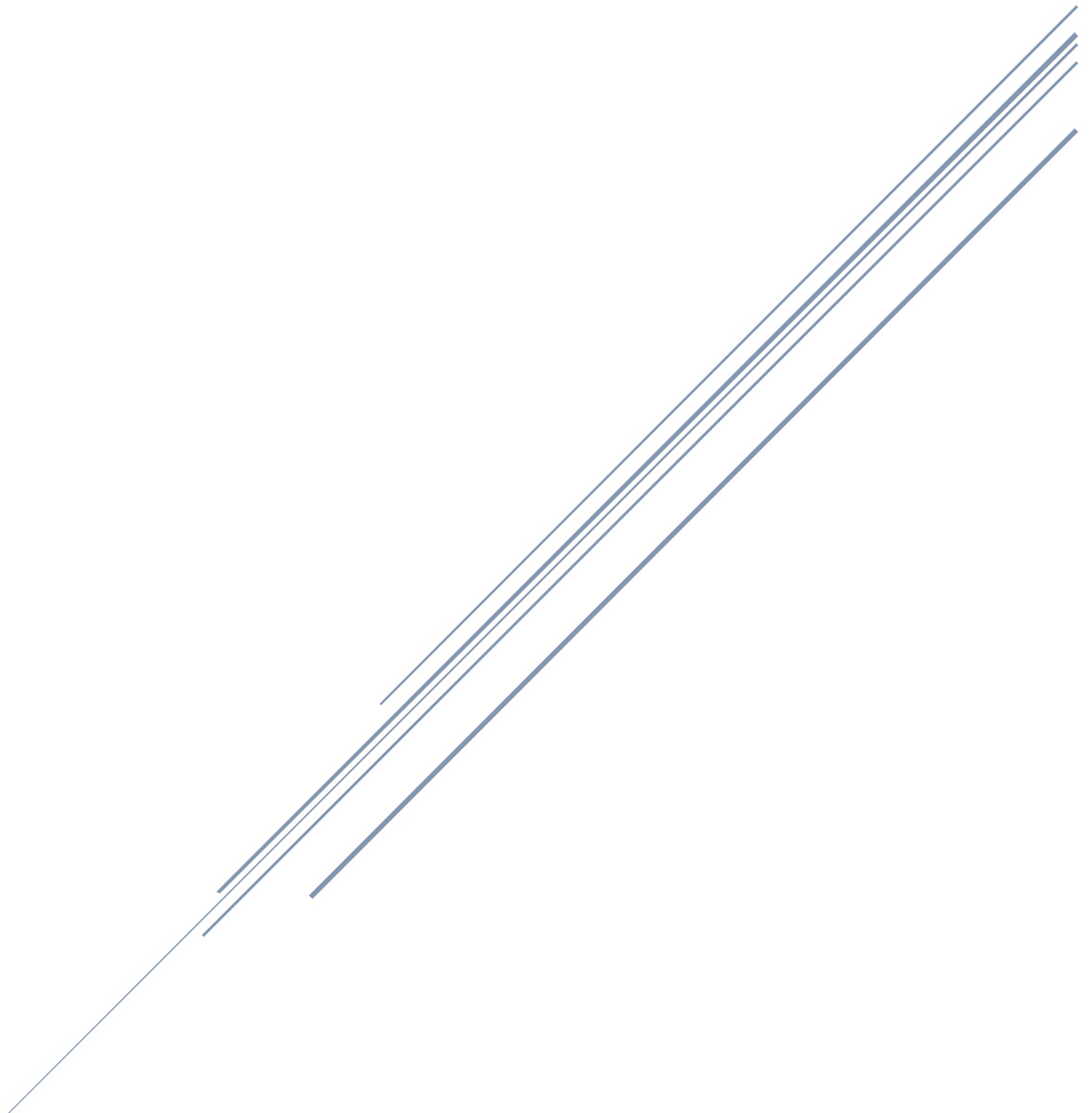


FEEDING PREFERENCE AND GROWTH EFFECTS FOR THREE TRICHOPTERA SPECIES

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Abstract:

Detritivores diets are very limited in key nutrients such as phosphorus, nitrogen, and fatty acids. This study looked at how detritivores caddisflies selected food based on nutrient quality. We set up controlled experiments to examine food preference and how that might affect overall growth. Our study found that there is the possibility for preference among different food types and that it could influence growth, but nothing has been confirmed. This study help us understand what types of nutrients were available in these aquatic systems.

Introduction:

It is common for many organisms to be deficient in important nutrients that are key to growth, survival and reproduction. Detritivores are especially vulnerable to nutrient limitation because vascular plant detritus alone doesn't provide substantial amounts of nitrogen and phosphorus. (Crenier et al. 2017, Demi et al. 2018, Urabe and Yamaguchi 2018). Some of the most important nutrients to detritivores are nitrogen, phosphorus, and fatty acids (Twining et al. 2016b, Crenier et al. 2017, Demi et al. 2018).

Another known limiting factor in growth is elemental stoichiometry, specifically phosphorus availability (Demi et al. 2018). Phosphorus is an essential nutrient to cell production and DNA replication. Since detritus is very limited in phosphorus it is important for detritivores to consume supplementary phosphorus (Urabe and Yamaguchi 2018). One of the primary ways that phosphorus becomes available to aquatic detritivores is through hyphomycetic fungus that grows on leaf detritus.

Detritavores that live in aquatic systems consume small amounts of algae which are a primary source of Polyunsaturated fatty acids (PUFAs); algae has been shown to promote growth

in detritivores (Crenier et al. 2017). PUFAs are an important component in the development of all animals. In aquatic systems algae is the primary source of PUFAs for consumers. Algae produces eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (types of omega-3 fatty acids) that are important biochemicals for regular bodily functions for aquatic insects and other organisms (Twining et al. 2016b). Algae play a significant role in transporting PUFAs to higher trophic levels in lakes and ponds and can be an important source of PUFAs for terrestrial organisms, such as swallows, that feed on emerging adult aquatic insects (Twining et al. 2016a Twining et al. 2017, Twining et al. 2018).

Our study proposes to examine these nutrient and elemental restrictions in several species of detritivorous caddisflies (Trichoptera) from the family Limnephilidae. We want to know if these animals exhibit feeding preference for sedge detritus with high phosphorus content, high algal biomass, or both and if this preference correlates to increased growth and performance in these caddisflies. If we see preference this would indicate that the insect's natural response would be to choose food that is limited and/or better for their development. We answered these questions by performing paired preference and growth experiments on three species of caddisfly using sedge conditioned under different light and phosphorus regimes. Based on the literature, our study proposes two hypotheses: (1) Caddisfly larvae will show preference for a specific type of sedge whether they be (a) limited in algal biochemicals, such as PUFAs, (b) phosphorus, or (c) both, and (2) caddisfly larvae will grow more mass on sedge treatments that they prefer. We predict that insects will prefer and grow better on sedge that has an increased amount of phosphorus, because it is limited in their diet but is needed for cell construction and the transfer of energy.

Methods:

Our experiments were conducted in a WeatherPort® within the Gothic townsite (38.958764°N, 106.991422°W, Fig.1). The experiments took place during the months of July and August and had a total run time of approximately 5 weeks. Sedge detritus and *Limnephilus externus* were collected from beaver ponds across the East River from the WeatherPort®. *Anabolia bimaculate* were both collected from ponds up valley from the townsite.

Sedge was incubated under different light (ambient and shaded) and phosphorus (ambient and added P) conditions in order to modify the amount of periphytic algae present on the sedge and the phosphorus content of the sedge. Four of eight incubation tubs were covered with shade cloth to inhibit the growth of algae; the other half was left uncovered to promote the growth of algae. Half of the shaded and half of the unshaded tubs were inoculated with phosphorus once a week, for two weeks to a target phosphorus concentration of 100 µg/L above ambient. Samples were taken every time we set up a preference experiment for a species so that we could see if we had any change in chlorophyll a (a surrogate for algal biomass) and phosphorus content over time, and between treated and non-treated tubs. Sedge was incubated under the different treatment conditions for two weeks prior to beginning the feeding preference and growth experiments.

Twentyfive plastic Tupperware® containers were used as the preference observation chambers. Each chamber was stocked with 1 g (wet mass; ± 0.1 g) of sedge from all four treatments, and each treatment was randomly assigned a quadrant of the chamber (Fig. 2). One insect was placed in each of the chambers along with pond water. Chambers were observed for 3

days at half hour intervals for 6-8 hours per day. The chamber label would be recorded on a data sheet if the insect was actively attached to the sedge. After the three days the sedge was weighed to calculate total mass loss and use it as another metric for preference. A wet to dry mass conversion of 0.935 was used to convert initial wet mass to dry mass for mass loss calculations.

Twenty plastic storage containers were used for the growth experiment chambers, with five replicate chambers used for each of the four treatments. Approximately 15 grams (wet mass; ± 0.1 g) of sedge was put into each container along with pond water and five bugs insects per chamber. Chambers that had shade treated sedge were covered with shade cloth as to not alter algal content for the duration of the experiment. *L. externus* was held in the growth chambers for thirteen days and *Anabolia* for nine days, then insects and sedge were weighed to calculate growth rates and mass loss, respectively. Additionally, growth and preference experiments for all three species of Trichoptera were conducted one week after the previous species experiments.

All data were ran through an analysis of variance (ANOVA) and followed up with a Tukey test. Data was processed using the program “R”.

Results:

Chlorophyll:

The shaded treatment varied significantly (ANOVA, $P < 0.05$) from the unshaded treatments in chlorophyll content (Fig. 3). When ran through a Tukey’s test the shaded-ambient phosphorus and the light-added phosphorus treatments varied the most ($P < 0.05$). These results prove that the shade treatment was highly effective at inhibiting the growth of photosynthetic algae.

Preference Experiments:

For *L. externus*, the mean proportion of time spent at quadrants “A”, “B”, “C”, and “D” were 0.114, 0.128, 0.337, 0.334, respectively. The mean proportion of observations at the light ambient phosphorus, light + phosphorus, shade ambient phosphorus, and shade + phosphorus treatments were 0.277, 0.245, 0.218, 0.172, respectively (Fig.4 & Fig.5). *L. externus* showed significant preference for quadrants “C” and “D” ($P < 0.05$) but didn’t show any significant preference for any of the treatments ($P = 0.636$). However, when we analyzed the mass loss data for the sedge there was a significant ($P < 0.05$) preference for the light + phosphorus treatment, and also we saw increased sedge mass loss of quadrants “C” and “D” over quadrants “A” and “B” (Fig.6 & Fig.7).

Anabolia mean proportion of time spent at quadrants “A”, “B”, “C”, and “D” were 0.206, 0.14, 0.218, 0.256, respectively. Mean proportion of time spent at the light ambient phosphorus, light + phosphorus, shade ambient phosphorus, and shade + phosphorus treatments were 0.186, 0.258, 0.106, 0.27, respectively (Fig.8 & Fig.9). *Anabolia* showed significant preference ($P < 0.05$) especially for the shade + phosphorus treatment over the shade ambient phosphorus treatment ($P < 0.05$). When we analyzed sedge mass loss, *Anabolia* showed significant preference for the shade + phosphorus over both ambient phosphorus treatments ($P < 0.05$).

Growth Experiments:

There was no significant difference in growth rates between all for treatments for both *L. externus* and *Anabolia* ($P = 0.225$, $P = 0.232$; respectively); however, *L. externus* showed slightly better growth on the light + phosphorus treatment compared to the other three treatments (Fig.10 & Fig.11). The growth experiment for *Anabolia* was confounded by a large number of individuals pupating during the experiment, which resulted in negative growth rates for all

treatments. However, individuals pupated at a higher frequency in both of the added phosphorus treatments relative to the ambient P treatments.

Discussion:

Our first hypothesis was not supported in the *L. externus* preference experiment. Our study saw a heavy preference for one side of the WeatherPort® and we believe that this was caused by having the chambers too close to one of the walls, thus making the insects move away from it. Conversely, the *Anabolia* preference experiment confirmed our first hypothesis and also showed *Anabolia* to have a preference for the high phosphorus sedge, regardless of light treatment. These results suggest that the *Anabolia* in this area may be phosphorus limited and have the ability to distinguish between foods with high amounts of phosphorus and foods with low amounts of phosphorus (Chung and Suberkropp 2009, Demi et al. 2018). We were unable to confirm if growth was correlated to food preference for either species because of stochasticity within the experiment, however; the multiple pupation suggests that phosphorus is beneficial for developing larvae. Furthermore, we still are processing data for the *Asynarchus* and thus did not include it in this report.

We had a couple of biases that had a significant effect on the experiments. The *L. externus* preference experiment was influenced by the wall of the WeatherPort® and thus invalidated the observations and mass loss calculated. We moved the chambers away from the wall for the next two species and it appeared to have worked for the *Anabolia* experiment. The other issue we found was in both of the *Anabolia* experiments. We collected larvae that were mostly in their fifth instar and many had pupated during our experiments. This resulted in negative growth rates. But, since the majority had pupated in the high-P treatments (Fig.12) our

results suggest that these insects met their nutritional demands faster than the individuals on the ambient-P treatments, therefore pupating at a lower body mass, but this has not been proven.

We propose that future studies take into account things like temperature (we have measure temperature for this study but have yet to retrieve the data) and physical structures in this simulated environment because they can heavily influence the results of the study. It would also be beneficial to investigate how dietary preferences vary among different instars. The research that we conducted gives insight to the nutritional limitation of these common aquatic detritivores, with implications that may propagate up the food web and across ecosystem boundaries through insectivorous birds (Twining et al. 2016a).

Tables and Figures:



Fig. 1: Map of the experiment site in relation to other areas around Gothic, CO.

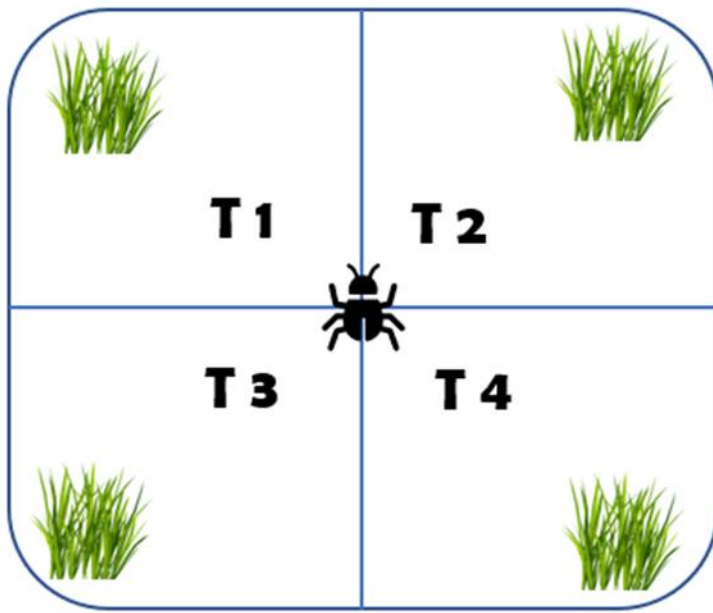


Fig. 2: A model of a microcosm used for the preference experiment. T₁-T₄ represent the four different types of treated sedge.

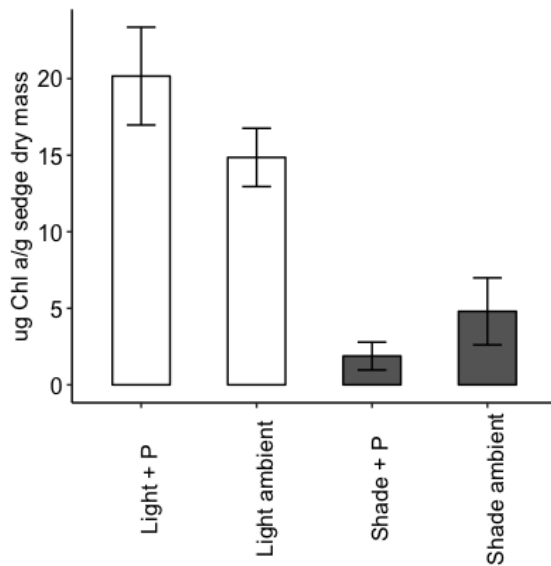


Fig. 3: Graph representing mean µg of chlorophyll per gram of sedge for each sedge treatment used in the preference and growth experiments.

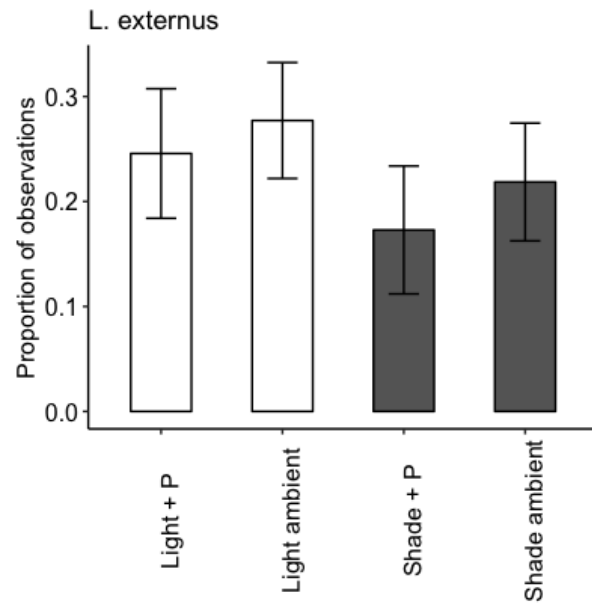


Fig. 4: Graph representing mean proportion of time spent at each treatment for *L. externus*.

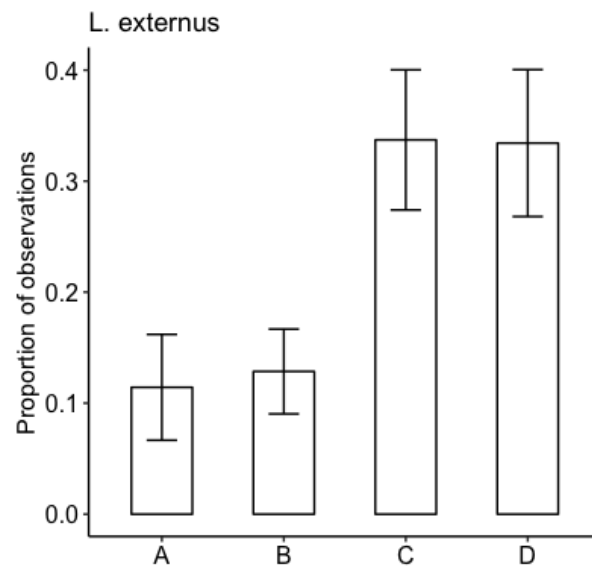


Fig. 5: Graph representing mean proportion of time spent at each quadrant for *L. externus*.

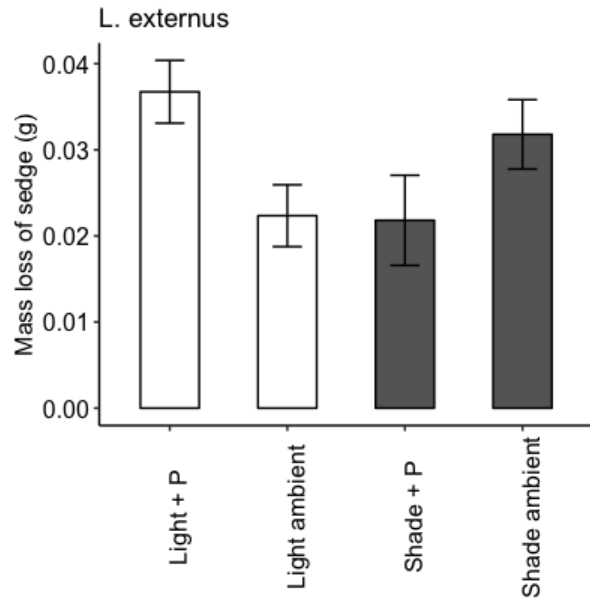


Fig. 6: Graph representing mean mass loss of sedge for each treatment for *L. externus*.

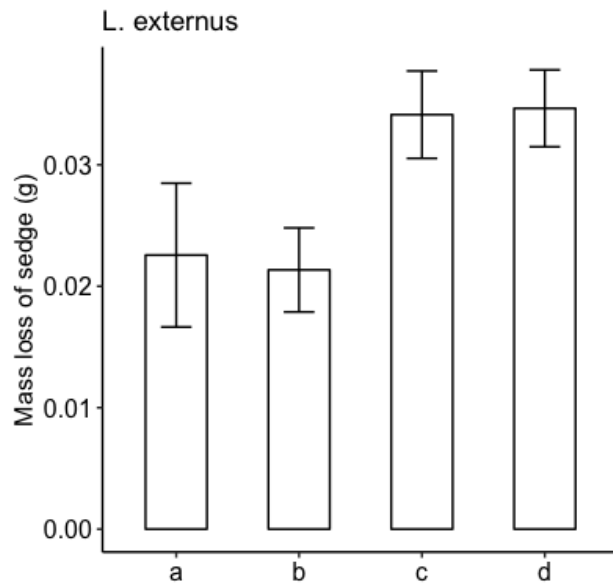


Fig. 7: Graph representing mean mass loss of sedge for each quadrant for *L. externus*.

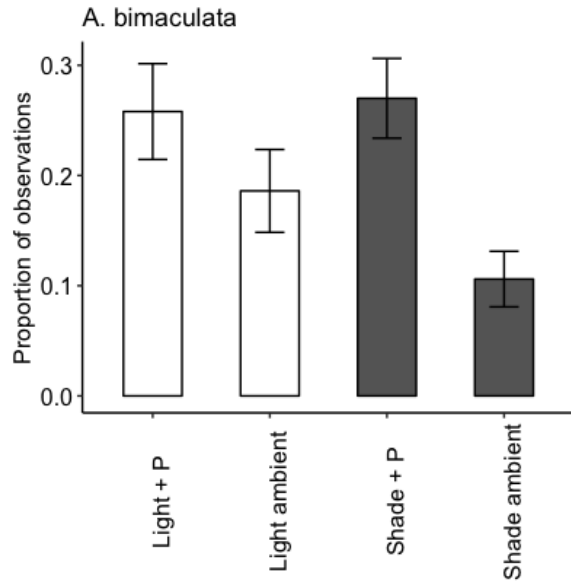


Fig. 8: Graph representing mean proportion of time spent at each treatment for *Anabolia*.

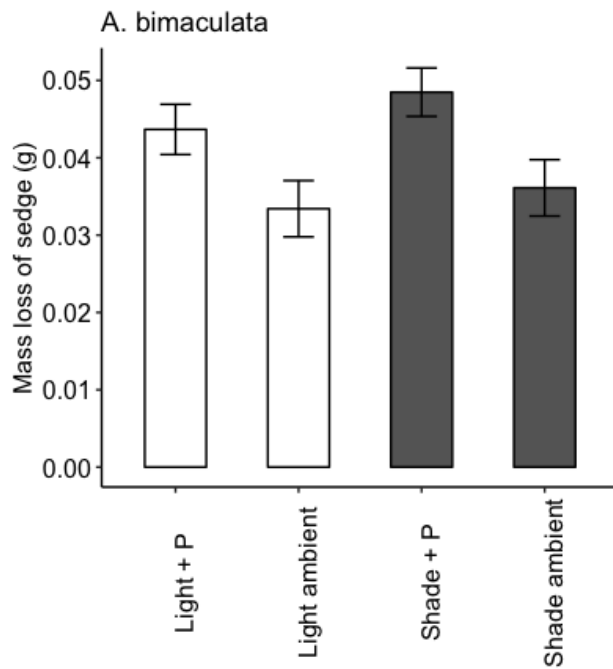


Fig. 9: Graph representing mean proportion of time spent at each treatment for *L. externus*.

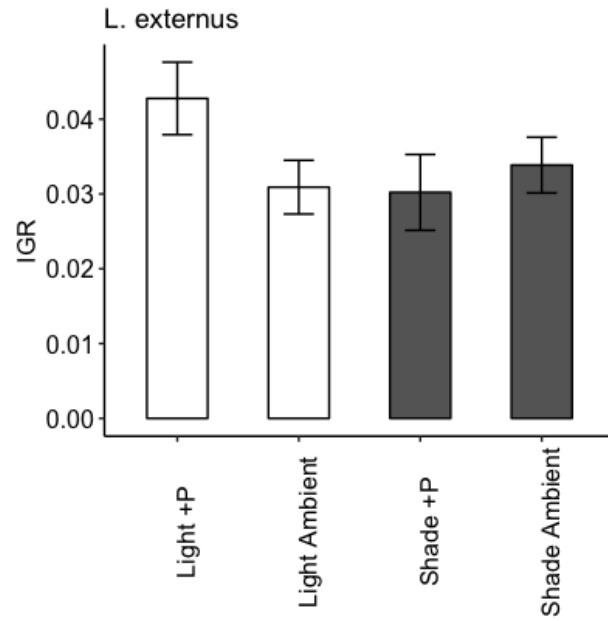


Fig. 10: Graph representing immediate growth rate for each treatment for *L. externus*.

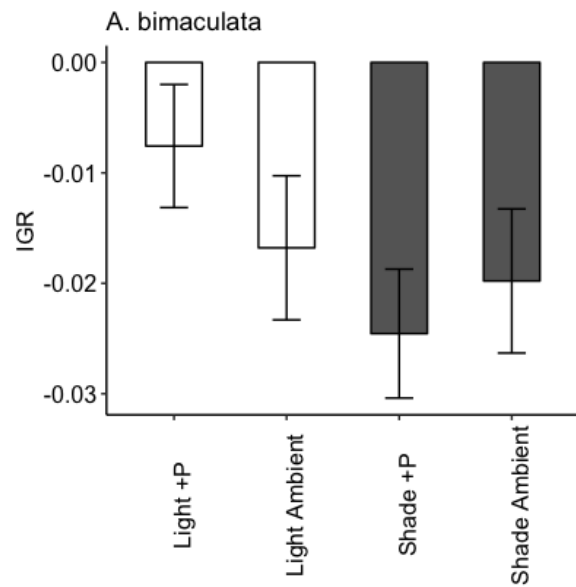


Fig. 11: Graph representing immediate growth rate for each treatment for *Anabolia*..

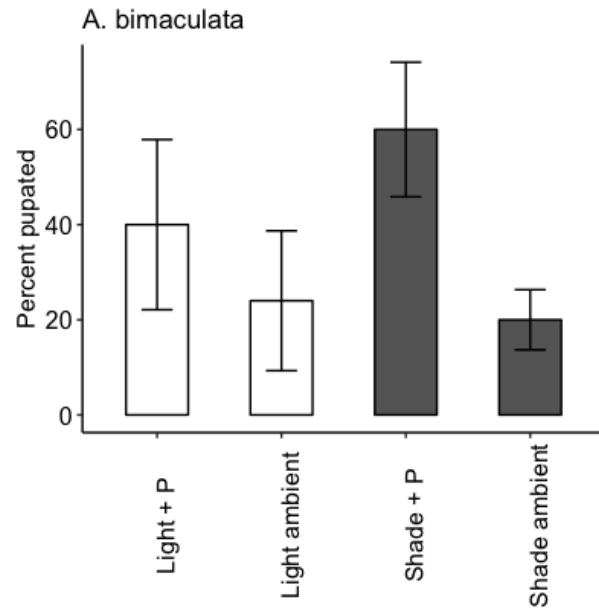


Fig. 12: Graph representing percent of *Anabolia* that pupated on each treatment.

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