

SUMMARY OF PROPOSED WORK

This is a CREST Partnership Supplement submitted under NSF 18-509

Title: CRESTropical: A thematic network studying the environmental-epigenetic linkages shaping phenotypic responses in tropical ecosystems

Overview: Global change profoundly impacts tropical ecosystems, particularly in areas with large human populations such as those represented by the NSF-CREST institutes involved in this proposal (U. Puerto Rico, Florida International U., and U. Hawaii). While such impacts are becoming increasingly well understood, the mechanisms governing organismal responses to these changes (e.g., promoting acclimated phenotypes) still remain obscure. This proposal builds on the research developed at three NSF-CREST centers located in the tropics to create a thematic network centered around understanding the mechanisms driving phenotypic responses in tropical ecosystems threatened by global change. This work will foster the incorporation of students from underrepresented groups to the study of critical environmental problems affecting their own home areas, heritage and traditions. In doing so, this network will facilitate the exchange of students across the three institutions, building on CREST and REU sites capacities as well as on the research projects from CREST investigators. Ultimately, the results expected from this thematic network will improve human resource development, positively impacting basic scientific progress as well as applied conservation and management.

Intellectual Merit: Global change leads to a range of environmental effects across all types of ecosystems. The negative impacts of global change are exacerbated in the case of tropical ecosystems, impacting species diversity, distribution, ecological interactions, and associated ecosystem services, particularly in areas with large human populations such as those represented by the 3 CREST institutes involved in this proposal. While the impacts of global change on tropical ecosystems are becoming increasingly well understood, the mechanisms governing organismal responses to these changes still remain obscure. Epigenetics, in combination with traditional ecological approaches, provides a framework for understanding the basis for such phenotypic responses. This proposal will contribute towards filling that gap by leveraging on the research currently being developed at three NSF-CREST centers located in the tropics. For that purpose, ecologically and environmentally relevant organisms will be used across local insular and peninsular ecosystems to address the following questions: Q1: What are the fundamental genetic building blocks involved in phenotypic responses to major drivers of global change in tropical ecosystems? Q2: What are the epigenetic determinants contributing to changes in gene expression in response to global change stressors? Q3: How do environmental cues, genetic information and transient epigenetic signals interact producing acclimatized phenotypic responses to global change in the tropics?

Broader Impacts: The proposed CREST thematic network will foster the incorporation of students from underrepresented groups to the study of critical environmental problems affecting their own home areas, heritage and traditions in the tropics. Areas of broader impacts include: 1) In the public field, this research assesses and predicts environmental risks. Findings of this research will be disseminated through planned publications and outreach activities from all three collaborating centers. 2) Graduate and undergraduate student training: Students exchanges across the three CREST centers will permit exposure to different laboratory techniques, facilities and analytical instrumentation, as well as faculty with different expertise. 3) Students from all three CREST centers can participate in the Bioinformatics workshop on RNAseq data analysis to be held in San Juan Puerto Rico on March 26-28, 2020 facilitating their ability to analyze their data. The outreach component of the present proposal will be strengthened by the participation of the NGO Sociedad Ambiente Marino, recruiting citizen volunteers to work in the field in Puerto Rico.

CRESTropical: A thematic network studying the environmental-epigenetic linkages shaping phenotypic responses in tropical ecosystems

1. Background and purpose: Global change profoundly impacts tropical ecosystems, particularly in areas with large human populations such as those represented by the NSF-CREST institutes involved in this proposal (U. Puerto Rico, Florida International U., and U. Hawaii). While such impacts are becoming increasingly well understood, the mechanisms governing organismal responses to these changes (e.g., promoting acclimated phenotypes) still remain obscure. This proposal builds on the research developed at **three NSF CREST centers located in the tropics to create a thematic network centered around understanding the mechanisms driving phenotypic responses in tropical ecosystems threatened by global change**. This work will foster the incorporation of students from underrepresented groups to the study of critical environmental problems affecting their own home areas, heritage and traditions. In doing so, this network will facilitate the exchange of students across the three institutions, building on CREST and REU sites capacities as well as on the research projects from CREST investigators. Ultimately, the results expected from this thematic network will improve human resource development, positively impacting basic scientific progress as well as applied conservation and management.

2. Rationale: Global change leads to a range of environmental effects across all types of ecosystems, including changes in temperature, nutrient loads, and turbidity. The negative impacts of global change are exacerbated in the case of tropical ecosystems, displaying higher susceptibility to alterations in atmospheric composition, carbon and nutrient assimilation, thermal changes, and water balance (Scholes and van Breemen 1997). Together, these drivers are impacting species diversity, distribution, ecological interactions, and associated ecosystem services (Worm et al. 2006), **particularly in areas with large human populations such as those represented by the 3 CREST institutes involved in this proposal (South Florida, Puerto Rico, and Hawaii)**. The struggle of key ecosystems in the face of global change is evident in terrestrial and freshwater habitats, including shifts in the timing and range of boundaries for both animals and plants (Parmesan and Yohe 2003), changes in animal migration patterns, plant flowering time, as well as on the extinction of vulnerable species (Falaschi et al. 2019). In the case of marine ecosystems, global change impact is best illustrated by alterations in “cradles of diversity” such as coral reefs and mangrove forests. Indeed, there is a growing literature describing changes at different levels, from molecules to ecosystems, regarding key species ranging from reef-building corals to reef fish [e.g. (Cheung et al. 2013; Wenger, Johansen, and Jones 2012; Nagelkerken and Munday 2016)].

While the impacts of global change on tropical ecosystems are becoming increasingly well understood, **the mechanisms governing organismal responses to these changes (e.g., promoting acclimated phenotypes) still remain obscure** (Franks and Hoffmann 2012), especially in the case of the genetic determinants promoting acclimated phenotypes (Franks and Hoffmann 2012; Hoffmann et al. 2019). Epigenetics, in combination with traditional ecological approaches, provides a framework for understanding the enigmatic crosstalk between heterogeneous environmental signals and genome function, generating the phenotype. Formally, epigenetics is defined as the study of phenomena and mechanisms that cause chromosome-bound, heritable (mitotically and/or meiotically) changes to gene expression that are not dependent on changes to DNA sequence (Deans and Muggert 2015). These mechanisms have functional outcomes that can generate significant phenotypic plasticity, but remain understudied and poorly understood in ecologically relevant organisms with high environmental variation (Eirin-Lopez and Putnam 2018). **Characterizing epigenetic triggers, mechanisms, and their role in organismal acclimatization and adaptation is therefore critical to understanding ecosystem function.**

3. Scope: This proposal will contribute towards filling that gap by leveraging on the research currently being developed at three NSF-CREST centers located in the tropics. Ecologically and environmentally relevant organisms will be used across local insular and peninsular ecosystems, including insects, molluscs, corals, and fish. **Question 1:** What are the fundamental genetic building blocks involved in phenotypic responses to major drivers of global change in tropical ecosystems? **Question 2:** What are the epigenetic determinants contributing to changes in gene expression in response to global change stressors? **Question 3:** How do environmental cues, genetic information and transient epigenetic signals interact producing acclimatized phenotypic responses to global change in the tropics?

4. Approach: The proposed questions will be addressed by leveraging on the ongoing research established and consolidated at the participating NSF-CREST centers, including funding from NSF's general, RAPID, and Rules of Life Programs, increasing the scope of the present work and providing additional opportunities for students. Terrestrial and freshwater ecosystem studies will be led by UPR, while marine studies will be developed by all three institutions.

Q1: What are the fundamental genetic building blocks involved in phenotypic responses to major drivers of global change in tropical ecosystems? This question will be studied using the honey bee, *Apis mellifera*, the snail *Biomphalaria glabrata*, and the stony coral *Acropora cervicornis* as model organisms to identify genetic tool kits used in responses to environmental stress. Comparative transcriptomics is a well-tested approach that has been used to identify shared molecular mechanisms of development, social behaviors such as territoriality and autism (Rittschof et al. 2014). In the present work, this approach will be used to elucidate how these organisms respond to taxa specific and commonly encountered environmental stressors.

Terrestrial model: *Apis mellifera* transcriptomic signature of exposure to contaminants: Honey bees provide pollination services in natural and human modified environments and are currently facing a world-wide health crisis due to multiple threats and fast pace of environmental change (Huang and Giray 2012; Seitz et al. 2016). Previous transcriptome studies have examined stressors such as diseases and pesticides (Johnson et al. 2009). Published genomic resources, along with ongoing genomic studies at UPR-CREST (Avalos et al. 2017) and the current sequencing of the genome of the Puerto Rico gentle honey bee strain and the genomes of the Africanized Texas honey bee and the African *Apis scutellata* among others, provides a strong basis of support for the work proposed herein. We will compare honey bees collected in agricultural and urban environments. Honey bees are useful environmental indicators due to the pollen and nectar gathering activity of foragers. As a result, they inadvertently sample the pollutants present in the aerial stream of their foraging area as well as concentrate these compounds in the products they generate. Both hydrophilic and lipophilic pollutants can concentrate in the hive, potentially including agrochemicals (Calatayud-Vernich et al. 2018) and heavy metals (Smith et al. 2019). Moreover, in the tropical and densely populated island of Puerto Rico, honey bees forage year round increasing exposure time and amount of toxins accumulated in the nest. Data collected through the USDA yearly honey bee health survey, which monitors the presence of pesticides and diseases, will be used as a basis for comparison. In addition to the above anthropogenic stressors, honey bees in PR are exposed to stressors resulting from severe and unpredictable extreme weather patterns which can create periodic dearth of resources for honey bees and other plant dependent organisms lasting several months.

Freshwater model: Responses to contaminants in *Biomphalaria* snails. *Biomphalaria* pond snails are the major intermediate hosts for *Schistosoma mansoni*, the causative agent of schistosomiasis. The publication of the *B. glabrata* genome (Adema et al. 2017) and our neural transcriptome for *B. alexandrina* (Mansour et al. 2017) provide new tools to examining parasite-host interactions in a system with major implications for global health and socioeconomic development. While *Biomphalaria* species are proposed as bioindicators of metal toxicity (Habib et al. 2016), large gaps remain in our understanding of the effects of contaminants on gene expression. This project will assess contaminant-induced changes in gene expression in the central nervous system, with a focus on neuropeptides, major regulators of gastropod behavior. Parasitization of *B. glabrata* by *S. mansoni*, which results in an increase in body size (parasitic gigantism), reduced interest in reproduction (parasitic castration) and other life supporting activities, is another stressor that can compound the detrimental effects due to toxin exposure. We will dissect and test the entire nervous system of *B. glabrata* with and without infection with *S. mansoni* and in the presence and absence of cadmium before and after infection.

Marine model: Responses to environmental stress and pollutants in reef-building corals. Corals are known to be highly susceptible to environmental stressors (e.g., elevated temperature and nutrient levels). However, their tolerance can vary significantly among species. Weedy species such as *Porites astreoides* can be readily found in sites impacted by sedimentation and nutrients, whereas susceptible species such as *Acropora cervicornis* are among the first to go locally extinct at impacted sites. Understanding the molecular mechanisms underlying the phenotypic responses of resilient and susceptible coral species to environmental stressors is instrumental to predicting the species mix and developing knowledge-based management practises for the coral reefs of the 21st century. This project will compare the transcriptomic response of a susceptible and resilient coral species in a pristine (i.e., low

sedimentation and low nutrient levels) and in an impacted site (high levels of sedimentation and nutrients) by performing a reciprocal transplant experiment. Survival and growth of transplanted and control colonies of both species will also be measured in order to relate the transcriptomic and phenotypic responses.

Experimental design: *Transcriptomic work-flow for hands on training and workshop.* *A. mellifera* (15 reps x 4 conditions; n=60 samples); *B. glabrata* (10 reps x 12 conditions; n=120 samples); corals *Porites astreoides* and *Acropora cervicornis* (15 reps x 4 conditions; n=60 samples); Reef building corals (10 reps x 2 conditions, n=20 samples). 1) Samples for each organism collected, 2) RNA extractions for each separate project done, 3) RNA quality control, 4) RT-qPCR check of gene expression of identified genes, 5) cDNA libraries constructed from total RNA prior to running RNAseq protocol using DNB-Sequencing technology, 6) RNAseq data quality controlled checked and low quality bases will be trimmed, 7) Reference genomes used for *A. mellifera* and *B. glabrata* and its *S. mansoni* parasite, and genome-free analysis for corals, 8) High quality trimmed read alignment against reference genomes (HISAT2) or into transcripts (Trinity), 9) Transcript abundance determination (Kallisto or Salmon), 10) Read count and differential expression analyses (DESeq2), 11) Metabolic pathways associated with differentially expressed genes determined and compared across taxa.

Q2: What are the epigenetic determinants contributing to changes in gene expression in response to global change stressors? The research developed on Q2 will be led by FIU, leveraging on the expertise of its NSF-CREST center in environmental epigenetics and marine ecology. For this purpose, this work will use coral reef ecosystems as model organisms currently being studied as part of the ongoing research, including reef-building corals, echinoderms, and reef fish.

Coral model: *Links between environment and dynamic epigenetic responses in reef-building corals.* Corals display high levels of plasticity in response to environmental variation. However, the extent to which epigenetic modifications respond to changes in the environment is still yet to be determined. The present work leverages on ongoing experiments funded by a RAPID award to characterize hurricane impacts on coral reefs in Puerto Rico (1810981), providing large numbers of samples subject to different environmental conditions for 6 months in the aftermath of hurricanes Irma and Maria. The present work will use these samples to develop epigenetic and demographic analyses providing insight into the temporal dynamics of epigenetic modifications in response to changes in environmental conditions. For that purpose, samples from coral nurseries before and after reciprocal transplants will be analyzed for DNA methylation patterns.

Echinoderm model: *investigating epigenetic-to-phenotype linkages under continuing climate change.* Among reef organisms, sea urchins have been extensively used as model systems for both ecological and molecular research, providing a broader insight into how epigenetic mechanisms govern responses in more complex organisms (i.e., as opposed to corals). The ecological role of these herbivores on coral reefs is critical, clearing space and facilitating the settlement of new coral recruits by feeding on macroalgae (Carpenter 1986; Ogden and Lobel 1978). This is best exemplified by the sea urchin *Diadema antillarum* which prevents macroalgal overgrowth in threatened Caribbean coral reefs. The present work builds on current fieldwork developed by UPR and FIU to investigate the role of epigenetic mechanisms as “sensors” able to translate environmental signals into regulatory modifications of genome function and modulating phenotypic plasticity under different thermal conditions. For that purpose, sea urchin samples will be collected at four different locations displaying heterogeneous thermal regimes in Puerto Rico and subsequently analyzed for epigenetic modifications and phenotypic responses.

Reef Fish model: *investigating the impacts of stress from decreasing water quality on fish planktivory.* Fish respond to environmental stressors in multiple ways, including changes in behavior, physiology, and demographics. In this project we will examine the effects of decreasing water quality (increasing turbidity) on the planktivorous damselfish *Stegastes partitus*. Increasing turbidity is a common contaminant on reefs driven by, for example, river run-off or dredging and is consistent with FIU’s NSF-CREST aims. During the proposed project, adult *S. partitus* will be collected from the wild, quarantined, and then acclimated in tanks containing a shelter and fed with *Artemia*. Individual fish will then be randomly assigned to treatments (seawater, low, medium or high turbidity). Turbidity will be controlled by varying the amount of commercially available clay, following the protocol of (Wenger, Johansen, and Jones 2012). After further acclimation, feeding rates and distance from protective shelter will be assessed during feeding periods. These observations will provide the phenotypic signal of environmental change.

The epigenetic signal will be investigated by quantifying histone modifications and DNA methylation in different fish tissues.

Experimental design: *Environmental epigenetic analyses.* This proposal will use coral and sea urchin samples collected at small-scale nurseries set at four reef locations differing in temperature and nutrient concentrations as part of our ongoing research on the island of Culebra (Puerto Rico). In addition, fish samples from laboratory experiments will be also incorporated. Coral samples collected after a 5-month growing period at each site (n=40 per site, 4 different genotypes), followed by a reciprocal transplant for additional 5 months, will be analyzed for epigenetic modifications. The same approach will be used in the case of sea urchins (*D. antillarum*, n=50 per site). In the case of fish, samples collected weekly (n=10 per treatment, 4 treatments) for a 2-month period will be analyzed. In all cases, a modified MSAP protocol (Reyna-López, Simpson, and Ruiz-Herrera 1997) will be used to generate DNA methylation epigenetic restriction enzyme profiles that will be analyzed with GeneMapper (Applied Biosystems) and the R package, *msap* (Pérez-Figueroa 2013). Principal coordinate analysis and an analysis of molecular variance will be used to test for genetic and epigenetic variation between sites. Epigenetic results will be compared with phenotypic performance in each case (demographic performance, coral symbiont densities, urchin righting responses). *Molecular level characterization.* We will take advantage of recent analytical tools developed at FIU CREST to study epigenetic changes by measuring post-translational modifications (PTMs) at the histone level. The histone analysis will provide a comprehensive characterization of the type and position of the PTM at the single organism level and can be complemented with state-of-the-art metabolomics (e.g. lipidomics) and proteomics studies. This expertise and analytical characterization capabilities will be available to all three CREST sites by the FIU CREST group.

Q3: Do the molecular signatures of corals exhibit distinct spatial patterns on coral reefs? The research to address this question will be led by UHH, leveraging on the expertise of UHH-CREST researchers in underwater 3D reconstruction and molecular characterization. This work will involve mapping entire study sites in 3D to conduct “multi-omic” characterization of coral responses to the physical environment.

Reef Model: *Linking environmental and molecular data in high resolution 3D habitat reconstructions.* Study sites will be mapped in 3D using structure-from-motion (SfM) photogrammetry techniques. Overlapping imagery will be acquired using single-lens cameras to survey entire study sites. Scale-invariant feature transform algorithms will be applied in Agisoft software to align the images and develop a sparse 3D point cloud representing the study sites. Extrinsic parameters of the camera focal properties will be utilized by the SfM software to perform bundle adjustments and accurately estimate camera positions to render a high-resolution dense 3D point cloud. The dense point clouds will be subsequently rendering into textured 3D mesh models with local coordinate systems. The resulting models will be annotated with the spatial locations of all colonies used for molecular analyses in Q2.

3D molecular cartography: *Multi-omic and 3D cartographic platforms will be used to characterize the molecular underpinnings of coral phenotypic responses.* The x,y,z coordinates for all sample locations from colonies used to address Q2 will be digitally annotated on the reef reconstructions to examine spatial relationships among patterns in molecular responses. Implementing this approach will allow for detecting spatial locations on colonies (e.g., competing borders, disease lesions, areas exposed to high irradiance) that have unique molecular expression patterns. Overlaying multiple molecular data layers onto the 3D reef reconstructions will identify epigenetic expression profiles associated with phenotypic patterns in coral on the reef system. This work will also allow for statistically examining clusters and associations of coral characteristics with physical environmental parameter. Data layers will be visualized using the ili platform to enable interactive examination of molecular profiles in a 3D context.

Experimental design: UHH will utilize the diver and ROV collected imagery to create large-scale 3D habitat maps of the study sites used to characterize corals in Q2. This approach can produce fine-scale georeferenced orthomosaic imagery and simultaneously be used to create 3D models of the study sites using SfM techniques. Ground control points will be placed throughout the study site and aligned with surface buoys so differential GPS points can be collected to ensure accurate spatial reconstruction of the resulting 3D habitat models. The resulting 3D habitat reconstructions will be exported as digital elevation models (DEMs) and orthophoto-mosaics into ArcGIS topographic software in order to classify the habitats and quantify structural features associated with fragmentation and disturbance (Burns et al. 2015). These comprehensive characterizations of 3D structural complexity will allow for statistically

comparing habitat structure and quality among sites, and furthermore, will elucidate how habitat structure may be affecting molecular profiles and phenotypic responses of coral. All molecular data collected in Q2 will be annotated onto the 3D habitat models to statistically examine spatial patterns in molecular profiles in corals. A combination of regression modeling and multivariate statistical procedures will be used to identify distinct patterns in molecular profiles and how they may be influenced by environmental parameters. Bayesian mixed effects models can identify the probability of various phenotypic responses based on the molecular characteristics and environmental parameters associated with a given coral. These results will provide transformative insight into how coral respond at a molecular and phenotypic level to the surrounding environment.

5. Management of Activities and Data Integration: **U. Puerto Rico:** 1, Characterization of transcriptomes across the three, model species (insect, mollusc, coral) in response to global change stressors; 2, Annotation of transcriptomes and identification of specific genes involved in responses and their association with metabolic/enzymatic pathways; 3, Characterization of common response gene networks across species from different tropical ecosystems. **Florida International U.:** 1, Dynamic changes in epigenetic responses across temporal scales in coral reef ecosystems; 2, Epigenetic-to-phenotype linkages under global change scenarios. **U. Hawaii:** 1, Spatially characterize molecular profiles of corals on 3D habitat reconstructions of coral reef ecosystems; 2, Statistically analyze 3D spatial patterns in molecular profiles of coral to determine how physical habitat characteristics influence epigenetic responses and phenotypic characteristics. **All Institutions (Data Integration):** 1) Coordination meetings (x3); 2) Graduate and undergraduate mentoring; 3) Transcriptome and epigenomic data integration; 4) Spatial model integration across ecosystems.

6. Broader Impacts: The proposed CREST thematic network will foster the incorporation of students from underrepresented groups to the study of critical environmental problems affecting their own home areas, heritage and traditions in the tropics. Areas of broader impacts include: 1) In the public field, this research assesses and predicts environmental risks. Findings of this research will be disseminated through planned publications and outreach activities from all three collaborating centers. 2) Graduate and undergraduate student training: Students exchanges across the three CREST centers will permit exposure to different laboratory techniques, facilities and analytical instrumentation, as well as faculty with different expertise. 3) Students from all three CREST centers can participate in the Bioinformatics workshop on RNAseq data analysis to be held in San Juan Puerto Rico on March 26-28, 2020 facilitating their ability to analyze their data. In addition, the proposed research benefits from ongoing research established and consolidated as part of the participating CREST centers including funding from NSF's RAPID and Rules of Life Programs, increasing the scope of the present work and providing additional opportunities for students. The outreach component of the present proposal will be strengthened by the participation of the NGO Sociedad Ambiente Marino, recruiting citizen volunteers to work in the field in Puerto Rico. **Ultimately, the results expected from this thematic network will improve human resource development, positively impacting basic scientific progress as well as applied conservation and management.**

7. Data Management Plan: Our data will be managed in accordance with FIU CREST established data management plans, submitted along with our initial NSF proposals for the three participating institutions.

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