How Heat Wave Simulation and Initial Body Mass Influences Arthropod Thermal Optimum, Molting Tendencies, and Evolution

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ABSTRACT

Arthropods are intriguing because they are the most abundant, diverse, and some of the most adaptable and crucial animals on earth. While arthropods have been a subject of research since ancient times, advancements in technology have increased the possibility for scientists to study the interconnection between arthropods and their environment. Because arthropods play such a vital role in the biodiversity of life on earth, studying them can help us learn the mechanisms that drive speciation and biodiversity to help create conservation strategies. This is important since there is scientific evidence of global warming, and an increase in heat waves with evidence that the earth will continue to increase in temperature as long as the emission of greenhouse gasses continues, possibly affecting arthropod's thermal optimum. For this project, data analysis on arthropod molting tendencies from prolonged exposure to high temperature and relation to initial body mass will be examined for two species of arthropods antlions, *Myrmeleon bore* and *Euroleon nostra*. Regression Analysis and an ANOVA showed that prolonged exposure to high temperature can cause extended molt length in *Euroleon nostras* instar stage 2 but not in *Myrmeleon bore* antlion arthropods. The number of days it takes for second instar larvae to molt during heatwave simulation did not have a correlation to the initial body mass of the larvae. Further research on the effects of prolonged high temperatures on arthropod instar development is needed and additional factors contributing to molting dysfunction should be explored.

INTRODUCTION

Arthropods make up the vast majority of the kingdom Animalia. They are invertebrates meaning they lack vertebrae. Instead, they have an external support system known as an exoskeleton. The word “arthro” means joints and “poda” meaning feet gives the meaning of “jointed feet" (Schmidt, 1991). The first arthropod is estimated to have appeared 520 mya. Today modern arthropods share the characteristics of some trilobites by having distinct body segments, jointed, appendages, and an exoskeleton. These traits allowed trilobites to adapt to terrestrial animals more simply since exoskeletons could provide protection and keep them from drying out (Dhara, 2017). Insecta a subclass of arthropods are characterized by having an abdomen, head, thorax, antenna, wings, and varying legs. Their exoskeletons made of chitin are shed in a process known as molting.

One family of molting Insecta includes antlions. These arthropods are characterized by the larval stage where antlions prey on small insects such as ants by creating pits in the soil or sand, trapping their prey. An open circulatory system is present. A dorsal vessel extends the entirety of the insect's body transporting hemolymph. An insect will contract the dorsal vessel to begin the molting process. Molting, termed ecdysis, happens when the insect's exoskeleton is shed to make room for more growth, development, and repair of cell damage (Krzysztof, 2020). It is an extremely important process for the organism. Before molting can begin, the insect will increase water absorbance to expand the body a critical on the exoskeleton. Epolysis is the first step of molting and peeling epidermal cells. This process is stimulated by the ecdysteroid hormone secreted by the prothoracic gland. While the hormone is regulated by a feedback system, environmental cues such as temperature or the insect's nutritional state can contribute to varying production (Krzysztof, 2020). Ecdysis occurs once the reabsorbed minerals push out the old chitin separating it by muscle contraction until a new exoskeleton is formed.

While molting isn't a direct product of selection it can contribute to an organism's phenotypic expression. Because arthropods are ectothermic, temperature changes could cause temporal variation adaptations to survive. Depending on the time it takes to adapt molting behavior to changing environmental conditions, an insect's survival could be increased or decreased. If molting is occurring between populations, it is possible that speciation can occur from reproductive isolation if the temperature is challenging the timing or duration in which a molting period occurs (Tawfik, 2012). Arthropods' thermal optimums are reviewed in a study of the impacts of climate change on terrestrial ectotherms. Thermal optimum is where the insect critical maximum is the temperature an organism reaches before issues occur (Deutsch, 2008). Many studies demonstrated that greater temperatures increased the production and release of ecdysone, causing more frequent molts (Tawfik, 2012).

Temperature is a key ecological factor in biological functions at every organizational level of life This means that for insects it could have effects on survival, reproduction, growth, and development rates (Deutsch, 2008). Heatwaves alter these significantly.One study found that even a 10-degree Celsius increase in temperature can increase ecdysteroid levels (Tawfik, 2012). The long-term effects of repeated increased temperatures remained unclear. Specifically, if the insect body mass of larvae is larger will influence the number of days to molt during a heat wave. Instar stage 3 is more developed and has a higher body mass than instar stage 2.

Past studies have tested the effects of increasing temperature on *Manduca sexta* arthropod molting in larvae. Specifically focused on measuring ecdysteroid concentration at high temperatures of 35-38 degrees Celsius for an acute period of 6 hours, and at more mild temperatures of 20-25 degree Celsius, the organism's thermal optima for days (Tawfik, 2012). Larvae size and weight were accessed as well as the time to determine temperature effects on growth and development. By using an ANOVA, researchers determined a significant correlation between ecdysteroid becoming triggered by temperature ($r = 0.6$, $P < 0.5$) (Krzysztof, 2020). The study determined that heat wave exposure caused antlions to become more sedentary, disrupting predatory habits and causing a decrease in body mass and mortality. It was inconclusive whether researchers studied the relationship between antlion species' instar stage initial body mass and if the days to molt are influenced by instar stage 2 larger body mass.

It can be hypothesized that prolonged exposure to high temperature will not only affect the antlion's days to molt but the number of days it takes for second instar larvae to molt after the simulation is longer when the initial body mass of the larvae is larger in *Myrmeleon bore* and *Euroleon nostra*. Regression analysis can compare the relationship between larvae' initial body mass and days to molt. A multiple regression or ANOVA can assess the effect initial body mass has on days to molt and the ANOVA can report the mean variances in addition to the r-squared value from the regression. For these multiple variables such as the initial body mass, days to molt, and heatwave treatment can be analyzed. Lastly, to meet the assumptions that this statistical analysis, residual and QQ plots are used to make sure the relationship between variables is linear and that data is distributed evenly.

MATERIALS AND METHODS

Myrmeleon bore and *Euroleon nostras' l*arvae developmental instar stages two and three experienced heatwave simulation of 40 degrees celsius for 10 hours and at a control of 25 degrees celsius, the arthropod's thermal optima for a week. The number of arthropods tested for each type of larvae was N=30. Antlions were transported to the laboratory from the Bledosa Desert in Poland (50º20'24′'N, 19º32′20′'E). Insects were weighed in milligrams (mg) upon arrival, raised in the laboratory to collect a timeframe between molting periods, and weighed again at the conclusion of the study.This was done by recording the time for every antlion's molt and what stage the arthropod was in. Thermal cabinets were then used to generate a heatwave simulation for both species (*M. bore* and *E. nostras*). The number of days in which molting ecdysis occurred after each simulation was assessed. Molting larvae were defined in three different stages: pre-ecdysis (instar 1), ecdysis (instar 2), and post-ecdysis (instar 3).

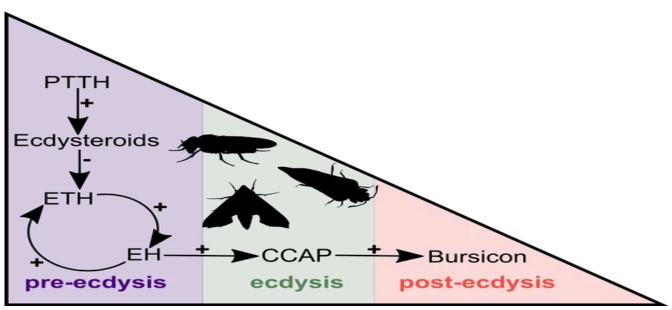


Figure 1: Simplified Map of Neuro/Hormone Insect Molting (De Oliveira, 2019)
PTTH signals ecdysteroids by environmental stimuli causing a cascade of hormones in a negative feedback loop. Each molting stage of insect larvae is referred to as an "instar" (Krzysztof, 2020).

In this study, Krzysztof's data of antlion molting was imported as a csv.file into R version 4.13 Programming Software from Dryad. The data frame was viewed and summarized using 'head()' and summary() functions. A summary table analyzed the central measures of the tendency for species, instar stage, simulation treatment, and days to molt. Datasets were merged. The 'lm()' function was used to create a linear regression. Data was reviewed in relation to molting, thermal simulation, and weight records. The 'aov()' function was used to create an analysis of variance (ANOVA) that replicated Krzysztof's. The 'qqnorm()' and 'qqline()' were used to check assumptions and limit bias. This was not included in Krystof's research. Each stage of ecdysis was recorded thoroughly and subjects were kept at thermal optima temperature as a control while others were subjected to fluctuating and experimental temperature ranges. This process reduces bias in data by ensuring that arthropods were randomly assigned to different treatments and that thermal optima of antlion species were used as a control to compare treatments.

RESULTS

The mass and molting activity of larvae at different instar stages for species is compared with constant and fluctuating temperatures. Treatment 1 is the control value, 2 is fluctuating treatment, and 3 is the heatwave simulation. Heatwave exposure increased the time to molt in the second instars of *E. nostras* but not of *M. bore*. The average time for both species *M. bore* and *E. nostras* to molt was 20.64 days (Figure 3). The maximum time till molt was recorded for instar stage 2 at 44 days in treatment 3 of prolonged heat exposure at 40 degrees Celsius. Antlion's at thermal optima, treatment 1 recorded the fastest days to molt with a mean of 15.75 days (Figure 2). Figures 2 and 3 only observed instar stages and excluded insect body mass.

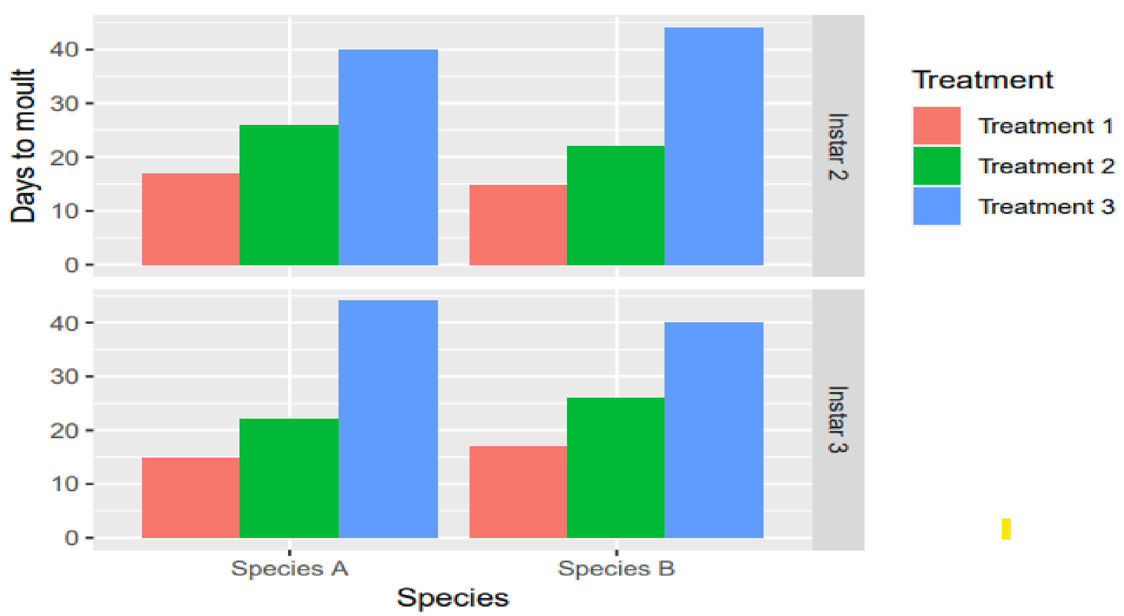


Figure 2: Molting Time between *M. bore*, Species A and *E. nostras*, Species B in Heatwave Simulation

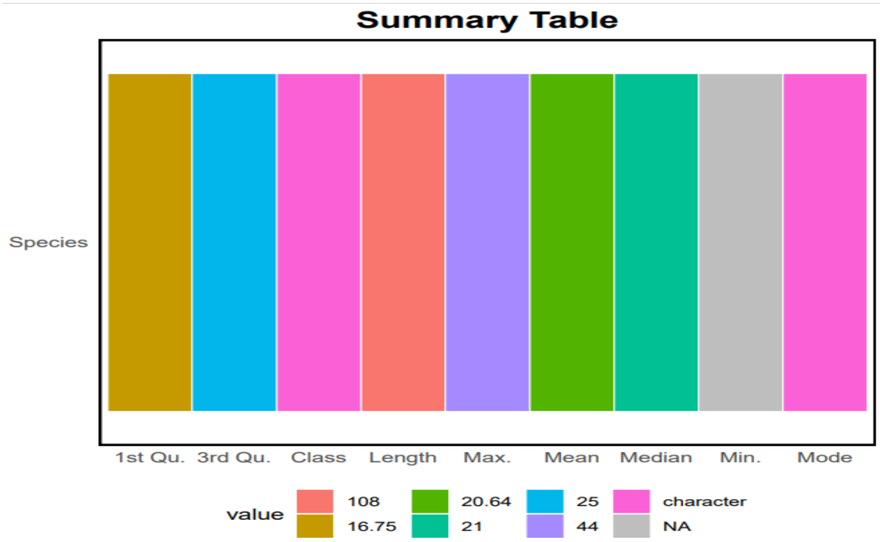


Figure 3: Measures of Central Tendency for Molting Time of Antlions

The findings of the linear regression analysis found that there is no relationship between initial body mass and days to molt in both *Myrmeleon bore* and *Euroleon nostras'* antlion species. The p-value has a greater significance than 0.05 and the coefficients of each intercept are at 0 (Table 1). The r-squared value was very small and did not show a linear relationship. (Table 1) Additionally, the model created in R supported no correlation with a horizontal line at zero between initial mass and days to molt.

Summary of Findings of Linear Regression Analysis in R
Residuals: **Minimum** -19.2, **1st Quartile** -4.78, **Median** -0.12, **3rd Quartile** 4.88, **Maximum** 22.9 **Residual Standard Error:** 8.335 on 64 degrees of freedom
Coefficients: **Estimated Standard Error t value Pr(>[t])** **Intercept** 21.222135, 1.650802, 12.856 **mass_prior** -0.001262, 0.012438, 0.101
Significance: **p-value:** 0.9195
R-Squared Value: **Multiple R-squared**: 0.0001609 **Adjusted R-squared:** -0.01546
F-Statistic: 0.0103 on 1 and 64 DF

Table 1: Results of Linear Regression of Relationship between Initial Mass and Days until Molting

The multiple regression proved similar results with an insignificant p-value of 0.07103 and a small r-squared value of 0.063129 (Table 2). There is a negative relationship between the initial mass and days to molt (Table 2). However, the p-value indicates that this relationship is not statistically clear. A significance of 0.02 is seen between “treatment x” and “y” in regard to the control and experimental simulations. In the heatwave simulation, treatment y took longer to molt than control treatment x.

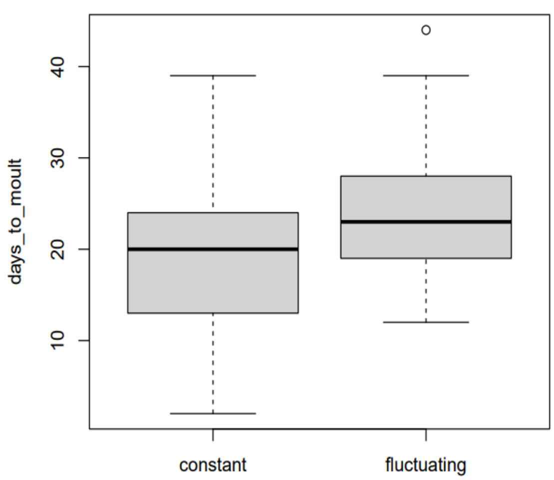
```
> str(merged_data)
'data.frame':   66 obs. of  12 variables:
 $ ID          : int  1 2 3 4 5 6 7 8 9 10 ...
 $ species.x   : chr  "bore" "bore" "bore" "bore" ...
 $ instar.x    : chr  "second" "second" "second" "second" ...
 $ treatment.x : chr  "constant" "constant" "constant" "constant" ...
 $ mass_prior  : int  89 94 99 102 106 122 129 143 91 94 ...
 $ mass_after  : int  115 119 127 124 137 144 136 151 98 106 ...
 $ days_active : int  7 7 7 7 7 6 6 7 7 7 ...
 $ change_mass : int  26 25 28 22 31 22 7 8 7 12 ...
 $ species.y   : chr  "bore" "bore" "bore" "bore" ...
 $ instar.y    : chr  "second" "second" "second" "second" ...
 $ treatment.y : chr  "constant" "constant" "constant" "constant" ...
 $ days_to_moult: int  11 19 9 39 5 25 6 44 19 17 ...
```

Figure 4: R Data Frame for Multiple Regression for Initial Body Mass, Days to Molt, and Treatment

Summary of Findings of Multiple Regression Analysis in R
Residuals: **Minimum** -15.81, **1st Quartile** -5.58, **Median** 0.14, **3rd Quartile** 5.10, **Maximum** 19.34 **Residual Standard Error:** 8.006 on 62 degrees of freedom
Coefficients: **Estimated Standard Error t value Pr(>[t])** **Intercept** 20.158004, 2.131585, 9.4571.23e-13 **mass_prior** -0.004538, 0.012382, -0.367, 0.715 **Treatment.xfluctuating** -2.099117, 2.082564, -1.008, 0.317 **Treatment.yfluctuating** 5.151349, 1.991024, 2.587, 0.012
Significance: **p-value:** 0.07103
R-Squared Value: **Multiple R-squared**: 0.1064, **Adjusted R-squared:** 0.06312
F-Statistic: 2.46 on 3 and 62DF

Table 2: Results of Multiple Regression of Relationship between Initial Mass, Days until Molting, and Treatment

Only a relationship between molting and simulation is present, rejecting that prior mass of antlions influences time to molt. An ANOVA was used in Krzysztof's et al. research but the assumptions of the ANOVA and other analyses are reviewed to limit bias by a residual and QQ plot. The ANOVA's p-value is statistically clear at a value of 0.0122 while evaluating the means of variance between the days to molt and heatwave simulation. The ANOVA is recorded in a box plot.



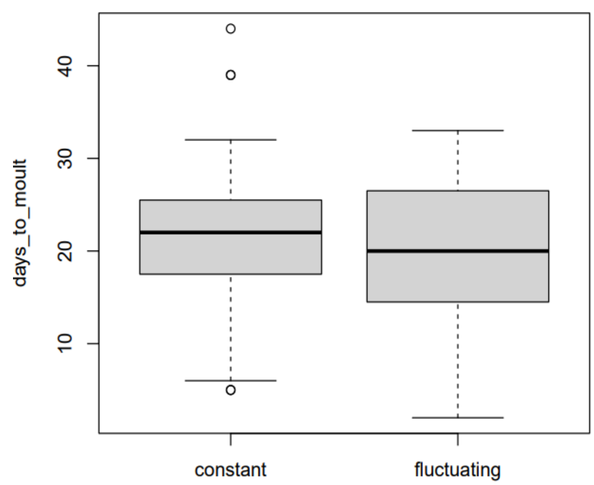
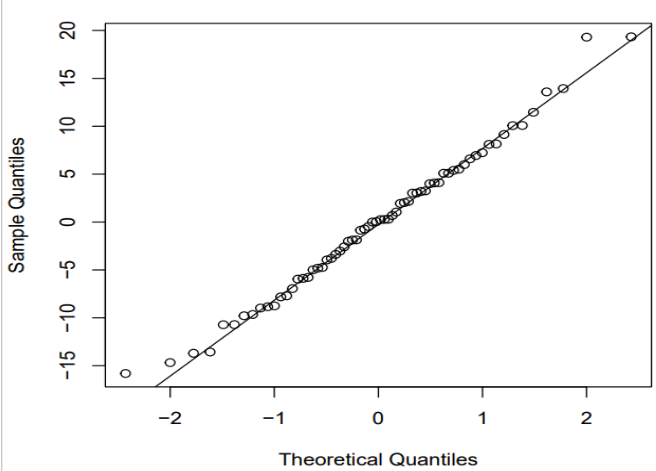


Figure 5: Variance between Control and Heat Wave Simulation to Days to Molt in *M. bore*
Figure 6: Variance between Control and Heat Wave Simulation to Days to Molt in *E. nostras*



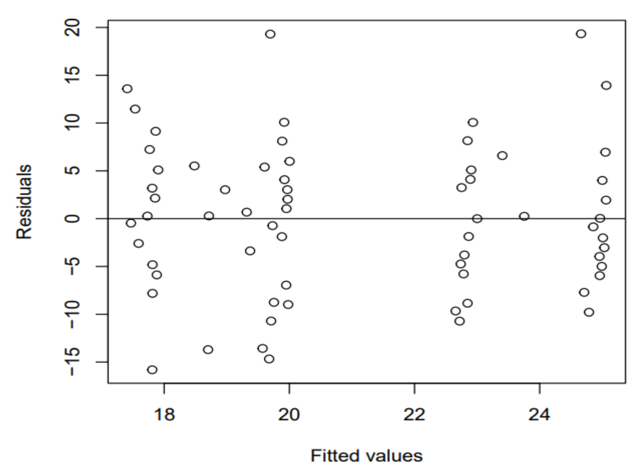


Figure 7: Q-Q Residual Plot

Figure 8: Residuals vs. Fitted Values

The Q-Q residual plot shows a linear relationship and normal distribution between the sample and theoretical quantities (Figure 7). The points in the Residual vs. Fitted values show a distribution around 0 but also have points that appear to be outliers of negative residuals and of positive residuals. The plot shows heteroscedasticity. Both of these statistical analyses assess the validity of the ANOVA.

DISCUSSION

Prolonged exposure to high temperature can cause an extended length of time to molt in *Euroleon nostras* instar stage 2 but not in *Myrmeleon bore* antlion arthropods. The number of days it takes for second instar larvae to molt during heatwave simulation did not have a correlation to the initial body mass of the larvae according to regression analysis. The QQ-test and residuals vs. fitted values showed ANOVA test validity. The QQ-test met assumptions for independence and normal distribution of data but the residual vs. fitted values showed a heteroscedasticity which is not an assumption. The multiple regression also read that mass was not a factor but there was a clear difference between the time to molt between the control and heatwave simulations.

Higher instar stages are more developed so they were assumed to have higher mass. High temperatures can disrupt instar stages causing higher mortality in heatwave simulation. Stage 2 instars exposed to heatwave simulation had the highest time to molt and most molting abnormalities. This type of disruption caused incomplete molting thus causing insect abnormalities (Krzysztof, 2020). However, the regression analysis indicates that relying on temperature and initial mass as the only determining factor of molting is not ideal. Reasons, why this test was not significant under laboratory conditions, could be a result of other environmental factors such as light exposure or nutrition. Statistical analyses have limitations and correlation tests do not always include other factors that could affect molting.

It is also important to understand the evolutionary forces that could influence this result.

While past studies demonstrate that arthropods in general are sensitive to temperature and have critical points within their thermal optima, the species referred to in this particular study may have thermal adaptation due to selection. These species were collected from a desert ecosystem, suggesting that over time they became better adapted to their environment enhancing fitness. This ability to adapt may be ideal if the species population had genetic variation. As a result of this, temperature could be dependent on the species within a phylum.

Another limitation of this project is the sample size as this project only focused on two species of the most abundant phylum of arthropods on Earth. To better understand the effects of prolonged high temperatures on arthropods, a wider variety of organisms needs to be studied. There is still evidence that temperature does have some effect on molting since ectotherms depend on external temperature to regulate their biological process.

One study examined that crustacean arthropods exposed to prolonged temperatures had claw deformities after ecdysteroid samples were reviewed in lobsters after being subjected to 30-degree celsius temperatures for 42 days. The lobsters that were exposed to higher temperatures had more abnormalities as a result of molting disruption than lobsters who were exposed to lower temperatures at 20 degrees Celsius (Schmidt 1991). Crustaceans use their claws for courtship and defense. With an increase in mortality or abnormality in any species, pre and post-zygotic barrier likelihood increases.

Regardless of the hypothesis, larvae development and the molting process can be better understood by analyzing data. Arthropods serve many ecological roles. Without them, ecosystems would become disrupted making conservation strategies for species essential. The next steps for researching the causes of molting that are not only related to prolonged temperature are identifying other factors such as exposure to pollution or precipitation. By identifying these factors temporal variation can be accessed more thoroughly. To continue this study, a phylogeny can be constructed to review the thermal optima between closely related species and a larger sample size collection can occur. This way thermal optima can be distinguished between different lineages. Time bias can be eliminated by studying larger samples in the population over periods of time. This can be achieved by monitoring the reproductive success and survival of molting insect populations.

Following these actions, researchers can gain additional knowledge of how temporal variation influences arthropod thermal optimum and evolution.

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