# A Growth Analysis and Review of Manduca sexta from Egg to Moth



Photograph of the Manduca Moth that was raised for this analysis

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#### **Abstract**

This growth analysis was completed as part of a semester long project for a UW-Madison entomology course. The purpose of the project was to raise at least one Manduca caterpillar from egg to moth. As part of the requirements a daily journal was made with observations of the Manduca caterpillars as well as weekly observations reported from 2 other group members completing the same project. As well as the daily journal, photographs were taken anytime the Manduca was changing, as well as of the setup and procedures followed in the project. Finally, volume and weight measurements were taken daily during the caterpillar stage to track the growth, which is what the main analysis focuses on. The growth trends were graphed and will be discussed. The main features of each instar as well as overall features were that both the weight and volume appear to be exponential functions of time. Finally, once the moth emerged, it was submitted for grading and then released.

#### Introduction

The goal of this project was to successfully raise an egg into a Manduca caterpillar, and then into a moth. Throughout the process, the eggs, caterpillars, pupae, and moths were photographed, logged daily and (when possible) weighed and measured for comparison. Within this analysis there are daily pictures of every stage, raw data from measurements, graphs fitting the data to curves, and a daily log that tracks the progress as well.

#### Method

## **Subjects**

The project began with three Manduca sexta eggs that came from one or more female moths. These eggs were only a few days old, but could be of different ages and from different females amongst themselves.

#### **Apparatus**

The eggs and caterpillars were kept in plastic Tupperware containers. All three were put into one container, until later in the project when they were separated which will be addressed in more detail in the procedures section. Once the caterpillars were ready to pupate, they were moved into three individual containers, 2 different, 2-liter soda bottles and a bug-terrarium approximately .016 cubic meters in volume. These containers would also serve as the vessel for which they emerged as moths in.

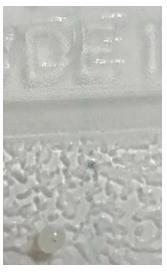
In addition to the housing, there were a number of other instruments used. A 20g scale with an uncertainty down to 1 mg was used to weigh the caterpillars daily. A standard ruler was used with marks down to the half millimeter to measure the length and width of the caterpillars daily. Forceps were used in the first instars while the caterpillars had horns to easily move them. Finally, wheat germ diet was used as the food and water source through out the instars, something that will be discussed further in the procedures section.

#### **Experimental Procedures**

The procedures section will be presented by sub-sections that are in chronological order, broken down by the natural stages of development.

#### **Egg Stage and Experiment Set-up**

Three eggs were received in a plastic vial on January 20th, 2017, as well as a plastic container to keep them in (as seen in Figure A). Once the environment that the caterpillars would be raised in was set-up, the eggs needed to be very carefully moved from the vial to the container. The eggs were put into a larger plastic container rather than the smaller one that was provided, so the caterpillars would have more room to grow and would be easier to clear out. The container needed to be in an area that had plenty of sunlight and a temperature that was approximately between 65 and 80 degrees Fahrenheit. In addition, it needed to be a relatively humid environment. The first set-up that was made was the one as seen in Figure B below. The relative humidity was around 25% and the temperature was 68 degrees. A desk lamp with an LED bulb (which would later be replaced) was set near the enclosure. When the eggs needed to be transferred to the larger container from the vial, very special care was needed, as the eggs are easily broken and lost. They have a tendency to bounce if dropped. Forceps were used very carefully to place the eggs into the container away from one another. Another major concern in this step, is that the eggs can easily drown in condensation on the side of the container. Therefore, the container had holes poked into the top for ventilation and was observed closely and wiped out if condensation started to form. The relative humidity made it so that this step was never needed in this experiment.



**Figure A:** One the eggs on the bottom of the plastic container. Note the size as compared to the lettering on the container

**Figure B:** The first general set up of the experiment. Note the desk lamp for light and warmth and the container.



The eggs were received a couple days old from several different mothers. They were supposed to hatch about 5-10 days after being received. After 7 days of having the eggs they still had not hatched. It is at this point that the set-up was altered. The container was placed in a larger bug-terrarium (as previously mentioned in the Apparatus section) and placed in a very small bathroom. The bathroom had tightly closing doors as well as a skylight. The LED bulb in the lamp was switched for an incandescent that would provide heat as well as light. The doors to the bathroom were kept shut constantly. This provided a temperature boost to around 78 degrees. The humidity was relatively low at about 28%. The very next day, on day 8 of the project, January 27th, 2017 the eggs had hatched. It should also be noted that a preliminary measurement of the egg's diameter was used to approximate its volume, this will be discussed more in the results section. The modified set-up can be seen in Figures C and D below.





**Figure C** (to the left): A close up of the modified setup in the closed bathroom. The plastic container is placed inside the bug-terrarium, and the light is placed over head with an incandescent bulb.

**Figure D** (above): A more general picture of the set-up. With the mirror lights on, the room reached around 83 degrees. This was too hot for the normal day, but if it was particularly cold, they could be turned on.

#### **Caterpillar Stage and Data Collection**

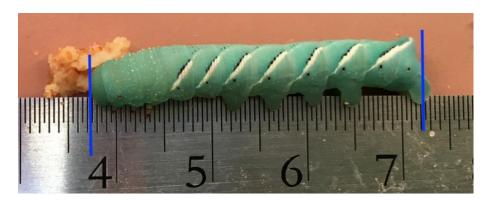
Once the eggs hatched into caterpillars they are put on a wheat-germ diet. The diet was provided. New diet was picked up every Monday, Wednesday and Friday and stored in a refrigerator in a sealed container. It was good for 1 week kept this way. The diet was switched out once it became dry, approximately every other day. The diet is

the only source of water for the caterpillars and therefore it was essential that it was kept moist. The caterpillars were free to roam around the container but mostly stayed on the diet cube.

Every day at the same time, the caterpillars were taken off of the diet cubes, weighed, and measured. They were then put back on the same diet if it was still moist, or put onto a new cube if it was not. This was also when the container was cleaned out. The frass would be thrown away in the trash and the container was wiped clean with a wet paper towel. It was important to be sure that the container was dried out completely before the caterpillars were put back in as in the beginning they were still small enough to drown in a drop of water.

The measurements were done in the same manner everyday. The caterpillars were weighed daily. Initially, they did not weigh enough by themselves to get an accurate reading, so they were weighed together and the average was taken as their individual weights. After a few days they were then weighed individually. They were also measured using a ruler. They were set on top of the ruler and their lengths and diameters were calculated from the picture taken of them on the ruler. This procedure can be seen in figures E and F below. The daily photographs can be seen in Appendix C, and the compellation of the raw data can be seen in the table in Appendix A.

**Figure E:** A photograph from Feb  $17_{\text{th}}$ , 2017 showing how the length measurements were done. The caterpillar was photographed while laying still along the ruler. The photo was then expanded on the computer, and lines were added to obtain a better measurement of the length.





**Figure F:** A photograph from Feb  $17_{th}$ , 2017 showing how the diameter measurement was done. This measurement is combined with the length to idealize the caterpillar as a cylinder, which will result in an approximate volume. It can be seen that it was very difficult to get the caterpillar to be perpendicular to the ruler. Thus, a different procedure was developed. The caterpillar was photographed anywhere on the ruler. On the computer a circle was lined up so that the diameter was the width of the caterpillar. The circle was then copied onto the ruler marks and read off.

The caterpillars completed 5 instars, with the first starting once they hatched from the eggs. Each instar was separated by the molting of the outer skin as well as some features of the caterpillar including the mandibles, spiracles, and horn on the tail. The timing for the 5 different instars can be seen in Figure 1 in Appendix A. At the point of the 5th instar, the caterpillars were about 1g in weight and would eat continuously for about 5-10 days and gain an order of magnitude in weight bringing them to about 10g before they buried themselves to pupate.

#### **Pupa Stage and Habitat Changes**

The fifth instar is the last instar before the caterpillars will bury themselves and pupate after which they will emerge as moths. During the fifth instar the new habitats had to be prepared and ready for when the caterpillars were ready to bury themselves.

In this experiment two different types of habitats were made, with 3 habitats in total. This was due to 2 different issues that could arise. First, the caterpillars will sometimes not want to bury themselves next to others, and so if there is already a pupa that is buried the second Manduca will not bury itself. Secondly, they needed to be taken out of the dirt 10-15 days after going in, causing an issue if two were going to be buried in the same habitat but needed to be taken out at different times. The two habitats can be seen below in figures G and H. Both were filled with organic potting soil that had no pesticides in it and only organic fertilizer. The soil was misted daily before they were ready to pupate to ensure that it was damp but not wet when the caterpillars were ready to go. The soil needed to be about 10-15 cm deep, as the caterpillars like to dig down far to bury themselves. On the third container a small soda can was emptied and placed in the middle as to prevent the caterpillar from going down the middle. This was in hopes that it would create its hole along the side and the progress could be tracked through out the pupal stages. Unfortunately, this did not work and the pupa still could not be seen.



**Figure G:** A photograph from Mar 3<sub>rd</sub>, 2017 showing the soda bottle that was used as one habitat. Another soda bottle identical was used as the third habitat.



**Figure H:** A photograph from Feb 17th, 2017 showing the Bug terrarium as previously mentioned in the set-up that was used as a habitat.

There were very distinctive signs that needed to be watched for to indicate that the caterpillar was ready to bury himself in the soil. They were to be around 10g. This is necessary as this is the last time that the caterpillar will eat before it completes metamorphosis into a moth and needs to be big enough to sustain itself during the process and for a short period after until the moth can get to a food supply. As well as being about 10g, the caterpillars when ready will have a very exposed backside, where it possible to see the heart beating. This is the most telling sign that the caterpillar is ready. This can be seen in figure I to the right. As well as these first two signs, the caterpillars will also become very restless and stop eating when they are ready to bury themselves. They will thrash their heads from side to side, and wander around a lot. In fact, this stage is often referred to as the wondering stage for this



**Figure I:** A photograph from Mar  $3_{rd}$ , 2017 showing the white color of the caterpillar that is ready to pupate. As well it is easy to see the heart along the line of the backside. In person the heart will clearly be beating. This sign is the most important for telling when it is ready to pupate.

reason. The final way to know if it is ready to pupate is that the color will be very white, which can also be see in

figure I as compared to the green-blue color in figure E. Once they are ready to pupate they need to be transferred to their new habitats as seen in figures G, H, and I.

The caterpillars were set on top of the soil and within a few minutes had begun to dig down to bury themselves. Once they were buried, there was no need to keep the light on anymore as they were under the soil. It is possible to observe them pupating if they make their hole near the side. However, none of the three caterpillars that pupated did this. Once they are in the soil they will remain there for 10-15 days, after which they will be unburied.

After 14 days the caterpillars were dug up. It is important not to dig them up prematurely, as they will not have formed properly and can be inhibited from doing so. They were gently dug up and set on top of a thin layer of



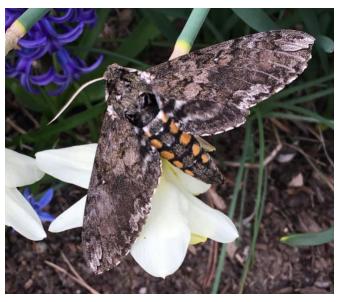
soil in the bottom of the large terrarium together. Two of the three caterpillars looked like they had formed a nice outer coat of a red-brown color that was solid all of the way around. However, one of them had not. The largest caterpillar who was also the first to pupate developed a defect on his coating as seen in figure J to the left. Once pulled out of the dirt it was important to put a few sticks into the enclosure so that the moths could climb up and inflate their wings once they emerged. This would take 2-4 weeks. Of three caterpillars, only one emerged. The issues with the other two will be discussed next. The one that successfully emerged was the medium sized one, who pupated on March 1st. This caterpillar then emerged on April 8th, 2017.

**Figure J:** A photograph from Mar 20th, 2017 of the largest caterpillar who was unburied. It had developed a defect in the outer coating as seen in the picture, below the proboscis. He lived for a couple weeks, but would die before becoming a moth.

Two of the three pupae died. The first to die was the one in figure J. It is plausible that they died from the defect in the coating. This caterpillar may have just not metamorphosed correctly. The second caterpillar that died was the smallest of the three. It was doing very well until one day it had cracked its outer coating open on the lower end where there are concentric creases. I do believe that this caterpillar would have been fine and turned into a moth if this had not happened. It is unknown why this occurred. It could be that it was too dry in the room. Or perhaps it got too cold at night. The specific reason is unknown, however.

#### **Emergence as a Moth**

The moth that did successfully emerge can be seen in figure K to the right. It was placed in one of the soda bottles and transported to the university for grading before being let go outside. It emerged on a Saturday and was not able to be transported until Monday. Because of this long time period, it was feared that it would not live. A dilute sugar-water mixture was placed in the cage for the moth to be able to eat while waiting. This mixture can be seen in figure L below in the orange dish. The moth did drink this, and was still alive on April  $10_{th}$ , 2017 when it was taken in. It also was alive when released in the gardens on campus.



**Figure K:** A photograph from Apr  $10_{th}$ , 2017 of the moth that is being released outside. This moth was raised from egg to caterpillar to pupa and moth over the course of a couple months.



**Figure L:** A photograph from Apr  $8_{th}$ , 2017. The moth can be seen on the middle of the left side. The orange container has a very dilute sugar water mixture in it for the moth to feed on. The other two pupae can be seen as well under the stick in the cage.

#### **Results**

The results that will be discussed will be in reference to the raw data collected as seen in Appendix A and the graphs that have been made as seen in Appendix B. While the graphs are for the largest Manduca caterpillar the data is for all three and each of the caterpillars followed similar growth trends just at slightly different paces.

The first graph that was made was for the density of the caterpillar. The purpose of this was to check the validity of the data. As can be seen from the graph, it appears that with the exception of the first 4 data points, the others are in agreement with each other. We expect that the density will be relatively constant with a slight slope possible as the caterpillars change body composition and consume more and more food. It is clear, though, that the first 4 data points are not very valid and the results from them should be taken lightly if at all.

The first set of six graphs are of the volume vs time. The time is measured in days since hatched and the volume is estimated as idealizing the caterpillars as cylinders and taking their diameter and length daily. The measurements were taken at the same time everyday, 5pm. What is expected is that the overall growth is exponential, which can be seen clearing in figure 3. The individual instars have a slightly different shape to them. They start off more level, grow rapidly and then level off. These leveling off trends are around the time that they are molting or have just molted. This is a characteristic that is known and expected to be seen. As such, it has been experimentally verified to be a trend in this study as well, as seen by all of the volume and weight graphs in Appendix B.

The second set of six graphs are of the weight vs time of the caterpillar. These graphs share the same trends as the volume, as one would expect if the density is relatively constant. The weight, overall, looks very exponential. As well, in the individual instars, we see the characteristic leveling off around the molts. Over the course of the study the caterpillars would go from about 3mg to 10g. This is a factor of about 3000!

The final set of six figures are of the natural log of the volume and weight as a function of time. This was to see if the growth was in fact exponential. If it is we expect to see a straight line on the plot of the natural log vs time. The trend does look, overall, linear. There are little wobbles in it, however that makes sense given that we see this levelling off trend during the molting and switching of instars. On the individual graphs of the instars however, the  $R^2$  values are close to 1, indicating that a linear fit was a pretty good approximation and they are about exponential.

It is also worth noting that the approximate volume for the egg was calculated, as well as the approximate volume of the caterpillar first after emerging. The later volume is very slightly less, as would be expected! The egg shell had a bit of volume to it as well the caterpillar was packed tightly in there but not perfectly. This is a nice reassurance on the approximations that were made for the egg size and the caterpillar size when first emerging. As well as the cylindrical approximation.

Overall, we did see the trends in the data that we expect to see. There is an overall tendency of exponential in both the volume and weight vs time graphs. As well there is the characteristic leveling off of the curves around the instars and the molting. Finally, it should be noted that from instar 5 they went from around 1 g to 10 g which is the characteristic magnitude of 10 that the caterpillars exhibit as they are getting ready to pupate.

#### **Discussion**

The results of the experiment confirmed trends that were expected to be seen as discussed in the results section. As well the goal of the project was to raise a caterpillar from egg to moth which was successfully done. While there was an opportunity to obtain new specimens if these failed, they did not. Although, it is quite a high fail rate that 2/3 of the pupae did not emerge as a moth, it is a high success rate that all three of the eggs that were provided entered into the pupa stage. Overall the project was a success and the analysis was as expected and enlightening.

#### **Appendices**

# **Appendix A:** Raw data from Instars

This appendix shows a table of the raw data from each day from egg to pupa.

# Appendix B: Graphs from raw data

This appendix shows graphs that were created from the raw data for the largest caterpillar. These graphs track growth trends throughout time as well as fitting trends to them with equations as seen on the graphs.

# **Appendix C:** Photo Log

This appendix shows photographs that were taken of the set ups, as well as other pivotal points in the project. There are also daily photographs of the caterpillars throughout the 5 instars.

# **Appendix D:** Daily Journal Entries

This appendix is of the daily log entries that were made to track the progress of the caterpillars and compile thoughts and observations on the project as well as procedures. It can be seen how some procedures were developed or changed as was needed. As well weekly entries were added from the other 2 group members who participated in the project.

# Appendix A: Raw data from Instars.

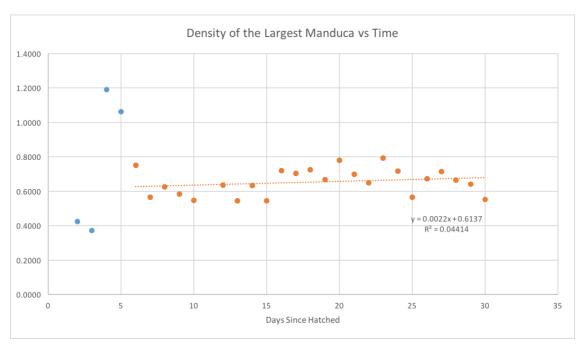
Appen	0.17	. ,		u vv	a	ato	1 11	011		150	.ui.	J.																								
Density of large one				0.4244	0.3725	1.1904	1.0610	0.7502	0.5649	0.6249	0.5838	0.5458		0.6371	0.5452	0.6334	0.5435	0.7202	0.7046	0.7255	0.6673	0.7790	0.6985	0.6478	0.7921	0.7167	0.5643	0.6714	0.7151	0.6640	0.6421	0.5514				
Weight bigRate of Change (mg/day)					1.0000	5.3333	-1.0000	1.0000	0.6667	6.3333	7.6667	-2.3333		16.3333	17.0000	24.0000	-6.0000	39.0000	67.0000	121.0000	21.0000	92.0000	308.0000	345.0000	496.0000	-67.0000	485.0000	1341.0000	2014.0000	1881.0000	2206.0000	1387.0000				
In(weightbig one)				1.0986	1.3863	2.2336	2.1203	2.2336	2.3026	2.7932	3.1781	3.0758		3.6376	4.0073	4.3694	4.2905	4.7185	5.1874	5.7038	5.7714	6.0234	6.5806	6.9717	7.3537	7.3099	7.5909	8.1080	8.5820	8.8841	9.1508	9.2881				
Weight big one (mg)				3.0000	4.0000	9.3333	8.3333	9.3333	10.0000	16.3333	24.0000	21.6667		38.0000	55.0000	79.0000	73.0000	112.0000	179.0000	300.0000	321.0000	413.0000	721.0000	1066.0000	1562.0000	1495.0000	1980.0000	3321.0000	5335.0000	7216.0000	9422.0000	10809.0000				
Weight percent difference					0.2857	0.8000	-0.1132	0.1132	0.0690	0.4810	0.2018	0.0800	-2.0000	2.0000	0.3930	0.3138	0.2430	0.0684	0.3862	0.5054	0.2119	0.2302	0.3290	0.3907	0.4436	0.2097	0.1963	0.3040	0.4266	0.3767	0.3271	0.1698				
Weight Rate of Change (mg/day)					1.0000	5.3333	-1.0000	1.0000	0.6667	6.3333	3.6667	1.6667		00000:6	15.0000	17.0000	17.3333	2.6667	41.0000	85.6667	50.3333	68.3333	130.3333	224.0000	390.6667	252.0000	289.0000	579.6667	1191.3333	1572.6667	1939.6667	1280.3333				
Average Weight (mg)				3.00	4.00	9.33	8.33	9.33	10.00	16.33	20.00	21.67		30.67	45.67	62.67	80.00	85.67	126.67	212.33	262.67	331.00	461.33	685.33	1076.00	1328.00	1617.00	2196.67	3388.00	4960.67	6900.33	8180.67				
Weights of biggest (mg)											24.00			38.00	55.00	79.00	73.00	112.00	179.00	300.00	321.00	413.00	721.00	1066.00	1562.00	1495.00	1980.00	3321.00	5335.00	7216.00	9422.00	10809.00				
Weight of middle (mg)											19.00			26.00	46.00	00.99		86.00	124.00	216.00	295.00	269.00	403.00	642.00	1078.00	1509.00	1492.00	1947.00	3253.00	5199.00	7127.00	8440.00	9176.00	9207.00		
Weight of smallest (mg)											18.00			38.00	36.00	43.00		29.00	77.00	121.00	172.00	311.00	260.00	348.00	588.00	00.086	1379.00	1322.00	1576.00	2467.00	4152.00	5293.00	6424.00	7388.00	9105.00	9732.00
Percent difference from previous day			1.0423	0.9091	0.4121	-0.3119	0.0018	0.4520	0.3491	0.3848	0.4453	-0.0351	0.0000	0.4016	0.5138	0.2115	0.0739	0.1463	0.4811	0.4778	0.1510	0.0971	0.6428	0.4581	0.1804	0.0562	0.5086	0.3401	0.4052	0.3719	0.2979	0.2877				
Volume Rate of Change (cubic mm/day)			1.8162	4.4179	3.6693	-2.8976	0.0137	4.5867	5.2622	8.4352	14.9736	-1.4176	0.000	19.9471	41.2353	23.8545	9.5720	21.2057	98.5183	159.4848	67.5442	49.0873	502.1297	613.3802	326.2345	114.1281	1422.6494	1437.8910	2513.5420	3407.9183	3804.5833	4930.9219				
Approx Vol Calculated (cumbic meters)	1.023E-09	8.34485E-10	2.65072E-09	7.06858E-09	1.07379E-08	7.84023E-09	7.85398E-09	1.24407E-08	1.77029E-08	2.6138E-08	4.11116E-08	3.9694E-08	3.9694E-08	5.96411E-08	1.00876E-07	1.24731E-07	1.34303E-07	1.55509E-07	2.54027E-07	4.13512E-07	4.81056E-07	5.30143E-07	1.03227E-06	1.64565E-06	1.97189E-06	2.08602E-06	3.50867E-06	4.94656E-06	7.4601E-06	1.0868E-05	1.46726E-05	1.96035E-05				
Ln(volume in mm)	0.0227	-0.1809	0.9748	1.9557	2.3738	2.0593	2.0610	2.5210	2.8737	3.2634	3.7163	3.6812	3.6812	4.0883	4.6139	4.8262	4.9001	5.0467	5.5374	6.0247	6.1760	6.2731	6.9395	7.4059	7.5867	7.6430	8.1630	8.5064	8.9173	9.2936	9.5937	9.8835				
Approx Vol Calc (Cubic mm)	1.0230	0.8345	2.6507	7.0686	10.7379	7.8402	7.8540	12.4407	17.7029	26.1380	41.1116	39.6940	39.6940	59.6411	100.8765	124.7309	134.3030	155.5087	254.0270	413.5118	481.0560	530.1433	1032.2730	1645.6532	1971.8877	2086.0158	3508.6652	4946.5562	7460.0982	10868.0164	14672.5997	19603.5216				
Uncert. in Diameter (mm)		0.25	0.1	0.1	0.15	0.15	0.15	0.1	0.15	0.15	0.1	0.15	0.1	0.25	0.15	0.25	0.5	П	0.5	0.5	1	0.5	0.25	1	1	1	1	1	1	1	1	2				
Diameter Measured (mm)		0.5	0.75	1	1.25	1.1	1	1.2	1.4	1.6	1.9	1.9	1.9	2.25	5.6	2.75	3	33	3.75	4.5	5	5	6.15	7.5	8.5	8	9.5	10.75	11	14	14.25	16				
Uncert. in Length (mm)		0.25	0.1	0.25	0.25	0.25	0.25	0.5	0.5	0.5	0.25	0.25	0.5	0.5	0.25	0.25	2	2	2	1	2	1.5	2	2	3	5	3	2	10	3	2	15				
Length Meas. (mm)		4.25	9	6	8.75	8.25	10	11	11.5	13	14.5	14	14	15	19	21	19	22	23	26	24.5	27	34.75	37.25	34.75	41.5	49.5	54.5	78.5	9.07	95	97.5				
Day Old (since Hatched)		0	1	2	3	4	2	9	7	8	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	56	27	28	53	30	31	32	33	34
Day of Project		8 u	0 ا	n 10	n 11	n 12	13	14	15	16	17	18	19	0 20	21	b 22	b 23	b 24	b 25	b 26	72 q	b 28	b 29	p 30	b 31	b 32	b 33	b 34	b 35	9E q	b 37	p 38	р 39	b 40	r 41	r 42
		27-Jan	28-Jan	29-Jan	30-Jan	31-Jan	1-Feb	2-Feb	3-Feb	4-Feb	5-Feb	6-Feb	7-Feb	8-Feb	9-Feb	10-Feb	11-Feb	12-Feb	13-Feb	14-Feb	15-Feb	16-Feb	17-Feb	18-Feb	19-Feb	20-Feb	21-Feb	22-Feb	23-Feb	24-Feb	25-Feb	26-Feb	27-Feb	28-Feb	1-Mar	2-Mar

Figure 1

**Figure 1**: A table of raw data that was collected over the course of the project. The specific processes in which the data was collected is discussed in detail in the procedures section of this report. The data was collected across 5 instars which are depicted above by different colors. The white is the egg stage. Red is the first instar, orange: second, yellow: third, green: fourth, blue: fifth. These instars correspond to the largest caterpillar initially, which is the one that has been graphed in all od the graphs in Appendix B, unless otherwise noted. The grey section is the data for the second and third caterpillars, who were not following the same instar dates and molted later than the colors indicate.

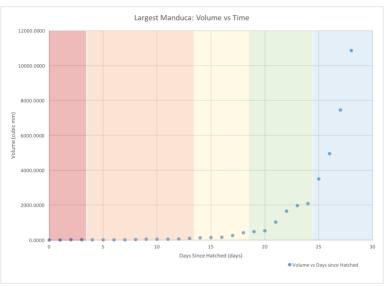
## Appendix B: Graphs from Raw Data

The graphs that are presented here are all for the largest caterpillar from the time that they hatched from the egg, unless otherwise noted. This same caterpillar was always measured as the largest, as is the same with the smallest and middle caterpillar. These graphs focus on the largest. However, as you can find from the data, the other two also followed very similar trends. The largest was chosen to focus on as it would overcome the bigger uncertainties in weight and size quicker and the data would be more reliable sooner.



**Figure 2**: A graph of the density. This graph was made to check the validity of the measurements. The density should stay about constant. With perhaps a slight change from the amount of diet that they are consuming. It is clear that the first 4 data points, especially points 4 and 5, are not reliable points and should not be considered in the analysis.

## **Graphs of the Volume vs Time**



Volume vs Time (Instar 1)

10,0000

8,0000

y = -0.5584x<sup>3</sup> + 2.9759x<sup>2</sup> - 0.6013x + 0.8345

R<sup>2</sup> = 1

Days Since Hatched (days)

Figure 3: Volume vs Time Graph, over all 5 instars

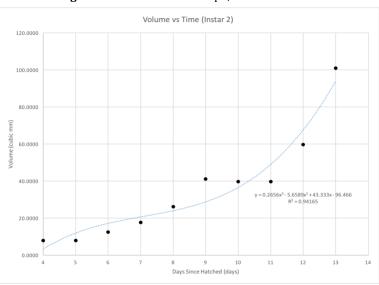
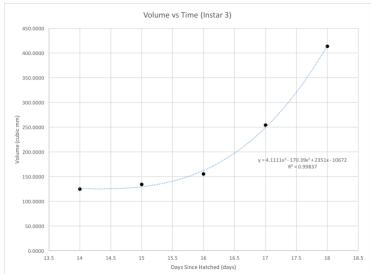
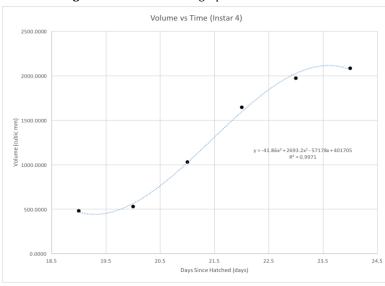


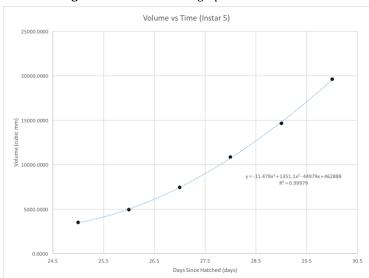
Figure 4: Volume vs Time Graph for the first instar



**Figure 5**: Volume vs Time graph for the second instar.



**Figure 6**: Volume vs Time graph for the third instar.



**Figure 7**: Volume vs Time graph for the fourth instar.

**Figure 8**: Volume vs Time graph for the fifth instar.

# **Graphs of the Weight vs Time**

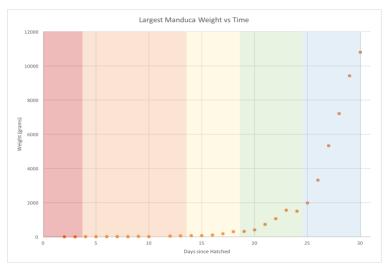


Figure 9: Weight vs Time graph for all of time

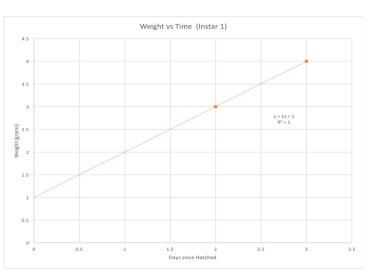


Figure 10: Weight vs Time graph for the first instar

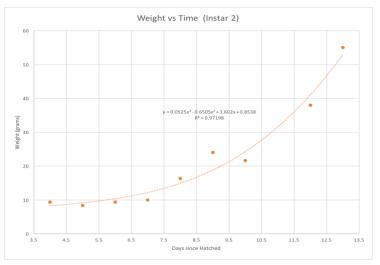


Figure 11: Weight vs Time graph for the second instar

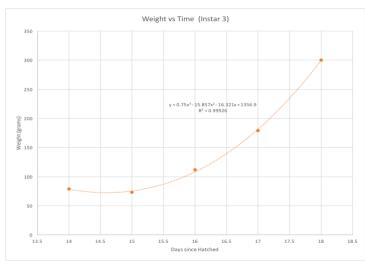


Figure 12: Weight vs Time graph for the third instar

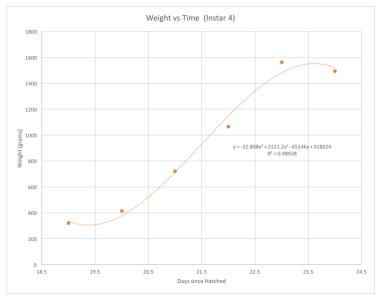
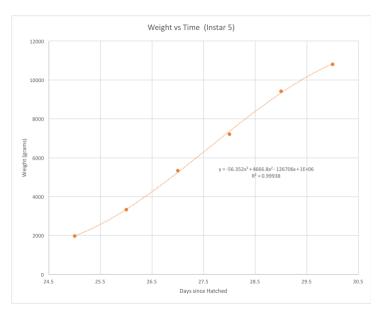


Figure 13: Weight vs Time graph for the fourth instar



**Figure 14**: Weight vs Time graph for the fifth instar

## **Graphs of the Natural Log of the Weight and Volume vs Time**



Figure 15: Natural log of weight and volume vs time: all of time

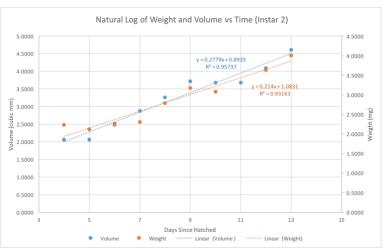


Figure 17: Natural log of weight and volume vs time: second instar

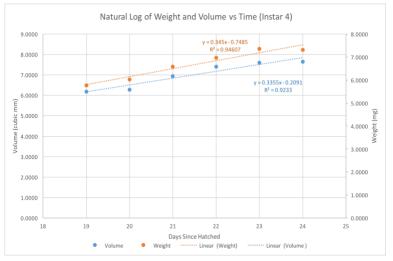


Figure 19: Natural log of weight and volume vs time: fourth instar

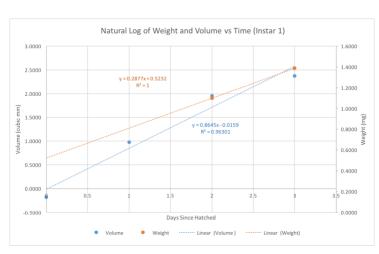


Figure 16: Natural log of weight and volume vs time: first instar



Figure 18: Natural log of weight and volume vs time: third instar



Figure 20: Natural log of weight and volume vs time: fifth instar

# Appendix C: Photographs from the Project

# Appendix D: Daily log and Partner Observations

## **Concluding Paragraph**

In conclusion of the project, I found this project very stimulating. While it seemed at first very cookie cutter, there were many chances to expand and adapt the project to your interests. It was very interesting to track the growth of the Manducas and to watch the trends, as it went towards the fourth and fifth instars it was easier to tell when they were going to molt. It was also very interesting to see the molted skin and to see the spiracles, as well as the mandibles in the head case. The caterpillars grew incredibly fast towards the end of their 5 instars and ate so much diet! At one point I had almost run out of diet as well and had to skip work to run home and feed them right after I had gotten more diet in class. I was sad that although 3 of the caterpillars pupated, only 1 survived. All three formed proboscises which was good. One formed a defect in it's shell however, and the other broke open before his time to emerge. The middle one was very good though, and emerged well! It was hard to transport him though, as to move him from the cage to the bottle was difficult, he lost a few scales on his wings sadly, but I think that he was probably OK when I released him after turning him in to be graded. Overall, I thought that it was a very fun project and enjoyed it a lot!