Membrane Composition Analysis of *Caenorhabditis* Genus Members

A Major Qualifying Project Report

Submitted April 26, 2018

in partial fulfilment of the degree requirements for the

Bachelor of Science in Biochemistry

Bri Heisler

with the support of Professor Carissa Olsen as faculty advisor

Table of Contents

Abstract

Lipidomic studies are essential to elucidating the biochemical mechanisms underlying cellular lipid metabolism, trafficking, and homeostasis. Changes in the lipid distribution and composition of the cell membrane are implicated in a variety of diseases; thus, understanding the processes that alter the membrane under normal physiological conditions or under controlled laboratory circumstances is crucial to eventually treating such ailments **[1, 2]**. In this research project, a comparative study across three *Caenorhabditis* species/strains was conducted to learn about the differential effects of native and growth environment on thermotolerance and the composition of the cellular membrane. An examination of the impact of varied growth temperature in conjunction with RNAi experiments defines the response to heat stress as the membrane is adapted and lays the foundation to explore the mechanisms or pathways responsible for varying thermotolerance in organisms as well as the genetic differences that cause such a phenomenon.

Acknowledgements

With my sincerest gratitude, I thank Professor Olsen for her support and guidance throughout the project. A special thank you to her lab group as well, for all their help inside and outside the laboratory.

Introduction

The genus *Caenorhabditis* is comprised of a range of constituent species spanning many different locations and environments throughout the world. The species *C. elegans* has long been used as a model organism for scientific study due to its compact structure, short and predictable lifespan, and adaptability to laboratory conditions. Other species, such as *C. briggsae*, are similarly useful research organisms. Despite diverging around 100 million years ago, both species are morphologically quite similar. They have retained a high degree of genetic overlap as well, with about 62% protein orthology reported. They also have the same ratio of coding to non-coding DNA and the same number of chromosomes. As soil-dwellers, they feed off decomposing fruits or other detritus. For their similarities and differences, and because both have had their genomes sequenced, they are used in comparison to one another for studies involving genetics **[3]**.

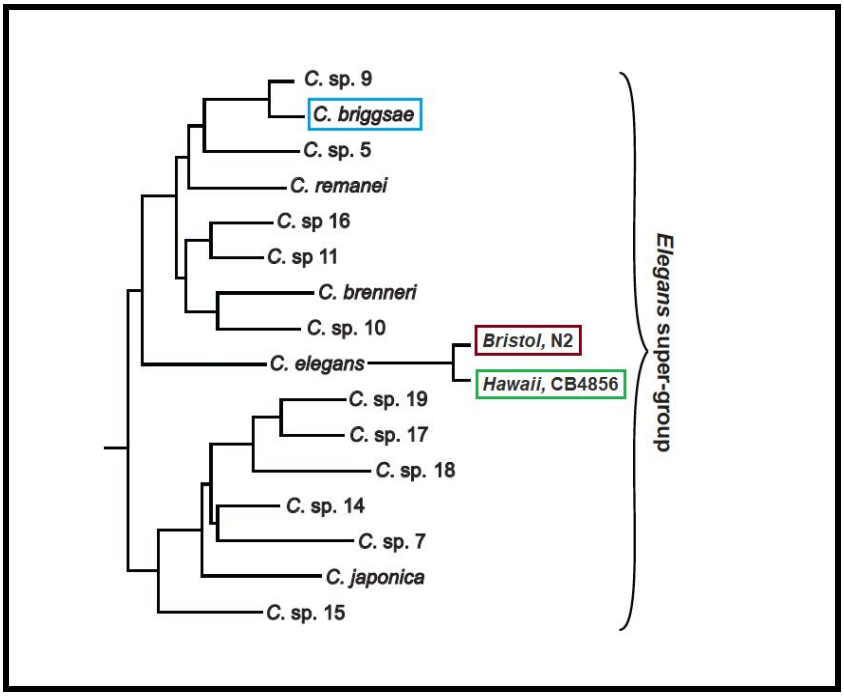

Figure 1. A diagram drafted to demonstrate phylogeny among *Caenorhabditis* species based on genes encoding RNA polymerase II **[4]**. The two *C. elegans* strains used in this project, N2 and CB4856, have been highlighted.

For all nematode species, health can be assessed in similar ways. Such methods include monitoring range of motion, pharyngeal pumping, and response to oxidative or heat stress **[5]**. Thermotolerance is a standard measure of health in *Caenorhabditis*, as thermal resistance correlates with longevity. During exposure to high temperatures, *Caenorhabditis* worms experience a multitude of biochemical changes, including adjustments to the composition of their cellular membranes. Different species and strains innately differ in resistance to heat shock due to the environments from which they originate or due to specific mutations that have been selected for under laboratory conditions **[6]**. It is documented that AF16 performs better at higher temperatures than N2 for a variety of metrics. The optimal temperature for reproduction and fertility is

18.2°C for N2 with an upper limit slightly under 27°C. For AF16, fertility is highest at 21.5°C, and it does not become sterile until after 30°C. Predictably, since thermotolerance is positively correlated with longevity, AF16 boasts a median and maximum lifespan of 3.3 and 3.0 days respectively higher than N2 when assayed at 20°C **[7, 8, 9]**. A number of processes account for these differences, among which are the mechanisms responsible for modulating the composition of the cellular membrane.

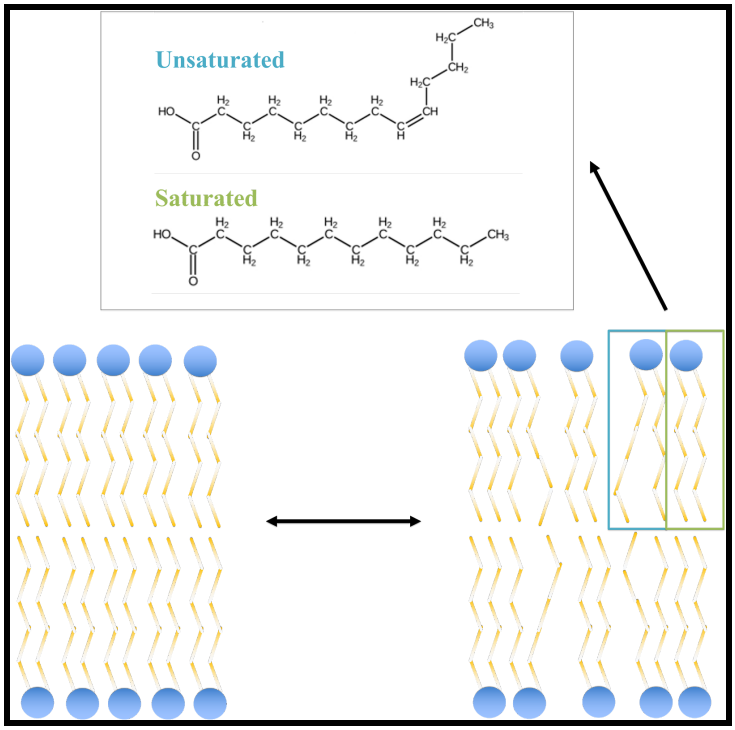

Figure 2. A simplification of membrane dynamics in different temperatures. Unsaturated members (left) are unable to pack tightly due to the kinks resultant from double bonds in their tails and are more representative of membranes at colder temperatures. This reduces contact with neighboring species, decreasing van der Waals forces that, in part,

hold them together. Thus, unsaturated fatty acids in the membrane increase fluidity, while a membrane with more saturated fatty acids will be rigid (left).

In warmer environments, organisms will upregulate the abundance of saturated fatty acids and shorter chain fatty acids, among other lipids, in order to combat increased fluidity caused by temperature. Conversely, in cold temperatures organisms must incorporate more unsaturated fatty acids to prevent stiffness. Of particular interest in to this project is the effect that native environment has on the lipid distribution in the cellular membrane, innately and under temperature stress. Because of differences in habitat, species may have evolved different mechanisms to alter the composition of the membrane in response to temperature changes in the environment to minimize other consequences. The environment in which the worms are reared could be a factor in this variability in response in that individuals “primed” during larval stages by long term exposure to warm temperatures may experience a significant impact on both the resting membrane composition as well as the response to later acute heat shock. Literature shows that priming can assist in later heat shock survival **[10]**, but research relating this finding to membrane adaptation or modulation are scarce.

Quantifying changes in the lipid composition is key to understanding the mechanisms by which the worms monitor for and adapt to changes in temperature. Strains for this study were chosen for the location from which they were isolated as well as the amount of prior research performed on them. The N2 strain of *C. elegans* was originally isolated from the United Kingdom and is the most commonly-used control for nematode research. Though it was grown in laboratory conditions for generations prior to being

frozen down (to reduce mutations and preserve the original, wild-type genotype) is it assumed to be representative of a temperate strain, adapted to colder environments and poorly-suited to heat stress. A closely-related strain of the same species, CB4856, was chosen for comparison. This strain, isolated from Hawaii, is obviously better adapted to a warmer climate, yet it retains enough genetic similarity to the N2 strain to be considered of the same species. Lastly, the AF16 strain of *C. briggsae* was used because it is well-studied as a comparison to *C. elegans* over a variety of inquiries and because it was originally isolated from an extremely hot region of India and likely has adapted to such weather. The lipid profile for N2 is well-characterized under standard laboratory conditions, but a baseline for the membranes of other strains and for a variety of temperature conditions was necessary to establish. Four main experimental conditions were tested.

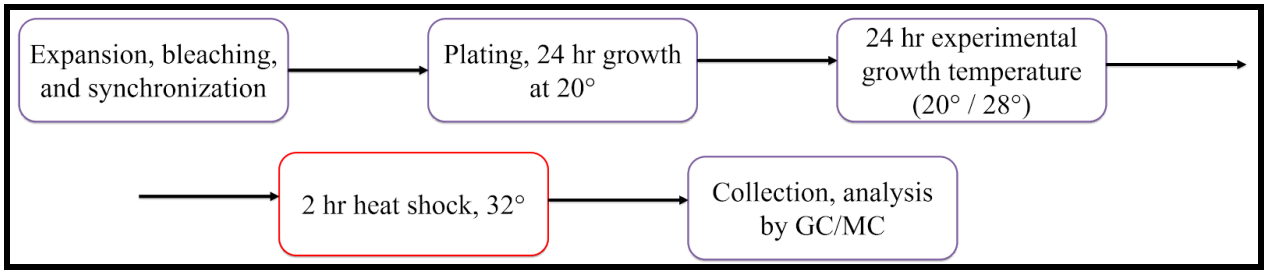

Figure 3. Outline of the growth regimen for the control condition (20°C) and for the heat-primed worms (28°C). The optional heat shock is shown in red, to which half of each growth condition were subjected. Further information about the growth and maintenance of the worms as well as fatty acid methyl ester (FAME) creation for analysis by GC/MS can be found in the section on materials and methods.

After establishing the expected profile of the worms under the various conditions, it became clear that an RNAi assay would be useful to probe further into the polyunsaturated fatty acid pathway, which seemed to be modulated differently in the three strains. Notably, AF16 relies more heavily on polyunsaturated fatty acid modulation whereas the N2s rely on monounsaturated fatty acid modulation. Interestingly, CB4856 appears adept at managing both classes of unsaturated fatty acids to compensate during heat stress.

Lipid by lipid, the polyunsaturated fatty acids changed the most dramatically during heat stress for all strains. Two genes, *fat-2* and *fat-3*, are important components of the polyunsaturated fatty acid synthesis pathway. They are both desaturases, and catalyze the following steps of the pathway:

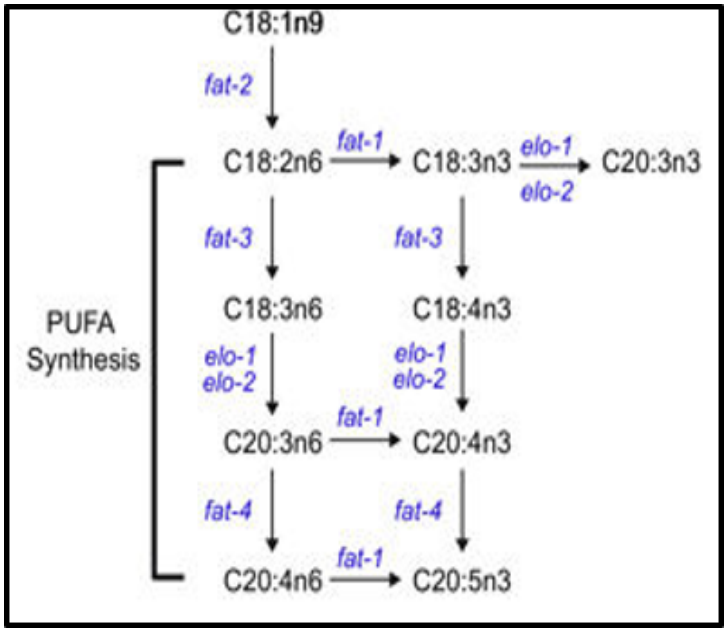

Figure 4. Pathway for the polyunsaturated fatty acids able to be synthesized by *Caenorhabditis* **[11]**. Fatty acids are written in black with the total number of carbons

first (C18, for instance), followed by the number of double bonds and the position of the first double bond. So, C18:1n9 has eighteen carbon atoms and one double bond which is located nine carbons in if counting from the carboxylic acid group. The genes coding the enzymes responsible for the conversions are listed in blue. First, *fat-2* codes the enzyme delta(12) fatty acid desaturase that converts oleic acid to linoleic acid (C18:1n9 → C18:2n6). From there, *fat-3* is implicated later on in the pathway, catalyzing two reactions (C18:2n6 → C18:3n6 and C18:3n3 → C18:4n3) that require addition of double bonds as well.

For the RNAi experiment, interfering RNAs are used to target and inhibit mRNAs, thus silencing genes by preventing their translation. Hereafter, an examination of the strategies used by *C. elegans* and those convergently arising in hot weather strains will lead to insights into phylogeny among *Caenorhabditis* worms as well as into the greater importance of membrane modulation in homeostasis and disease.

Results

To assess the lipid profile of the worms under the experimental conditions, synchronization was necessary to control the worms for growth stage, as the lipid composition of the membrane will change greatly through the life stages and as adults age **[12]**. For every experimental condition, the first 24hrs of growth post-plating was at 20°C to allow normal progression through the early larval stages. Thereafter, some plates were moved to 28°C for heat priming for 24 hours while their counterparts remained at 20°C for the duration. As worms reached adulthood, control and heat primed worms were subjected to a 2 hour heat shock at 32°C. At the end of the growth period regardless of condition, worms were collected simultaneously and frozen for analysis.

The worm pellets were then subjected to fatty acid methyl ester creation, which cleaves the fatty acid from the headgroup yielding a population of molecules that are small enough to be analyzed via gas chromatography and mass spectrometry **[13]**. In the gas chromatograph, samples are vaporized and transferred to a chromatographic column by a carrier gas. When flowing through the column, all of the components of the sample are separated out according to their relative interactions with both the stationary phase (the surface of the column) and the gaseous phase (the carrier gas). Thereafter, the compounds going through the column are ionized, which often fragments larger molecules. Identification and quantification of these fragments can provide enough information to identify the larger molecule. After ionization, the molecules are separated based on mass and detected by the machine to produce output to the user **[14]**.

Following processing by GC/MS, the data was collected and analyzed to yield the relative abundance of each lipid species for all worms and conditions. For complete data showing the results for every lipid species, worm strain, and experimental condition, see **Fig. 11** in the appendix.

Qualitative Assessment of Competence in Different Growth Environments

Qualitative assessment of adaptability to each temperature was performed by examining each plate of worms at all timepoints (every 24 hours, and pre- and post-heat shock). For the worms reared at 28°C, both morphological and behavioral differences across the different types of worms were observed. Consistently, the Hawaiian strain of *C. elegans* was the most robust and active at the high temperature; its growth was accelerated compared to other Hawaiian worms reared at 20°C and compared to the two other populations at 28°C. *C. briggsae* did not appear to be greatly affected by rearing at 28°C. The N2 population of *C. elegans* suffered from long-term exposure to warmer temperatures, experiencing reduced motility, but also undergoing maturation at a slightly increased rate. The approximate time needed in ambient temperature for the worms to recover their motility post-heat priming is between 1 - 2 hours.

Polyunsaturated Fatty Acid Abundance Across all Strains

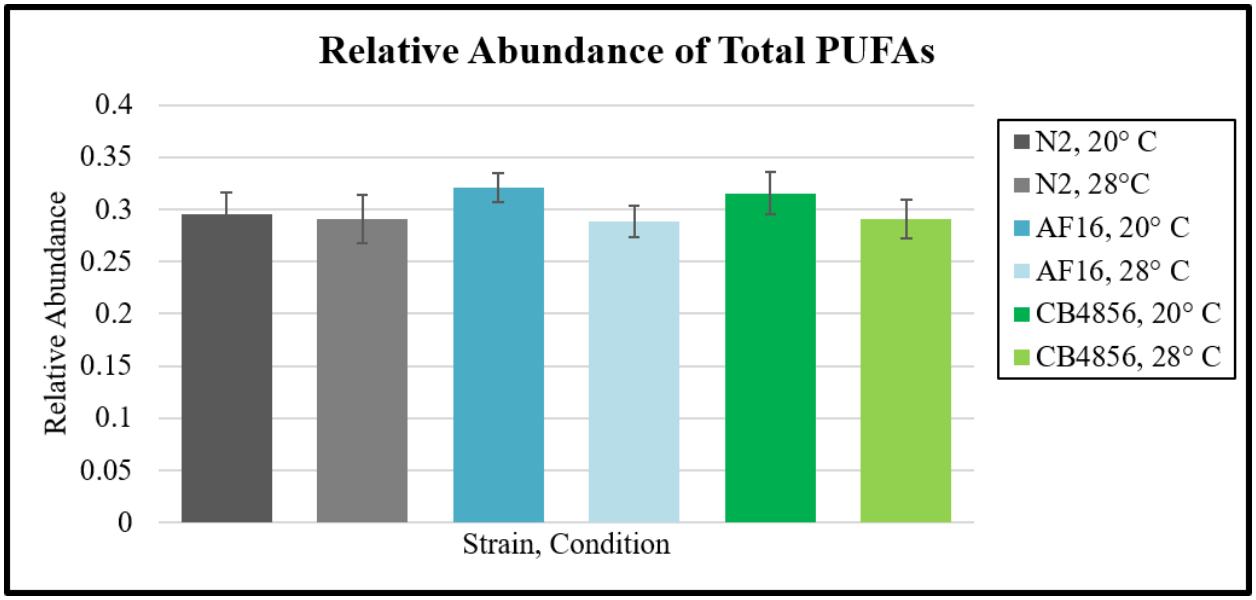

Figure 5. The relative abundance of pooled polyunsaturated fatty acids for control and heat primed strains. This preliminary evidence suggests that possibly the two hot weather strains (AF16 and CB4856) are better able to modulate polyunsaturated fatty acid levels in response to heat stress, which would allow them to more rapidly or efficiently compensate in warm environments. As noted before, unsaturated fatty acids contribute to membrane fluidity, but moreover, these longer-chain, polyunsaturated fatty acids contribute more to fluidity than do the monounsaturated fatty acids.

Monounsaturated Fatty Acid Distribution After Heat Priming

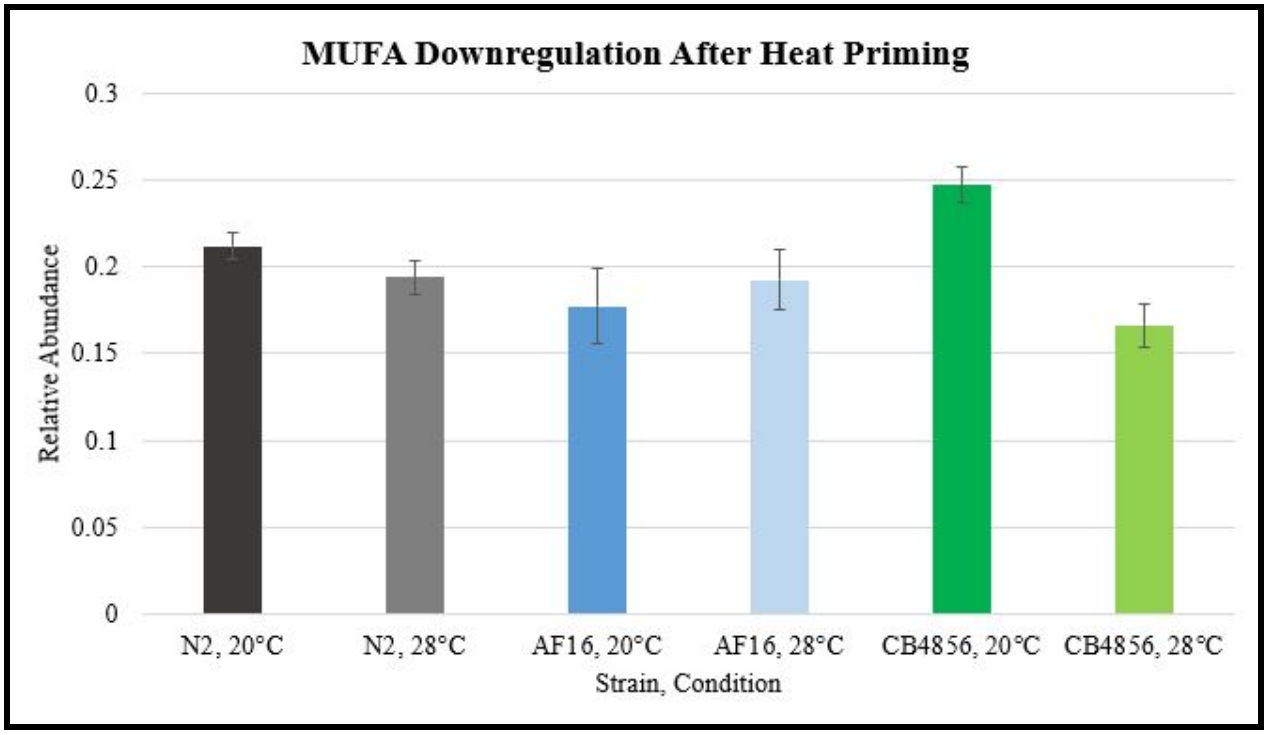

Figure 6. Downregulation of monounsaturated fatty acids in the *C. elegans* species and the *C. briggsae* species. Here, it is evident that the two strains of *C. elegans* (left and right) modulate total monounsaturated fatty acid levels to compensate during heat priming, whereas *C. briggsae* (center) appears to not. All strains demonstrate an increase in saturated fatty acid to some degree when faced with heat stress, but regulation of other classes of lipids changes from strain to strain.

Regulation of Polyunsaturated Fatty Acid Composition by Species

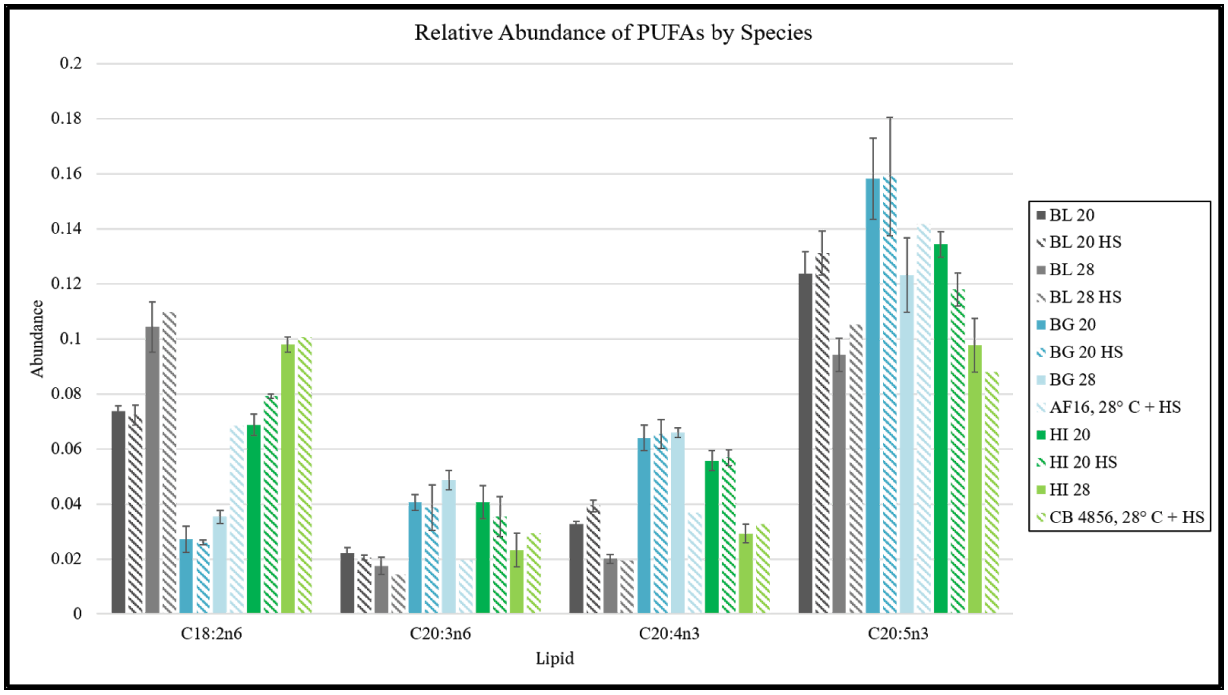

Figure 7. Downregulation of many polyunsaturated fatty acid species in response to heat priming. For most lipids affected by the *fat-2* and *fat-3* pathways (i.e. those highlighted here), a sharp decrease in abundance occurs during heat stress **(Fig. 4)**. Across all strains, downregulation is most pronounced for the 20:5n3 species, which would yield the most effective reduction in fluidity. For all species, innate lipid levels are most similar between the two *C. elegans* members. It also appears that heat priming uniformly elicits a more significant response than does heat shock, suggesting more than a few hours are needed for the membrane to acclimate to environmental changes. Only very rarely would organisms in the wild encounter such severe temperature swings so quickly in their environment, especially because the soil around them mitigates ambient temperature change. Therefore, it would not be necessary to adapt to such rapid, drastic temperature change.

Knockdown of *fat-2* and *fat-3* Impacts Survival During Heat Shock

Some of the greatest changes among both different strains and different conditions occurred in the downregulation of polyunsaturated fatty acids. Especially notable were the changes occurring to the species controlled by *fat-2* and *fat-3* in the polyunsaturated fatty acid synthesis pathway **(Fig. 4)**. RNAi assays were performed to determine what role these genes had in modulating response during the 2 hour heat shock at 32°C and why *C. elegans* and *C. briggsae* differ. N2 and AF16 worms were monitored for survival when exposed to RNAi of *fat-2* or *fat-3*, and the results are quantified below.

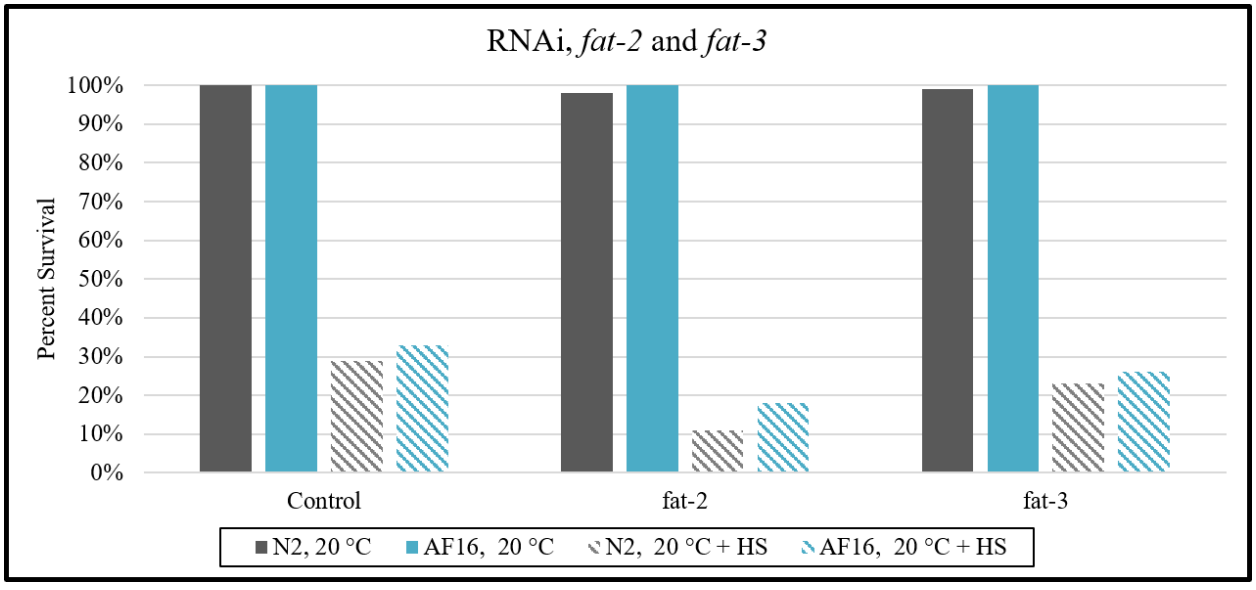

Figure 8. Differential response to *fat-2* or *fat-3* knockdown in *C. elegans* and *C. briggsae*. Preliminary data here suggests *fat-3* knockdown may be more easily compensated for than *fat-2* knockdown, though it is unclear why inhibiting polyunsaturated fatty acid synthesis would seem to increase lethality during heat shock.

During the RNAi assays, the worms were also monitored for behavioral and developmental changes. Knockdown of either gene did not appear to affect motility or pharyngeal pumping in adult worms. All new-generation L1s were impaired in their movement, sluggish, and, rarely, reluctant to move even with prodding. For N2s, RNAi fatally arrested larval development at the L1 stage, as evidenced by a small number of bodies remaining on the plate among the healthy adult worms. For AF16, development was retarded but not fatally; a gradient of larval and adult stages was present at the end of the experiment. The proper functioning of the polyunsaturated fatty acid pathway is important at all stages of development, as evidenced by the impairment of survival on RNAi-exposed adult worms during heat shock, but evidently it is integral to growth and even survival at early developmental stages.

Cyclopropyl Fatty Acid Incorporation During Heat Stress

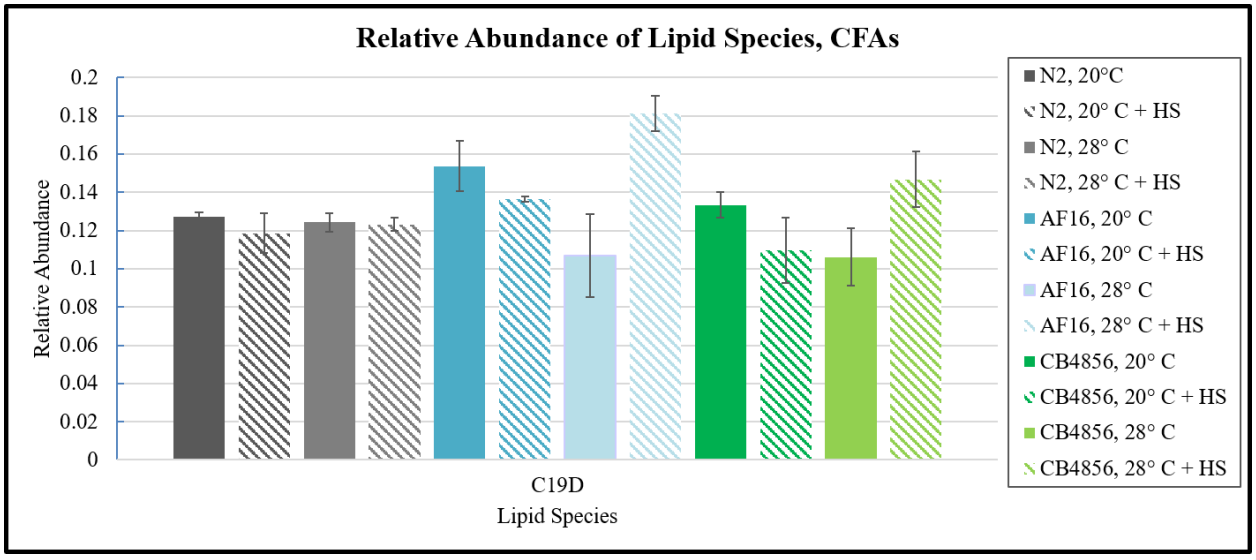

Figure 9. Heat-primed tropical strains increase the rate of incorporation of cyclopropyl fatty acids into the membrane during heat shock. N2s retain the same proportion of C19D throughout all four conditions tested, whereas the hot weather strains are able to upregulate cyclopropyl fatty acid incorporation during heat shock post-priming. cyclopropyl fatty acids are only available through dietary means; thus, there is no accompanying enzymatic or synthesis pathway to modulate their abundance.

Discussion

Targets of Membrane Modulation

The cellular membrane is a dynamic and diverse structure that requires constant maintenance to maintain proper function through an ever-changing extracellular environment. Fluidity is a key property requiring maintenance. The modulation of many components is necessary to achieve homeostasis. When subject to a hot environment, the excess thermal energy causes lipids in the membrane to move about more quickly, increasing the fluidity irrespective of its composition. To mitigate this and prevent cellular damage, organisms can differentially regulate many membrane constituents. Cholesterol is one of many lipid molecules in the membrane of eukaryotes and comprises up to 20% of the membrane by mass. Its sterol group is closely attracted to the fatty acid tail of neighboring phospholipids, which functions to anchor it strongly into the membrane, thereby stiffening it. Fatty acids with shorter chains are less stiff than their longer counterparts because they are more susceptible to changes in kinetic energy and they lack the surface area requisite to maximizing van der Waals forces. Longer chains cause more rigidity in the membrane, as these species are not as easily influenced by kinetic or thermal energy increases and have stronger intermolecular forces. Saturated fatty acids pack tightly together due to their tail structure which increases van der Waals forces and subsequently intermolecular attraction; conversely, the kinks formed in fatty acid tails by the presence of double bonds prevents close packing and causes an increase in fluidity for membranes with a high proportion of unsaturated fatty acid species **(Fig. 2)**.

Polyunsaturated Fatty Acid Modulation

It was among the polyunsaturated fatty acid species that some of the greatest changes occurred through the various conditions, though not necessarily congruously with expectations. It would be expected that a nearly uniform downregulation of polyunsaturated fatty acids would be seen upon increasing the temperature, since decreased polyunsaturated fatty acid abundance would theoretically aid in balancing out membrane viscosity, but this is not observed. Of the lipid species analyzed, C20:5n3 would be expected to contribute most significantly to membrane fluidity due to its length and its level of saturation; therefore, it is consistent with expectations that it is markedly downregulated in all strains (**Fig. 7**).

Other polyunsaturated fatty acids are downregulated as well, but 18:2n6 expression deviates from this, and is increased in all worms, especially for both *C. elegans* strains. This is could be due to the relative decrease of all other polyunsaturated fatty acid species, though it could also be because 18:2n6 is actually upregulated. Further inquiry into the synthesis pathway could help determine what occurs.

Overall, the amount of total polyunsaturated fat decreases for CB4856 and AF16, but there is no significant change in the abundance of polyunsaturated fatty acids overall for the N2s **(Fig. 5)**. This would suggest that perhaps the hot weather strains have an easier time swiftly downregulating polyunsaturated fatty acids in response to increase in temperature. Also of note, for the species C20:3n6 and C20:4n3, downregulation is occurring in both *C. elegans* strains, but the abundance of these

species is relatively unaffected in *C. briggsae* **(Fig. 7)**. For CB4856, C20:5n3 downregulation alone is nearly totally responsible for reducing the overall amount of polyunsaturated fatty acid in the membrane.

Of note, even if the relative abundance for these is different between the two *C. elegans* strains, the Hawaiian strain still follows the same pattern of change as its brother strain (albeit, more dramatically); so, even if innate levels of a lipid at 20°C are different, the response and therefore, possibly the mechanism governing that response, are conserved. There could simply be a regulatory element that causes the same, conserved pathway, to be more active in hot temperatures in CB4856 since it is accustomed to adaptation in warm weather. For instance, the Hawaiian strain could have a larger number of copies of a heat-responsive gene.

Monounsaturated Fatty Acid Modulation

In lieu of modulating the total polyunsaturated fatty acid content, N2 downregulates total monounsaturated fatty acid levels **(Fig. 6)**. While N2s downregulated certain types of polyunsaturated fatty acid, pooling results seems to suggest that in upregulating species that stiffen the membrane (saturated fatty acids), monounsaturated species are excised (or converted) to compensate. Here, it is not the hot weather strains that behave similarly, but the two strains of *C. elegans*. AF16 does not modulate total monounsaturated fatty acid abundance in the membrane; CB4856 does significantly. This is another example of a process that could be mechanistically similar in the two *C. elegans* strains but different in magnitude due to increased gene copies or altered genetic regulatory elements.

Cyclopropyl Fatty Acid Modulation

The N2 worms do not experience any changes to the relative abundance of either two species (C17D, C19D) of cyclopropyl fatty acid through any of the four conditions **(Fig. 9)**. Cyclopropyl fatty acids are only available dietarily for the worms, as their bacterial food source alone possesses the enzyme required to add methylene groups to double bonds in lipids. Unless the worms were excising other species in huge quantities out of the membrane entirely or converting existing species into something not addressed in these analyses, it is likely that cyclopropyl fatty acid incorporation is upregulated in response to heat shock. It is noted in literature that having less cyclopropyl fatty acid is associated with greater lethality during heat stress **[15]**. Perhaps regulating the rate of incorporation of cyclopropyl fatty acids is an important element in modulating membrane composition during temperature change. Interestingly, the two *C. elegans* members respond differently with regard to cyclopropyl fatty acid changes. More so than with the unsaturated fatty acids, the hot weather strains begin to exhibit similar changes in the membrane composition. Because of the N2 strain's dissimilarity in reaction compared to CB4856, it could be hypothesized that the similarities between AF16 and CB4856 here are due to convergent evolution and not necessarily a conserved or analogous pathway or mechanism. It could be as simple as an adaptation consume more to incorporate more during heat-stressed times, but it could also be that they have independently figured out how to upregulate incorporation.

Materials and Methods

Worm Strains and Maintenance

Worm strains included two members of the *C. elegans* species, N2 and CB4856, as well as *C. briggsae* strain AF16. Stocks of worms were grown and maintained at 20°C on HG plates seeded with *E. coli* strain OP50. Aliquots were kept frozen at -80°C for preservation and as a backup in case of contamination **[12]**.

Experimental Growth Regimen

Worm populations were synchronized by bleaching (20% bleach, 10% KOH, 70% M9) and eggs were allowed to incubate 20-24 hours at 20°C in M9 buffer. Approximately 5,000 of the resultant L1 larvae were transferred to each seeded HG plate and incubated another 24 hours at 20°C after which time experimental samples were moved to growth temperatures of 28°C, or maintained at 20°C for 24 hours. At the three day mark, select samples were subjected to 2 hour heat shocks at 32°C. Heat shocked samples were allowed to recover a few minutes before visual analysis and collection and freezing. Certain samples were also selected for extended heat shocks, varying in duration. After these three days, all worms were washed in M9 and collected in preparation for FAME creation and subsequent analysis by GC/MS. At each timepoint, worms were monitored for survival, as well as qualitative markers for health such as motility, fertility, and development **[12, 13, 14]**.

FAME Creation

To each whole worm sample, 975 µL methanol and 25 µL sulfuric acid was added. Samples were heated at 80°C for an hour and periodically vortexed. Then, 1.50

mL dH_2O and 200 µL hexane were added and snap frozen in a bath of ethanol and dry ice. The fluid hexane layer that remained after freezing was poured into a smaller vial for analysis by GC/MS **[13]**.

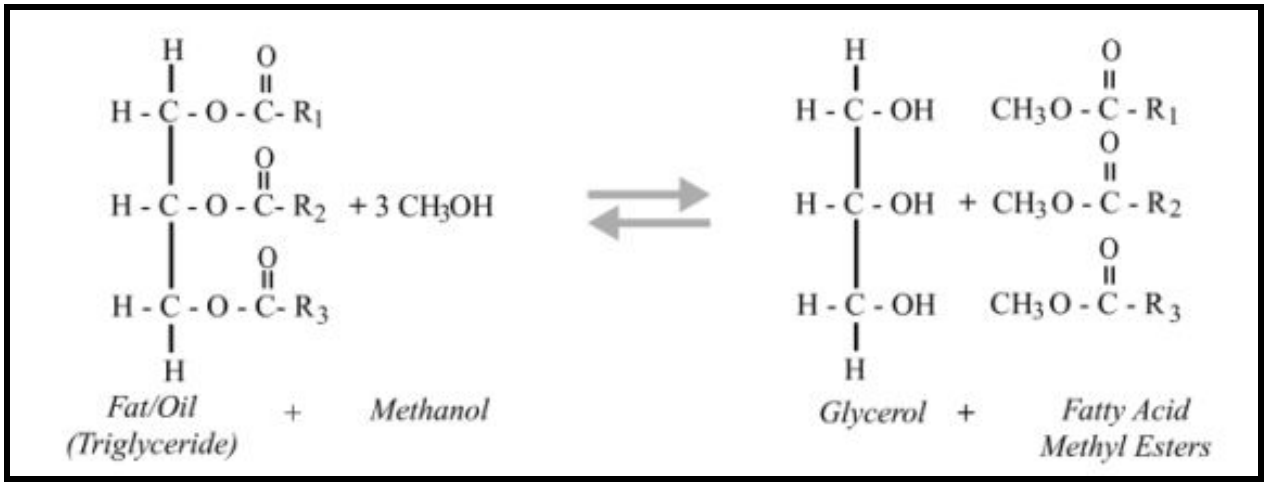

Figure 10. Simplified example of FAME creation **[16]**. The transesterification yield glycerol and fatty acid methyl esters, derived from the tails of the membrane lipid species. In this procedure, sulfuric acid was used for catalysis, but strong bases can work as well.

RNAi Assay

Preparation of RNAi Feeding Plates

Obtained the sequence name for each gene of interest and removed appropriate plates from -80°C freezer. The parafilm was pierced with sterile needle and a 20 µL pipet was used to help streak bacteria onto an LB + Carb + Tet plate. The plate was then incubated for 16-18 hours at 37°C.

Make RNAi Starter Culture

5 mL of LB + Carb + Tet culture was inoculated (final antibiotic concentrations are carb: 50 ug/mL and tet: 15 ug/mL) with 2-3 colonies from the stock plate and incubated for 16 hours at 37°C

Grow RNAi culture

For each RNAi treatment, a minimum of two RNAi plates are needed. 80-100 mL of LB was added plus the appropriate amounts of RNAi starter, Carb, and Tet (see table below).Then, samples were shaken at 37°C overnight.

LB (mL)	RNAi bacteria (µL)	Carb (µL)	Tet (µL)
50	50	25	37.5
60	60	30	45
80	80	40	60
100	100	50	75

Table 1. Components needed for RNAi treatment.

Make RNAi plates

RNAi culture was harvested and spun at 4000 rpm for 10 minutes, after which the LB was discarded. The remaining culture was added to same tube, spun at 4000 rpm for 10 minutes, and the LB was again discarded. Lastly, one quick spin at high speed was performed and the remaining LB was aspirated to ensure it was completely discarded. The pellet was resuspended in 0.15 g/mL of M9. Then, 1 mL of concentrated RNAi stock was added to a 10 cm NGM + CI plate and gently distributed to cover the entire plate and dried briefly. This was incubated for 48 hours at 25°C.

Stock Solutions

LB + Carb + Tet can be premade and stored at 4°C

Antibiotic Stocks

100mg/mL Carbenicillin

- Add 1 g to 10 mL sterile dH2O

- Vortex to mix; filter sterilize

- Aliquot 1mL stocks; store at -20C

20 mg/mL Tetracycline-HCl

- Add 200mg to 10mL sterile dH2O

- Vortex to mix (may need to gently heat); filter sterilize

- Aliquot 1mL stocks; store at -20C

References

1. Ying-Yong Zhao, Xian-long Cheng, Rui-Chao Lin, Chapter One - Lipidomics Applications for Discovering Biomarkers of Diseases in Clinical Chemistry, Editor(s): Kwang W. Jeon, In International Review of Cell and Molecular Biology, Academic Press, Volume 313, 2014, Pages 1-26, ISSN 1937-6448, ISBN 9780128001776, https://doi.org/10.1016/B978-0-12-800177-6.00001-3. (http://www.sciencedirect.com/science/article/pii/B9780128001776000013)
2. Lipidomics: Techniques, Applications, and Outcomes Related to Biomedical Sciences. Yang, Kui et al.Trends in Biochemical Sciences , Volume 41 , Issue 11 , 954 - 969.
3. Kiontke, K. and Fitch, D.H.A. The Phylogenetic relationships of *Caenorhabditis* and other rhabditids (August 11, 2005), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.11.1, http://www.wormbook.org.
4. Kiontke et al. 2011 BMC Evolutionary Biology 11: 339.
5. A. Rollins, Jarod & C. Howard, Amber & K. Dobbins, Sarah & H. Washburn, Elsie & Rogers, Aric. (2017). Assessing Health Span in Caenorhabditis elegans: Lessons From Short-Lived Mutants. The Journals of Gerontology: Series A. 72. 10.1093/gerona/glw248.
6. Fatt HV, Dougherty EC. (1963). Genetic control of differential heat tolerance in two strains of the nematode, *Caenorhabditis elegans*. Science. *141*, 266-267.

7. Amrit, F. R. G., Boehnisch, C. M. L., & May, R. C. (2010). Phenotypic Covariance of Longevity, Immunity and Stress Resistance in the *Caenorhabditis* Nematodes. *PLoS ONE*, *5*(4), e9978. http://doi.org/10.1371/journal.pone.0009978
8. McCulloch, D. and Gems, D. (2003), Evolution of male longevity bias in nematodes. Aging Cell, 2: 165-173. doi: 10.1046/j.1474-9728.2003.00047.x
9. Steven N. Austad, Kathleen E. Fischer, Sex Differences in Lifespan, Cell Metabolism, Volume 23, Issue 6, 2016, Pages 1022-1033, ISSN 1550-4131, https://doi.org/10.1016/j.cmet.2016.05.019. (http://www.sciencedirect.com/science/article/pii/S1550413116302376)
10. Parida L, Neogi S, Padmanabhan V (2014) Effect of Temperature Pre-Exposure on the Locomotion and Chemotaxis of *C. elegans*. PLOS ONE 9(10): e111342. https://doi.org/10.1371/journal.pone.0111342
11. Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans.* Jennifer L. Watts, John Browse. Proceedings of the National Academy of Sciences Apr 2002, 99 (9) 5854-5859; DOI: 10.1073/pnas.092064799
12. Stiernagle, T. Maintenance of *C. elegans* (February 11, 2006), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.101.1, http://www.wormbook.org.
13. Sekora, Nicholas S.; Lawrence, Kathy S.; Agudelo, Paula; van Santen, Edzard; McInroy, John A. (2009). "Using FAME Analysis to Compare, Differentiate, and Identify Multiple Nematode Species". *Journal of Nematology*. **41** (3): 163–173. PMC 3380492 . PMID 22736811.

14. Pavia, L., Gary M. Lampman, George S. Kritz, Randall G. Engel (2006). *Introduction to Organic Laboratory Techniques (4th Ed.)*. Thomson Brooks/Cole. pp. 797–817. ISBN 978-0-495-28069-9.
15. Yuan Yao Chen, Michael G. Gänzle, Influence of cyclopropane fatty acids on heat, high pressure, acid and oxidative resistance in Escherichia coli, International Journal of Food Microbiology, Volume 222, 2016, Pages 16-22, ISSN 0168-1605, https://doi.org/10.1016/j.ijfoodmicro.2016.01.017. (http://www.sciencedirect.com/science/article/pii/S0168160516300186)
16. Homemade Biodiesel. “The Chemical Reactions in Biodiesel.” *Homemade Biodiesel-Research*, 2012, www.odec.ca/projects/2012/besoic/research.html.

Appendix

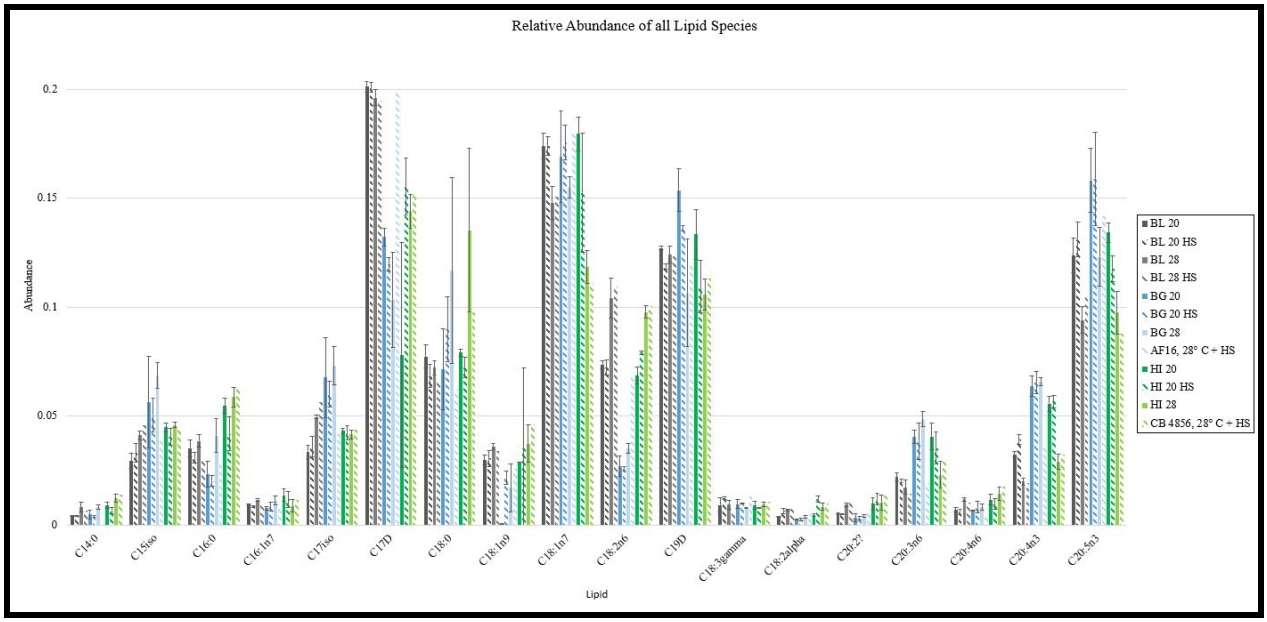

Figure 11. The relative abundance of all lipid species examined during experimentation.