Reviewer 1

Minor Essential Revisions:

-*In the reporting of the qPCR results, the authors provide p-values for both*

*transformed and non-transformed data. If the data fail normality testing, only*

*transformed results are necessary, if they did not fail, there is no reason to report*

*transformed results. Also why were specific transformations chosen?*

REPLY: We have removed the p-values for the untransformed data so only p-values post-transformation remain for the genes where a transformation was necessary (acid ceramidase and ceramide glucosyltransferase). Transformations were chosen based on the maximum value of lamda found in the boxcox plot. This information has now been added to the manuscript. In the methods, we have added: “The need to transform the data and the transformation used were determined from the lamda (λ ) corresponding to the maximum log likelihood in the boxcox plot. If λ = 1 fell within the 95% confidence interval of the maximum log likelihood, then no transformation was used.” In the results section, we have added the values of lamda that corresponded to the maximum log-likelihood and which transformation was chosen based on that value for the two genes that were transformed.

Major Compulsory Revisions

-*The authors provide qPCR efficiencies below the acceptable values. Can the*

*authors provide reasonable explanation why qPCR primers and conditions were*

*not further optimized? Were these the best primer sequences for the each gene?*

*Was primer optimization attempted? Is it possible that the low reaction efficiency*

*was due to inhibitors in the oyster tissue?*

*Although Miner is a robust and noise resistant program, the values seem low to*

*me. Can the authors provide the variation of efficiencies between samples*

*(proved in Miner’s output)? Could the authors also provide the melting curve*

*analysis of each gene used or a gel showing a clear single band?*

REPLY: We put a lot of effort into designing primer sequences that were specific to the genes that we were looking at and would not result in primer dimer. Primer optimization was attempted and these were the conditions at which the primers performed best. We agree that the efficiencies are not perfect, but we are confident that we were amplifying the correct product and getting true expression values. For each primer set, single melt peaks were observed.

We have included the standard deviation of efficiencies for each gene to give an idea of the range. We do not feel that a melting curve as a figure would enhance the manuscript, but we have included in the methods that “each qPCR primer pair amplified a single product verified by a single melt peak for each gene”.

Reviewer 2

Discretionary Revisions

1*. If there is one thing I think the study really lacks it is a perfectly clear pathway*

*diagram of ceramide metabolism. Fig 1 in ref 15 is a nice example of format. For*

*this study, what I would most like to see is a similarly formatted pathway diagram*

*showing all categories from the current version of Figure 1.*

*Many of the genes were found in this study. At least a few were not. Please*

*include these in the pathway diagram. It would be interesting to see which genes*

*could not be found, whether this results from lack of expression in larvae, low*

*absolute expression levels, or unusually high sequence divergence.*

REPLY: Figure 1 has been revised to include more genes in the ceramide metabolism pathway.

*2. Lines 194-195 list two genes not found in the transcriptome. The subsequent*

*text lists possible reasons for this absence. I suggest also reminding the reader*

*that this is a larval cDNA dataset, so developmental stage-specific expression*

*differences might explain absent genes.*

REPLY: As stated in line xxx, both the larval data set and ESTs from NCBI were downloaded. The gene mining was therefore performed in both transcriptomic data from larvae and adults.

*3. Line 183 says “almost all the genes coding for enzymes necessary for de novo*

*ceramide synthesis were identified”. Which ones were not found? I had thought*

*all the biosynthetic genes were found.*

REPLY: The desaturase that transforms dihydroceramide into ceramide was not found. We have added this detail to the manuscript.

*4. I would greatly condense the presentation of sequence homology searches – it*

*doesn’t seem necessary to present such full descriptions in the text (e.g. the*

*central point of lines 100-107 could be conveyed in a single short sentence;likewise for 117-122, etc.)*

REPLY: The text has been condensed and the details have been summarized in a new table, Table 3.

*5. Its unclear why a square-root transformation was used for Cg-GlcCer (lines*

*142-144) but not other genes. Please add a brief explanation of this decision to*

*the text.*

REPLY: Decisions regarding what transformations to perform were based on the results of a boxcox analysis. This explanation has been added to the methods.

*6. The efficiency of qPCR primers was fairly low for 3 of the 4 genes. Were any*

*alternative primers tested to improve this? Any experience you can share*

*regarding primer design and efficiencies for these genes would be valuable.*

REPLY: The primer pairs were designed to be specific to the genes being studied and to not have primer dimer. For some of the genes, multiple primer pairs were tested. Even though some of the efficiencies are low, a single melt peak was observed for each product. It is possible that more efficient primers could be designed, but we feel that our results are accurate despite the relatively low efficiencies.

*7. Figure 1 legend says the pathway was modified from references 9 and 27. I’m*

*not sure if these numbers are accurate, especially 27. Please check.*

REPLY: The reviewer is correct, the references should be 2 and 15 (Ballou et al. 1996 and Hannun & Luberto 2000).

*8. Lines 265-268 are unclear; these statements seem to be contradictory. The*

*phrasing “changes in transcriptomics” seems unusual. Please clarify these two*

*sentences.*

REPLY: The phrasing has been changed to be more clear: “Assays of gene expression are sometimes merely suggestive of the true functional organismal response effected by shifts in protein expression. Changes in global gene expression, however, are indicative of a significant physiological response, at least at the cellular level, and frequently these changes are correlated with changes in protein expression.”

*9. Its not clear to me what e-value threshold was used. The text lists E-30 (line*

*285), while table 2 includes higher e-values. I have no objection to these higher*

*e-values, my only suggestion is to clarify the thresholds.*

REPLY: A threshold of 1E-30 was used for identifying genes that were the focus of this study. This is a very conservative cut-off, however, and so in our extended table of other discovered genes (Table 2) we included those that were found with higher e-values (for this we used a liberal cut-off of 1E-5). We have clarified this discrepancy in the text of the methods.

*10. Line 348 “water water”*

REPLY: This typo has been fixed.

*11. Lines 361-362. “EF1# did not show differential expression between*

*treatments”. Please briefly state how this control was conducted.*

REPLY: We have added the following qualification to clarify: “EF1a did not show differential expression between treatments as verified by a t-test done on expression values of the qPCR run in duplicate”