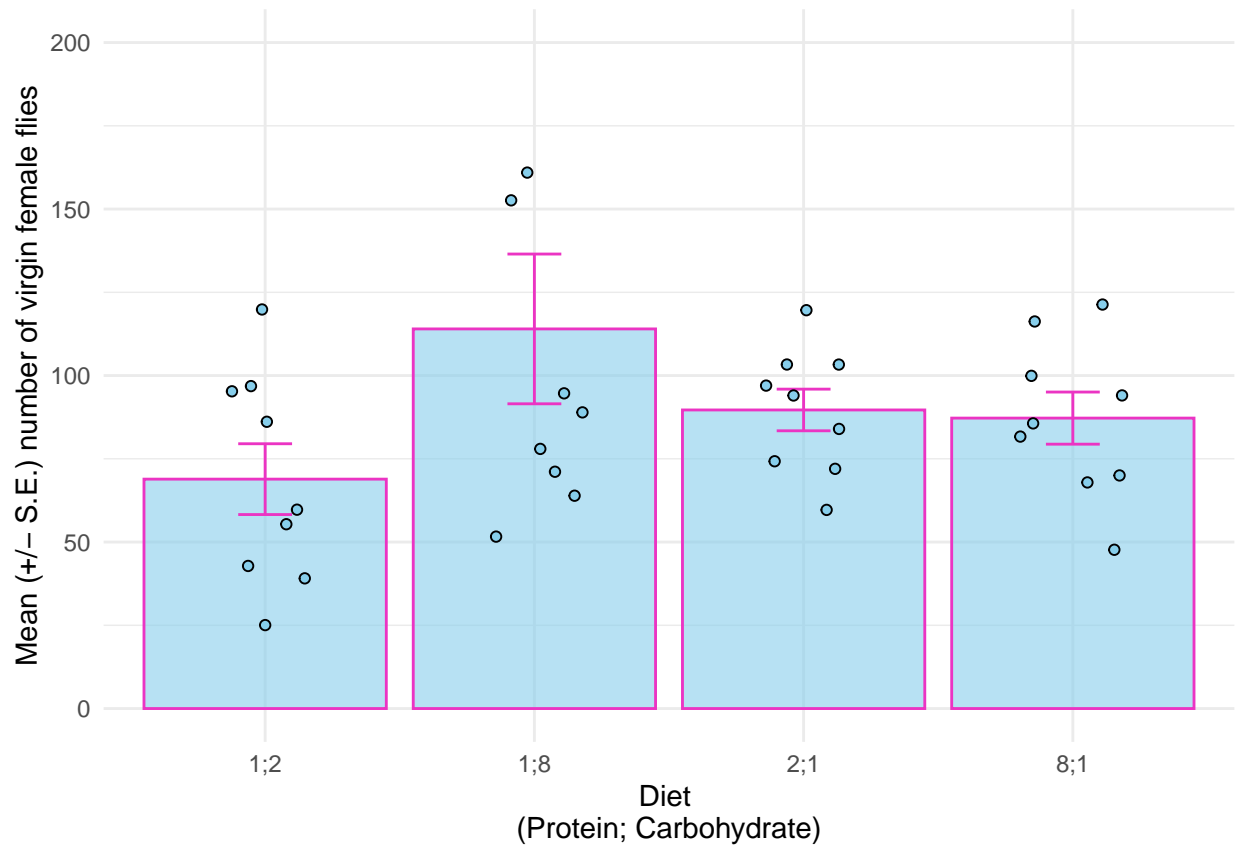

Figure 3: A boxplot of the offspring counted from mated females. Boxplot shows mean offspring +/- S.E. that emerged from four varying protein: carbohydrate diet patches.

Data was also collected in a preliminary experiment, where the egg counts of where both virgin females and mated females laid their eggs was collected. It was found that there was no significant differences found in where virgin females laid their eggs and where mated females laid their eggs in this experiment. This experiment also showed however, that there was significantly more eggs laid on the 1:8 diets compared to the 1:2 diets (P = < 0.001), with a mean average of 121 +/-13.7 S.E. eggs laid on 1:8, and a mean average of 40.6 +/- S.E. 14.1 laid on the 1:2 diets. There was also a slight significant difference in eggs laid on the 1:2 diet, and eggs laid on the 8:1 diet (P=0.025), with a mean average of only 4.21 +/- S.E. 1.52 eggs laid on the 8:1 diets.
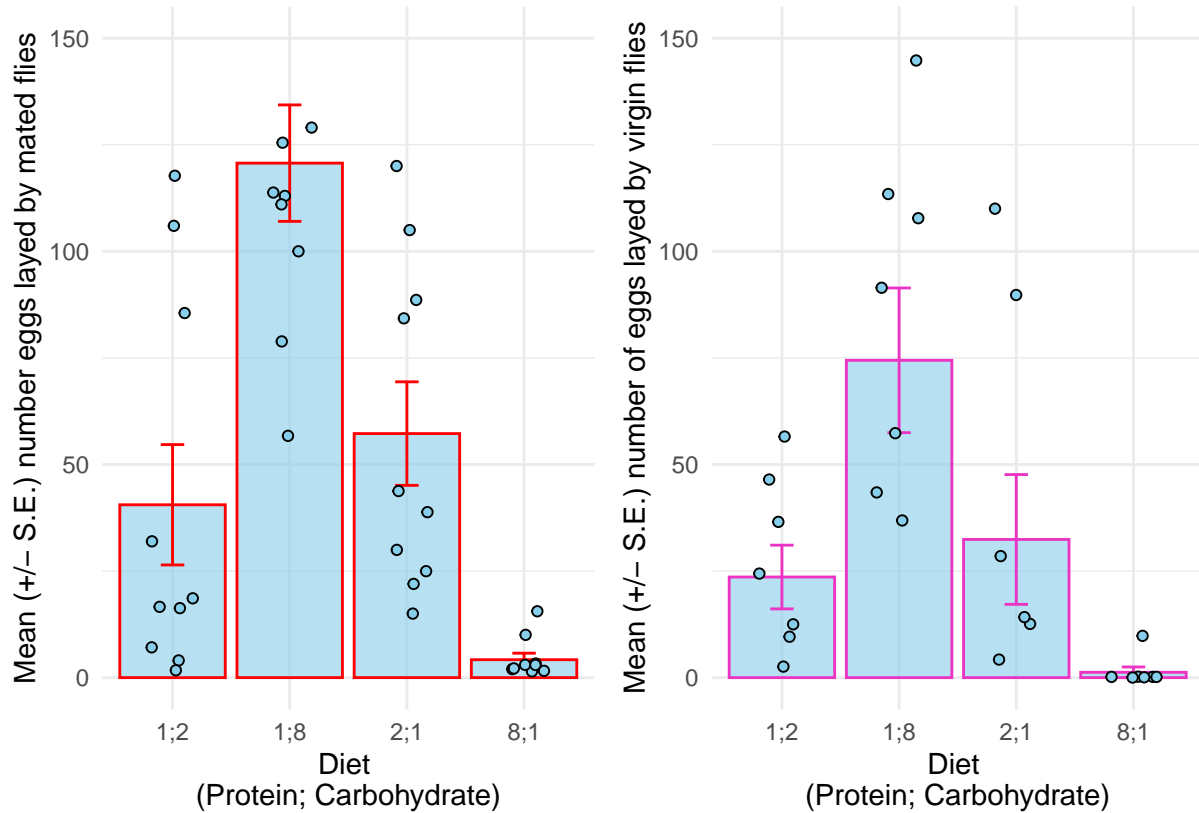
Figure 4: A boxplot showing the eggs counted from mated females and virgin females. Boxplot shows the mean average +/- S.E. that emerged from the varying protein: carbohydrate food patches.

Mated Female behaviour alone vs Mated Female behaviour with Males

When looking at female feeding behaviour, and if this changed with females alone in a feeding assay, to females who were in a feeding assay with males. There was a small interaction effect of day with diet and feeding choice, however this was not significant from not having day as an interaction effect, ($F_{1,0.91}$ = 0.941, P = 0.27), and was therefore dropped from the full model.

A generalized linear model with quasipoisson was used (as there was over-dispersion), which showed there was no significant difference in dietary choice between mated females who were alone on a plate and mated females who were on a plate with males.
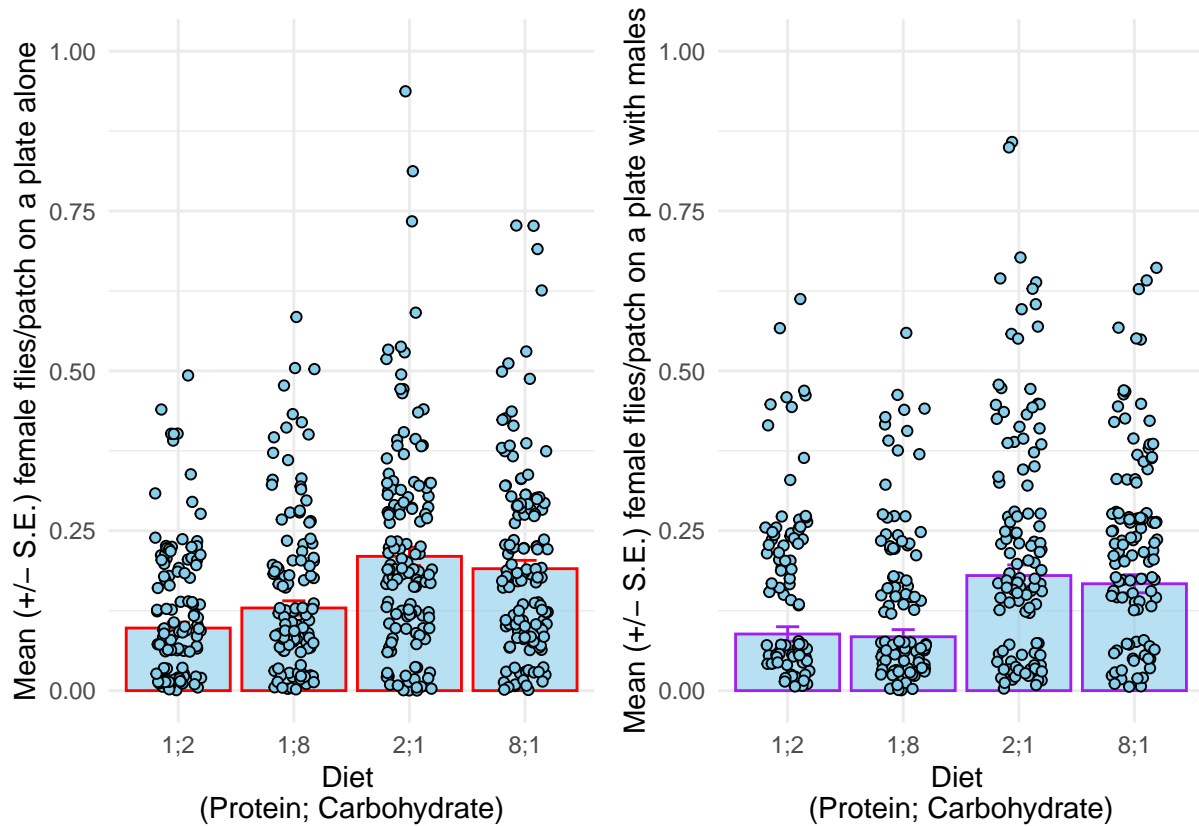
Figure 5: A boxplot showing the feeding behaviour females on a plate alone and females on a plate with males. Boxplot shows the mean average +/- S.E. flies on the varying protein: carbohydrate food patches

Offspring counts

A general linear model was used to test the significance of whether oviposition preference changed depending on if the mated females were in a plate alone or in a plate with males. There was no significant difference found between the diets chosen depending on the conditions (ANOVA: P = 0.44).

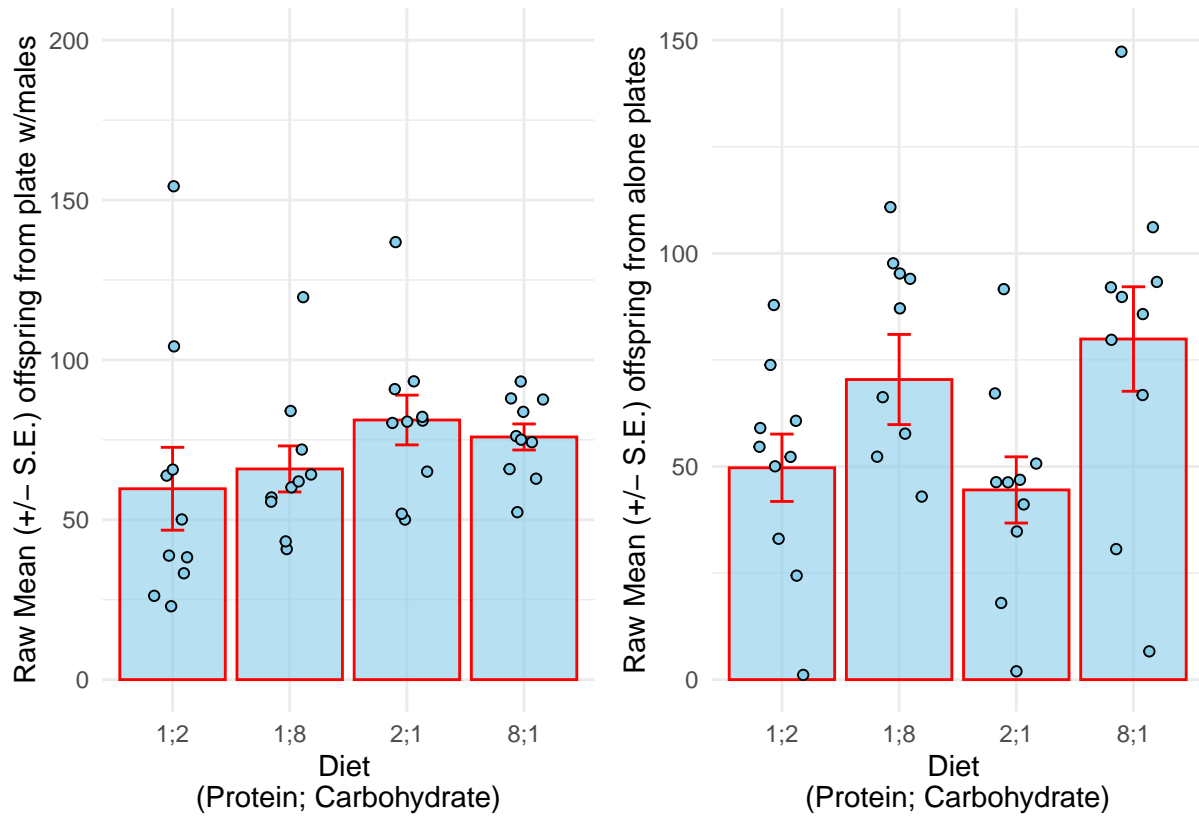**Figure 6: A boxplot showing the offspring count from females in a plate alone and females in a plate with males.** Boxplot shows the mean average +/- S.E. of offspring that emerged from the varying protein: carbohydrate food patches