Occurrence Data Analysis of Calliphorid Flies in Indiana.

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Abstract

Occurrence data collection is one of the many techniques still used today for statistical analysis of a species. For forensics occurrence data of calliphorid flies, also known as blow flies, are of great importance to the field, especially in time of death approximation. The United States of American does not have any published calliphorid occurrence data from consistent collection. The collection from the Purdue University forensics entomology department, known as Indiana Carrion Fly Occurrence ID or ICFOD, is used for this analysis. Management and identification of flies are put into a three-stage process. The allocation of identifications per day, the data input of the identifications into an electronic data sheet, and the analysis of occurrence data with local weather data. The two most prominent species in Indiana are *Phormia regina* and *Lucilia coeruleiviridis*, however in the older data under another system of identification found that *Lucilia illustris* is also prominent in the area. *Phormia regina* and *Lucilia coeruleiviridis* show a possibility of bivoltism in Indiana. These findings raise more questions about the older identification process in assessing occurrence data due to L. illustris preference for colder temperatures.

Introduction

Occurrence data is the most widely used data to analyze populations of concern. The technique is commonly used in pest management, agriculture, conservation, ecology, parasitology, and forensics. When it comes to blow flies of the family Calliphoridae, forensics is the focus for occurrence data. There are well over 1,000 species of calliphorid flies around the earth, with only a handful commonly found in the United States of America (Kosmann 2013). In Indiana alone, there is less than 10 different common species under Calliphoridae. The most commonly known is the Black Blow fly, *Phormia regina*, and the Green Bottle Fly, *Lucilia coeruleiviridis*. The importance of these species for forensics is that their instar stages of their lifecycle is used to calculate the window of death for a cadaver just by knowing the weather data to grow a brood sample from the body (Mohr 2014).

The lifecycle of most calliphoridae consist of a female going to a carrion to lay 150-200 eggs (Byrd 2001). This can occur rapidly due to chemicals produced before death as portrayed in Edgar Allan Poe's literary pieces. The first larval instar last up to 8-24 hours before the next 2 instars cycle through. Once at the third instar the larvae leave the carrion to pupate in the soil. It is approximately 14 days later when the adult stage emerges from the soil to go mate and find another carrion to start the cycle again. Not all species are purely carrion feeders as the less common to trap species, Pollenia, is a parasite on earthworms as well as other hosts (Capinera 2008).

This data will be the first to be publicly published in the United States solely as occurrence data analysis. It will be a stepping stone in the research that will benefit forensic analysis in the lab and out in the field.

Materials and Methods

Sample Collection and Labelling

The fly survey was conducted by Purdue University research areas around Indiana. These traps would be set with tainted chicken and pork parts in a cone trap. Each trap would be set to be checked between 7 to 14 days to be collected and transported back in 95% alcohol to slow down the decay process of the specimens. Adult flies would be separated from larvae as well as other insects that may have got into the traps, such as carrion beetles. Adult flies would be pinned once dry enough to be able to be handed to be placed in trays for labelling and identification by students in the lab. The labels on the flies would be markers for databasing the identifications starting with IAA0001 from 2014 to the most current 2018 specimens, however this paper focuses on just 4997 specimens from 2014 to 2015.

Identification

Unidentified flies would be put in trays each day to be identified by students through a three-person identification process. The three-person identification process is to ensure precision and accuracy in the identifications before putting them into the electronic sheet for databasing and analysis. A program with picture verification of the fly traits to guide identifications was used as a tool for more efficient identifications under a microscope. Lab notes would be recorded by hand for archiving for verification and unusual characteristics. Flies that were too damaged to identify due to decomposition, too immature of features, or poor pinning techniques were set as outliers if not identified in the three-person identification process.

Database and Analysis

Lab notes from the identification process are hand imputed into an electronic spreadsheet for analysis of data and local weather data. The method to find and verify the significance of weather data as well as each fly group is the correlation coefficient. Data calculation was done in Excel 2016 for the data to be consistent with prior work on the ICFOD project.

Results

Identification data

The results showed out of seven species of calliphorid fly of interest, there were three species that filled most of the data. These three species listed at highest to lowest is *Phormia regina* at 2447 specimens identified, *Lucilia illustris* at 989 specimens identified, and *Lucilia coeruleviridis* at 563 specimens identified. Of the three only *Phormia regina* data showed bivoltism in population fluctuation (Figure 1). *Lucilia illustris* and *Lucilia coeruleiviridis* shows univoltism in late summer (June - September) in comparison (Figure 2, Figure 3).

Analysis

There was no significant correlation between precipitation and the flies with negative correlation coefficient results. Negative correlation coefficient results pointing to weak relationships between variables when compared (Table 1-3). Similarly, high temperature data also showed negative correlation data between the flies, however low temperature data had one positive correlation between *Phormia regina* and minimum temperature with a P-value of .00701, which is significant at $P < .05$ (Table 2).

Discussion

The results show a skew within the data when it comes to the Luciliinae. This is especially the case with *Lucilia illustris* in which the data shows a staggering spike in population during a period that is not preferential temperature for the species (Wang 2016). Looking back on prior data in the electronic spreadsheet, the older identifications listed before this experiment began does not differenciate the Luciliinae from other genera such as *Lucilia coeruleiviridis*, *Lucilia sericata*, and *Lucilia silvarum*. With a high certainty the identifications of older data will likely need to be reexamined to be consistent with current taxonomical standards. Other obstacles that arose during the analysis of the data was the issue of non-SI units being given for not only the weather data but also the information on the biological cycle of the calliphorids.

The data does suggest that low temperatures do affect Phormia regina, a less cold tolerant species of calliphorid. This aligns with greater confirmation of developmental data from other research (Byrd 2001). Prospects for this data are the usage of occurrence data analysis of calliphorids in various regions of the United States as a stepping stone. The importance of larger occurrence maps in forensics is to have detailed reports of the minimum post-mortem interval for death cases.

Overall, this experiment will need replication and more annual data identified to give more accurate data as to the exact dates of emergence and disappearance of the calliphorid flies. Modifications that would need to be done is the identification system currently is being updated and will continue to do so, thus a plan for reassessment every few years should be necessary for upkeep of the collection

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Figure 1. Cluster graph of *P. regina* occurrence over 2014 and 2015.

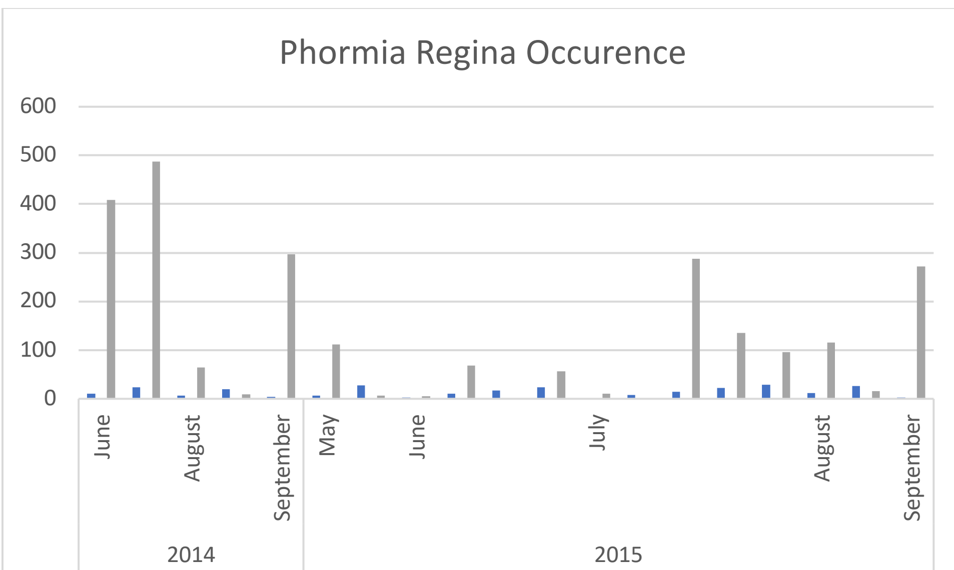

Figure 2. Cluster graph of *L. illustris* occurrence over 2014 and 2015.

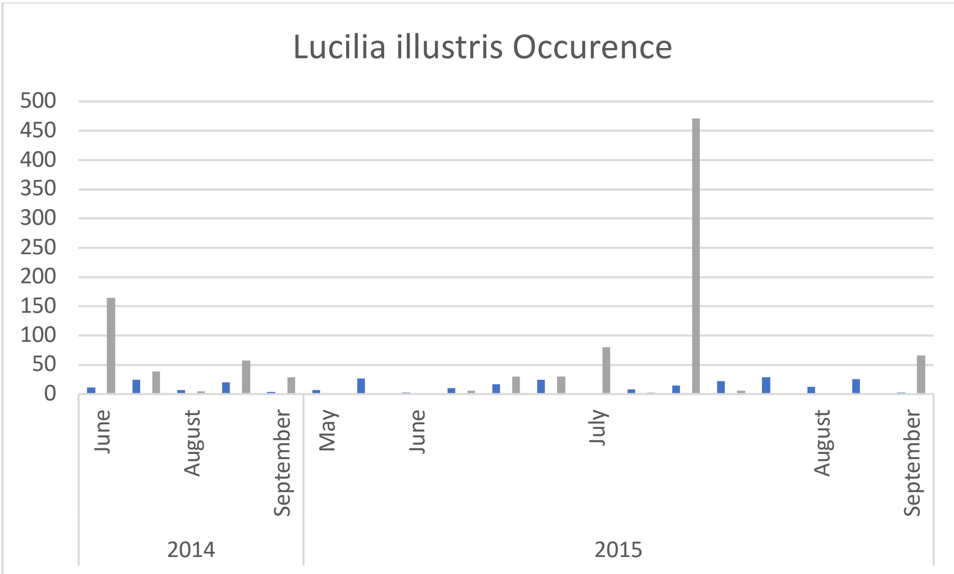

Figure 3. Cluster graph of *L. coeruleiviridis* occurrence over 2014 and 2015.

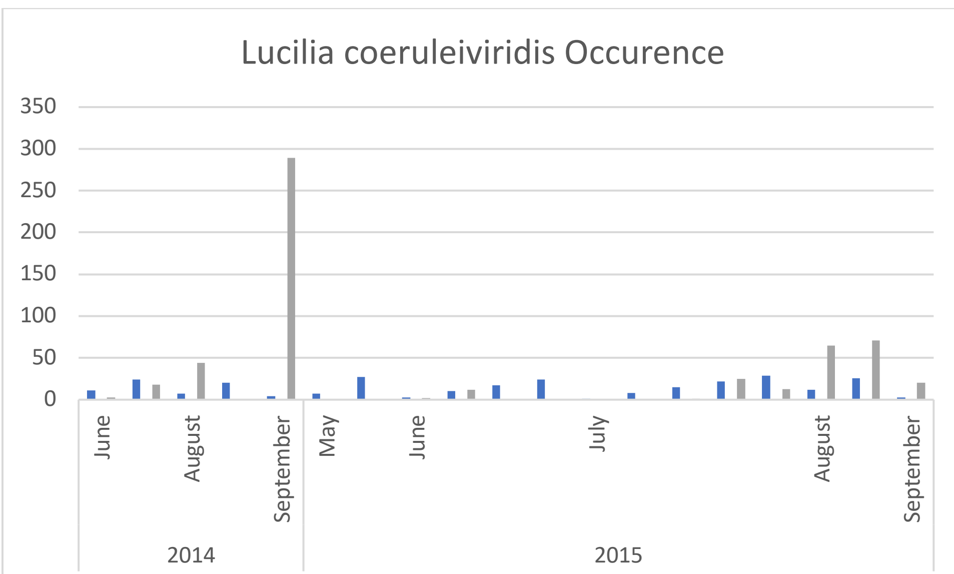

Figure 4. Precipitation data over 2014 to 2015 data.

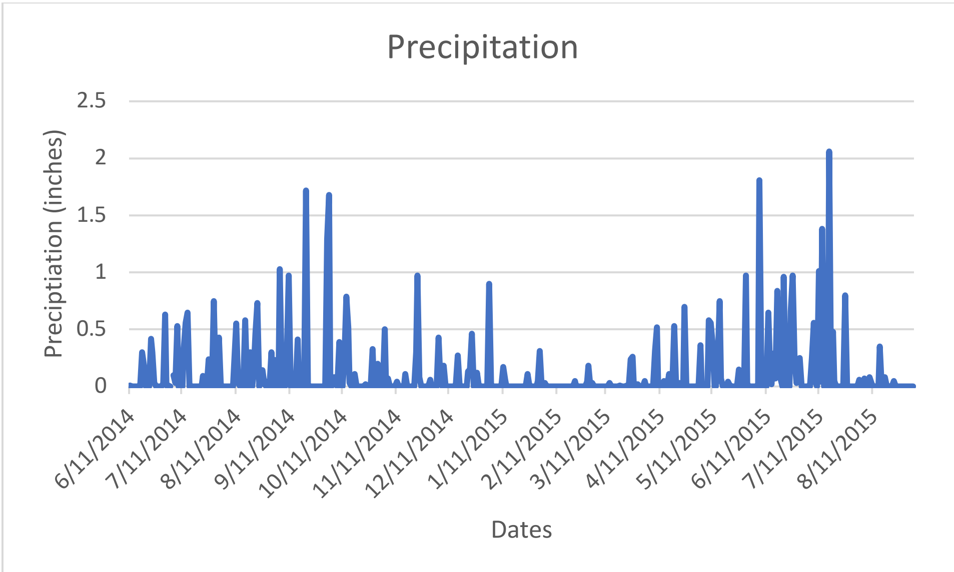

Figure 5. Temperature variation of 2014 and 2015 data range.

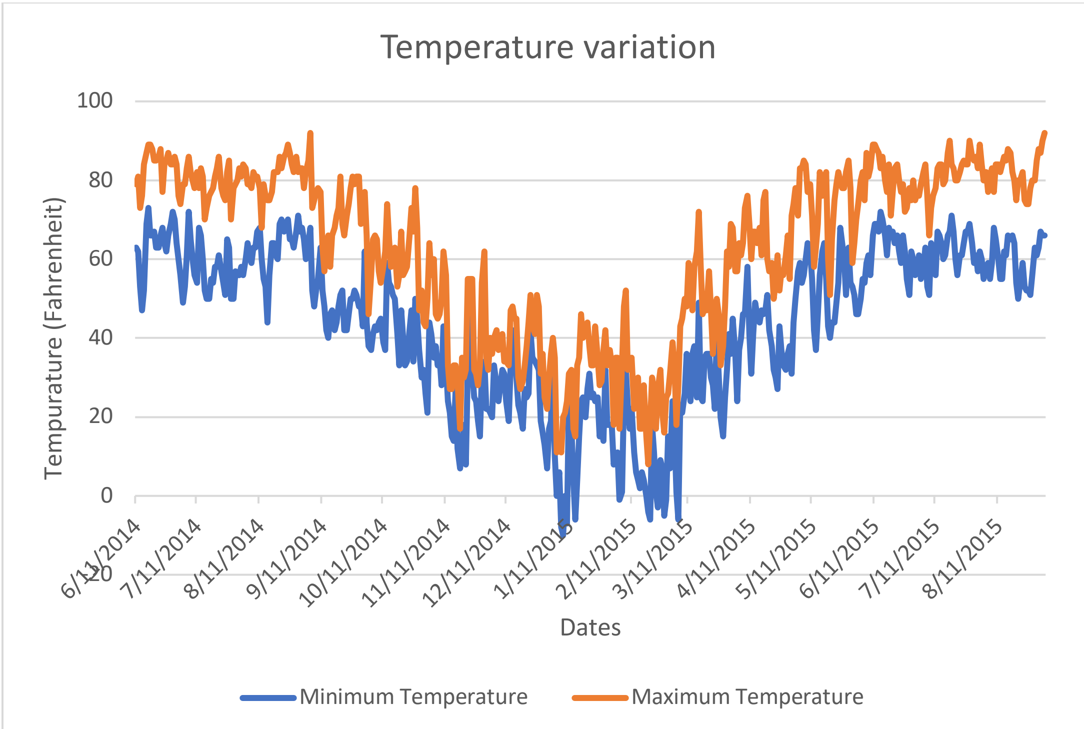

Table 1. Table depicting correlation coefficient data for species vs precipitation.

Regina (X)	Precipitat	X-M	Y-M	(X-M)^2	(Y-M)^2	(X-M)(Y-M)		R-value	N	P-value
408	0.01	279.211	-0.081	77958.52	0.007	-22.631		-0.11693	19	0.653942
488	0.3	359.211	0.209	129032.2	0.044	75.056				
65	0	-63.789	-0.091	4069.097	0.008	5.808				
9	0	-119.789	-0.091	14349.52	0.008	10.907				
297	0	168.211	-0.091	28294.78	0.008	-15.316				
111	0	-17.789	-0.091	316.465	0.008	1.62				
7	0.02	-121.789	-0.071	14832.68	0.005	8.653				
5	0	-123.789	-0.091	15323.83	0.008	11.271				
68	0	-60.789	-0.091	3695.36	0.008	5.535				
0	0.84	-128.789	0.749	16586.73	0.561	-96.457				
56	0	-72.789	-0.091	5298.307	0.008	6.628				
10	0	-118.789	-0.091	14110.94	0.008	10.816				
0	0.56	-128.789	0.469	16586.73	0.22	-60.395				
288	0	159.211	-0.091	25347.99	0.008	-14.497				
135	0	6.211	-0.091	38.571	0.008	-0.565				
96	0	-32.789	-0.091	1075.15	0.008	2.986				
116	0	-12.789	-0.091	163.571	0.008	1.165				
16	0	-112.789	-0.091	12721.47	0.008	10.27				
272	0	143.211	-0.091	20509.26	0.008	-13.04				
2447	1.73	128.789	0.091	400311.2	0.952	-72.186				

Illustris (X	Precipitat	X-M	Y-M	(X-M)^2	(Y-M)^2	(X-M)(Y-M)		R-value	N	P-value
165	0.01	112.947	-0.081	12757.11	0.007	-9.155		-0.1111	19	0.650986
39	0.3	-13.053	0.209	170.371	0.044	-2.727				
5	0	-47.053	-0.091	2213.95	0.008	4.284				
57	0	4.947	-0.091	24.476	0.008	-0.45				
29	0	-23.053	-0.091	531.424	0.008	2.099				
1	0	-51.053	-0.091	2606.371	0.008	4.648				
1	0.02	-51.053	-0.071	2606.371	0.005	3.627				
0	0	-52.053	-0.091	2709.476	0.008	4.74				
6	0	-46.053	-0.091	2120.845	0.008	4.193				
30	0.84	-22.053	0.749	486.319	0.561	-16.516				
30	0	-22.053	-0.091	486.319	0.008	2.008				
80	0	27.947	-0.091	781.055	0.008	-2.545				
2	0.56	-50.053	0.469	2505.266	0.22	-23.472				
471	0	418.947	-0.091	175516.9	0.008	-38.146				
6	0	-46.053	-0.091	2120.845	0.008	4.193				
0	0	-52.053	-0.091	2709.476	0.008	4.74				
1	0	-51.053	-0.091	2606.371	0.008	4.648				
0	0	-52.053	-0.091	2709.476	0.008	4.74				
66	0	13.947	-0.091	194.529	0.008	-1.27				
989	1.73	52.053	0.091	215856.9	0.952	-50.361				

Coerule. (	Precipitat	X-M	Y-M	(X-M)^2	(Y-M)^2	(X-M)(Y-M)		R-value	N	P-value
3	3	-26.632	-0.081	709.241	0.007	2.159		-0.1664	19	0.497017
18	18	-11.632	0.209	135.294	0.044	-2.43				
44	44	14.368	-0.091	206.452	0.008	-1.308				
0	0	-29.632	-0.091	878.03	0.008	2.698				
289	289	259.368	-0.091	67271.98	0.008	-23.616				
0	0	-29.632	-0.091	878.03	0.008	2.698				
0	0	-29.632	-0.071	878.03	0.005	2.105				
2	2	-27.632	-0.091	763.504	0.008	2.516				
12	12	-17.632	-0.091	310.873	0.008	1.605				
0	0	-29.632	0.749	878.03	0.561	-22.192				
0	0	-29.632	-0.091	878.03	0.008	2.698				
0	0	-29.632	-0.091	878.03	0.008	2.698				
0	0	-29.632	0.469	878.03	0.22	-13.896				
1	1	-28.632	-0.091	819.767	0.008	2.607				
25	25	-4.632	-0.091	21.452	0.008	0.422				
13	13	-16.632	-0.091	276.609	0.008	1.514				
65	65	35.368	-0.091	1250.925	0.008	-3.22				
71	71	41.368	-0.091	1711.346	0.008	-3.767				
20	20	-9.63	-0.091	92.767	0.008	0.877				
563	1.73	29.632	0.091	79716.42	0.952	-45.833				

Table 2. Table deicting correlation coefficient with minimum temperatures vs species.

Regina (X)	Low Temp	X-M	Y-M	(X-M)^2	(Y-M)^2	(X-M)(Y-M)		R-value	N	P-value
408	63	279.211	3.789	77958.52	14.36	1058.061		0.5966	19	0.007082
488	68	359.211	8.789	129032.2	77.255	3157.271				
65	59	-63.789	-0.211	4069.097	0.044	13.429				
9	60	-119.789	0.789	14349.52	0.623	-94.571				
297	64	168.211	4.789	28294.78	22.939	805.64				
111	56	-17.789	-3.211	316.465	10.307	57.114				
7	60	-121.789	0.789	14832.68	0.623	-96.15				
5	46	-123.789	-13.211	15323.83	174.518	1635.324				
68	66	-60.789	6.789	3695.36	46.097	-412.729				
0	61	-128.789	1.789	16586.73	3.202	-230.465				
56	59	-72.789	-0.211	5298.307	0.044	15.324				
10	56	-118.789	-3.211	14110.94	10.307	381.377				
0	51	-128.789	-8.211	16586.73	67.413	1057.429				
288	60	159.211	0.789	25347.99	0.623	125.693				
135	56	6.211	-3.211	38.571	10.307	-19.939				
96	64	-32.789	4.789	1075.15	22.939	-157.044				
116	55	-12.789	-4.211	163.571	17.729	53.85				
16	55	-112.789	-4.211	12721.47	17.729	474.903				
272	66	143.211	6.789	20509.26	46.097	972.324				
2447	1125	128.789	59.211	400311.2	543.158	8796.842				

Illustris (X	Low Temp	X-M	Y-M	(X-M)^2	(Y-M)^2	(X-M)(Y-M)		R-value	N	P-value
165	63	112.947	3.789	12757.11	14.36	428.011		0.1625	19	0.506259
39	68	-13.053	8.789	170.371	77.255	-114.726				
5	59	-47.053	-0.211	2213.95	0.044	9.906				
57	60	4.947	0.789	24.476	0.623	3.906				
29	64	-23.053	4.789	531.424	22.939	-110.41				
1	56	-51.053	-3.211	2606.371	10.307	163.906				
1	60	-51.053	0.789	2606.371	0.623	-40.305				
0	46	-52.053	-13.211	2709.476	174.518	687.643				
6	66	-46.053	6.789	2120.845	46.097	-312.673				
30	61	-22.053	1.789	486.319	3.202	-39.463				
30	59	-22.053	-0.211	486.319	0.044	4.643				
80	56	27.947	-3.211	781.055	10.307	-89.726				
2	51	-50.053	-8.211	2505.266	67.413	410.958				
471	60	418.947	0.789	175516.9	0.623	330.748				
6	56	-46.053	-3.211	2120.845	10.307	147.853				
0	64	-52.053	4.789	2709.476	22.939	-249.305				
1	55	-51.053	-4.211	2606.371	17.729	214.958				
0	55	-52.053	-4.211	2709.476	17.729	219.169				
66	66	13.947	6.789	194.529	46.097	94.695				
989	1125	52.053	59.211	215856.9	543.158	1759.789				

Coerule. (	Low Temp	X-M	Y-M	(X-M)^2	(Y-M)^2	(X-M)(Y-M)		R-value	N	P-value
3	63	-26.632	3.789	709.241	14.36	-100.92		0.1741	19	0.475947
18	68	-11.632	8.789	135.294	77.255	-102.235				
44	59	14.368	-0.211	206.452	0.044	-3.025				
0	60	-29.632	0.789	878.03	0.623	-23.393				
289	64	259.368	4.789	67271.98	22.939	1242.238				
0	56	-29.632	-3.211	878.03	10.307	95.133				
0	60	-29.632	0.789	878.03	0.623	-23.393				
2	46	-27.632	-13.211	763.504	174.518	365.028				
12	66	-17.632	6.789	310.873	46.097	-119.709				
0	61	-29.632	1.789	878.03	3.202	-53.025				
0	59	-29.632	-0.211	878.03	0.044	6.238				
0	56	-29.632	-3.211	878.03	10.307	95.133				
0	51	-29.632	-8.211	878.03	67.413	243.291				
1	60	-28.632	0.789	819.767	0.623	-22.604				
25	56	-4.632	-3.211	21.452	10.307	14.87				
13	64	-16.632	4.789	276.609	22.939	-79.657				
65	55	35.368	-4.211	1250.925	17.729	-148.92				
71	55	41.368	-4.211	1711.346	17.729	-174.183				
20	66	-9.632	6.789	92.767	46.097	-65.393				
563	1125	29.632	59.211	79716.42	543.158	1145.474				

Table 3. Table depicting correlation coefficient between high temperture and species.

Regina (X)	High Temp	X-M	Y-M	(X-M)^2	(Y-M)^2	(X-M)(Y-M)		R-value	N	P-value
408	79	279.211	-1.158	77958.52	1.341	-323.296		0.2442	19	0.313675
488	77	359.211	-3.158	129032.2	9.972	-1134.35				
65	78	-63.789	-2.158	4069.097	4.657	137.651				
9	82	-119.789	1.842	14349.52	3.393	-220.665				
297	85	168.211	4.842	28294.78	23.446	814.493				
111	85	-17.789	4.842	316.465	23.446	-86.139				
7	78	-121.789	-2.158	14832.68	4.657	262.809				
5	74	-123.789	-6.158	15323.83	37.92	762.283				
68	89	-60.789	8.842	3695.36	78.183	-537.507				
0	77	-128.789	-3.158	16586.73	9.972	406.704				
56	77	-72.789	-3.158	5298.307	9.972	229.861				
10	75	-118.789	-5.158	14110.94	26.604	612.704				
0	66	-128.789	-14.158	16586.73	200.446	1823.388				
288	79	159.211	-1.158	25347.99	1.341	-184.349				
135	80	6.211	-0.158	38.571	0.025	-0.981				
96	86	-32.789	5.842	1075.15	34.13	-191.56				
116	82	-12.789	1.842	163.571	3.393	-23.56				
16	82	-112.789	1.842	12721.47	3.393	-207.77				
272	92	143.211	11.842	20509.26	140.235	1695.914				
2447	1523	128.789	80.158	400311.2	616.526	3835.632				

Illustris (X	High Temp	X-M	Y-M	(X-M)^2	(Y-M)^2	(X-M)(Y-M)		R-value	N	P-value
165	79	112.947	-1.158	12757.11	1.341	-130.781		-0.0362	19	0.883674
39	77	-13.053	-3.158	170.371	9.972	41.219				
5	78	-47.053	-2.158	2213.95	4.657	101.535				
57	82	4.947	1.842	24.476	3.393	9.114				
29	85	-23.053	4.842	531.424	23.446	-111.623				
1	85	-51.053	4.842	2606.371	23.446	-247.202				
1	78	-51.053	-2.158	2606.371	4.657	110.166				
0	74	-52.053	-6.158	2709.476	37.92	320.535				
6	89	-46.053	8.842	2120.845	78.183	-407.202				
30	77	-22.053	-3.158	486.319	9.972	69.64				
30	77	-22.053	-3.158	486.319	9.972	69.64				
80	75	27.947	-5.158	781.055	26.604	-144.15				
2	66	-50.053	-14.158	2505.266	200.446	708.64				
471	79	418.947	-1.158	175516.9	1.341	-485.097				
6	80	-46.053	-0.158	2120.845	0.025	7.271				
0	86	-52.053	5.842	2709.476	34.13	-304.097				
1	82	-51.053	1.842	2606.371	3.393	-94.044				
0	82	-52.053	1.842	2709.476	3.393	-95.886				
66	92	13.947	11.842	194.529	140.235	165.166				
989	1523	52.053	80.158	215856.9	616.526	-417.158				

Coerule. (	High Temp	X-M	Y-M	(X-M)^2	(Y-M)^2	(X-M)(Y-M)		R-value	N	P-value
3	79	-26.632	-1.158	709.241	1.341	30.837		0.2705	19	0.262681
18	77	-11.632	-3.158	135.294	9.972	36.731				
44	78	14.368	-2.158	206.452	4.657	-31.006				
0	82	-29.632	1.842	878.03	3.393	-54.584				
289	85	259.368	4.842	67271.98	23.446	1255.889				
0	85	-29.632	4.842	878.03	23.446	-143.479				
0	78	-29.632	-2.158	878.03	4.657	63.942				
2	74	-27.632	-6.158	763.504	37.92	170.152				
12	89	-17.632	8.842	310.873	78.183	-155.9				
0	77	-29.632	-3.158	878.03	9.972	93.573				
0	77	-29.632	-3.158	878.03	9.972	93.573				
0	75	-29.632	-5.158	878.03	26.604	152.837				
0	66	-29.632	-14.158	878.03	200.446	419.521				
1	79	-28.632	-1.158	819.767	1.341	33.152				
25	80	-4.632	-0.158	21.452	0.025	0.731				
13	86	-16.632	5.842	276.609	34.13	-97.163				
65	82	35.368	1.842	1250.925	3.393	65.152				
71	82	41.368	1.842	1711.346	3.393	76.205				
20	92	-9.632	11.842	92.767	140.235	-114.058				
563	1523	29.632	80.158	79716.42	616.526	1896.105				

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