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Supplementary Materials for

Species-specific molecular responses of wild coral reef fishes during a marine heatwave

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/12/eaay3423/DC1)

Data file S1 (Microsoft Excel format). Final list of orthologous genes shared across the five sampled species and their corresponding annotation.
Data file S2 (Microsoft Excel format). Significantly diverged genes (i.e. higher difference between species) in the Expression Variance and Evolution (EVE) analysis for all samples collected in our study, and their corresponding annotation.
Data file S3 (Microsoft Excel format). Enriched Gene Ontology Categories for the significantly diverged genes (i.e. higher difference between species) in the Expression Variance and Evolution (EVE) analysis.
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Data file S5 (Microsoft Excel format). Differentially expressed genes (DEGs) between fish collected at different months for the five focal species of the study. The dashes represent comparisons that were not possible, as samples from February for three of the species (*C. quinquelineatus*, *O. cyanosoma*, *O. doederleini*) were not available.
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Data file S11 (Microsoft Excel format). Significantly enriched Gene Ontology terms between December and March for *O. cyanosoma*, *O. doederleini* and *C. quinquelineatus*, based on the analysis of differential expression.
Data file S12 (Microsoft Excel format). Overlapping differentially expressed genes between the three species of cardinalfishes when comparing samples collected in December and March.
Data file S13 (Microsoft Excel format). Significantly enriched GO terms for the "CYAN" gene module, for March samples of three species of cardinalfishes.
Data file S14 (Microsoft Excel format). Significantly enriched Gene Ontology processes for the "RED" gene module for March samples of three species of cardinalfishes.
Data file S15 (Microsoft Excel format). Average Resting Oxygen consumption (MO_2Rest), Standard Deviation (SD in parenthesis), number of samples and temperature quotients (Q_{10}), from studies by Nilsson *et al*. (2009) and Rummer *et al*. (2014; for *C. quinquelineatus*). Estimates for four of the species correspond to Lizard Island populations (i.e. same location as genetic analyses in the main text), while the samples of *C. quinquelineatus* correspond to Nago Island, Papua New Guinea. Only measures between 29°C and 31°C are presented in this comparison, as these resemble the temperatures during the 2016 heatwave. The values of Q_{10} were calculated with the equation $Q_{10} = (MO_2Rest2/MO_2Rest1)^{[10/T2-T1]}$.

Data file S16 (Microsoft Excel format). Overlap in the enriched Gene Onthology categories based on the differentially expressed genes of *A. polyacanthus* and *P. moluccensis* between samples collected in February and March.
Data file S17 (Microsoft Excel format). Heat shock proteins that were differentially expressed in the comparison of samples from February and March for *A. polyacanthus* and *P. moluccensis*.
Data file S18 (Microsoft Excel format). Significantly enriched GO terms for the "GREEN" gene module, corresponding to samples of damselfishes collected in March.
Data file S19 (Microsoft Excel format). Overlapping differentially expressed genes that were exclusively found in the comparisons of prolonged exposure (February vs. March) in *A. polyacanthus* and *P. moluccensis*.
Data file S20 (Microsoft Excel format). Significantly enriched GO terms for the "RED" gene module, resulting from the contrast of damselfishes collected in March and July.
Data file S21 (Microsoft Excel format). Significantly enriched GO terms for the "BLACK" gene module, corresponding to samples of cardinalfishes collected in March.
Data file S22 (Microsoft Excel format). Differentially expressed genes between samples of March and July for *O. cyanosoma* (OCYA) and *O. doederleini* (ODOE).
Data file S23 (Microsoft Excel format). Differentially expressed genes between samples of March and July of *C. quinquelineatus*.
Data file S24 (Microsoft Excel format). Common differentially expressed genes across all five species, when comparing March and July samples.
Data file S25 (Microsoft Excel format). Table of collected individuals, dates and fish sizes.
Data file S26 (Microsoft Excel format). Number of reads per individual before and after trimming and decontamination.
Data file S27 (Microsoft Excel format). Statistics of de novo assemblies of transcriptomes determined with BUSCO.
Data file S28 (Microsoft Excel format). Results from the quality assessment using the program Transrate, for contigs assembled with Trinity and summarized with Transdecoder (A) and the orthologous sequences (B).
Data file S29 (Microsoft Excel format). Representative transcript for each species in our study, for all orthologous genes.
Data file S30 (Microsoft Excel format). Number of transcripts in WGCNA modules.

Supplementary Figures

Figure S1

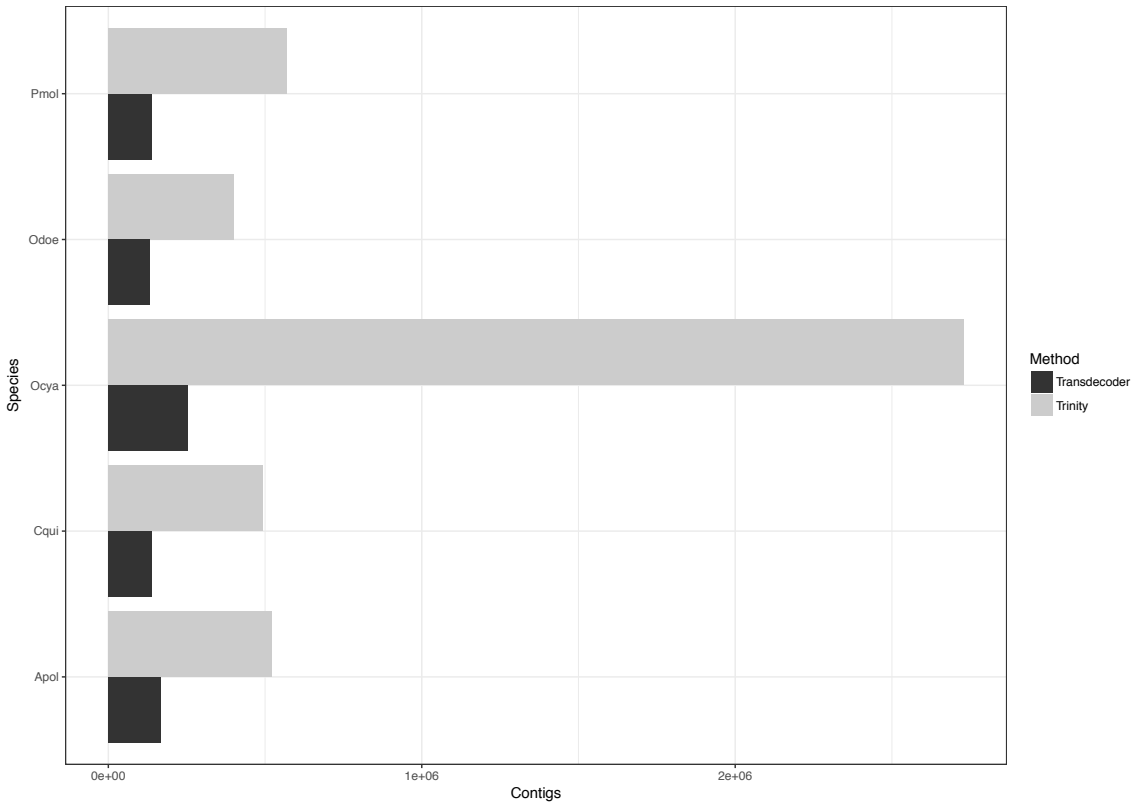

Fig. S1. Number of transcripts obtained after de novo transcriptome assembly with Trinity (gray) and retained transcripts after predicting open reading frames with TransDecoder (black).

Cqui= *Cheilodipterus quinquelineatus*, Odoe = *Ostorhinchus doederleini*, Ocya= *Ostorhinchus cyanosoma*, Apoly= *Acanthochromis polyacanthus*, Pmol= *Pomacentrus moluccensis*

Figure S2

A

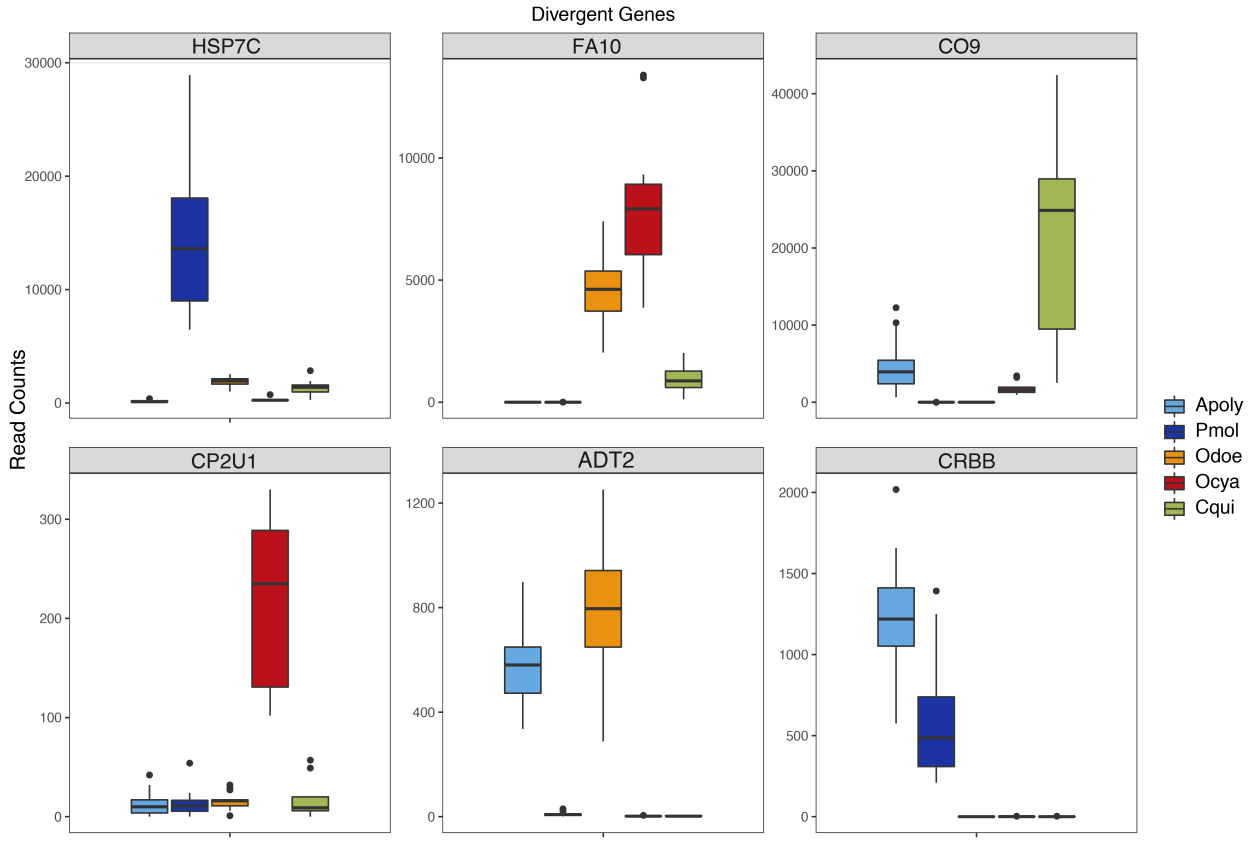

Fig. S2. Normalized read counts for six of the genes that showed divergent expression (i.e., larger differences between species) in the analysis of EVE.

Patterns of divergence were extremely varied, showing different patterns of activation: species of the same family (CRBB and FA10), different families (CO9 and ADT2), just one species (CP2U1 and HSP7C).

Figure S3.

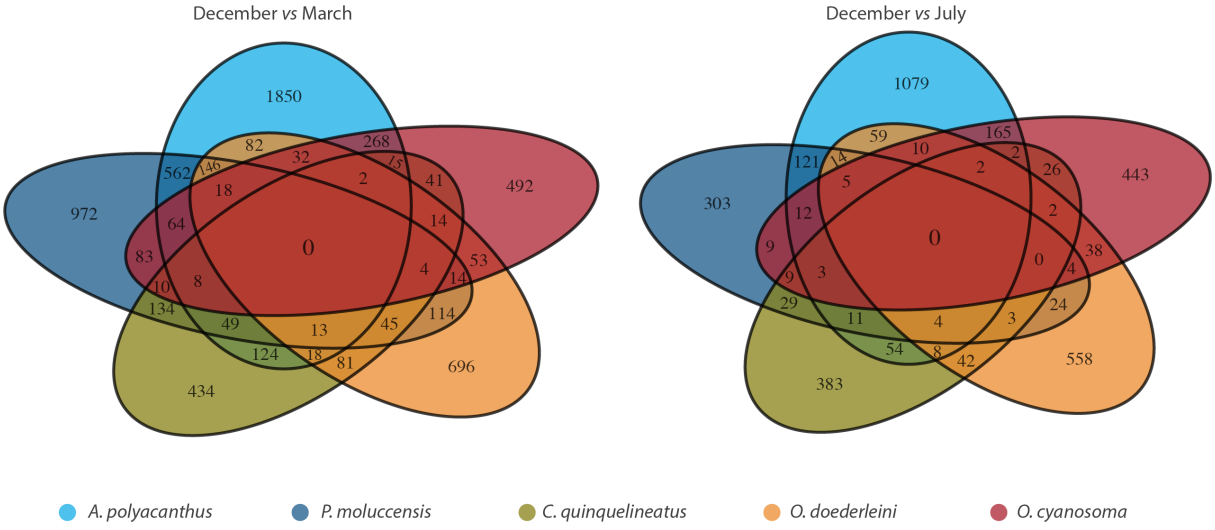

Fig. S3. Venn diagram showing the overlap of differential expressed genes in the pairwise comparisons between December and March and December versus July for the five species of interest.

Figure S4.

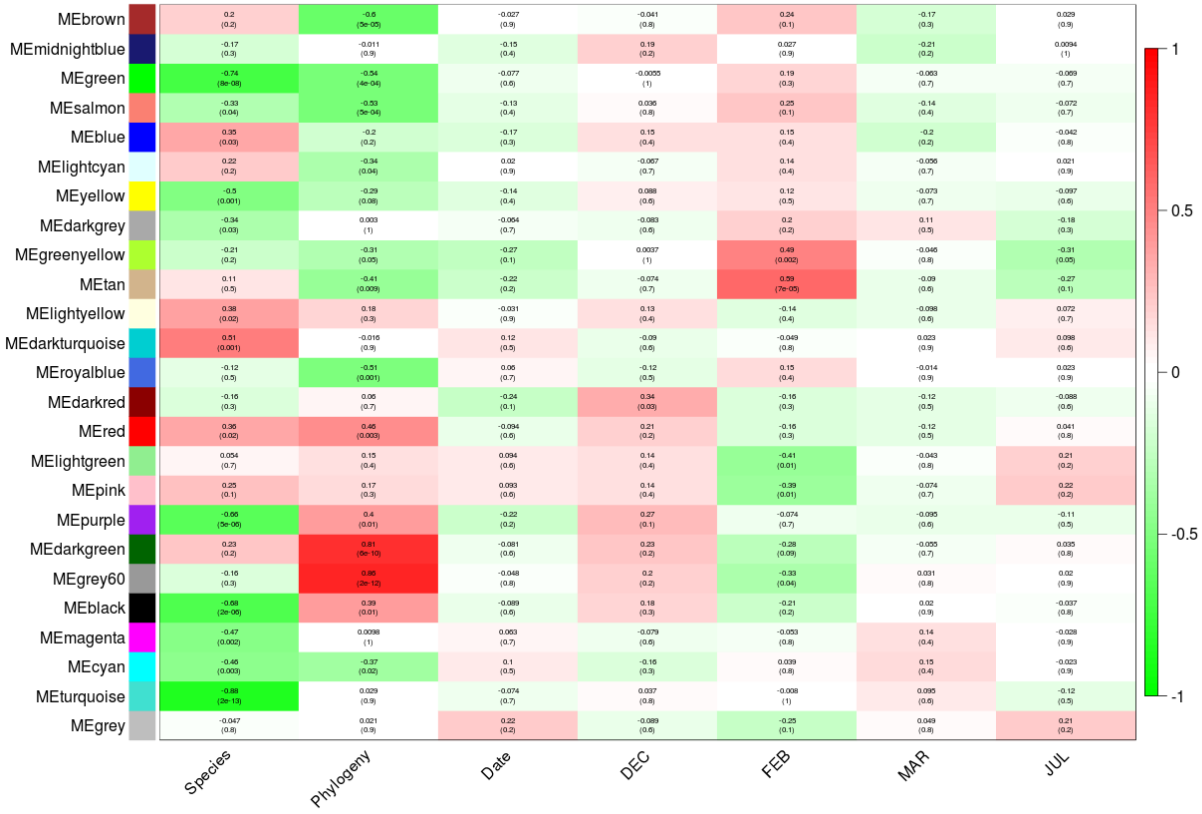

Fig. S4. Module-trait correlation matrix for all species showing correlation value with *P* value in brackets for each module and trait. Green and red rectangles represent negative and positive correlations of gene expression in the modules with a particular trait, respectively. Module (ME) and the colour are represented on the y=axis. Traits are represented on the x-axis where "Species" represents the five species separately; "Phylogeny" is if the sample was a cardinalfish or damselfish; "Date" are all four collection points and "DEC" (December), "FEB" (February), "MAR" (March) and "JUL" (July) represent correlations with specific collection points.

Figure S5.

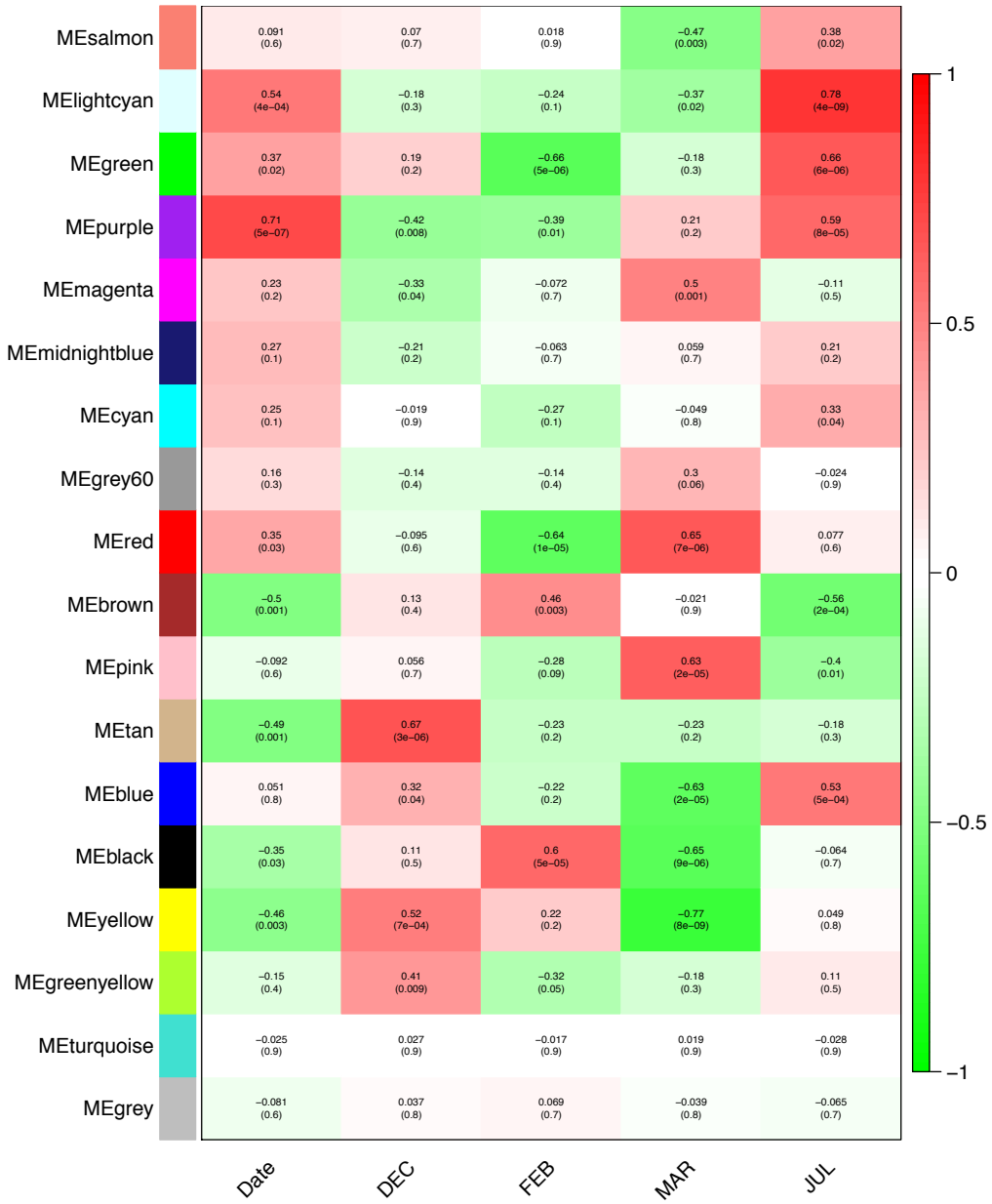

Fig. S5. Module-trait correlation matrix for *A. polyacanthus* and *P. moluccensis* (damselfish species). Green and red rectangles represent negative and positive correlations of gene expression in the modules with a particular trait, respectively. ME= Module and the colour are represented on the y=axis. Traits are represented on the x-axis where "Date" stands for all four collection points and "DEC" (December), "FEB" (February), "MAR" (March) and "JUL" (July) represent correlations with specific collection points.

Figure S6.

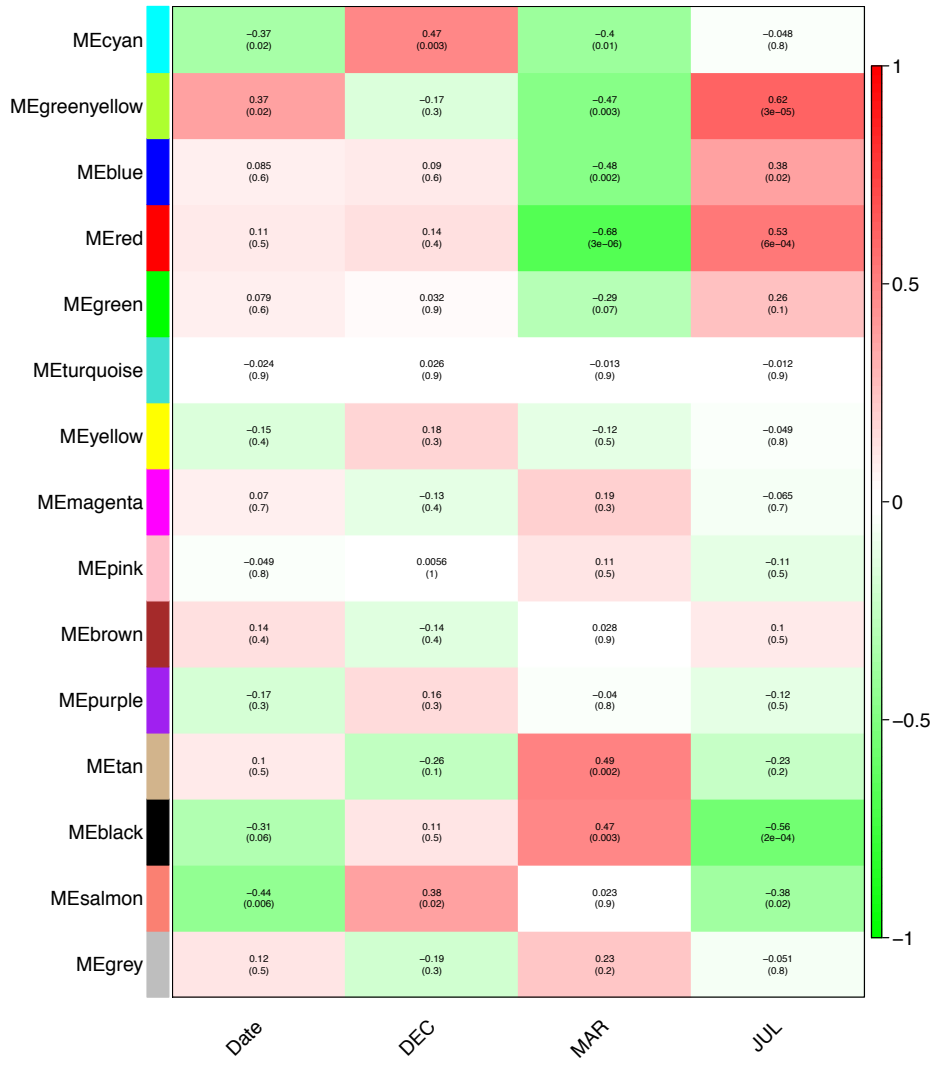

Fig. S6. Module-trait correlation matrix for specimen of all three cardinalfish species (*C. quinquelineatus*, *O. doederleini*, and *O. cyanosoma*). Green colour represents negative correlation and red positive correlation of the gene expression in the networks with a particular trait. ME= Module and the colour are represented on the y=axis. Traits are represented on the x-axis where Date stands for all four collection points and DEC (December), FEB (February), MAR (March) and JUL (July) represent correlations with specific collection points.

Figure S7

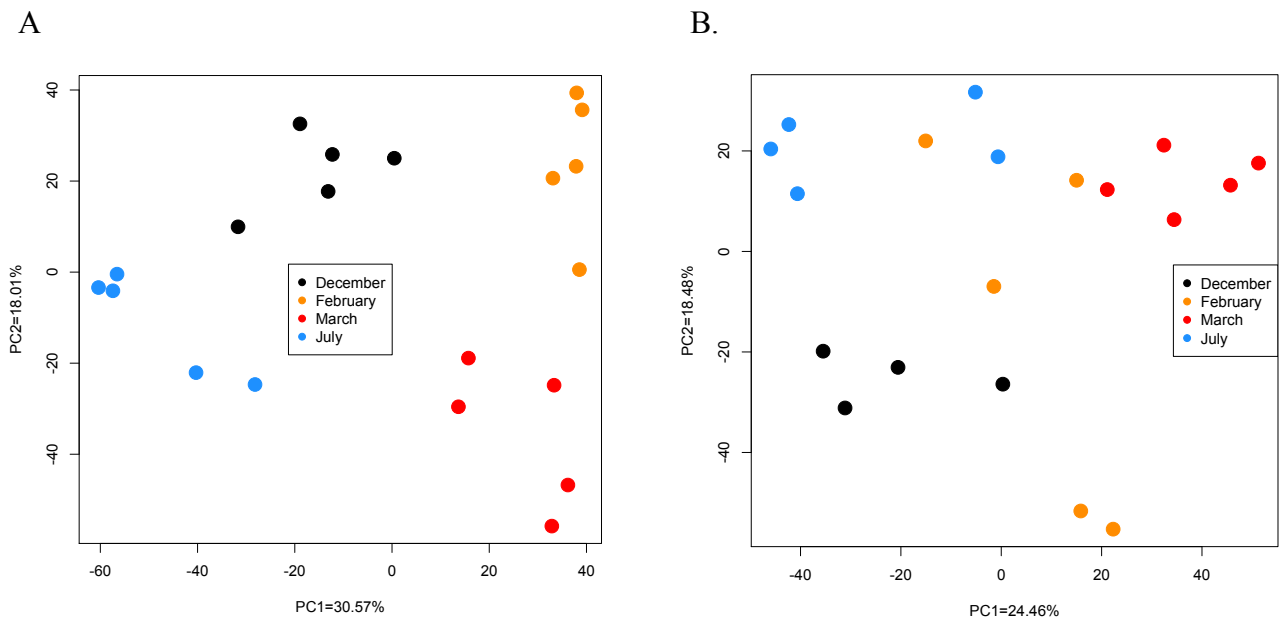

Fig. S7. Principal Coordinate Analysis for damselfishes, based on liver gene expression. Principal Coordinate Analysis for the damselfish species *Acanthochromis polyacanthus* (A) and *Pomacentrus moluccensis* (B) based on the differentially expressed genes of all months, as determined by the Likelihood-Ratio Test.

Figure S8

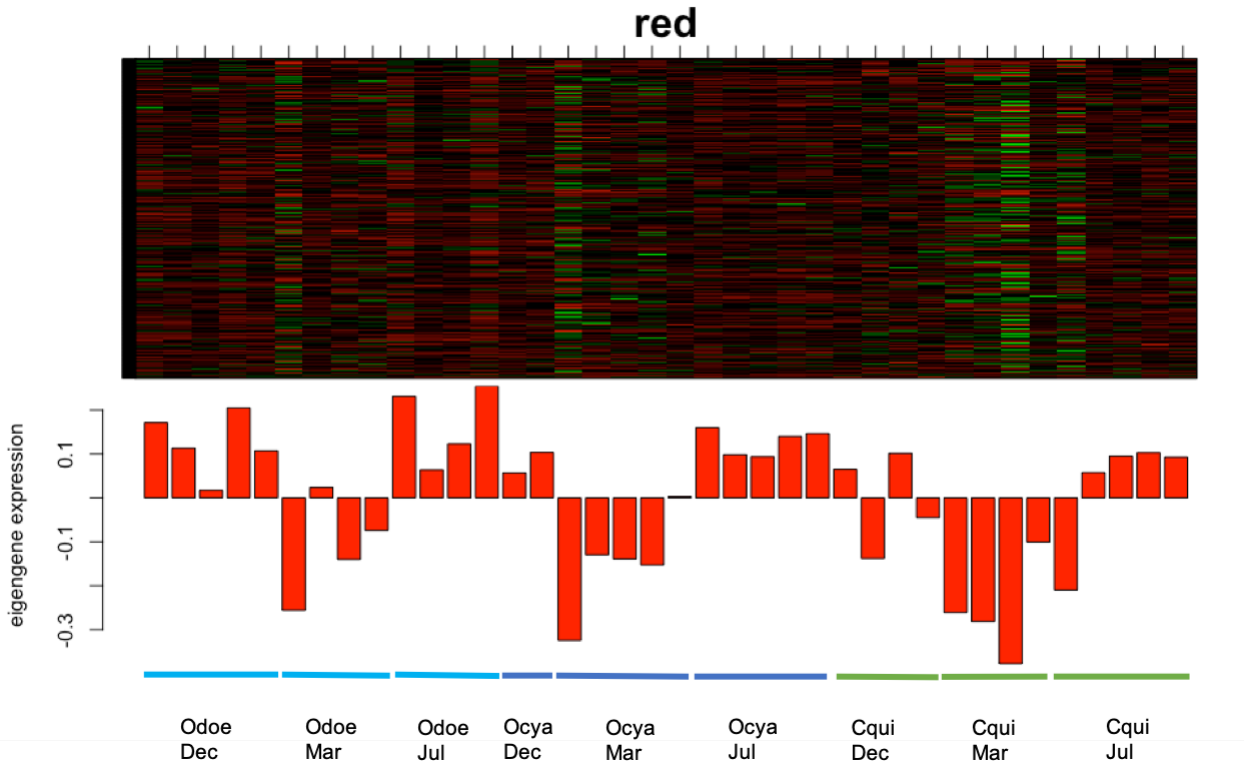

Fig. S8. Eigenvalue of expression data for each biological replicate in the red module for cardinalfishes. Dec = December, Mar = March, Jul = July. Odoe = *Ostorhinchus doederleini*, Ocya = *O. cyanosoma*, Cqui = *Cheilodipterus quinquelineatus*.

Figure S9

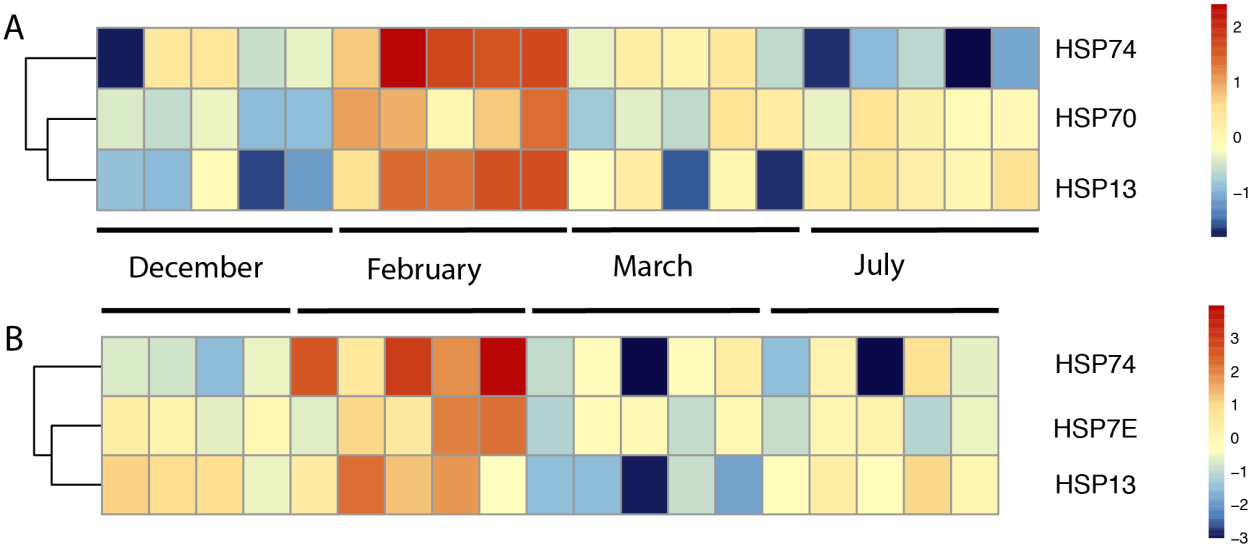

Fig. S9. Heatmaps for the expression of heat-shock proteins in damselfishes. Heatmaps for the expression of shared differentially-expressed genes coding for heat shock proteins for (A) *Acanthochromis polyacanthus* and (B) *Pomacentrus moluccensis* across all samples. Blue shades represent lower expression levels and red shades represent higher expression levels. Highest expression for these genes was observed in February, i.e. the beginning of the warming period.

Figure S10

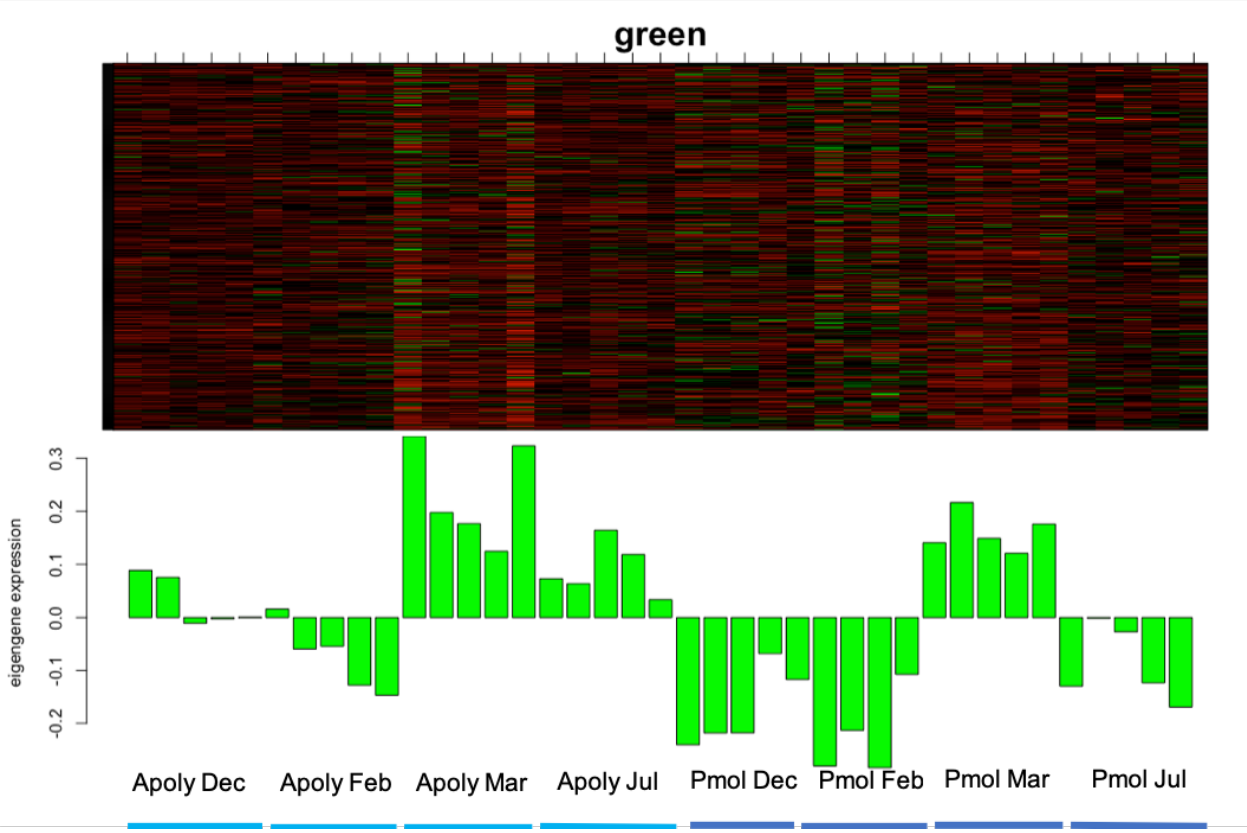

Fig. S10. Eigenvalue of expression data for each biological replicate in the green module for damselfishes. Apoly = *Acanthochromis polyacanthus*, Pmol = *Pomacentrus moluccensis*. Dec = December, Feb = February, Mar = March, Jul = July.

Figure S11

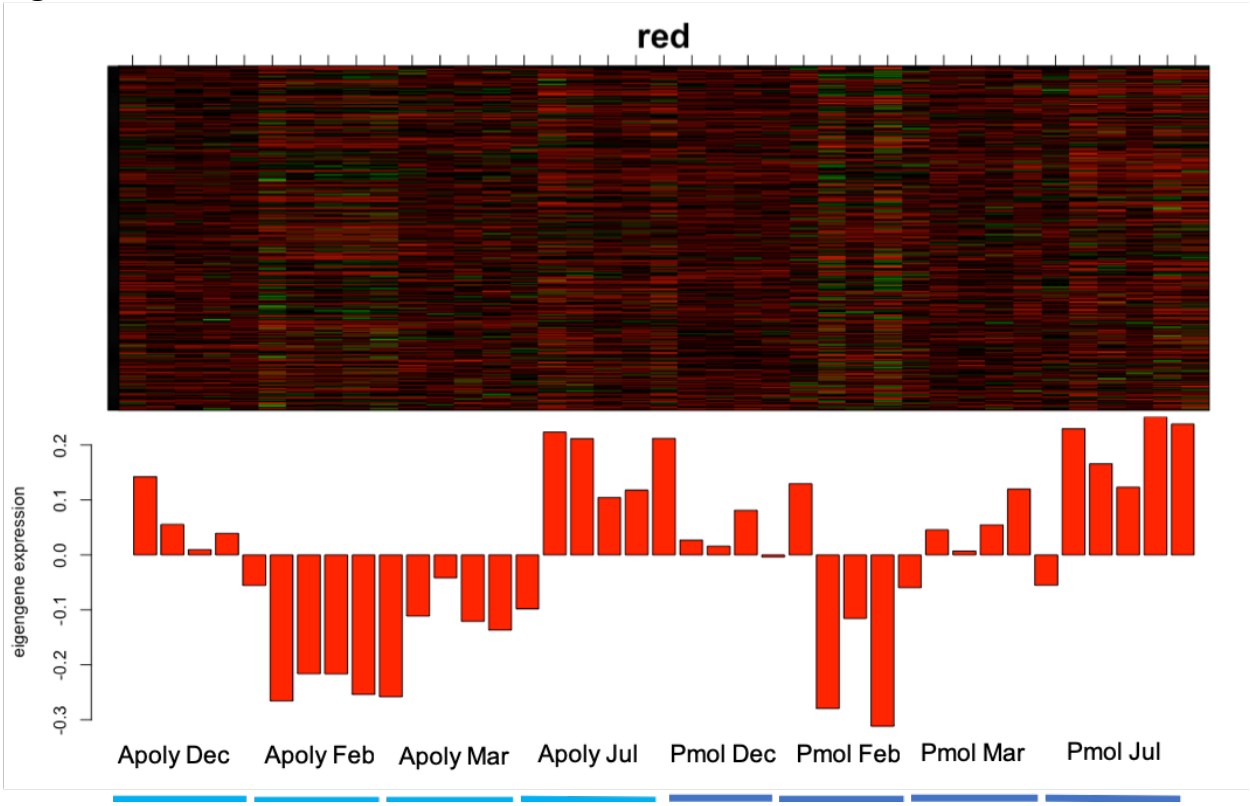

Fig. S11. Eigenvalue of expression data for each biological replicate in the red module for damselfishes. Apoly = *Acanthochromis polyacanthus*, Pmol = *Pomacentrus moluccensis*. Dec = December, Feb = February, Mar = March, Jul = July.

Figure S12

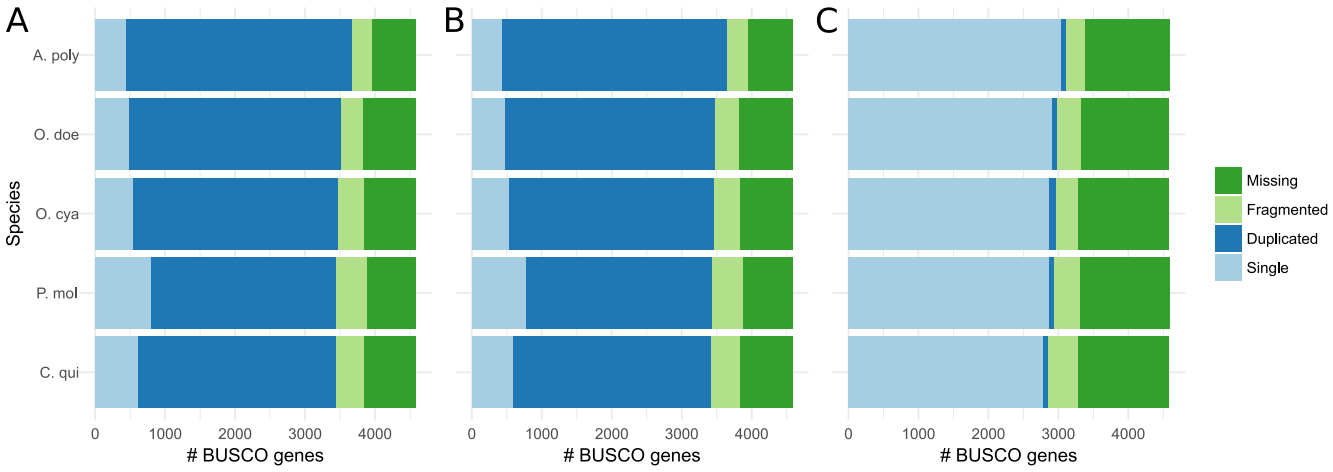

Fig. S12. Transcriptome completeness estimates for all five species with BUSCO. A) BUSCO completeness for the primary Trinity assemblies, B) the assemblies after filtering with Transdecoder, and C) after filtering only orthologous transcripts. C. qui= *Cheilodipterus quinquelineatus*, O. doe= *Ostorhinchus doederleini*, O. cya= *Ostorhinchus cyanosoma*, A. poly= *Acanthochromis polyacanthus*, P. mol= *Pomacentrus moluccensis*

Figure S13

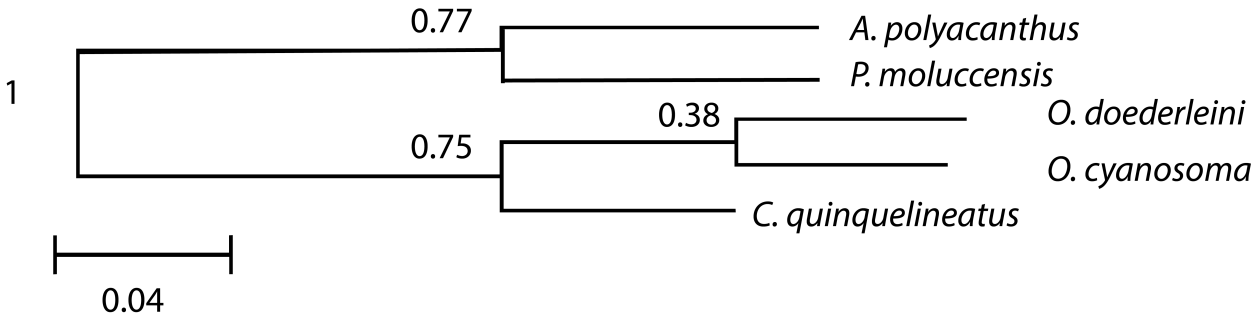

Fig. S13. ML tree showing the relationship between the five species analyzed in this study, generated with RAXML. The ML tree was used to define relationships for the analysis of Evolutionary Variation Estimates (EVE).