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Response to Editor

Dear Dr. Jacob,

We have submitted a manuscript entitled “Gene expression plasticity and frontloading promote thermotolerance in Pocilloporid corals” (https://doi.org/10.1101/398602), for recommendation to the editorial board of PCI Ecology.
We would like to thank you for your constructive comments and opportunity to propose a revised version of the manuscript. We are grateful for the work done by PCI and two reviewers on our manuscript. As suggested, we nuanced the statements made on the link between thermal regime and response to experimental stress. We added a new analysis of differential gene expression for each colony independently. We also modified Figures 1, 2 and 5 and provided a new figure to clarify the experimental setup. The discussion has been considerably streamlined and long discussion on functional analysis has been moved to supplementary materials.
You will also find below our responses (in blue) after each point raised by the reviewers. We slightly changed the title for “Gene expression plasticity and frontloading promote thermotolerance in *Pocillopora* corals” (instead of Pocilloporid that represent numerous genera).

We hope that the new version of the manuscript will be suitable for recommendation by PCI Ecology.

We look forward to hearing from you.

Yours sincerely,

Eve Toulza for all co-authors

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Decision
by Staffan Jacob, 2018-10-26 12:02
Manuscript: https://doi.org/10.1101/398602
Decision on manuscript

Dear authors,
Two reviewers have read your manuscript entitled "Gene expression plasticity and frontloading promote thermotolerance in Pocilloporid corals" and provided detailed and constructive reviews. As you will see, they both appreciated the topic, the experimental approach used and the impressive diversity of measures performed. However, they also raised a number of important concerns and requests, from the theory concepts to clarification of experimental design and important changes into the way analyses were performed. The authors should also modify throughout the manuscript the statements made about the link between thermal sensitivity and the traits quantified to make clear that the study consist in contrasting two populations (and even species), that differ in thermal regime but certainly not only. I need to make clear that, although your manuscript potentially represent an interesting contribution to the literature, that the conclusions hold on a comparison between two populations/species is a critical specificity of this study that should be outlined and made clear throughout the manuscript.
I encourage the authors to consider these detailed reviews to carefully revise their manuscript. Please keep in mind that the correct integration of the reviewers' requests is compulsory for the acceptance of this manuscript, but that I am uncertain at this step whether all these serious objections can be adequately addressed.
Thank you for submitting your work to PCI Ecology. I look forward to see the revised version of your manuscript soon.
Sincerely Staffan

We would like to thank you for your constructive comments and really appreciate the work done by PCI and two reviewers on our manuscript that have been acknowledge in this new version. As suggested, we nuanced the statements made on the link between thermal regime and response to experimental stress. We added a new analysis of differential gene expression for each colony independently. We also modified Figures 1, 2 and 5 and provided a new figure to clarify the experimental setup. The discussion has been considerably streamlined and long discussion on functional analysis has been moved to supplementary materials. We also added elements of discussion on environmental priming, plasticity and adaptation.
In addition, please find below our detailed answers (in blue) to reviewers' specific comments.
To ease the responses to the editor, the comments have been numbered and reported in the revised manuscript to locate the corrections corresponding to the comments, rather than referring to the line or page numbers because they differ according to the manuscript version (with or without changes indicated). The comment numbers can be easily found in the manuscript using the "search" command.

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Review #1
Reviewed by Mar Sobral, 2018-10-19 10:51
Comments by Mar Sobral on the manuscript submitted to PCI "Gene expression plasticity and frontloading promote thermotolerance in Pocilloporid corals" by Brener-Raffalli et al. and submitted by Eve Toulza

I believe that studying gene expression plasticity and frontloading in face of a realistic and important environmental change (heat stress), for corals (which sustain a big amount of marine diversity) is a wonderful task to undertake. Both, because of the fundamental implications of this research (advances on the knowledge of evolutionary processes) and because of the potential practical use of this information. Even more, the fact that these organisms are holobionts and that bacterial and algae communities associated to the cnidarian hosts were investigated is a wonderful characteristic of this study. I also commend the authors for using so many alternative molecular approaches for ascribing the hosts to the species level, studying gene expression via transcriptomes, and studying bacterial and algae communities' compositions. Not only that, they used bioinformatics to search the proteins and function for each gene with a differential expression in the experiment. Additionally, I agree with the sampling and experimental design of the study. Because of all the previous reasons I believe this work has a huge potential.
However, there are other reasons -which I believe are very important- to make me think that, before publication, this work should go trough a major revision. Particularly regarding data analyses and result interpretation. In summary; I believe that the work is really good but the manuscript is very immature yet.

We thank the reviewer for pointing out the relevance of the topic and the integrative approach used in this work, and for her useful comments. We regret that the manuscript was not clear enough in its previous version. We have modified the text and streamlined the discussion to focus the attention of readers on our main results.

Here I explain some of my concerns.

Line 39 (and affecting the whole manuscript): With only 2 populations, each belonging to one of contrasting environments, this hypothesis is not tested. To test whether differences among populations from contrasting environments are due to environmental characteristics at the population level, it would be necessary to have several populations per contrasting environment or several populations along an environmental gradient (so to test a statistical relationship between environment and gene expression at the population level). Additionally, 3 different coral species were studied (one in Oman, with 3 colonies), another in New Caledonia (with one colony) and another in NC (with two colonies). These means that colony is not the same level of organization in OM and in NC, and that the comparison between NC and OM is not ok, because different coral species are being compared.

1.1. We agree that after genetic analysis, it ended up that the 3 colonies sampled in New Caledonia corresponded indeed to two different species hypothesis although much more closely related to each other than the other population (single species) from Oman. We now clarify in the

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introduction that *Pocillopora damicornis* is a species complex. Nevertheless, the results in terms of gene expression analysis were very similar for the different colonies (genotypes) within each population. Thus for clarity purpose, we presented in the previous version of the manuscript only the comparison between the two populations as a whole whatever the genetic identity of the colonies. We also present in this new version the differential gene expression analysis for each colony independently (Supplementary Figure S6 and Supplementary File S7). In addition, it is clear from the DEseq2 transcriptome clustering as well as the DAPC analysis that the response is very different between localities and similar within colonies from the same locality whatever their species identity. This has been added to the discussion.

Lines 76-78 (and affecting the whole paper): The dilemma is not between acclimatization via intra generational plasticity and intergenerational Darwinian adaptation. As you say later, both processes can occur at the same time and complement each-other. But even more important is that there is also trans-generational plasticity, which is the clearer link between acclimatization and Darwinian microevolution. This is because natural selection acts on phenotypes, therefore transgenerational plasticity will change phenotypes available for natural selection to act upon, changing the evolutionary course of a population (see for example Day, Troy, and Russell Bonduriansky. "A unified approach to the evolutionary consequences of genetic and nongenetic inheritance." The American Naturalist 178.2 (2011): E18-E36, and other works by these authors).

1.2. We totally agree with your point, sorry for the confusion and opposition between evolutionary scales in this sentence. It has been modified as “Natural variation in thermal tolerance exists among coral populations (Oliver &Palumbi 2010; Palumbi et al. 2014), especially along a latitudinal gradient (Polato et al. 2010; Dixon et al. 2015), hence providing some hope for coral survival based on their capacity to cope with heat stress.”

Additionally, the frontloading discussion (all over the manuscript) should have into account the “priming” processes explained in epigenetics (see works by Eva Jablonka).

1.3. We added the transgenerational priming concept (which is a more general process that may eventually involve frontloading of gene expression) to the discussion.

Regarding analyses of gene expression (L 393-400), I believe that expression differences between before and after the stress, should be the response variable in a model with locality, colony (nested within locality) and treatment and interaction between treatment and locality as factors. Another possibility is substitute localities and colonies by species, regrouping data in a different manner but probably the balance won’t be ok to do that.

1.4. As you mentioned before, colony is not the same level of organization in Om population (three genotypes of the same species) and in NC (three genotypes belonging to two different species). We now provide differential gene expression analysis for each colony. Their responses are much more linked to their locality of origin even if it is very difficult to disentangle the effect

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of genotype/species. Still, the main results of higher plasticity as well as gene frontloading for the colonies from the most variable thermal regime remain true.

I am not convinced that there were not differences between bacterial communities from hosts from different treatments. First, I believe that Fig. 2 needs to be changed in such a way that it is easier to understand. The legend and combination of colors and shapes makes it difficult to read.

1.5. We have changed the color scheme in order to make it easier to read: all NC samples in cold colors and Oman samples in hot colors. The shapes have also been modified to clearly distinguish the different conditions. We hope the new version of Figure 2 is now easier to interpret.

In any case, I can clearly see that for the first four genotypes in the legend there's always one of the stressed samples with a very different bacterial community than others in same genotype. I believe that an additional analysis should be made to test that. I believe that it is also needed a statistical procedure linking distances among bacterial communities with gene expression differences in hosts from control and stressed samples. That result (if there is a relationship) would prove that plastic and epigenetic effects not only affect evolution of involved populations but shape entire communities (in an analogous manner of works about community genetics by Thomas G. Whittam but with epigenetics) which is a very important potential results that goes overlooked in the current version of the manuscript. Additionally, (L 260-262) I believe this analysis is wrong. Alfa and Beta diversity should be response variables in a model including simultaneously locality, colony nested within locality, treatment (and if power enough the interaction between locality and treatment). I don't understand why one-way ANOVAS were chosen.

1.6. This microbiota analysis is rather descriptive and aimed at looking for major shifts between control and stress conditions. We hope that it is now clear on the new figure 2 that samples do not cluster by conditions but rather by species and locality. We already showed that different species display specific microbiota compositions (see our previous work Brener-Raffali et al. Microbiome 2018 which is now discussed and cited). The fact that the *in situ* samples cluster close to the experimental samples for each colony (after 5-6 months in artificial seawater) support our conclusions. We also believe that inter-individual variability in complex community composition as well as a small dataset does not allow deeper statistical analysis in the present work.

Some doubts about design: All the nubbins bleached at the same time? It would have been necessary to use survival rates as a fitness measure at a point during the experiment to measure the costs of frontloading and plasticity to fitness (as you say in L 578). Why didn´t you do that? Do you have the data to test that idea?

1.7. Before bleaching and necrosis, we first observed polyp closure that was considered as the first macroscopic sign of heat stress as specified in the methods section, and that was consistently observed for all nubbins of each population at the same temperature threshold. This

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has been now clarified. We unfortunately do not have the data to test fitness effect of plasticity, as we had limited biological material with not enough nubbins left after the experiment to measure survival rates in our experimental setup. To avoid any confusion, we nuanced speculations on possible trade-offs that we could not test experimentally.

In L 193-194 I see that four nubbins per colony were placed in each tank, right? That would mean there were 12 nubbins per tank (8 tanks total). So, there were 96 nubbins, why later only 36 were studied? (L. 208). Explanation about this is needed. From the nubbins remaining in the experiment, were the 3 stressed ones per colony and population belonging to different tanks? same tank? etc? I believe it is necessary to include a figure explaining the experimental design. (It is possible that some of my comments reflect a lack of perfect understanding of what exactly was done with samples and data. That would mean that the writing should be much clearer).

1.8. For a more robust molecular analysis we used true biological triplicates for each condition and for each colony. These replicates corresponded thus to three different tanks individually monitored and adjusted for the temperature. Three nubbins (one per tank) were sampled just before the first temperature increase (control condition) as well as before each new step of temperature increase (Figure 1, which legend has been clarified in this regard). There were actually more additional nubbins that we did not sample (5 to 8 per tank for each colony) as we did not know precisely the number of temperature increase steps before the physiological collapse. In addition, a fourth tank was maintained at the control temperature to verify that the stress we observed at the end of the stressful treatment was not due to other potential confounding effects or water cues. As suggested, we added an additional figure displaying the experimental setup to clarify this (Supplementary Figure S1). In addition, legends in Figure 1 have been modified to avoid confusion.
This experimental design finally allowed us to sample three nubbins at each timepoint (control temperature + four temperature increase steps) after photographic monitoring and visual inspection of polyp state. In order to characterize the coral response to heat stress before more profound homeostasis breakdown, we only analysed nubbins that were sampled at the timepoint before the collapse became visible (together with control nubbins) corresponding to 36 RNAseq datasets.

MINOR COMMENTS -I believe that a better explanation on how the Go analysis works is necessary, and also, that too much detail is given on that section, especially given that those results are partly speculative. I would move the function result section to supplementary materials (leaving only a general paragraph about patterns found) and the same is true for the functional section in the discussion.

1.9. A few words were added in the material and methods section to explain the goal of the GO analysis. The long discussion on functional analysis has been moved to Supplementary File S11 and replaced by a short synthesis on the main results.

It makes the discussion unnecessarily long and takes space for potentially more interesting discussion on the evolution of plasticity (see works by Kathleen Donohue). For example, I would

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discuss a bit differences between predictable, variable and predictably variable environments and which strategies could be expected in each case.

1.10. We agree with the reviewer comment and we have added a paragraph in the discussion to discuss how frontloading and plasticity may fit with offspring's environment predictability.

-Line 52: What do you mean by highly enriched?

1.11. It refered to Gene Ontology term enrichment (Fisher test). To clarify, it has been replaced by "overrepresented in the Oman colonies" in the abstract.

-Line 53: Advance which insights.

1.12. The whole sentence has been modified to be more factual and precise.

-Line 99-100, I believe you mean via transgenerational epigenetics processes, right?

1.13. Yes indeed the sentence was misleading and replaced by "it could also reflect an acclimation via epigenetic processes leading to constitutive gene expression (...) eventually enabling trangenerational plasticity".

-Line 86-87, typo? Something lacking? Sentence is not understandable.

1.14. This has been modified.

-Often, specially between text and figures you mix the term colony and genotype, please use the same throughout the manuscript. Same occurs for population and locality (Locality is changed for population in the supplementary materials, and supplementary materials are refereed to as additional materials).

1.15. This has been unified to colony, which is the sampling unit, and locality to avoid confusion throughout the text. Additional material have been renumbered and renamed as Supplementary Figures/Tables/Files.

-L. 166: With this aim.

1.16. This has been modified.

-L. 181: How many clones?

1.17. Depending on the colony size, from 15 to 20 nubbins were produced by cutting branches. This has been specified.

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-L 186. As I said, no implications can be made from environmental differences because only two localities are studied, but even more important because different species are studied per locality and in NC 2 different species are mixed.

1.18. We now provide an additional differential gene expression analysis considering each colony independently rather as replicates for the same population (Supplementary Figure S6 and Supplementary File S7). We hope now to address the effect of locality vs. colony/species more clearly.

-L 45 and 203 seem contradictory.

1.19. The sentence in the abstract refers to the timepoint that has been chosen for molecular analysis and that corresponded to the increase step before the first signs of compromised health were visible. Nevertheless, we did pursue the heat stress until the polyp closure but did not sequence that last sample as we were willing to study the response to heat stress before physiological collapse. We hope the experimental design is now clearer (see also response 1.8).

-L 295. I believe the use of a posteriori is wrong in this context

1.20. The sentence has been modified.

-L 264. The comparison should be made per colony so that we avoid the problems of mixing different species and localities.
-L 296. As I said, comparing localities with different species is not ok.

1.21. This has been done and as results are consistent we hope to convince the reviewer of the robustness of our conclusions on the differences in response to heat stress between colonies of the two localities (see also response 1.1).

-L414-415. From these lines I don´t see what the analysis was about. It seems wrong, unless I miss something.

1.22. This analysis is about testing for differences in transcriptomic plasticity at the whole-genome level between colonies from NC and Om when exposed to stress as first described in Kenkel & Matz (2016). In the previous version of the manuscript, coordinates in the DAPC space were used as a responsive variable, the origin of colonies, the treatment (control and stressed), and the interaction between these two variables were used as explicative variables. Using this approach, differences in plasticity between colonies from NC and Om is expected if the interaction term between the origin and the treatment variables is significant. However, according to a previous comment raised by reviewer 1, we modified the statistical model to account for a possible (random) effect of colonies within regions (NC and Om) nested within the locality of origin. Note that the results obtained remain strictly the same, but the new model is described in the new version of the manuscript.

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-L 415-417. Having the result of the GLM why you would need to perform Wilcoxon tests. Again, I am not sure if I miss something.

1.23. We agree with this comment that the two analyses (GLM and Wilcoxon tests) are redundant. Our initial intention was to validate this result by using two independent approaches (that lead to the same conclusion). But there is no justification in doing so and we thus removed the Wilcoxon test in the new version of the manuscript.

-L 479. Which analysis?

1.24. This paragraph refers to the comparison of the constitutive (basal) gene expression level, i.e. the comparison of the gene that has been performed using differential gene analysis between NC control condition and Om control condition. This has been clarified in the new version of the manuscript.

-L504. I disagree, the experimental design doesn't allow to focus results this way as I comment previously. Same for line 525. L 520. Remove to.

1.25. We replaced "study the evolution of adaptive abilities" by "compare the phenotypic plasticity in terms of transcriptomic response to heat stress" to stick to our results and avoid overstatements in terms of evolutionary strategies.

-L 556. This comparison should be made within genotype, not possible to mix the responses of different genotypes and specially such in the case of NC if they belong to different species.

1.26. We argue that these results are consistent with the individual analysis for each colony (see also previous comments).

-L568: Which stats were used for this comparison?

1.27. This analysis is based on differential gene analysis performed using DEseq2 as is the case for the comparison between control and stress condition for each locality. The significant gene expression level differences that we considered had an adjusted p-value < 0.05.

-L 586, Very few what?

1.28. Our RNA extraction method resulted in very few algal transcripts. This has been added.

-L 600. Eliminate also.

1.29. This has been done (now in supp file S11).

-L 628. I don't understand what you mean, explain where it comes from please.

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1.30. This has been clarified (now in supp file S11).

-L741-742. That is great, I would focus this entire section on this idea making it less descriptive and shorter.

1.31. Our transcriptome analysis suggests possible trade-offs between response to stress and growth and reproduction as we identified opposite patterns in terms or differential gene expression. This has been widely discussed in the literature, but as we could not test here the actual effect of heat stress on fitness, we nuanced our interpretations as recommended (see also response 1.7).

-Fig 6. In the text it is said that there are 24 genes which were overexpressed in OM and under-expressed in NC (L 406-407), why they aren´t show in Fig 6? Am I missing anything?

1.32. These genes are visible between the two groups "NC Specific over-expressed genes (272)" and "Om Specific over-expressed genes (2082)" but were not annotated on the figure. This was also the case for the 22 genes with the opposite pattern. This has been added to the figure.

-Fig 7. Interestingly the transcriptomes from stressed colonies were more like another than non-stressed transcriptomes, I believe that result is very relevant and I didn´t find anything about it in the text.

1.33. We thank Reviewer 1 for this interesting comment. This pattern was also observed in previous studies on coral (e.g. Kenkel & Matz 2016) but not necessarily well discussed. We believe that this convergence in the functional responses of colonies from different habitats to heat stress reflects the fact that some common molecular pathways are turned-on when colonies are facing stressful conditions although the magnitude of such responses is different. This is now stated in the new version of the manuscript.

Review #2
Reviewed by anonymous reviewer, 2018-10-24 11:32

The manuscript by Kelly Brener-Raffalli and colleagues describes how laboratory experimental increase of temperature affect the transcription of genes and the diversity of the associated symbionts in colonies of Pocillopora corals. Metabarcoding and metatranscriptomics are effectively useful but demanding technologies to get insights into the effect of heat stress on a worldwide in-danger taxon, greatly considered here as anholobiont. This work is based on a big amount of data and certainly implied big efforts to extract from bioinformatics' outputs a significant and biologically-relevant wider picture of what is going on.
Unfortunately, there are reservations with the experimental design and how far data are analyzed that limited my enthusiasm as a reviewer. I need major justifications from authors to better appreciate the value of this study. Here are the points that need to be addressed:

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1- Corals were collected at two different localities at two different time points; No initial information is given about the local water temperature and abiotic parameters (pH, photoperiod, ion concentration, c.f. l.167 & l.178 p.6 & 7) when collection was made.

2.1. Indeed corals were collected at two different localities and timepoints. This was designed deliberately to limit putative factors of variation between populations. Indeed, the different periods were not randomly selected, both of them correspond to the warm season (June for Oman and December for NC). The temperature on the field during sampling was 30.8°c and 27.1°c for Oman and NC, respectively. These information were added in the Coral sampling and Maintenance section. We did not measure other physio-chemical parameters since the two populations were then subjected to a long period of acclimatization where they were maintained under the same physico-chemical conditions.

I have read table 1 but I have no idea how stressful was the transfer by aircraft and the delayed maintenance at 26 degrees in the cited lab conditions.

2.2. The transfer by aircraft was indeed a critical part in this project together with aquaria maintenance of the corals (see below). To limit the effect and length of transportation the gold standards used in the aquarium trade industry were applied (note that the Banyuls Public Aquarium is involed in the project). Brielfy, after sampling in the field, the colonies were fragmented and stored for one week at the Al-Hail field station of the Sultan Qaboos University and the Public aquarium of Noumea for Om and NC localities, respectively. The day of transportation, the nubbins were placed in large oxygenated plastic bags with a ratio of 800 mL of filtrered (2µm) seawater for 1600 mL of medical oxygen. At the arrival, 100% of the exported nubbins were alive and no subsequent mortalities were observed. Since we still believe that the travel represent a stress, and because we used artificial seawater, the corals were let to recover and acclimatize to these new conditions for 3 months for Oman and 7 months for New Caledonia. The best indicator of these recovery and acclimatization process resides in the growth obtained during these 5-6 months' period.

How similar is the lab filtered seawater to the in situ water properties? Then, I guess that lab experiments were run separately for the Om (collected in June) and the NC colonies (collected in November) as acclimatation runs for 2 months. This point is not mentioned. Is this acclimatation period deals with potential parental effect? More importantly, if I understood well, acclimatation was made at 26 °Cs but when the experiment of heat stress started, control temperatures were set at 27 or 31 degrees. If I am getting it right, this made already a change for the Om colonies of +5 degrees. Can the authors clarify this point and with respect to the biology of organisms?

2.3. As mentioned above, the corals were acclimatized to the lab conditions (including filtered and artificial seawater parameters) at 26°C for several months before the heat stress experiment. Since the sampled colonies were big adult colonies of several years old, we believed that putative parental effect were either phenotypically assimilated or lost. Two weeks before the experiment, an additional acclimation to the control temperatures (mean temperature of summer months in the field) was performed (27°C or 31°C for NC and OM respectively); there was a

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mistake regarding the temperatures that were inconsistent between the methods section and the Figure 1. This has been clarified and an additional figure for the experimental setup is now provided (Supplementary Figure S1). Thus for the two localities, there were 2°C difference between the temperature for the control condition and the stress condition (see also response to first reviewer 1.8 and below 2.7).

2- It seems to me that both barcoding and transcriptomics analyses could be more deeply conducted. On the bacterial metabarcoding side, analysis of OTU richness is not sufficient to claim that community remains stable.

2.4. Not only the alpha diversity was not significantly different between control and stress conditions, but beta diversity using Bray-Curtis distance (reflecting dissimilarities in community structure) was not significantly different between these conditions as well as mentioned in the results and additional table 2 (now renamed Supplementary Table S4). To avoid confusion the term stable was changed for "no major shift".

By analogy with what is done in RNAseq analysis, authors could use normalization and DESeq2 or edgeR package tools (available in phyloseq) to identify differentially abundant OTUs. Change or no change in relative abundances of OTUs are valuable information, not only during the heat stress experiment in lab conditions but also when transferring field-collected corals to lab conditions. My feeling is that authors could observe changes in relative abundance between field and lab conditions, with a bias toward the already abundant OTUs in the field. Although authors may observe many more less abundant OTUs once in lab, abundance analysis of this "rare biosphere" may be informative after the transfer or during experiment as shown in other coral studies.

2.5. This microbiota analysis aimed at looking for major shifts between control and stress conditions, which was not the case, supporting the importance of host transcriptomic response to temperature increase. We now provide a new version of Figure 2 with new colour scheme and shapes to make the clustering of the samples more clear. We do not exclude that changes have arisen, especially in the rare biosphere, but samples do not cluster by control vs. stress condition, which was our focus in this work. We feel that a more detailed microbiota analysis would be out of the focus of this paper and would require more samples, and we published last year a more complete work on that topic considering a more considerable number of samples (Brener-Raffali et al. Microbiome 2018).

On the other side, it is still widely accepted that transcriptome data need quantitative PCR experiments to confirm the accuracy of the results on a selected set of genes, in particular differentially expressed genes.

2.6. Indeed with the advent of NGS and RNA sequencing, most transcriptome analysis results were validated on a subset of genes using qPCR. This is less and less the case because RNAseq analysis is based on very high numbers of actual and quite long sequences (paired-end reads of 2x100 bp) that are mapped to a reference genome and cannot appear as an artefact. In contrast,

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qPCR is not a perfect technique and is prone to errors and biases depending on the primer specificity and efficacy. We argue that biological replicates are much more important. We used here three replicates by colony (from independent tanks) as well as three colonies per locality to strengthen our results. In addition, we discuss here general patterns in terms of transcriptome plasticity rather than differential expression for very specific genes.

About differentially expressed genes, is there any potential link between the greater variance in the Om and the above cited point 1 of control temperature?

2.7. We hope that the experimental design is now clearer: Om nubbins were placed at the control temperature of 31°C for two months before the experiment (whereas NC nubbins were placed at the control temperature of 27°C). In addition, the differential gene expression analysis was performed between control and heat stress conditions for the two populations, corresponding in both cases to a two degrees increase (27°C vs. 29°C for NC and 31 vs. 33°C for NC). We are thus confident that the pattern we observe was not linked to an experimental bias.

3- There are confounding factors that may limit the interpretation of results. Here, the study deals with one genus made of 3 species (at least 2 genotypes with the NC and 1 more corresponding to the Om).

2.8. Indeed, the different colonies were sampled as *Pocillopora damicornis sensu lato* morphotypes, but actually corresponded to three different haplotypes (one in Oman, two – much more close to each other – in NC). This point was also raised by the reviewer #1, thus we added in the new version of the manuscripts the differential gene expression analysis considering each colony independently rather as replicates for the same population. The conclusions remain similar as the different colonies form the same locality display the same pattern.

By the way, the Species Hypothesis is not that obvious for a non- initiated person. Gelin et al. 2017a and this concept could be detailed.

2.9. We modified the Material and Methods section and changed the paragraph title to make easier to follow to non-initiated readers. We defined what a species hypothesis is and also how Primary and Secondary Species Hypotheses are delimited.

So, in this study, locality can merely be confounded with coral genotype.

2.10. This point was also raised by the reviewer #1. In the new version of the manuscript, differential gene expression analysis for each colony (corresponding to three different species hypotheses) is now provided and confirm the differences in terms of transcriptome plasticity whatever the genotype (Supplementary Figure S6 and Supplementary File S7). Nevertheless, we nuanced the statements made on the link between thermal regime and response to experimental stress.

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Genotypes of Symbiodinium are also well different between localities. By the way, heat tolerance of these two Symbiodinium genotypes are not presented although authors have arguments (c.f. Brener-Raffalli et al. 2018 Microbiome). This added to the above points make it hard to generalize on plasticity and frontloading.

2.11. Notice that in the new version of the manuscript we replaced Symbiodinium by Symbiodiniaceae family to stick to recent systematic revision of this taxonomic group (Lajeunesse et al. Current Biol. 2018). Indeed the Symbiodiniaceae clades associated with each of the two populations are different and in addition, are known to display contrasted levels of heat tolerance. Mode details regarding our recent work have been added. Nevertheless, we argue that even the Symbiodiniaceae associates can confer different tolerance levels to heat stress, changes in the community composition are not involved in the short term response to heat stress when comparing here control and stress temperature samples within each population.

The authors talked about plasticity but never mentioned local adaptation. For the constitutive upregulation or frontloading, it is even harder to me. It also raises more general questions that are likely not the scope of the study. How does the frontloading gene expression response happen in normal populations (e.g. not under controlled lab conditions), and how is it turned on over time in response to environmental variation?

2.12. The concept of local adaptation was not discussed in the discussion but its putative involvement in the frontloading and gene expression plasticity was highlighted in the introduction (line 127-128). To circumvent the lack of discussion we have added a paragraph about environmental prediction which fall into the concept of local adaptation

Given that some of the identified genes are involved in multiple cellular pathways, how do gene expression changes ultimately (and simultaneously) affect both coral health and stress tolerance?

2.13. Our results indeed suggest that response to heat stress likely affects morpho-anatomic functions (growth, reproduction) as well as control of transposable elements via a possible trade-off mechanisms. This has been underlined in the general discussion but as we could not test here the actual effect of heat stress on fitness, we nuanced our interpretations as recommended by the first reviewer (see also response 1.7).

4- Another point is the format of the discussion and the long text on the functional aspect of the study. It is in between an analysis/result and a discussion. Do the authors plan to submit this paper to a specific journal afterwards and made a specific formatting for that reason? Although super interesting, I think it would more valuable to incorporate data from other studies made on corals. At the moment, many studies have investigated the effect of heat stress on different coral models. Moreover, Barshis et al. (2013) observed that heat-tolerant populations of Acropora hyacinthus displayed front-loading of 60 genes associated with stress and immune response following simulated bleaching. Haslun et al. 2018 (Marine Biology) also focused on a meaningful set of genes. Maybe for these genes in particular or others, it is worth bringing details, this would prevent from reading a too long catalogue of what is moving.

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2.14. The results have been more focused on the most meaningful functions and the discussion has been considerably streamlined as the detailed part concerning functional analysis has been moved to the new Supplementary File S11.

In addition, and related to point 2 and line 586 p.22, can authors anyway get some information from some of the *Symbiodinium* genes?

2.15. We agree that our interpretations are limited at the community composition and do not take into account potential changes in Symbiodiniaceae gene expression. It would indeed have been very interesting to go deeper in the symbiont functions that could be involved in the holobiont response to thermal stress, but the soft procedure that we use for RNA extraction greatly limits the lysis of algal cells. As very few sequences of cDNA could be aligned to the *Symbiodinium* genome, we think our dataset is not suitable for such analysis. This has been added to be discussion.