Effect of varying light exposure on the cell growth of *Licmophora abbreviata*

Prabhleen Dhami, Jeremy Fong, Jasmandeep Kaur, Faraz Kazi

Abstract

Licmophora abbreviata are a light-sensitive organism that serve as oxygen providers for fish, including salmon, in marine ecosystems. For this reason, *L. abbreviata* growth can directly impact salmon abundance in a specific stream. The purpose of this experiment is to study which light conditions provide optimal growth for *L. abbreviata* with all other conditions being constant. Three treatments were set up as light, dark, and control conditions each with three samples containing 1.0 x 10^4 cells/mL of *L. abbreviata*. Our null hypothesis suggested that all three conditions would exhibit the same level of cell growth and our prediction was that we would reject the null hypothesis. All three treatments were placed in incubators with the light treatment receiving 21 hours of light per day, the control receiving 8 hours of light per day, and the dark treatment receiving no light. Samples were taken five, seven, eight, nine and thirteen days following inoculation and counted using a hemocytometer. Our results indicated the greatest overall cell growth occurred in the light condition, and with a *p*-value of 0.0004, these results can be considered statistically significant. We were able to reject our null hypothesis; thus, our prediction of finding higher cell growth under more light exposure was correct.

Introduction

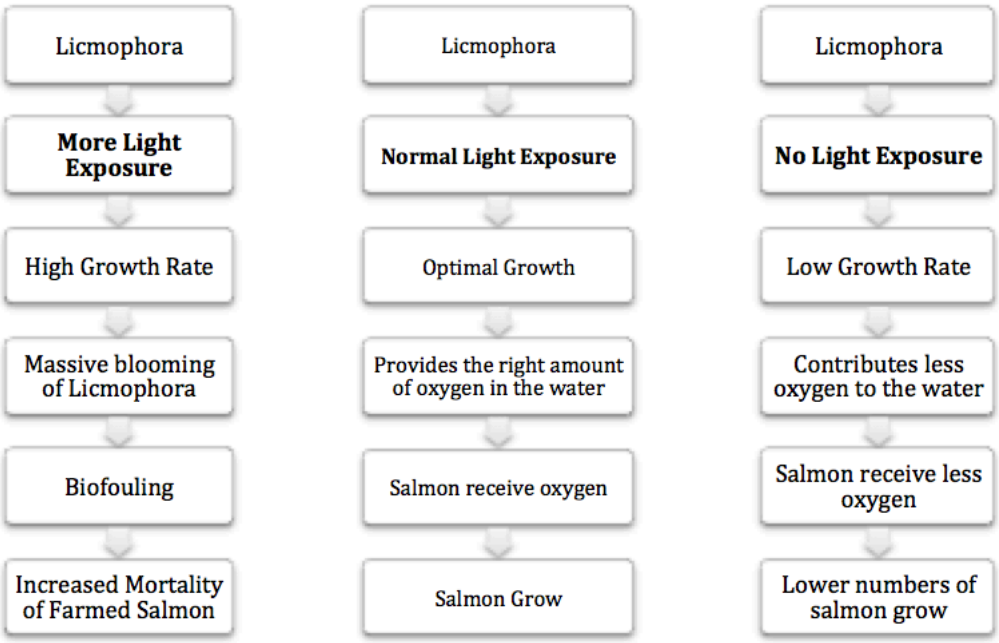
Licmophora are photosynthetic diatoms that grow in well-lit environments (Ravizza and Hallegraeff, 2015). In this experiment, we wished to assess the growth of the licmophora diatom under various light conditions to determine which environment produces the highest growth rate. *Licmophora abbreviata*, the species being used in this experiment, vary between 7-13 μm in length (Honeywell, 1998), and tend to grow well in temperatures ranging from 15-25 ˚C, with a pH between 7.8-8.5 (Ohgai et al., 1984).

Licmophora are very similar to other diatoms because they act as an oxygen source in water for marine organisms, such as fish. However, during periods of bloom, licmophora grow in large concentrations that result in the biofouling of various organisms (Cembella et al., 2002). Biofouling is the build-up of different microorganisms and living matter on wet, artificial

surfaces. This could be very harmful for farmed salmon, as licmophora frequently foul around salmon nets (Ravizza and Hallegraeff, 2015), which can cause high mortalities in farmed salmon (Cembella et al, 2002). Most of the licmophora biofouling tends to occur during periods of bloom, which happen in the warmer months when there is more sunshine (Cembella et al, 2002) because licmophora experience higher growth rates in more light.

Our experiment investigated the optimal lighting conditions of *L. abbreviata* growth by cultivating the organisms in three different lighting conditions. The first light treatment will expose *L. abbreviata* to 21 hours of light, the control treatment will expose *L. abbreviata* to 8 hours of light and the dark treatment will keep the *L. abbreviata* away from light. Our null hypothesis predicts that *L. abbreviata* will experience similar cell growth between the three different light exposure conditions. Our alternative hypothesis would suggest that they would not have similar cell growth under the three different light exposure conditions. Rather, the alternative hypothesis suggests a likelihood of higher cell growth with more light exposure and lower cell growth with less light exposure. Figure 1 further explains the consequences that could result from the rejection of our null hypothesis. We chose to model our experiment on a previously conducted study which found that licmophora growing under more light exposure had a lifespan of up to 21 days, whereas the ones that grew under optimal light grew for 3-7 days (Ravizza and Hallegraeff, 2015). Depending on the cell growth of *L. abbreviata* under the three different light conditions, we can estimate the growth of licmophora diatoms under different light exposures in the wild.

The growth of licmophora is directly related to the environment the salmon grow in. An excess of licmophora growth can lead to mortality; hence the right amount is required for the



optimal growth of salmon, as shown in Figure 1. Furthermore, diatoms such as licmophora are able sink into the sediment of the environment allowing researchers to track the average growth of salmon at different time periods in history (Bradbury, 1999). Overall, identifying the growth of licmophora is imperative to helping us better understand the environment they grow in.

Figure 1: How different light exposure affect the growth of *L. abbreviata* and salmon.

Methods

Preparation of L. abbreviata solution

A solution of *L. abbreviata* with a concentration of 1.0 x 10^4 cells/mL was needed to prepare the replicates for each treatment. In order to do this, the concentration of the initial stock solution was determined by swirling the flask of *L. abbreviata* and collecting 100 µL using a micropipette. This volume was transferred into a 500 µL counting tube along with 10 µL of Lugol's iodine fixative (IKI). The resulting solution was thoroughly mixed using a micropipette before adding 20 µL to a hemocytometer. The hemocytometer was mounted upon the stage of an Axiostar Plus microscope and the cells were counted with the help of a click counter. The cell

count and appropriate dilution factors were used to determine the concentration of the initial *L. abbreviata* solution, which called for further dilution using media to reach the required concentration of 1.0 x 10^4 cells/mL

Preparation of samples

Sample preparation began with labelling test tubes with the appropriate identification. As shown in Figure 2, each light treatment contained three replicates, which were labelled with "L" for light, "C" for control, or "D" for dark, followed by a number from one to three. Using sterile techniques, 10 mL of the 1.0 x 10^4 cells/mL *L. abbreviata* solution was added to each of the nine test tubes. The replicates for each light condition were placed in three separate test tube racks and assigned to their respective environments. The three replicates of the light condition were placed in a 21-hour light cycle incubator while the three replicates of the control were placed in an 8-hour light cycle incubator. The three replicates of the dark condition were also placed in 21-hour cycle incubator; however, they were covered by a cardboard box to prevent any light exposure.

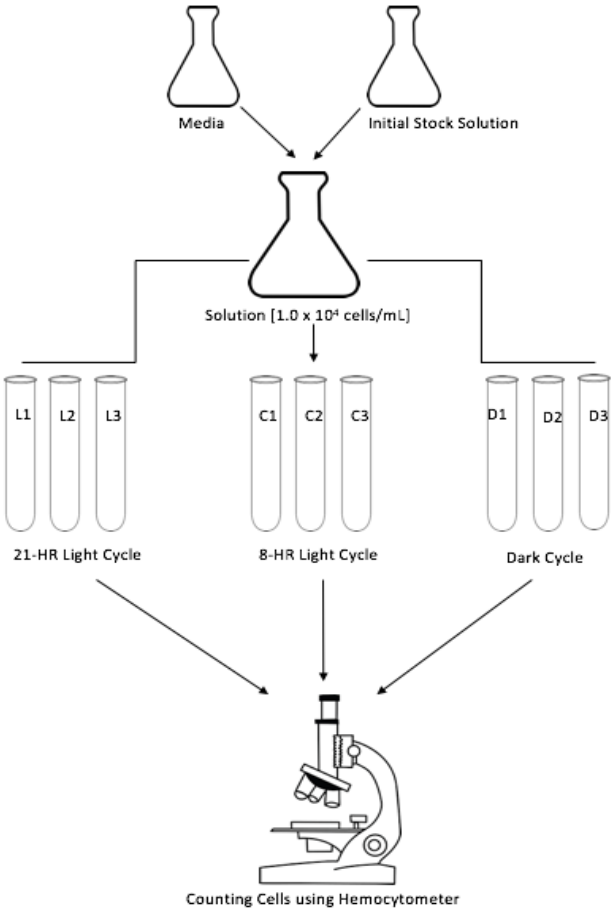


Figure 2: Preparation of *L. abbreviata* samples.

Counting Cells

The samples were counted five, seven, eight, nine and thirteen days following inoculation. The test tube racks containing the samples were obtained from their respective incubators. Observations regarding the samples' appearance, such as colour, transparency and location of cloudiness, were recorded. Each of the nine test tubes were vortexed to ensure equal distribution of *L. abbreviata* cells before collecting 100 µL using a micropipette and sterile techniques. This volume was transferred into a 500 µL counting tube along with 10 µL of Lugol's iodine fixative (IKI). The resulting solution was resuspended before adding 20 µL to a hemocytometer. The hemocytometer was then mounted upon the microscope and the cells were counted as described above. The cell counts were recorded and the concentration of each tube was calculated using the appropriate dilution factors. After 100 µL from all nine test tube samples were combined with IKI fixative, the test tubes were returned to their respective environments.

Statistical Analysis

To determine if there were any statistically significant differences in cell growth between the different light conditions, a one-way ANOVA was performed on the collected data and a box-plot was generated for each light condition.

Results

Over the course of 14 days, the licmophora were left under the three light exposure conditions. Cell concentration was calculated by multiplying the cell count by the dilution factor of the haemocytometer (3.125×10^2) and the dilution factor of the IKI fixative (x1.1)

As shown in Figure 3, the *L. abbreviata* grown under the light conditions experienced the highest cell growth while those in dark conditions experienced the lowest cell growth. The light condition had a maximum cell growth concentration of 1.10 x10^4 cells/mL and a minimum of 7.62 x 10^3 cells/mL. Similarly, the control condition had a maximum cell growth concentration of 7.27 x10^3 cells/mL and a minimum of 4.57 x 10^3 cells/mL. The dark condition had a maximum cell growth concentration of 9.29 x 10^2 cells/mL and a minimum of 1.10 x 10^2 cells/mL. Based on the one-way ANOVA analysis, the *p-value* was found to be 0.0004 between the different light exposure conditions.

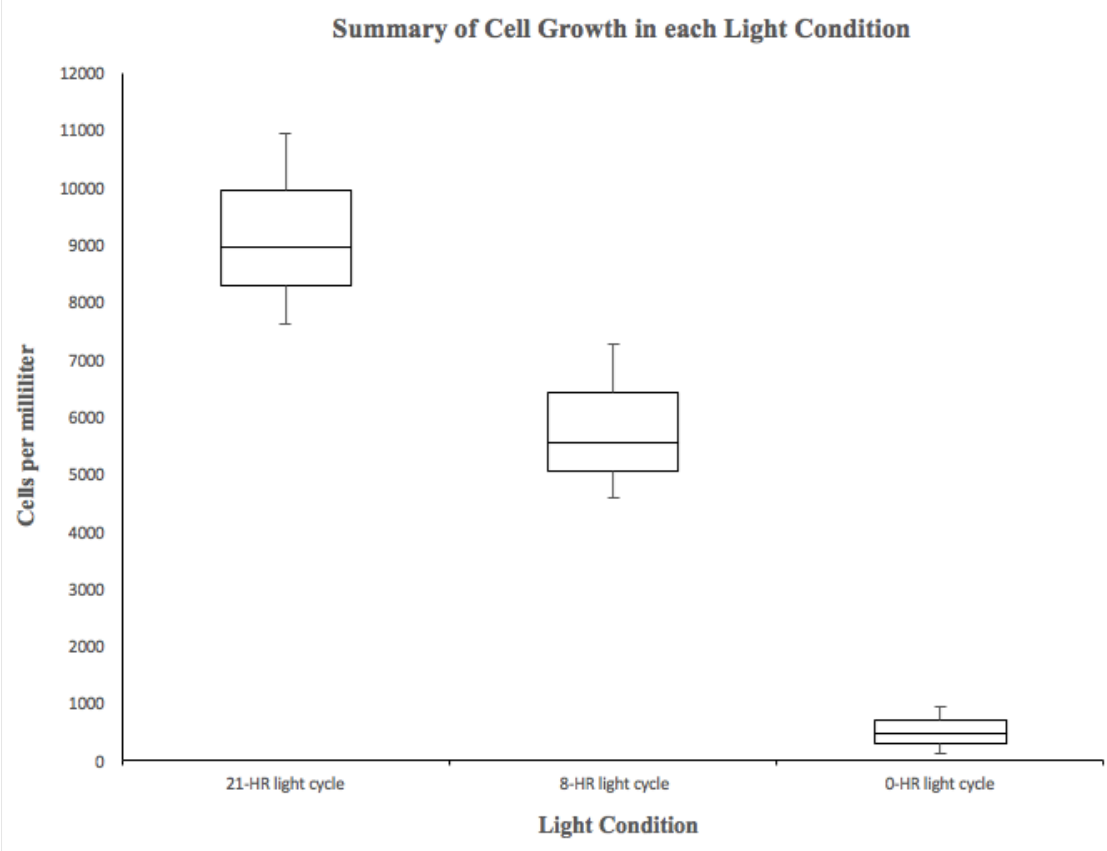


Figure 3: Summary of *L. abbreviata* growth under 21, 8 and 0 hours of light exposure

In Figure 4, we can see on Day 8, we accumulated growth for light (3A) and control (3B) condition. However, dark (3C) condition did not exhibit any. As the days progressed to Day 14, we can see in Figure 5 that there is an increase of cloudiness in light (4A) and control (4B) conditions. Evidently, the dark condition did not exhibit any growth due as we can see from the transparency and lack of colour in their test tube.

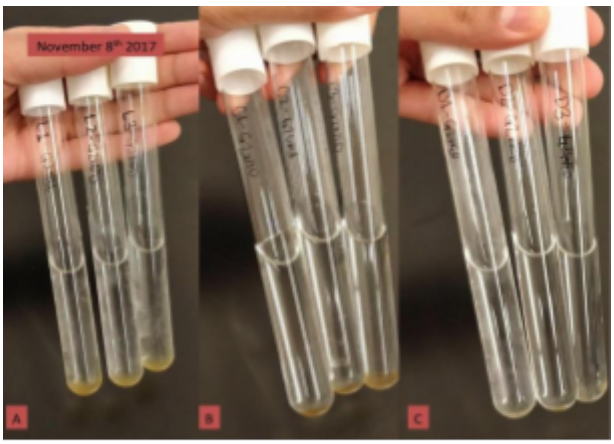


Figure 4: L. abbreviata under high (A), control (B), and dark (C) conditions on Day 8.

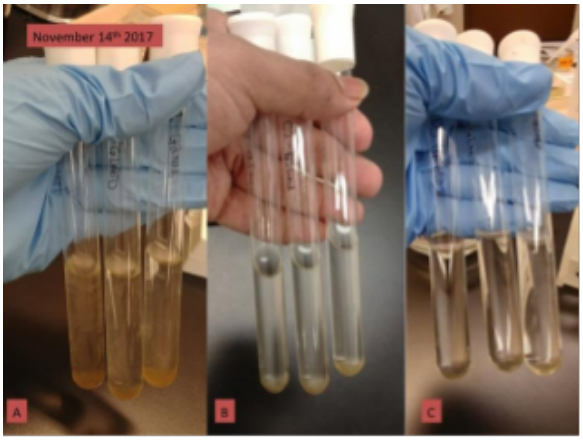


Figure 5: L. abbreviata under Light (A), control (B), and dark (C) conditions on Day 14.

Discussion

The one-way ANOVA determined a *p*-value of 0.0004, which is less than 0.05, thus allowing us to reject the null hypothesis. In other words, we determined that there was a statistical significance in cell growth of *Licmophora abbreviata* under different light exposure conditions.

The findings from our study are similar to the outcomes presented by previous research studies. For example, Stockner and Shortreed (1976) determined that when *L. abbreviata* grew under more light exposure, they tended to grow over a longer period of time compared to when

they grow in their optimal light exposure. This is similar to the findings contained in our raw data, and after our data analysis there is statistical significance that would suggest this to be true.

Furthermore, other studies mentioned how diatom blooms result in higher mortality of farmed salmon. Ravizza and Hallegraeff (2015) mention how licmophora cause lots of problems to artificial structures due to their ability to act as a biofouling organism. This is important because the netting surrounding the farmed salmon tend experience biofouling during periods of bloom. Another study performed by Cembella et al. (2002) supports this conclusion, as they explain how biofouling from dinoflagellates during periods of high bloom results in high mortality of farmed salmon. However, they also reported high volumes of licmophora being present with the dinoflagellates during the bloom. If we compile the information from previous research studies, it can be suggested that licmophora biofouling around the salmon farm nets results in mortality of salmon during periods of rapid licmophora growth.

Since our research agrees with the results from other researchers, we can relate it to the proposed model shown in Figure 1 for the alternative hypothesis. The model shows how growth of salmon would vary with the response of licmophora growth rate under different light exposure, with more light exposure resulting in mortality, optimal light exposure providing optimal conditions, and no light exposure resulting in fewer numbers of salmon. Our data suggests that the growth of licmophora was affected by the light exposure, thus agreeing with previous research findings.

Although our results were sufficient to reject the null hypothesis, there were still errors made in our methodology. One of the primary sources of error in our experiment could have been overexposure of our samples to lab conditions. During our sampling periods, we removed

our test tubes from the incubators and brought them into the lab. We then extracted 100µl of each test tube individually and added them to counting tubes with fixative. While performing this procedure, each test tube was left exposed to the temperature and lighting conditions of the lab for anywhere from 20-40 minutes per sampling period. While this may be a very small period of time, the disruption of conditions could have affected our samples and caused some of the licmophora to die, especially in the control samples where they were confined to a strict 8-hour light period.

Another source of error may have stemmed from our counting procedure. In order to increase efficiency, we had up to three different people counting at a time. Having a greater number of individuals counting organisms allowed us to count more in a shorter period of time. However, there could have been variations in how each individual counted the *L. abbreviata*. These variations are not measurable, so the only way for this to be accounted for would have been to have a single individual do all the counts or assign each individual to count a specific set of samples for the entirety of the study to keep consistency in our numbers.

Conclusions

The results of our study indicated that the overall cell growth was greater in *L. abbreviata* samples exposed to a 21-hour light cycle. Furthermore, our statistical analysis allowed us to reject our null hypothesis, which stated *L. abbreviata* would experience similar growth between all three different light conditions. This supports our initial prediction, as we expected more light exposure would result in higher cell growth in licmophora. Since we were able to reject the null hypothesis, we can predict the growth of salmon by analyzing the effects of light exposure on licmophora in the oceans, as shown in Figure 1.

Acknowledgements

We would like to express our greatest appreciation to our instructor, Dr. Celeste Leander, for her continuous support and insight during the planning and execution of our research project. We also extend our thanks to our lab technician, Mindy Chow, for her helpful critiques and providing us with all the necessary resources we needed to implement our research. In addition, we would like to thank our teaching assistant, Harmen, for answering our many, many questions. Lastly, we are grateful to the University of British Columbia for providing us with the opportunity to conduct our research through such an enriching course like BIOL 342.

References

Battarbee, R.W., Charles, D.F., Dixit, S.S., and Renberg, I. 1999. Diatoms as indicators of surface water acidity. *In* The Diatoms: Applications for the Environmental and Earth Sciences. *Edited by* E.F. Stoermer and J.P. Smol. Cambridge University Press, Cambridge.

Bradbury, J.P. 1999. Continental diatoms as indicators of long-term environmental change. *In* The Diatoms: Applications for the Environmental and Earth Sciences. *Edited by* E.F. Stoermer and J.P. Smol. Cambridge University Press, Cambridge.

Cembella, A.D., Quilliam, M.A., Lewis, N.I., Bauder, A.G., Dell'Aversano, C., Thomas, K., Jellett, J., and Cusack, R.R. 2002. The toxigenic marine dinoflagellate Alexandrium tamarense as the probable cause of mortality of caged salmon in Nova Scotia. Harmful Algae. **1**(3): 313-325.

Honeywill, C. 1998. A Study Of British Licmophora Species And A Discussion Of Its Morphological Features. Diatom Research. **13**(2): 221.

Ohgai, M., Tsukahara, H., Matsui, T., And Nakajima, K. 1984. The Effect of the Environmental Factors on the Growth of two Epiphytic Diatoms Licmophora abbreviata AGARDH And L. Paradoxa (LYNGBYE) AGARDH in vitro. Nihon Suisan Gakkai Shi. **50**(7): 1157.

Ravizza, M. and Hallegraeff, G. 2015. Environmental conditions influencing growth rate and stalk formation in the estuarine diatom Licmophora flabellata (Carmichael ex Greville) C.Agardh. Diatom Research. **30**(2): 197.

Stockner, J.G. and Shortreed, K.R.S. 1976. Autotrophic Production in Carnation Creek, a Coastal Rainforest Stream on Vancouver Island, British Columbia. Journal of the Fisheries Research Board of Canada. **33**(7): 1553-1563.

Appendices

Appendix A. One-Way ANOVA input data and analysis

	Treatment		
Replicate Number	L	C	D
1	8946.4	5536.1	472.16
2	10940	7272.2	107.63
3	7618.6	4571.1	929.28

Anova: Single Factor

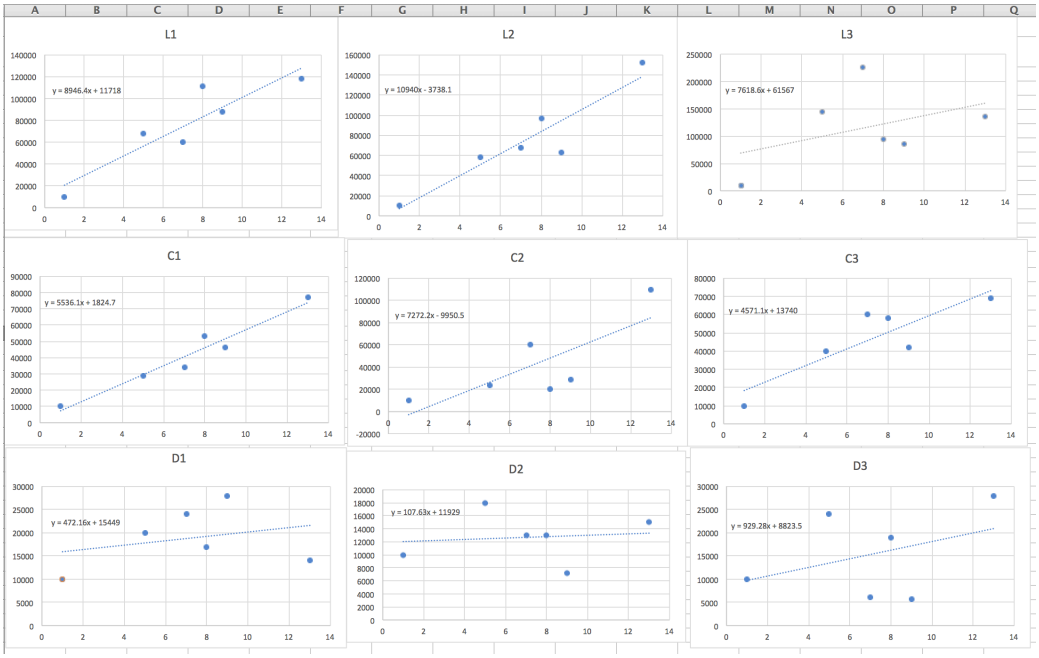
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	27505	9168.3333	2794865.3
Column 2	3	17379.4	5793.1333	1873534.9
Column 3	3	1509.07	503.02333	169491.59

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	114464836	2	57232418	35.490098	0.0004735	5.1432528
Within Groups	9675783.6	6	1612630.6			
Total	124140620	8				

Appendix B. Scatterplots of Raw Data (Cell Counts)



Day	L1	L2	L3	D1	D2	D3	C1	C2	C3
1	10000	10000	10000	10000	10000	10000	10000	10000	10000
5	68000	58000	145000	20000	18000	24000	29000	24000	40000
7	60000	68000	226000	24000	13000	6100	34000	60000	60000
8	111000	97000	94000	17000	13000	19000	53000	20000	58000
9	88000	63000	86000	28000	7200	5800	46000	29000	42000
13	118000	152000	136000	14000	15000	28000	77000	110000	69000

Appendix C. Box Plot Summary

	21-HR	8-HR	0-HR
MAX	10940	7272.2	929.28
MIN	7618.6	4571.1	107.63
MEDIAN	8946.4	5536.1	472.16
Q1	8282.5	5053.6	289.895
Q3	9943.2	6404.15	700.72
	21-HR	**8-HR**	**0-HR**
Q1 - Minimum	663.9	482.5	182.265
Q1	8282.5	5053.6	289.895
Median - Q1	663.9	482.5	182.265
Q3 - Median	996.8	868.05	228.56
Maximum - Q3	996.8	868.05	228.56