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Microevolution of the oxidative stress response: organismal and transcriptomic effects of peroxide exposure in the starlet sea anemone Nematostella vectensis

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MICROEVOLUTION OF THE OXIDATIVE STRESS RESPONSE: ORGANISMAL AND TRANSCRIPTOMIC EFFECTS OF PEROXIDE EXPOSURE IN THE STARLET SEA ANEMONE *NEMATOSTELLA VECTENSIS*

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ABSTRACT

The starlet sea anemone, *Nematostella vectensis* (phylum Cnidaria, class Anthozoa), inhabits tidal marshes along the Atlantic coast of North America. These shallow coastal habitats are subject to extreme environmental variability, including fluctuating levels of reactive oxygen species (ROS). High ROS concentrations can be cytotoxic and lead to oxidative stress. Given the importance of tidal marshes for marine biodiversity and their susceptibility to oxidative stress, it is important to study how resident organisms counteract oxidative stress. *N. vectensis* is an excellent model system for addressing the evolution of oxidative stress mechanisms because (1) this anemone exhibits tolerance to a variety of environmental stressors, (2) there is clear evidence of local adaptation to stress in different populations and sub-populations, and (3) protein-coding polymorphisms have been identified, some in proteins that are implicated in stress response. I exposed fifteen different clone lines of *N. vectensis* collected from four estuaries to biologically relevant levels of hydrogen peroxide. Pronounced differences are

apparent between clone lines collected from Meadowlands, NJ, Baruch, SC, and Kingsport, NS, as well as among twelve clones collected at a single Cape Cod marsh. This is the first study that demonstrates intraspecific variation in the oxidative stress response of *N. vectensis*. To understand how the peroxide response of these clones might differ at the level of gene expression, I sequenced the transcriptomes of 7 clone lines under control conditions and when exposed to survivable levels of peroxide for 24 h. I found survivable peroxide exposure induces robust and repeatable changes in gene expression, including the up-regulation of genes shown to be associated with cellular responses to oxidative stress, thermal stress, and UV stress, and down-regulation of genes that promote generation of ROS, including the enzymes involved in oxidative phosphorylation. Additionally, I identified pronounced differences in the peroxide-induced transcriptional profiles between genets of *N. vectensis*. The existence of a conserved oxidative stress response has major implications for studies on how other cnidarians, particularly corals, will withstand the impacts of climate change. Indeed, the proximal cause of coral bleaching—the major threat to corals worldwide—is oxidative stress.

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LIST OF ABBREVIATIONS

8-oxo-dG 8-oxo-dihydroguanine

AP-1 ..... Activator Protein 1

AP-2 ..... Activating Protein 2

ANOVA ..... Analysis of Variance

ASW ..... Artificial Seawater

B[a]P ..... Benzo[a]pyrene

BLAST ..... Basic Local Alignment Search Tool

°C ..... Degrees Celsius

CCS ..... Copper Chaperone of Superoxide Dismutase

C/EBP ..... CCAAT-Enhancer-Binding Proteins

cm ..... Centimeter

CO_2 ..... Carbon Dioxide

CuZnSOD ..... Copper Zinc Superoxide Dismutase

DNA ..... Deoxyribonucleic Acid

ELISA ..... Enzyme-Linked Immunosorbent Assay

ERK ..... Extracellular Signal-Regulated Kinase

EST ..... Expressed Sequence Tag

F1 ..... First Filial Generation

F2 ..... Second Filial Generation

GAPDH ..... Glyceraldehyde 3-Phosphate Dehydrogenase

GO ..... Gene Ontology

GPx ..... Glutathione Peroxidase

H_2O_2 ..... Hydrogen Peroxide

JNK ..... c-Jun-N-terminal Kinase

MAPK ..... Mitogen-Activated Protein Kinase

Mb ..... Megabase

MnSOD ..... Magnesium Superoxide Dismutase

NADH ..... Nicotinamide Adenine Dinucleotide

NADPH ..... Nicotinamide Adenine Dinucleotide Phosphate Oxidase

NF-κB ..... Nuclear Factor Kappa-light-chain-enhancer of activated B cells

nM ..... nanoMole

NO ..... Nitric Oxide

N. vectensis ..... *Nematostella vectensis*

O_2 ..... Molecular Oxygen

OGG1 ..... 8-oxo-dG DNA glycosylase

PI(3)K/AKT ..... Phosphatidylinositol-3-Kinase and Protein Kinase B

ppt ..... Parts per thousand

qPCR ..... Quantitative Polymerase Chain Reaction

RNA ..... Ribonucleic Acid

ROS ..... Reactive Oxygen Species

RSOH ..... Sulfenic Acid Group

SNPs ..... Single Nucleotide Polymorphisms

SOD ..... Superoxide Dismutase

Sp1 .....Specificity Protein 1

TCA cycle .....Tricarboxylic Acid Cycle

UV..... Ultraviolet

µM..... Micromolar

CHAPTER ONE: OXIDATIVE STRESS RESPONSE IN COASTAL MARINE ECOSYSTEMS

Introduction

In this chapter, I review current literature on how species respond to chemical challenges in their environments by altering gene expression. In particular, I focus on how coastal marine invertebrates respond to reactive oxygen species (ROS). This review summarizes recent literature on (1) ROS levels in coastal marine habitats, including what is known about sources of oxidative stress in the habitat of my model organism *Nematostella vectensis*, (2) marine invertebrates' genetic tolerance to ROS, and (3) the cellular pathways affected by oxidative stress. In marine invertebrates, gene expression changes induced by ROS exposure likely include adaptive mechanisms to mitigate oxidative effects. However, exposure to ROS can also trigger apoptosis and otherwise cause the breakdown of cellular pathways, cellular events that would be accompanied by non-adaptive changes in gene expression.

Reactive oxygen species (ROS)

Molecular oxygen first appeared in the oceans approximately three billion years ago as a metabolic byproduct of oxygenic photosynthesis, and it later began to accumulate in the atmosphere as marine oxygen sinks (such as elemental iron) became saturated (Crowe et al., 2013). The presence of atmospheric oxygen facilitated the evolution of aerobic life forms that use molecular oxygen (O_2) as the final electron

acceptor during oxidative phosphorylation and allowed more highly efficient energy production in cells (Bubici et al., 2006; Crowe et al., 2013).

Today a variety of normal cellular processes use O_2 for energy production and oxidative reactions and many of these processes, as well as environmental factors, create reactive oxygen species (ROS) as a byproduct. ROS byproducts are generated endogenously during the mitochondrial electron transport chain, mainly from complex I (NADH dehydrogenase) and complex III (ubiquinone–cytochrome c reductase), through numerous enzymes (including dehydrogenases in the TCA cycle, NADPH oxidases, lipoxygenases, and cycloxygenases) and by cytokines, growth factors, ionizing radiation, UV exposure, and other environmental factors (Acharya et al., 2010; Finkel and Holbrook, 2000; Sies, 2014).

Different ROS vary in their origins, their cellular half-lives, and their reactivity. Superoxides are short-lived and highly unstable metabolic byproducts, which can serve as signaling molecules within cells and between pathways (Finkel and Holbrook, 2000). Superoxide is biologically common and highly reactive. It can react with nitric oxide (NO) to form toxic peroxynitrite. Alternatively, superoxide can be converted to hydrogen peroxide (H_2O_2) by spontaneous dismutation or superoxide dismutase (SOD) (Gloire et al., 2006; Saragosti et al., 2010). H_2O_2 is a metabolite of respiration with normal intracellular concentrations of approximately 10 nM (Sies, 2014). H_2O_2 is a relatively long lived ROS compared to superoxide and is freely diffusible (Finkel and Holbrook, 2000). Gradients of H_2O_2 in cells are used for transport across membranes along with

transport protein aquaporins called peroxiporins. Peroxide can further be broken down to hydroxyl radicals in the presence of metal ions (Richier et al., 2003; Sies, 2014).

Toxicity of reactive oxygen species

ROS can oxidize biological macromolecules, including DNA, lipids, and proteins, resulting in cellular damage (Carillon et al., 2013; Han et al., 2008). Here, I focus on the deleterious effects of ROS on DNA and proteins. ROS have been shown to damage both genomic and mitochondrial DNA (Finkel and Holbrook, 2000). DNA damage resulting from oxidation by ROS includes depurination, depyrimidination, single- or double-stranded DNA breaks, base and sugar modifications, and DNA-protein crosslinking. ROS induced DNA damage can arrest or induce transcription, interfere with signal transduction pathways, and lead to replication errors or genomic instability (Acharya et al., 2010; Han et al., 2008).

As a result of its pervasive cellular effects, oxidative damage to DNA has been shown to play a role in cancer, aging, diabetes, arthritis, and neurodegenerative diseases (Acharya et al., 2010; Zhang et al., 2013). Mitochondrial DNA damaged during replication has been shown to impair cellular energy metabolism. Immune system responses to inflammation are regulated by macrophages and ROS induced regulatory disruption in differentiation of these cells is implicated in cancers and autoimmune diseases (Zhang et al., 2013). The accumulation of oxidized DNA in mitochondrial and nuclear DNA is correlated with increased susceptibility to cancer and neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington's disease (Ventura et al.,

2010). In addition to its damaging effects on DNA, sustained exposure to elevated ROS can inactivate many cellular enzymes and play a role in cellular senescence (Bubici et al., 2006; Finkel and Holbrook, 2000; Wu et al., 2009).

ROS are known to modify amino acid side chains containing cysteine, methionine, proline, histidine, and tryptophan. One of the main sites of ROS damage to proteins is the thiol group of cysteine. A variety of modifications can be made to cysteine that can change a protein's conformation and functions. Effects include formation of disulphide bridges connecting cysteine groups (-S-S-), oxidation of a single thiol to form a sulfenic acid group (RSOH), and S-nitrosylation reactions between nitrous oxide and a thiol group. By this epigenetic mechanism, ROS can impact protein function directly without changing the underlying DNA or RNA. ROS can have a variety of roles in gene expression, including the modification of transcription factor activation. For example, the NF-κB pathway is activated in response to a range of environmental stressors including pathogen exposure, ultraviolet light, and oxidative stress. Nuclear localization of NF-κB, which is a critical step in the activation of the NF-κB signaling pathway, can be activated by H_2O_2 in certain cell types (Gloire et al., 2006; Shi and Gibson, 2007).The presence of ROS in cells is hypothesized to activate NF-κB nuclear localization and stress response mechanisms (Gloire et al., 2006).

Beneficial roles of ROS

While high ROS concentrations can harm cells, at low levels, ROS are important effectors in a variety of signal transduction pathways as secondary messengers that can

help regulate transcription, differentiation, tissue repair, inflammation, circadian rhythms, aging, proliferation, activation of apoptosis, and oncogenesis. For example, H_2O_2 acts as a secondary messenger in cell signaling, because it inhibits tyrosine phosphatases by oxidizing cysteine residues in their catalytic domains, which in turn activates tyrosine kinases and a cascade of signaling events. ROS also play a role in regulating a variety of redox sensitive transcription factors, some of which activate pathways that mediate ROS cytotoxicity such as the c-Jun-N-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) cascade and the NF-κB signaling pathway (Carillon et al., 2013; Gloire et al., 2006; Saragosti et al., 2010; Sies, 2014; Zhang et al., 2013).

Oxidative stress and cellular defenses against ROS

Oxidative stress occurs when oxidative balance in a cell is disturbed by excessive exposure to or production of ROS relative to the antioxidant defenses in a cell (Acharya et al., 2010; Anjos et al., 2014; Carillon et al., 2013; Finkel and Holbrook, 2000; Shi and Gibson, 2007). The generation of a certain amount of ROS is required to maintain homeostasis, and antioxidant enzymes help maintain this cellular balance between ROS production and removal (Anjos et al., 2014; Carillon et al., 2013; Finkel and Holbrook, 2000). However, excess ROS have cytotoxic effects as described above. Importantly, oxidative stress can result from or be exacerbated by other environmental stressors including thermal stress and UV stress. As sea surface temperatures and solar irradiation have been increasing in recent decades (Herman, 2010; Khan et al., 2013) , the already pervasive problem of oxidative stress has been growing in importance.

The introduction of oxygen in the oceans and the atmosphere caused the extinction of many species, while others were able to evolve defense mechanisms to mitigate oxygen toxicity. Aerobic cells evolved specific antioxidant enzymes to convert superoxide anions to hydrogen peroxide, and enzymes to degrade H_2O_2. ROS enzymatic scavengers include DNA repair enzymes, SODs, glutathione peroxidases (GPx), peroxiredoxins, and catalases (Carillon et al., 2013; Han et al., 2008; Sies, 2014). SOD is an important enzyme that can have both pro-oxidant and anti-oxidant effects. In addition, studies have shown that when SOD is administered exogenously, ROS effects on cells decrease and endogenous antioxidant defenses increase (Carillon et al., 2013). Non-enzymatic systems have also evolved to sequester ROS in reduced glutathione, ascorbate, pyruvate, flavonoids, carotenoids, and vitamins C and E. In addition, ROS stress response pathways include ERK, JNK, MAPK, PI(3)K/AKT, NF-κB, p53, and heat shock responses. These pathways link apoptosis or pro-survival responses to oxidative stress (Acharya et al., 2010; Bubici et al., 2006; Finkel and Holbrook, 2000).

Oxidative stress in marine environments

Elevated levels of ROS are a common problem in a wide range of marine ecosystems, from salt marshes to deep-sea hydrothermal vents (Lesser, 2006). One important mechanism for the production of ROS is solar radiation acting on dissolved organic matter (Mopper and Kieber, 2000), which tends to cause elevated ROS in the well-lit surface layers of the ocean, particularly in nutrient rich waters. Pollutants, such as run-off from industrial processes, including textile bleaching and chemical manufacturing, are

also a major source of ROS in coastal marine environments. In the open ocean, vertical mixing tends to mitigate the accumulation of ROS, but in some coastal habitats, such as salt marshes, high levels of ROS can accumulate due to the combined effects of photochemical production and anthropogenic introduction of ROS as well as a lack of mixing. In a single marsh, H_2O_2 concentrations in the water have been shown to vary from 0.05 µM to approximately 4.0 µM (Abele-Oeschger et al., 1997). Concentrations in sediments can be up to four times higher than this (Abele-Oeschger et al., 1997). By a different set of mechanisms, ROS are also known to accumulate in hydrothermal vents. In these vents, oxidation of sulfide compounds leads to the production of superoxide radical (Tapley et al., 1999), which is then thought to be quickly converted to H_2O_2. Indeed, among the ROS found in seawater, H_2O_2 is probably of paramount concern to marine organisms because it is the most concentrated, has the longest half-life, and can pass through lipid bilayers (due to its lack of charge) (Mopper and Kieber, 2000).

Importance of tidal marsh ecosystems

As described above, salt marshes are subject to elevated ROS; they are also among the most highly productive marine ecosystems, and they contribute substantially to marine biodiversity. Wetlands filter out pollution, buffer coastal areas from floods and storms, and are good at building up soil to counteract sea level rise. They provide a permanent home for endemic species and serve as vital nurseries or temporary feeding grounds for numerous transient occupants. Tidal marshes are currently suffering significant degradation from both global forces (e.g., climate change, sea level rise) and

local forces (e.g., eutrophication, point sources of pollution). Human effects include groundwater withdrawal, artificial drainage, nutrient inputs, building dams and reservoirs, seawalls and development. Due to the patchy distribution of tidal marshes, populations of marsh-endemic organisms are limited in their effective dispersal ability and are therefore under strong selection pressure to evolve tolerance to their changing environment. To understand how marsh organisms may be adapting to environmental change, there is an urgent need to study model species that are endemic to salt marshes and tractable both in the laboratory and in the field (Darling et al., 2005; Kirwan and Megonigal, 2013; Reitzel et al., 2008b).

The starlet sea anemone—a leading salt marsh model system

The starlet sea anemone, *Nematostella vectensis*, is a small basal eumetazoan species endemic to marshes and other estuarine habitats. As a member of the phylum Cnidaria (along with corals, jellyfish, hydras), *N. vectensis* last shared a common ancestor with bilaterian animals (e.g., vertebrates, arthropods, annelids) some 600-800 million years ago (Peterson and Butterfield, 2005). *Nematostella* is a good model system for both field and laboratory studies. It is easy to collect and monitor natural populations, and can be cultured easily under laboratory conditions (Darling et al., 2005; Harter and Mattews, 2005; Technau and Steele, 2011). The *Nematostella* genome is sequenced to 6.5-fold coverage, and it is estimated to be approximately 450 megabases (Mb) spread over 30 chromosomes (Putnam et al., 2007). In comparison, the coral *Acropora millepora* has 28 chromosomes, an estimated genome size of 200 Mb, and about 80% of its genes

have homologs in *Nematostella*. The genome of the closely related coral *Acropora digitifera* has an estimated genome size of 420 Mb with 23,668 gene models and 93% of genes with counterparts in other metazoans. The sequencing of the *Nematostella* genome, along with 150,000 ESTs, has revealed abundant protein-coding polymorphisms, some in stress-response proteins (Putnam et al., 2007; Shinzato et al., 2012; Sullivan et al., 2009; Wang et al., 2009b; Wang et al., 2009c).

Nematostella's *environmental tolerance and genetic diversity*

All known *Nematostella* populations exhibit tolerance to a wide range of environmental variation, such as temperature and salinity changes. *Nematostella* prefer salinities between 10-26 parts per thousand (ppt) but can tolerate up to 51 ppt. Their temperature tolerance ranges from 2-28.5°C (Darling et al., 2005; Harter and Mattews, 2005; Putnam et al., 2007; Sheader et al., 1997; Technau and Steele, 2011). *Nematostella* populations have shown evidence of local adaptation including temperature preferences (Reitzel et al., 2013a). *Nematostella* are benthic organisms with relatively low mobility, so they are especially vulnerable to pollutants that settle in sediments of estuaries (Anjos et al., 2014; Harter and Mattews, 2005).

N. vectensis' range includes estuarine habitats of the United Kingdom, the US Pacific coast, and the Atlantic coast of North America from Nova Scotia to the Gulf of Mexico. Atlantic coast populations show a high degree of genetic differentiation and regional population genetic structure that is not correlated to relative geographic distances. It is hypothesized that *Nematostella's* population genetic structure is the result

of limited gene flow between *Nematostella* populations, adaptation to local environmental conditions, and episodic anthropogenic dispersal over large distances (Darling et al., 2009; Darling et al., 2005; Darling et al., 2004; Reitzel et al., 2008a; Technau and Steele, 2011).

The varying environmental conditions that *Nematostella* encounter may confer differing selection pressures, and the ability of this species to tolerate rapid and dramatic variation in these environmental conditions implies that *N. vectensis* populations must exhibit wide environmental tolerances. At the same time, the pronounced environmental differences between and even within estuaries inhabited by *N. vectensis* suggest the potential for local adaptation to differing selection pressures. The potential for local adaptation is magnified by the fact that *Nematostella*'s natural dispersal ability appears quite limited, as evidenced by significant genetic structure between and even within estuaries (Darling et al., 2004; Reitzel et al., 2008a; Reitzel et al., 2009; Reitzel et al., 2008b).

Nematostella *SNPs*

Single nucleotide polymorphisms (SNPs) are relatively common and can occur in both coding and non-coding regions of the genome. SNPs in eukaryotic genomes are the most abundant type of genetic variation and can be used as genetic markers for linkage mapping, mapping of complex traits, and population genomic studies. SNPs in functional genes are particularly useful in non-model organisms, because they help identify genes underlying complex traits in these species. Single-nucleotide polymorphisms can have

functional consequences if they change the amino acid encoded at a functionally significant position in a protein. In addition, SNPs can influence promoter activity, splice site recognition, exon skipping, and mRNA binding or localization (Li et al., 2011; Wang et al., 2009b).

The human genome contains three billion base pairs, and a SNP occurs on average at one nucleotide per 300-500 base pairs. In the Pacific coral *Acropora millepora*, relatively high levels of polymorphisms have been found among individuals within the same reef. These polymorphism rates are similar in other cnidarian species. In *Nematostella*, large-scale genome sequencing projects have identified at least 555 genomic SNPs with a high degree of confidence, with 343 in coding regions and only 78 of these being nonsynonymous polymorphisms. It is estimated that in *Nematostella*, the rate of generating new nucleotide polymorphisms is about ten times higher than in humans (Li et al., 2011; Reitzel et al., 2009; Wang et al., 2009a; Wang et al., 2009b).

Previous studies have correlated a variety of allelic variations with a species' geographic distribution. Studies of long-term adaptation on specific nucleotide substitutions have shown evidence that geographic differences correlate to variation in allelic distribution (Velez and Feder, 2006). Relative fitness in specific environmental conditions has also been correlated with the effects of selection pressures on nonsynonymous mutations already present. These environmental conditions impose selection, since individuals with alleles that are favored in specific conditions are more likely to survive and reproduce (Hoffmann et al., 1995). The relative fitness of an allele at a polymorphic position in the *Nematostella* genotype (such as the cysteine or serine

polymorphisms at position 67 in the DNA binding loop of the NF-κB locus) depends on how that allele interacts with the genetic background to affect the phenotype and how alternate phenotypes fare in a given environment. Previous studies have begun to investigate relative allele distributions and have observed differences in *Nematostella* NF-κB allele frequencies among populations at different locations (Sullivan et al., 2009; Wolenski et al., 2011a; Wolenski et al., 2011b).

Summary of my experimental approach

Given the importance of tidal marshes for marine biodiversity and their susceptibility to oxidative stress, it is important to study how estuarine organisms counteract oxidative stress and whether there is variation in this trait within and between species. *Nematostella vectensis* is an excellent model system for addressing these questions. In the chapters that follow, I use survival and regeneration assays to demonstrate that there is significant variation in peroxide resistance within and between populations of *N. vectensis*. In order to characterize *Nematostella's* transcriptomic response to peroxide exposure and potentially identify molecular differences between peroxide resistant and peroxide sensitive individuals, I sequenced RNA from seven different genets exposed to peroxide or control conditions. A bioinformatics analysis of approximately 390 billion filtered reads of transcriptomic data identified hundreds of genes that exhibit elevated expression in response to peroxide exposure, including many genes known to be involved in the oxidative stress response of other species. Importantly, my analyses revealed pronounced differences in peroxide's effect on the expression of

individual genes, including superoxide dismutase. These transcriptomic differences may partially explain the phenotypic differences in peroxide tolerance that I documented. In the final chapter, I discuss the broader significance of these findings, and I suggest a research program to identify the genetic variation that is critical to the observed variation in peroxide tolerance.

CHAPTER TWO: INTRASPECIFIC VARIATION IN PEROXIDE SENSITIVITY IN NEMATOSTELLA VECTENSIS

Introduction

The starlet sea anemone, *Nematostella vectensis,* is a leading laboratory model organism in the phylum Cnidaria due to its ease of culture, amenability to a wide range of experimental protocols, and the availability of abundant genomic resources (Darling et al., 2005; Hand and Uhlinger, 1991, 1992; Stefanik et al., 2013a; Stefanik et al., 2014; Stefanik et al., 2013b). In its natural habitat—estuaries along the Atlantic coast of North America—this animal experiences dramatic variation in key environmental variables over short temporal and spatial scales (Darling et al., 2005; Hand and Uhlinger, 1994). Furthermore, there is extensive genetic and phenotypic variation between populations of *N. vectensis* (Darling et al., 2004; Reitzel et al., 2013a; Reitzel et al., 2013b). Atlantic coast populations have a high degree of genetic differences and of regional population genetic structure that is not correlated to relative geographic distances. These differences within local populations are hypothesized to be the result of limited gene flow within and between *N. vectensis* populations, adaptation to local environmental conditions, and episodic anthropogenic dispersal over large distances. The estuarine habitats inhabited by *N. vectensis* are often physically isolated, and these habitats can differ dramatically with respect to key environmental parameters, such as temperature, pH, and salinity. Moreover, the early stages of the *N. vectensis* lifecycle exhibit limited dispersal ability. For example, the egg mass is negatively buoyant (Hand and Uhlinger, 1992), and the

larvae are negatively geotaxic [Braff, Lever, and Finnerty, unpublished data]. The combination of diverse environments and limited dispersal will tend to promote adaptive genetic differentiation between populations. For this reason, *N. vectensis* has been advocated as a useful model for studying adaptive microevolution in a coastal invertebrate beset by rapid climate and environmental changes (Darling et al., 2005; Reitzel et al., 2013a).

One environmental stressor that varies dramatically in salt marshes and other estuarine habitats is hydrogen peroxide (Abele et al., 1999; Abele et al., 1998b). Peroxide can accumulate in shallow coastal habitats due to natural inputs such as photochemical production and atmospheric wet deposition (Abele et al., 1999) as well as anthropogenic inputs such as runoff from industrial processes (e.g., mining, industrial manufacturing) (Aseervatham et al., 2013). Salt marshes are particularly susceptible to peroxide accumulation because large amounts of organic matter can fuel peroxide production through photo-degradation, and isolated pools may seldom be flushed. In a single marsh, hydrogen peroxide concentrations in the water have been shown to vary from 0.05 µM to approximately 4.0 µM, with concentrations in sediment higher than concentrations in the adjacent water (Abele-Oeschger et al., 1997). *N. vectensis* is a benthic organism with relatively low mobility, so this species is readily exposed to compounds that accumulate in sediments of estuaries (Anjos et al., 2014; Harter and Mattews, 2005).

In its natural habitats, *N. vectensis* can be exposed to a wide range of exogenously produced reactive oxygen species (ROS) including hydrogen peroxide. It must, therefore, be under strong selection to counteract oxidative stress. *N. vectensis* is known to tolerate

a wide range of other highly variable environmental parameters. For example, the species can tolerate salinities from ~0-50 ppt and temperatures from 2-28.5°C (Darling et al., 2005; Hand and Uhlinger, 1994; Harter and Mattews, 2005). The ability to tolerate rapid and dramatic variations in these environmental conditions implies that individual *N. vectensis* have wide environmental tolerances. At the same time, the pronounced environmental differences between and even within estuaries inhabited by *N. vectensis* suggest the potential for local adaptation to differing selection pressures. The potential for local adaptation is magnified by the fact that *N. vectensis*' natural dispersal ability appears limited, as evidenced by significant genetic structure between and even within (Darling et al., 2004; Reitzel et al., 2008a). Specifically with reference to peroxide tolerance, we might expect all *N. vectensis* to exhibit a wide tolerance to peroxide, with no observable differences between or within populations. However, given that a recent study identified substantial differences in temperature-specific growth and regeneration rates in *N. vectensis* (Reitzel et al., 2013a), we sought to investigate whether different clone lines exhibit differential peroxide tolerance. Indeed, as elevated temperatures promote oxidative stress, we hypothesized that the same clone lines that are best adapted to high temperatures would also be most resistant to oxidative stress.

In this study, we exposed fifteen different clone lines of *N. vectensis* collected from four different estuaries to biologically relevant concentrations of hydrogen peroxide. H_2O_2 was used to induce oxidative stress because (1) it is persistent in marine waters; (2) it is uncharged and penetrates cellular membranes, and (3) it is easy to work with in the laboratory (Finkel and Holbrook, 2000; Richier et al., 2003; Sies, 2014). The

study included three clone lines (one each from Baruch SC, Meadowlands NJ, and Kingsport Nova Scotia) known to vary in their growth and/or regeneration rates at 21 and 29°C as well as 12 previously uncharacterized clone lines established from founder individuals collected in Great Sippewissett Marsh, Falmouth, MA (Fig. 2.1). We monitored the effects of peroxide on adult animal survival and on the ability of animals to regenerate a new "head" following bisection through the body column. We identified substantial differences in the effects of peroxide exposure on survival and regeneration rates between the SC, NJ, and NS clone lines and among clone animals collected from the single estuary in Sippewissett. To our knowledge, this is the first demonstration of intraspecific variation in oxidative stress tolerance in any animal.

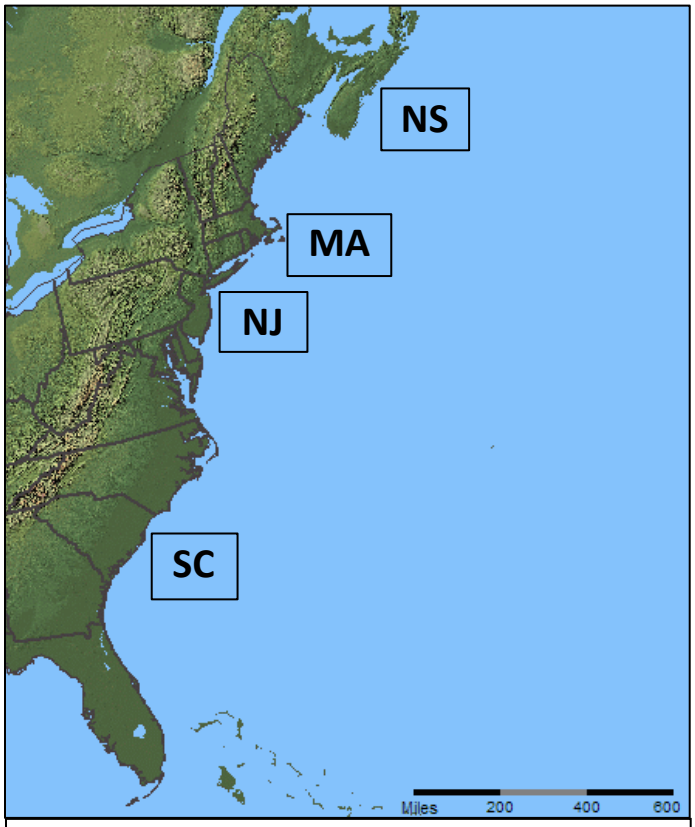


Figure 2-1. *N. vectensis* collection sites. Founder individuals for *Nematostella vectensis* clone lines used in this study were collected from Nova Scotia, Massachusetts, New Jersey, and South Carolina.

Methods

Animal collection and husbandry

N. vectensis animals were collected from estuaries in four locations: Kingsport, Nova Scotia; Great Sippewissett Marsh, Falmouth, Massachusetts; Meadowlands, New Jersey; Baruch, South Carolina, as previously described (Fig. 2.1) (Darling et al., 2004; Reitzel and Darling, 2003). Within Sippewissett Marsh, founder individuals were collected from three distinct pools labeled Pool A-C. Shortly after capture, individual anemones were used to establish clone lines by repeatedly bisecting the animals and allowing them to regenerate (Stefanik et al., 2013a). All clone lines were housed in the laboratory using standard culture conditions (Stefanik et al., 2013a) for a period of several years before being used in this study.

Peroxide survival experiments

Several individual anemones from each clone line (n=5-10) were maintained in 0.0005% H_2O_2/11 ppt artificial seawater (0.00021M H_2O_2) or under control conditions (11 ppt artificial seawater, ASW; Instant Ocean™)) for a period of two weeks. The ASW/H_2O_2 or ASW media was changed daily. Over the course of these experiments, each individual anemone was housed in a single well of a 12-well plate (Thermo Scientific Nunc) and maintained at 20°C on a 12 h-light/12 h-dark diel cycle. Once per day, individual anemones were scored for tentacle number and for the ability to contract the tentacles and body column in response to mechanical stimulation. Single factor ANOVAs were performed to identify significant differences in average tentacle number

between peroxide exposed and control individuals on each day of the study ($p<0.05$). By the end of the study, animals that had lost the ability to respond to mechanical stimulation were scored as deceased. All surviving anemones that had been subjected to peroxide exposure were retired from any future stress-tolerance studies due to concerns over potential lingering epigenetic effects of exposure.

Peroxide regeneration experiments

Individual anemones from each clone line (n=10-20) were bisected along the transverse axis at the midpoint of the body column, approximately equidistant from the tip of the foot and the opening of the mouth. The aboral halves of 50% of the bisected anemones (n=5-10) from each clone line were then transferred to 0.00025% H_2O_2/11ppt ASW (i.e., half of the H_2O_2 concentration used in the survival experiments). These animals were then allowed to regenerate for 14 days, while the other 50% were allowed to regenerate for the same period under control conditions (11 ppt ASW). The H_2O_2/ASW or ASW media were changed daily. The anemones were sorted between H_2O_2 and control treatments so that mean body length was approximately equal between treatments. Individuals used in these experiments were between 0.5-3.0 cm in length from the tip of the foot to the oral opening. Regenerating anemones were scored daily for the presence and number of tentacles, and after 14 days, they were scored for their ability to feed. If the bisection site of the regenerating anemones was not a clean cut, then the individual was not included in the data. Previous studies have shown the regeneration process is slowed when the bisection is not clean or the mesenteries are exposed (Reitzel

et al., 2008a). ANOVAs were performed to determine significant differences in average tentacle number during regeneration between H_2O_2-exposed and control individuals. As with the H_2O_2 survival experiments, all surviving anemones that successfully regenerated when exposed to H_2O_2 were retired from any future stress-tolerance studies due to concerns over potential lingering epigenetic effects of exposure.

Results

Clone lines of adult anemones show differences in survival upon treatment with H_2O_2

We scored adult polyps in peroxide and control conditions for the number of tentacles in the extended position to measure survival over a period of 14 days (Fig. 2.2). We used this metric as an indicator of the anemones' condition because tentacle number is easy to score, the tentacles are particularly sensitive to tissue damage from elevated peroxide, and tentacle extension, which is necessary for feeding, is characteristic of unstressed animals. In all clone lines tested, there was no change in mean tentacle number in control conditions. However, there was a significant decrease in tentacle number in several clone lines under conditions of H_2O_2 exposure (Fig. 2.3, 2.4). In the clone line from Nova Scotia (NS), mean tentacle number showed a significant decrease in peroxide -exposed animals beginning on day 9 and continuing to the end of the study on day 14. In the clone line from New Jersey (NJ), peroxide -exposed and control animals began to diverge in tentacle number at day 12, and the difference between treatments became statistically significant by day 14. By contrast, the South Carolina clone line may be able to tolerate indefinite exposure to the tested level of peroxide, as we observed neither a

significant decline in tentacle number nor any visible tissue disfigurement over the course of the treatment period.

Differences between the NJ, NS, and SC clone lines in H_2O_2 tolerance were not surprising given previously demonstrated differences in their response to elevated temperatures (Reitzel et al., 2013a). However, we also observed significant and equally substantial differences in H_2O_2 tolerance among 12 genetically distinct *N. vectensis* clone lines generated from individuals collected from three pools in Sippewissett Marsh, MA in 2008 (Figs. 2.3,2.4). In particular, three Sippewissett clone lines appeared minimally affected by H_2O_2 exposure (MA A1, MA C2, and MA C4), while the other nine clone lines displayed varying levels of disfigurement and loss of tentacles over time (Figs. 2.3, 2.4). The three clone lines minimally affected by H_2O_2 exposure had been collected from two different pools in Sippewissett Marsh. Two of the resistant clone lines came from Pool C, which also contained three clone lines that were sensitive to H_2O_2 exposure. The other resistant clone line came from Pool A (MA A1), and there were four sensitive clone lines also from that pool (MA A2, A3, A4 and A5). Unfortunately, we are unable to directly compare the performance of MA A1 under H_2O_2 -exposed versus control conditions because the clone line perished, apparently due to disease, before the control could be performed.

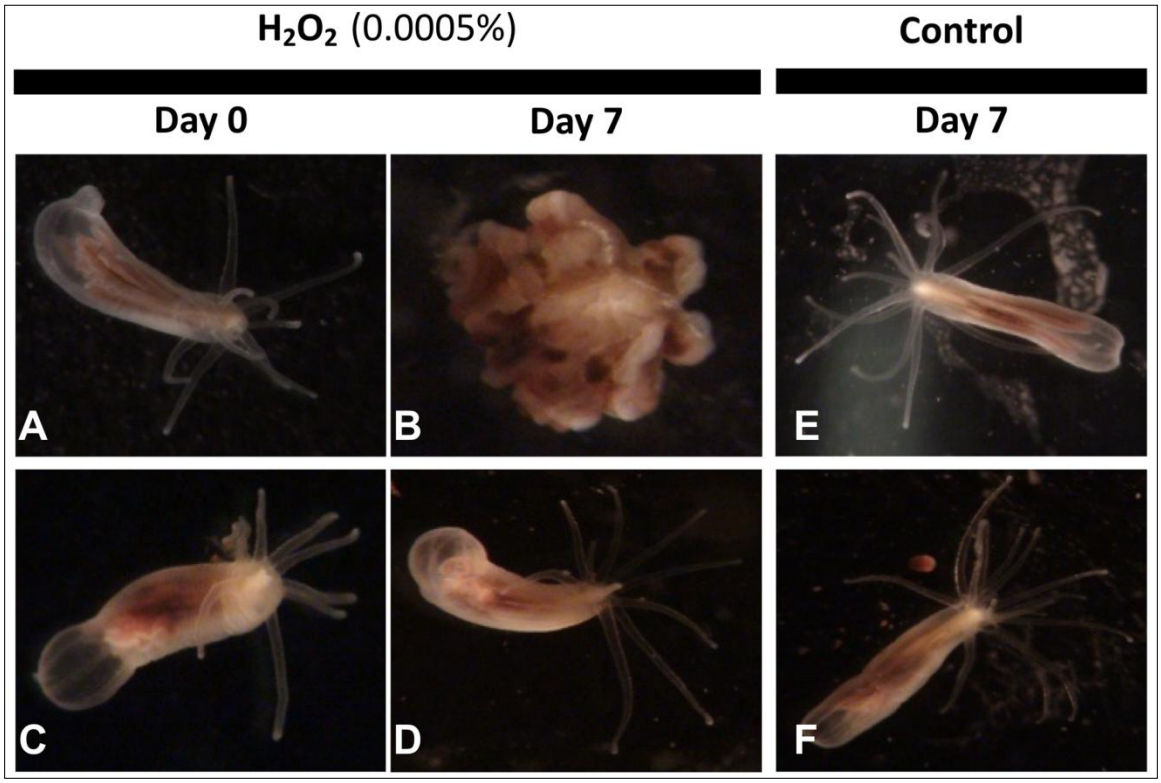
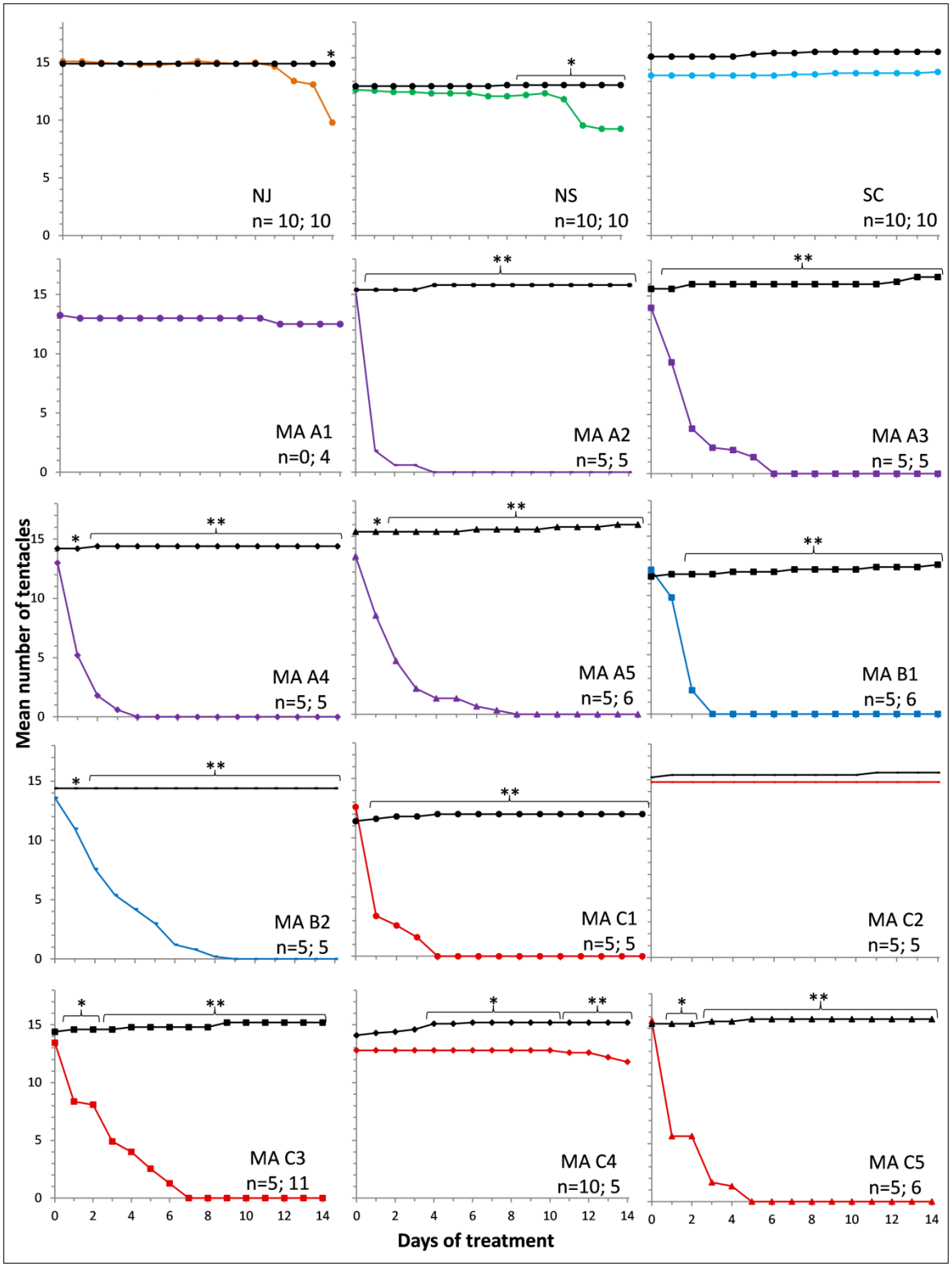


Figure 2.2. Representative adult tolerance to peroxide exposure. Representative individuals from Sippewissett MA (top row: MA B1; bottom row: MA A1) during exposure to 0.0005% H_2O_2 (A-D) or control conditions (E-F) over 7 days. After a week, the majority of the clone lines showed severe disfigurement and loss of tentacles but we also found clone lines that were resistant to H_2O_2 exposure.

Figure 2.3. Adult tentacle number over the course of 14-days exposure to peroxide or control conditions for 15 *N. vectensis* clones. In all of the graphs, days of exposure are given along the X-axis, and tentacle number is shown on the Y-axis. The control condition is shown using black lines, while the H_2O_2 exposure is shown in the colored lines. The number of individuals tested under control conditions and H_2O_2 exposure is provided for each clone line (n=#/#). Statistically significant differences between H_2O_2 and control conditions on a given day are indicated using asterisks (* = $p<0.05$; ** = $p<0.005$).


Mean number of tentacles
Days of treatment
NJ
n= 10; 10
NS
n=10; 10
SC
n=10; 10
MA A1
n=0; 4
MA A2
n=5; 5
MA A3
n= 5; 5
MA A4
n=5; 5
MA A5
n=5; 6
MA B1
n=5; 6
MA B2
n=5; 5
MA C1
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MA C3
n=5; 11
MA C4
n=10; 5
MA C5
n=5; 6

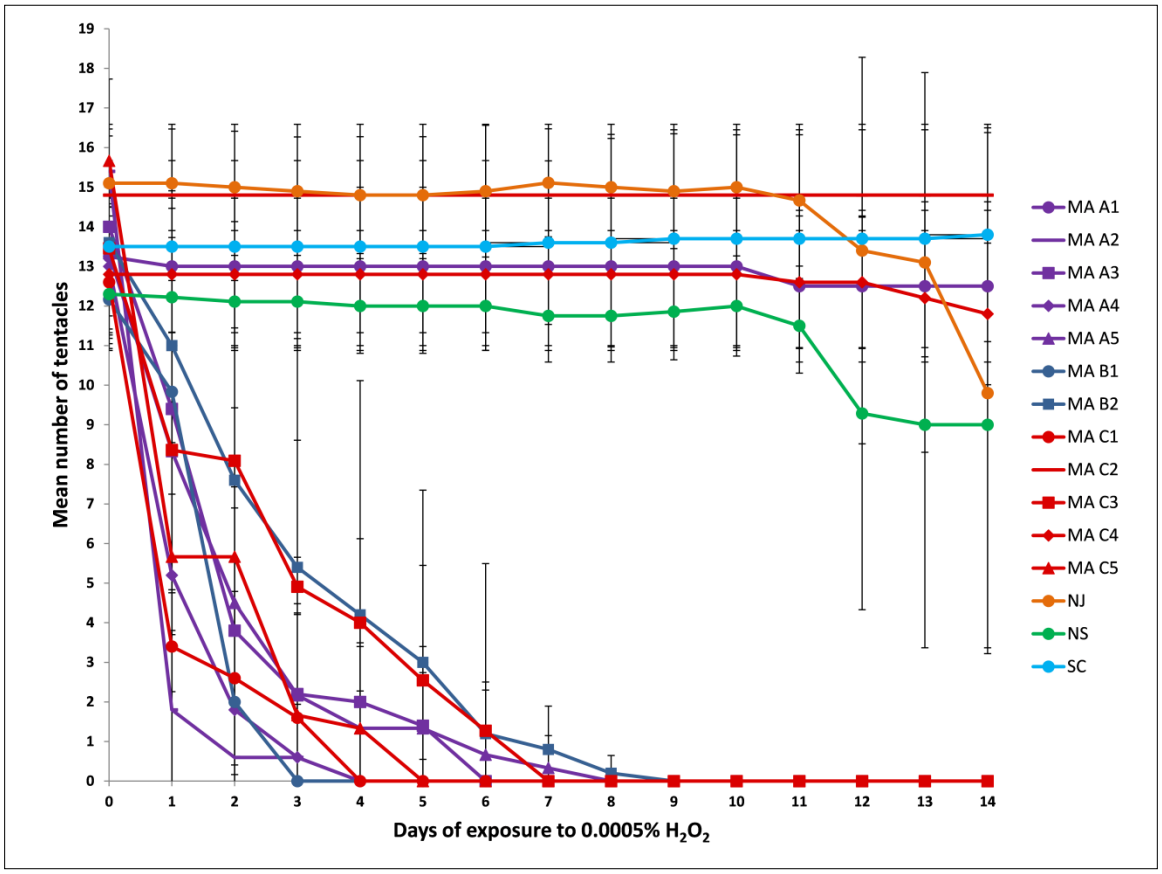


Figure 2.4. Adult peroxide sensitivity during peroxide exposure for 15 *N. vectensis* clone lines. Adult H_2O_2 sensitivity as measured by mean tentacle number over time (n=4-11) during exposure to 0.0005% H_2O_2 in 15 clone lines from MA pool A (purple), MA pool B (blue), MA pool C (red), New Jersey (orange), Nova Scotia (green), and South Carolina (teal). Six clone lines appear minimally affected by H_2O_2 and this does not seem to be a pool-specific effect within the populations. (*$p<0.05$; **$p<0.005$).

Regeneration is differentially affected by H_2O_2 in clone lines of anemones

To determine whether H_2O_2 had an effect on regeneration, we bisected animals, isolated the aboral halves, and then counted the number of tentacles in these regenerating animals once per day over a 14-day period under control conditions or in the presence of H_2O_2 (Fig. 2.5). The rate of regeneration was significantly affected by H_2O_2 exposure and clone population (Fig. 2.6). The NS clone line showed a significant effect of H_2O_2 exposure on average number of tentacles present on days 5-12 ($p<0.05$), with highly significant effects on days 5, 11, and 12 ($p<0.005$). The H_2O_2 exposed NJ individuals showed significantly lower average tentacle numbers on day 7 as well as days 11-14 of regeneration ($p<0.05$), with highly significant differences apparent by day 14 ($p<0.005$). The SC clone line showed no statistically significant effects of H_2O_2 exposure on tentacle number throughout the 14-day regeneration period.

Continual H_2O_2 exposure had varying effects on regeneration in 11 clone lines from Sippewissett Marsh (Fig. 2.6). In general, the ability to regenerate in 0.00025% H_2O_2 was correlated with their ability to survive in 0.0005% H_2O_2. From MA pool A, the four clone lines that were sensitive to H_2O_2 in the adult survival assay (MA A2, A3, A4, A5) also showed delayed regeneration in the presence of H_2O_2 at some time points. In clone lines MA A4 and A5, a significant detrimental effect of H_2O_2 was observed until the end of the study, becoming increasingly severe over the 14-day period. In MA A2 and A3, the number of tentacles on individuals undergoing H_2O_2 treatment had similar mean tentacles number as the control specimens by the end of the study. Unfortunately, the clone line from pool A that exhibited the greatest tolerance to H_2O_2 in the adult survival

assay (MA A1) was lost due to an apparent disease before it could be subjected to the H_2O_2 /regeneration assay. While neither of the two clone lines from pool B was able to tolerate 0.0005% H_2O_2 as an adult, both were able to regenerate without any effect in 0.00025% H_2O_2. From pool C, the two clone lines that demonstrated the greatest H_2O_2 tolerance as adults (MA C4 and C2) showed negligible or minor effects of H_2O_2 on regeneration; regeneration in MA C2 was not affected by H_2O_2, whereas MA C4 was affected by H_2O_2 on days 3 and 4, before H_2O_2 treated individuals had similar tentacle numbers as control individuals on day 5-14. Some of the less resistant clones (MA C1 and C3) showed delayed rates of tentacle development early in regeneration (e.g., day 4), but were similar to control animals by the end of the study. By contrast, clone line MA C5 showed the most persistent and severe detrimental effects of H_2O_2 on regeneration, with a highly significant ($p<0.005$) decrease in average tentacle number versus control from days 8-14.

If we compare the performance of all clone lines in the presence of H_2O_2 (Fig. 2-7), we observe the greatest variation in tentacle number between H_2O_2 and control condition on days 4-6. For example, on day 4, the clone line with the lowest mean tentacle count (MA C1) averaged fewer than two tentacles per individual anemone, whereas the clone line with the highest mean tentacle count (MA C4) averaged approximately ten tentacles per individual. By day 14, many clone lines appeared to achieve a steady-state number of tentacles ranging between ~9 (MA B1) and ~14 (NJ).

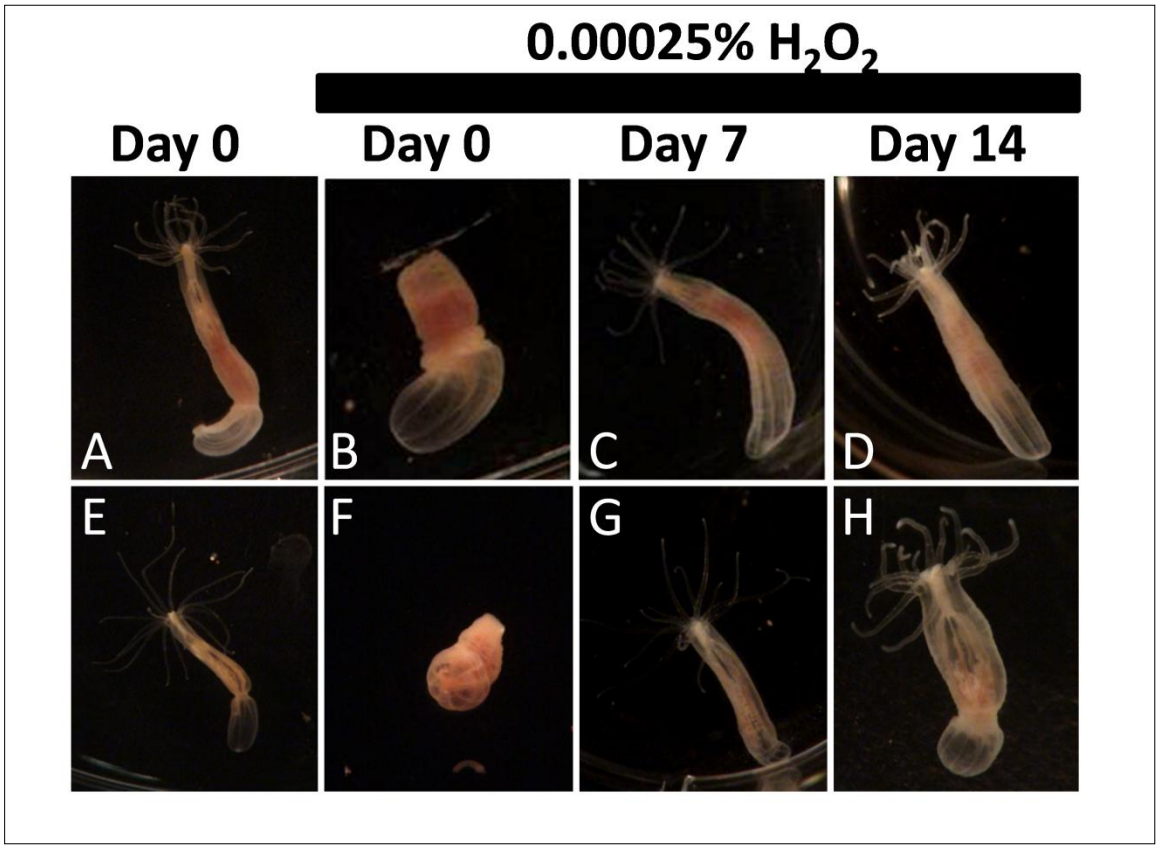
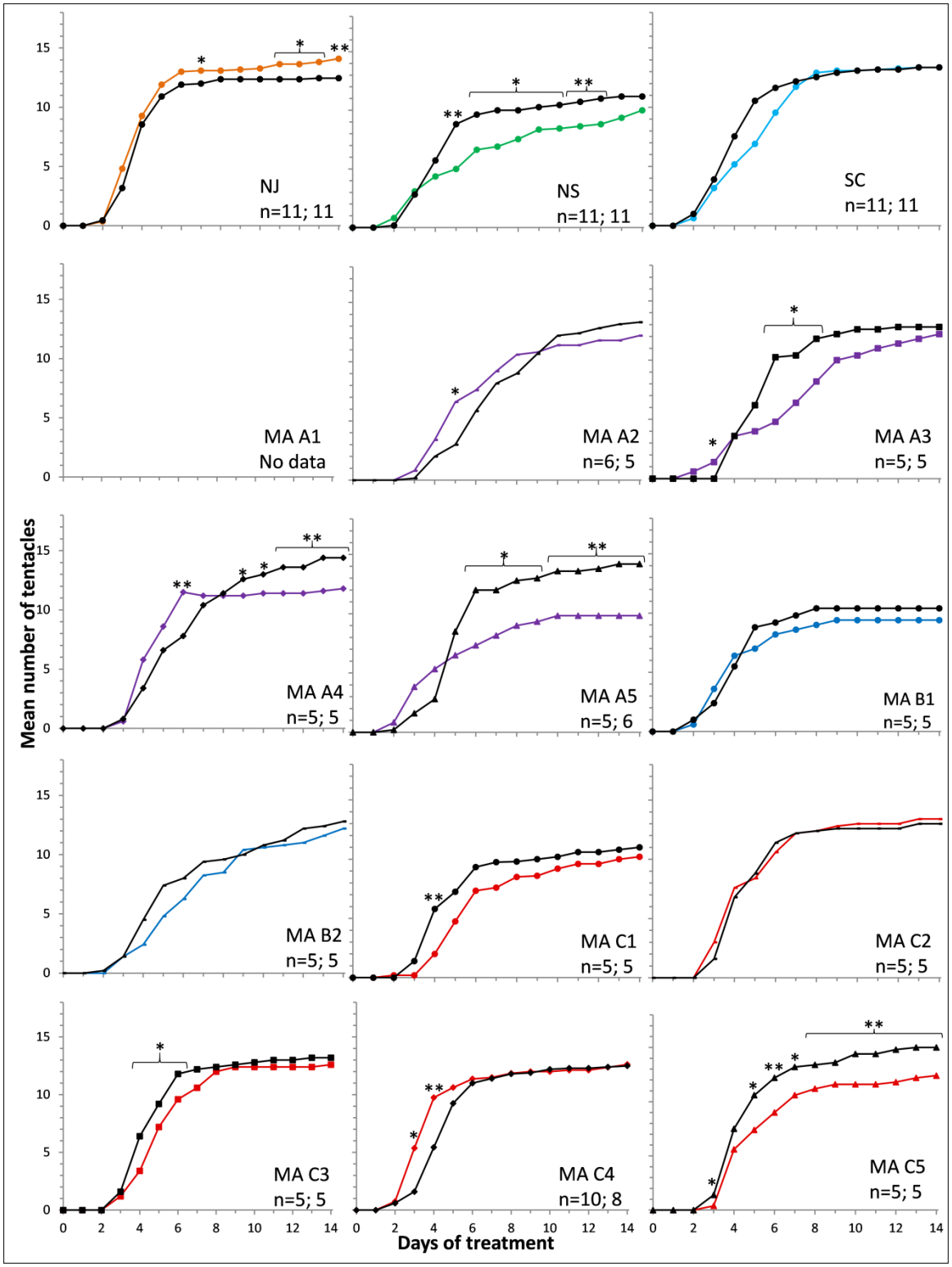


Figure 2.5. Representative regenerating *N. vectensis* during peroxide exposure. Representative *N. vectensis* individuals from New Jersey (A-D) and Nova Scotia (E-H) regenerating during exposure to 0.00025% H_2O_2 over 14 days. Normal tentacle number ranges from 12-14 tentacles, and the regeneration process typically takes between 7 to 10 days.

Figure 2.6. Regenerating tentacles during peroxide exposure for 15 *N. vectensis* clone lines. Regeneration ability as measured by mean tentacle number over time (n=5-11) during exposure to 0.00025% H_2O_2 in 14 clone lines from MA pool A (purple), MA pool B (blue), MA pool C (red), New Jersey (orange), Nova Scotia (green), and South Carolina (teal). (*$p<0.05$; **$p<0.005$).


NJ
n=11; 11
NS
n=11; 11
SC
n=11; 11
MA A1
No data
MA A2
n=6; 5
MA A3
n=5; 5
MA A4
n=5; 5
MA A5
n=5; 6
MA B1
n=5; 5
MA B2
n=5; 5
MA C1
n=5; 5
MA C2
n=5; 5
MA C3
n=5; 5
MA C4
n=10; 8
MA C5
n=5; 5
Mean number of tentacles
Days of treatment

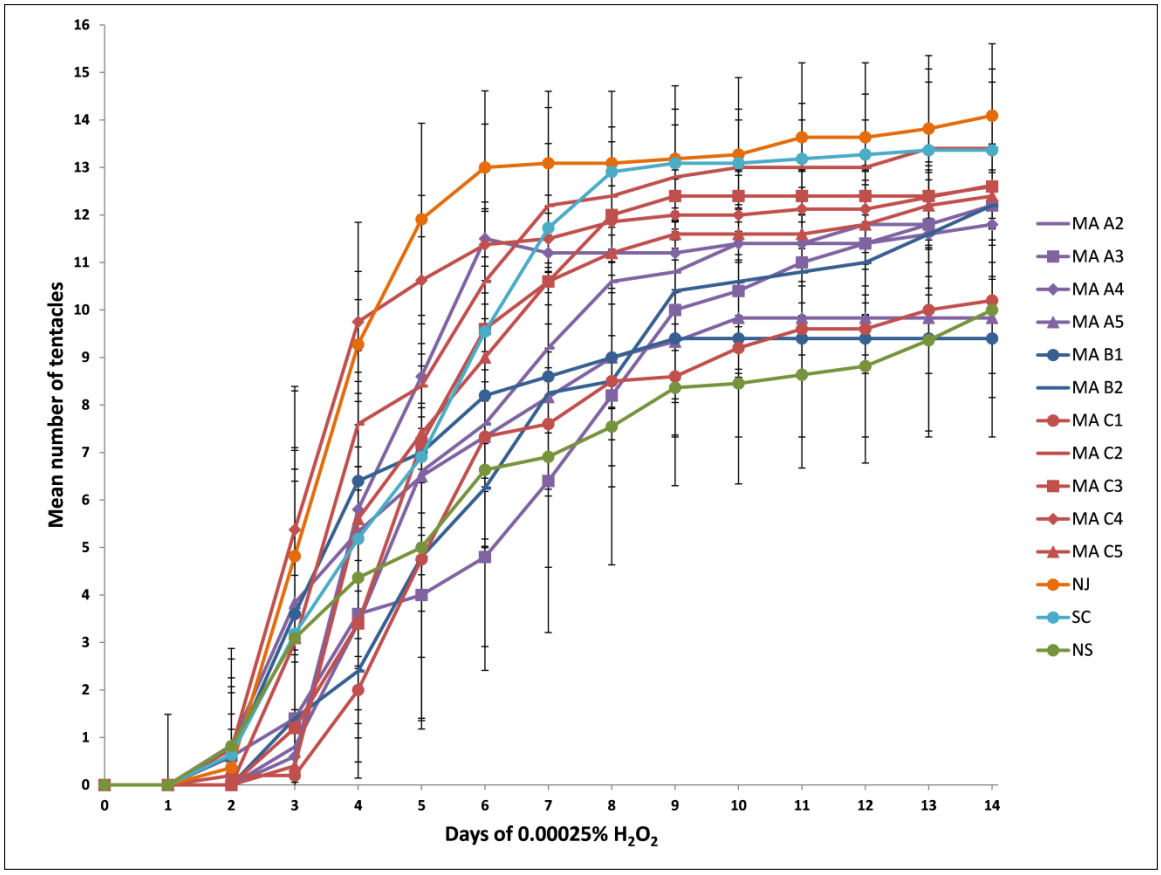


Figure 2.7. Regeneration ability during peroxide exposure for 15 *N. vectensis* clone lines. Regeneration ability as measured by mean tentacle number over time (n=5-11) during exposure to 0.00025% H_2O_2 in 15 clone lines from MA pool A (purple), MA pool B (blue), MA pool C (red), New Jersey (orange), Nova Scotia (green), and South Carolina (teal) (*p<0.05; **p<0.005).

Discussion

In this study, we describe significant differences in the response of various genotypes of the estuarine sea anemone *N. vectensis* to peroxide exposure (Table 2.1). The fifteen clone lines tested were divided into four categories based on their peroxide resistance. Highly resistant clones showed minimal tentacle loss (0-3 tentacles) over the course of the 14 day survival assays (Figure 2.4). Moderately resistant clone lines lost between 4-7 tentacles over two weeks. Moderately sensitive clones took more than 7 days to lose all of their tentacles and highly sensitive clones lost all of their tentacles in fewer than 7 days (Table 2.1).

H_2O_2 is a straightforward and biologically relevant way to challenge the oxidative stress response of a coastal marine invertebrate such as *N. vectensis*, because the species regularly confronts elevated H_2O_2 levels in its natural environment. Unlike other ROS, H_2O_2 is uncharged and can diffuse freely across membranes. It is a long-lived ROS compared to superoxide and is a strong oxidant in both acidic and alkaline conditions. Elevated levels of H_2O_2 are generally observed during the spring and summer months in intertidal sandflats. Previous studies have shown that intertidal habitats where *N. vectensis* live have higher variations in chemical stresses (including oxidative stress) and physical disturbances than subtidal zones (Finkel and Holbrook, 2000; Perkins, 2007; Storch et al., 2001). In previous studies on coastal and estuarine invertebrates and fishes, elevated peroxide concentration has been shown to compromise tissue integrity, impair physiological function and induce mortality (Abele et al., 1998a; Storch et al., 2001).

In addition to contributing to the chemical stress variation in intertidal zones, ROS also play a significant role in the degradation and bleaching of coral reefs. In recent years, increasing CO_2 has been accumulating in both the atmosphere and ocean. Coral bleaching is initiated by photosynthetic dysfunction leading to oxidative stress and can be triggered by numerous stressors including temperature fluctuations, UV light, pollution, and changing seawater conditions (e.g., sedimentation, salinity, nutrients) (Downs et al., 2002; Merle et al., 2007; Shinzato et al., 2012; Weis, 2008). Bleaching is the result of a breakdown in the symbiotic relationship between the host cnidarian and unicellular photosynthetic dinoflagellate symbionts (e.g., Symbiodinium). Oxidative stress has been shown to be a major role in bleaching events and apoptosis in corals, with ROS playing a central role in injury in both the corals and symbionts (Downs et al., 2002; Merle et al., 2007; Nii and Muscatine, 1997; Richier et al., 2008; Saragosti et al., 2010; Weis, 2008). Corals and other cnidarians are in peril due to climate change, so understanding cnidarians' response to environmental effectors is increasingly important (Lewis et al., 2013; Shinzato et al., 2012; Shinzato et al., 2011; Wolenski et al., 2013).

Organismal resistance to heat stress has been shown to be correlated with resistance to oxidative stress in several organisms. For example, in the mud crab *Scylla serrata*, a species native estuarine ecosystems of the Indo-Pacific, a positive correlation was found between thermal and oxidative stress and the levels of antioxidants (Paital and Chainy, 2014). Furthermore, temperature fluctuations in either direction are known to negatively affect mitochondrial respiration and oxidative metabolism in several marine organisms (Abele et al., 2002; Downs et al., 2002; Heise et al., 2003; Paital and Chainy,

2014; Weis, 2008). Given this, it is perhaps not surprising that the three clone lines of *N. vectensis* previously shown to differ in their tolerance of elevated temperature (SC, NJ and NS; Reitzel et al., 2013a) also differed in their sensitivity to H_2O_2 (Table 2.1). In the previous study, the SC, NJ, and NS clone lines were among 14 different clone lines sourced from seven different estuaries along the Atlantic seaboard of North America whose growth and regeneration was compared at 9°C, 21°C and 29°C. Our findings mirror the relative temperature tolerances in the previous study with the NS clones being more sensitive to temperature variation and the SC population being more resistant. For example, the SC clone line, which was found to be most tolerant of high temperatures (29°C), was also least affected by H_2O_2, whereas the NS clone line, which was most sensitive to high temperature, was most affected by H_2O_2.

Perhaps the most surprising result of this study is that we observed significant differences in survival and regeneration during H_2O_2 exposure among clone lines taken from the single marsh in Sippewisset, MA. The Sippewisset clone lines used in our experiments are derived from wild-caught individuals that lived within a hundred meters of each other, some collected from the same small pool where they would have presumably encountered similar environmental stressors; i.e., each of the genets collected from the same pool would have been subjected to the same daily and seasonal variation in temperatures, which suggests that they would not differ widely in their temperature preferences. Nevertheless, there was variation among these clone lines in their ability to tolerate H_2O_2 exposure.

One explanation for this is may be that within each pool there is an opportunity for an individual to select a microhabitat to which it is highly adapted. Habitat selection is one of the natural pressures that shape ecosystems, and in coastal ecosystems, this is influenced by spatial and temporal heterogeneity in the microhabitats. Habitat selection by organisms also influences ecosystem dynamics and can be affected by both biotic and abiotic factors including resource quality, risk of predation, competition, reproductive benefits and physical constraints including temperature, salinity, and oxygen tolerance. Other abiotic factors that can have effects on peroxide levels include sediment composition and shifting sediment distribution, the amount of organic matter, water depth and tidal action, the amount of pollution and heavy metal contamination, primary production, flooding events and the associated interconnectedness of tidal pools, solar radiation, and other microbial plant and algal species present (Fujii, 2012; Iken et al., 2010; Ross and Adam, 2013; Sheaves and Johnston, 2008). The same intertidal pools have been shown to have oxygen saturation and high levels of photosynthesis during the day but suboxic or anoxic conditions at night. For example, changes in vertical gradients of oxygen, H_2S, temperature, and pH have been shown to effect changes in molecular response to ROS in the marine polychaete burrower *Heteromastus filiformis*. Gradients of pH have also been shown to affect both oxygen consumption and H_2O_2 levels (Abele et al., 1998b; Storch et al., 2001).

One model to explain differences in heat and peroxide tolerance between individual anemones is that they differ in a stress response pathway. Our results have not identified genetic differences between peroxide sensitive and resistant anemones. We

note, however, that there are two functionally distinct alleles (cysteine or serine) of the NF-κB gene in natural populations of *N. vectensi*s (Table 2.1). These allelic variants of NF-κB differ in their DNA-binding and transactivation abilities (Sullivan et al., 2009; Wolenski et al., 2011a; Wolenski et al., 2011b). Transcription factor NF-κB can counteract biotic and abiotic stresses in insects and mammals by increasing the expression of resistance genes (Morgan and Liu, 2011). Related to the studies conducted herein, in mammals, transcription factor NF-κB can be activated by ROS, which can enable it to activate antioxidant genes such as MnSOD (Das et al., 1995; Djavaheri-Mergny et al., 2004). Whether NF-κB variants or other genetic or epigenetic factors influence heat or peroxide tolerance in *N. vectensis* is not known. Further studies are needed to investigate the genetic basis of the heat and peroxide tolerance discovered among *N. vectensis* individuals.

A recent study showed variation in temperature tolerance in the coral *Acropora hyacinthus* collected from pools with highly and moderately variable temperature ranges. Furthermore, transplant experiments between the pools showed that the corals acquired at least part of the gene expression and temperature resilience profiles of the pool they were transplanted into (Palumbi et al., 2014). *N. vectensis* shows similar natural variation in response to induced oxidative stress. Adaptation over many generations may be too slow for dealing with widespread climate changes. Therefore, the ability of these cnidarians to acclimate to changing conditions will have an important role in acquiring the needed resistance to deal with stressors in their environments.

Table 2.1. Overview of resistant and sensitive clone line collection sites, peroxide tolerance, and temperature tolerance. Peroxide sensitivity based on mean tentacle number during 14 day exposure to 0.0005% H_2O_2. Over the course of the 14 day survival assays, highly resistant clones showed minimal tentacle loss (0-3 tentacles), moderately resistant clone lines lost between 4-7 tentacles, moderately sensitive clones lost all tentacles in > 7 days and highly sensitive clones lost all of their tentacles in < 7 days. Thermal sensitivity estimates taken from Reitzel et al., 2013. The polymorphic NF-κB residue at position 67 of DNA binding loop is also noted. nd = no data available.

Clone Line	Location	Pool	H_2O_2 Tolerance	Thermal Tolerance	NF-κB allele
NJ	Meadowlands, NJ	NA	moderately resistant	sensitive	C/C
SC	Baruch, SC	NA	highly resistant	resistant	C/S
NS	Kingsport, NA	NA	moderately resistant	sensitive	C/C
MA A1	Sippewissett, MA	A	highly resistant	nd	nd
MA A2	Sippewissett, MA	A	highly sensitive	nd	nd
MA A3	Sippewissett, MA	A	highly sensitive	nd	nd
MA A4	Sippewissett, MA	A	highly sensitive	nd	nd
MA A5	Sippewissett, MA	A	highly sensitive	nd	nd
MA B1	Sippewissett, MA	B	highly sensitive	nd	nd
MA B2	Sippewissett, MA	B	moderately sensitive	nd	nd
MA C1	Sippewissett, MA	C	highly sensitive	nd	C/S
MA C2	Sippewissett, MA	C	highly resistant	nd	C/C
MA C3	Sippewissett, MA	C	moderately sensitive	nd	nd
MA C4	Sippewissett, MA	C	highly resistant	nd	C/C
MA C5	Sippewissett, MA	C	highly sensitive	nd	C/C

CHAPTER THREE: TRANSCRIPTIONAL RESPONSE TO OXIDATIVE STRESS IN *NEMATOSTELLA VECTENSIS*

Introduction

Rationale for a transcriptomics approach

A principal goal of my dissertation research is to illuminate the genetic basis for the observed differences in peroxide tolerance both within and between populations of *N. vectensis*. The function of any gene consists of two major elements: spatiotemporal expression (when and where the gene is expressed) and interactional architecture (how the gene or gene product interacts with other molecules, including other genes) (Arthur, 1997). A shift in peroxide tolerance that has a genetic basis could therefore be explained by changes in the expression of one or more genes and/or changes in the sequence of one or more genes that impact interactional architecture. Both kinds of changes could be identified by sequencing the transcriptomes of peroxide-exposed animals that differ in their peroxide tolerance. It is important to note that knowledge of the transcriptome cannot directly reveal changes in the sequence of cis-regulatory elements that may be responsible for differences in gene expression.

The recent advent of high-throughput DNA sequencing makes it possible to compare entire transcriptomes under control conditions and under various environmental perturbations (Costa et al., 2010; Ekblom and Galindo, 2011; Martin and Wang, 2011; Shendure and Ji, 2008). A transcriptomics approach can be used to catalogue all types of RNA transcripts and quantify expression levels under various conditions. Where the

genome sequence is known (as in *N. vectensis*), RNA sequencing can also reveal gene structure, the existence of alternative splice forms, and post-transcriptional modifications. RNA sequencing provides a more precise measure of transcript and isoform abundance than other methods (such as qPCR and microarray analysis), and it does not suffer from experimental limitations that confound microarray-based approaches, including high background noise and the inability to detect previously unknown genes (Ekblom and Galindo, 2011). RNA sequencing is also more efficient at detecting variation in gene expression and rare transcripts than microarrays (Head et al., 2014; Ramsköld et al., 2012; Wang et al., 2009c). It is also a good way to investigate species, like *N. vectensis*, that have incomplete genomes or that are sequenced with relatively low coverage where tiling arrays are not an option.

Elevated ROS have been shown to induce stereotyped changes in gene expression in bacterial cells, plant cells, and mammalian cells, including the up-regulation of genes that constitute the oxidative stress defense system (Chen et al., 2013; Enjalbert et al., 2003; Mostertz, 2004; Raj et al., 2014; Röhrdanz and Kahl, 1998; Vandenabeele et al., 2003). However, no previous study has examined the transcriptional response to elevated ROS in a cnidarian, and no previous study has compared the transcriptomic response of individuals from the same species that are known to differ in their phenotypic response to ROS. When anemones are challenged with survivable levels of ROS, genes that are differentially expressed in treated vs. control anemones will be candidates for involvement in oxidative stress response. Presumably, the genes that are involved in the oxidative response of *N. vectensis* will overlap extensively with those known to mitigate

oxidative stress in other species, which include genes involved in apoptosis regulation, response to endogenous stimuli, and stress response genes (Martin and Wang, 2011; Wolenski et al., 2011b). Genes that are differentially expressed under conditions of peroxide exposure in resistant versus sensitive individuals may provide clues as to the genetic basis of peroxide resistance. As no similar study has been conducted, it is difficult to anticipate the possible genetic basis for differences in peroxide tolerance.

Hypotheses on the origin of differential peroxide sensitivity

The *Nematostella* populations examined may have varying strategies to deal with peroxide exposure. My initial phenotypic assays demonstrated that genets collected from South Carolina, New Jersey and Nova Scotia differ significantly in peroxide tolerance. One explanation for this difference is that some populations (e.g., South Carolina) have been subjected to higher levels of peroxide than other populations (e.g., Nova Scotia), and have therefore been selected for greater peroxide tolerance. However, my subsequent studies showed that differences in peroxide tolerance of a similar magnitude could be found within a population. The Sippewisset clone lines are derived from wild-caught individuals that lived within a hundred meters of each other, some collected from the same small pool where they presumably would have encountered similar levels of peroxide exposure. Thus, peroxide tolerance varies within and between populations.

At a genetic level, how do we explain the presence of resistant and sensitive individuals in multiple, genetically isolated populations? There are three possible explanations, though they are not necessarily mutually exclusive: (1) inheritance of

ancestral genetic variation; (2) independent but parallel evolution of resistance and/or sensitivity; (3) independent and divergent evolution of resistance and/or sensitivity. In the first scenario, an ancestral population harbored variation in peroxide tolerance. The variability in this source population was represented in the founders of new populations and has since been retained in these descendant populations. The genetic basis of peroxide tolerance would then be shared across populations by virtue of being inherited from an ancestral population. In the second scenario, peroxide resistance and/or susceptibility evolved independently in multiple populations, but the genetic basis was the same. In other words, different populations converged on the same genetic solution to the same environmental stressor (e.g., evolution of the same residue at the same amino acid position in an anti-oxidant protein). In the third scenario, peroxide resistance and/or susceptibility evolved independently in multiple populations, but the genetic basis was different. In other words, different populations invented different genetic solutions to the same environmental stressor (e.g., one population evolved a modified anti-oxidant protein, while another population altered the expression of an entirely different anti-oxidant protein.).

To investigate how the *N. vectensis* transcriptome responds to peroxide exposure and how this may differ in peroxide sensitive vs. peroxide resistant clone lines, I performed deep mRNA sequencing on whole anemones exposed to peroxide or control conditions. I hypothesized that (1) peroxide exposure would induce stereotypic and highly conserved gene expression changes in *Nematostella*, but (2) that the gene expression profile of a peroxide resistant individual would differ from that of a sensitive

individual. This study examined both the commonalities in the conserved oxidative stress responses and possible variations in the stress response mechanisms among the various populations from South Carolina, New Jersey, and Nova Scotia and Sippewissett, MA. By comparing the gene expression profiles of individuals exposed to a survivable peroxide challenge with control animals I was able to identify a conserved transcriptional response to oxidative stress shared between multiple *N. vectensis* genets and other model systems. Additionally, I was able to show that resistant individuals differ from sensitive individuals in the expression of genes known to be involved in oxidative stress responses of other organisms. However, there were differences among resistant genets and among sensitive genets, which suggest that differences in peroxide tolerance have evolved independently in multiple populations.

Methods

Peroxide treatments

Individuals from each of seven different clone lines (Baruch, SC [n=1]; Meadowlands, NJ [n=1]; Kingsport, Nova Scotia [n=1]; Sippewissett, MA [n=4]) were exposed to 0.0005% hydrogen peroxide or control conditions for 24 h. Clones were selected to exhibit a range of peroxide tolerance (Table 2.1; percentage surviving 14 day exposure to 0.0005% H_2O_2), as determined in chapter 2.

RNA isolation, library preparation and sequencing

Total RNA was isolated from whole anemones in each of the treatments using a modified TRIzol protocol (Stefanik et al., 2013b). RNA samples were used to produce 47 separate cDNA libraries using the Illumina mRNA TruSeq v2 kit. This included 3 to 4 replicates for each of the seven populations (NJ, SC, NS and 4 from MA Pool C) and each of the two conditions (peroxide exposure vs. control) (Table 3.1). Different bar-coded primers were used for each library, and multiple libraries were pooled for sequencing on individual flow cells of an Illumina High Seq instrument (Harvard University, Boston MA). Prior to sequencing, the quality of cDNA libraries was assessed using a BioAnalyzer High Sensitivity microfluidic DNA chip (Agilent) to evaluate the cDNA size distribution. I also used qPCR (Kappa Biosystems' NGS library qPCR kit) to determine the concentration of cDNA fragments with adapter sequences.

Differential expression analysis

All sequencing reads were mapped to a recently-generated reference transcriptome for *N. vectensis* (Lubinski et al., 2014a) using Bowtie 2.0 (Langmead and Salzberg, 2012). RSEM (Li and Dewey, 2011) was then used to quantify the expression of particular transcripts based on the read alignments generated by Bowtie. RSEM was chosen because it accounts for variance in expression counts due to uncertainty in mapping reads to alternate genes or alternate isoforms. This method has been used recently to determine transcriptional profiles and characterize differential expression in a number of non-model organisms (Cooper et al., 2014; Cornet et al., 2014; Janbon et al.,

2014; Lee et al., 2014; Yeaman et al., 2014). The gene-isoform relationship data produced by RSEM was then analyzed in EBSeq to identify differentially expressed genes between two or more biological conditions in a dataset (Li and Dewey, 2011; Robinson et al., 2010). A false discovery rate of 1% was used to identify significant differences in expression between peroxide exposed (n=3-4) and control (n=3-4) conditions for each clone line. I also tested for significant differences between peroxide resistant vs. peroxide sensitive individuals in their transcriptional profiles under conditions of peroxide exposure. Once the differentially expressed genes were identified, we could search the genes by Gene Ontology (GO) because all of the transcripts in the reference transcriptome had previously been assigned provisional ontology based on BLAST vs. the non-redundant database at NCBI and extraction of ontology terms using Blast2Go (Lubinski et al., 2014a). The differential expression and gene ontology analyses were performed by Tristan Lubinski.

Results

Sequencing yield

In this study, I sequenced the transcriptomes of seven clone lines under control conditions and 24 h exposure to survivable levels of peroxide. I sequenced 3-4 replicates for each of the seven populations (NJ, SC, NS and 4 from MA Pool C) and each of the two conditions (peroxide and control), which amounted to 47 libraries. The total sequencing yield was 389,738,649 filtered reads, or approximately 8.3 million reads per library (ranging from 2.4-16.5 million reads per library; Table 3.1). On average, 93% of

the reads (86-95% per library) align to the updated *Nematostella* transcriptome (Lubinski et al., 2014a), while the reads generated from each library aligned to ~55% of the nucleotide positions in the reference transcriptome (Table 3.1). As in other studies [e.g.,(Jiménez-Gómez, 2011)], we noted a correlation between the number of sequencing reads obtained for each library and the resulting coverage of the reference transcriptome (Figure 3.1).

Table 3.1 Sequencing yield.

Clone line	Treatment	Reads (millions)	% map to NvT1	% coverage of NvT1
South Carolina	H_2O_2	4.91	86.6	24.8
		3.06	86.2	22.5
		8.45	94.4	63.1
		12.66	94.2	74.0
	Control	7.41	94.3	54.7
		8.54	94.1	57.7
		2.41	93.9	35.2
		4.61	92.6	36.6
Nova Scotia	H_2O_2	12.38	93.0	66.0
		6.32	94.7	56.1
		5.88	94.9	53.3
	Control	8.30	92.4	56.5
		6.23	95.0	50.3
		3.26	94.9	38.4
		8.78	95.1	59.3
New Jersey	H_2O_2	4.48	87.6	26.1
		4.03	87.8	24.1
		9.65	94.6	68.0
		9.51	94.7	67.4
	Control	7.41	94.7	54.7
		6.90	94.4	53.3
		9.85	94.6	66.6
		4.23	94.4	47.8
Massachusetts C1	H_2O_2	8.45	90.7	55.7
		7.60	93.5	55.2
		8.71	95.0	59.7
	Control	8.47	94.2	55.3
		7.79	92.0	57.4
		6.14	92.1	47.9
Massachusetts C2	H_2O_2	12.55	91.9	65.7
		16.53	92.0	68.8
		9.20	92.1	60.8
	Control	7.38	92.6	53.1
		8.40	92.7	55.2
		7.48	92.9	53.3
Massachusetts C4	H_2O_2	7.59	95.2	56.3
		6.02	94.5	50.3
		7.30	95.2	56.2
	Control	13.18	91.3	69.4
		7.00	94.9	54.8
		9.38	94.1	58.5
Massachusetts C5	H_2O_2	13.06	91.5	65.9
		13.50	92.0	65.6
		8.15	92.7	57.6
	Control	12.95	91.7	67.9
		7.10	93.7	54.2
		16.55	91.6	72.7

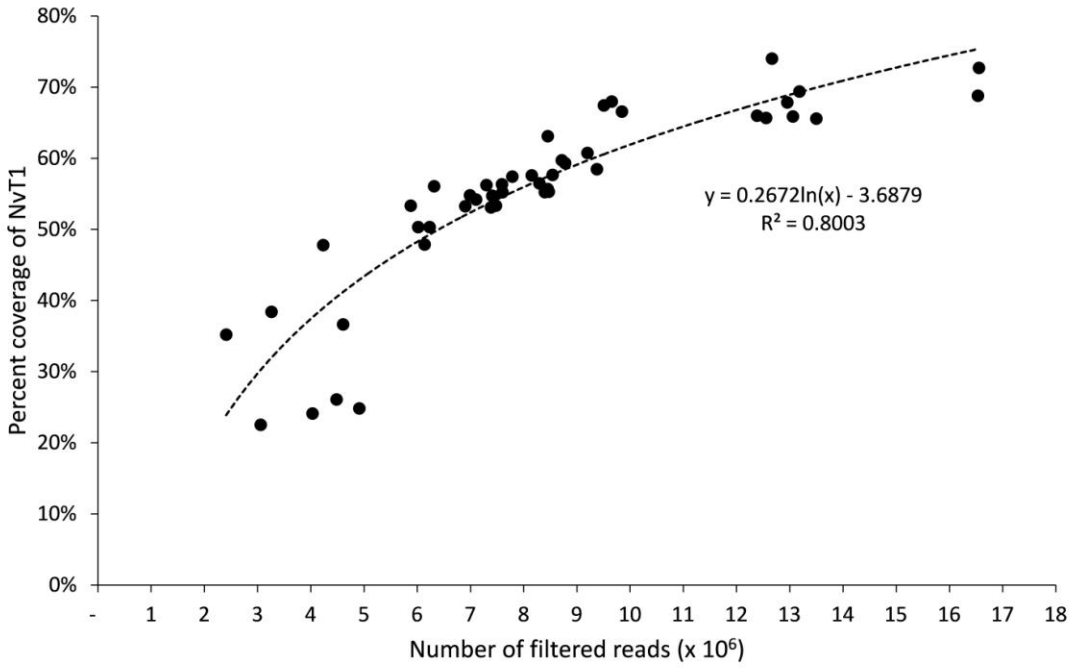


Figure 3.1. Number of filtered reads (in millions) vs. percent coverage of the reference transcriptome for each of seven clone lines exposed to H_2O_2 (n=3-4) or control (n=3-4) conditions.

All of transcriptomic data generated in this study will be added to StellaBase—The *Nematostella vectensis* Genomics Database (accessible at stellabase.org), which was established in 2006 to facilitate the use of *Nematostella* as a model organism and disseminate genomic and transcriptomic data (Reitzel et al., 2009; Sullivan et al., 2008; Sullivan et al., 2006). The database has been upgraded to include transcriptomic data from multiple populations and multiple environmental treatments (e.g., peroxide exposure) (Lubinski et al., 2014a; Lubinski et al., 2014b). The sequencing conducted for this study has enabled the production of seven new clone-line specific reference assemblies. These assemblies as well as the sequencing data generated from each individual library are being made publicly available and searchable via gene ontology terms and BLAST at the upgraded StellaBase. A test version of the upgraded database is currently accessible at http://cnidarians.bu.edu/stellabase.

Differential expression analysis: variation among individual clone lines

For all seven clone lines, we compared the mRNA expression profiles of peroxide exposed vs. control anemones. For each of the 14 conditions (seven clones lines times two treatments), the mean number of filtered reads obtained over 3-4 replicates ranged from 5.7 million to 12.8 million reads, and for all but one clone line (MA C2), we obtained an overlapping number of reads from peroxide and control trials (Figure 3.2).

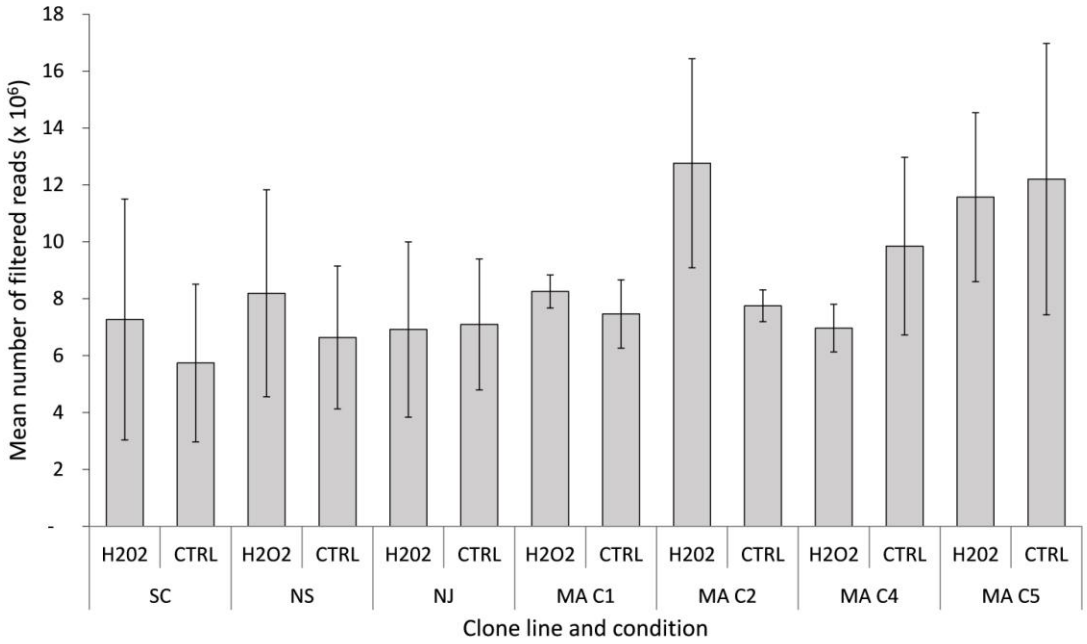


Figure 3.2. Number of filtered reads (in millions) for peroxide and control conditions for each of seven clone lines. The average number of filtered reads was taken for H_2O_2 (n=3-4) and control (n=3-4) conditions.

Variation in the number of differentially expressed transcripts

In each clone line, thousands of genes were differentially expressed in response to peroxide exposure (Figure 3.3; produced with assistance from Tristan Lubinski) however, the number of differentially expressed contigs varied substantially between clone lines, from 2,500 in MA C1 to 14,100 in MA C2 (Table 3.2). As greater sequencing yield could allow us to identify a greater number of differentially expressed contigs (particularly for rare transcripts), we plotted the total number of reads per clone line versus the number of differentially expressed contigs that were identified (Figure 3.4). This graph indicates a significant positive correlation ($p = 0.0301$) between the number of differentially expressed contigs identified and the number of sequencing reads, but there appears to be a substantial amount of variation that cannot be explained by differences in sequencing yield alone.

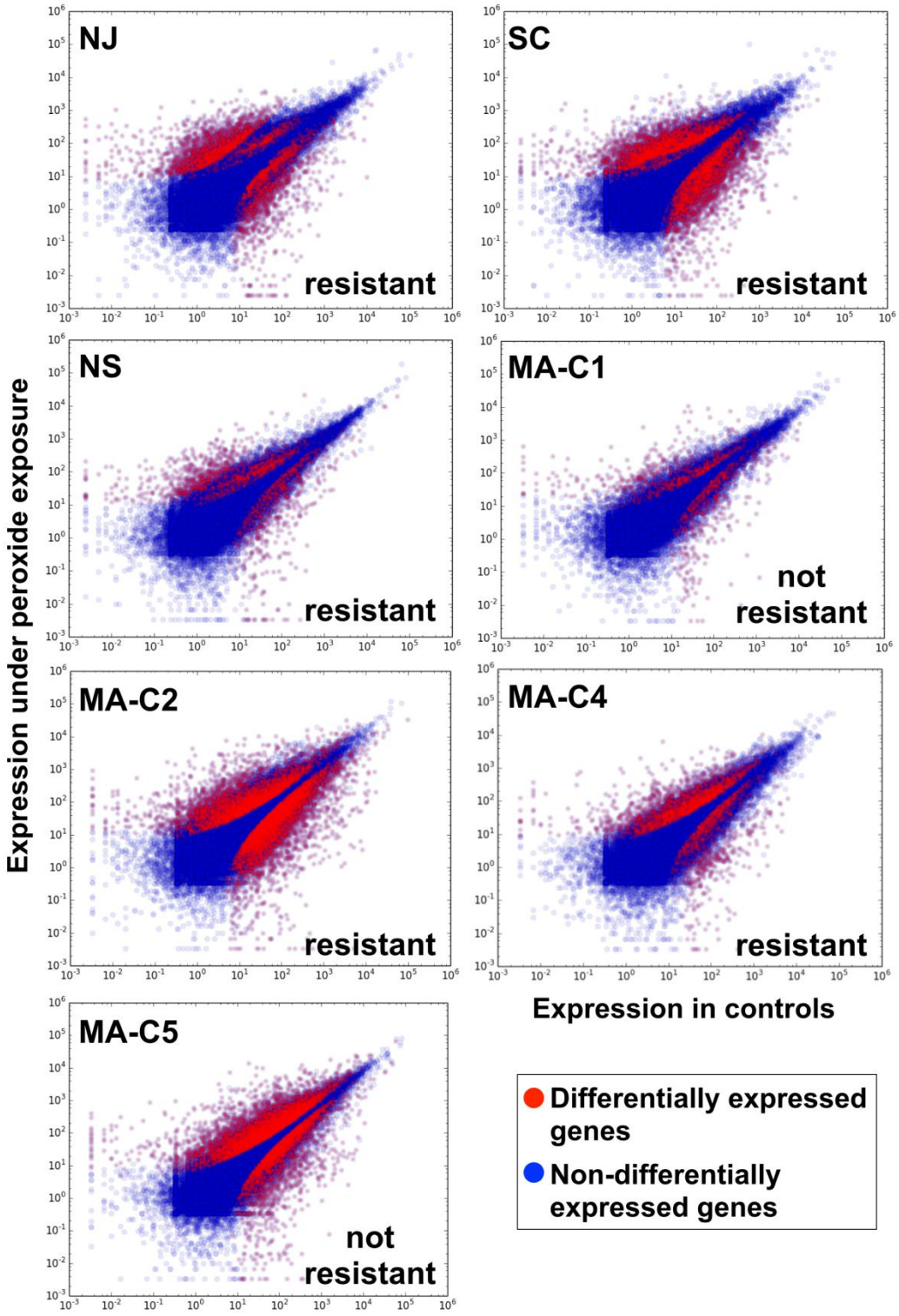


Figure 3.3 Contig expression for control vs. peroxide exposure for each of 7 clone lines.

Table 3.2 Number of contigs exhibiting increased or decreased expression in each clone line.

Clone line	Filtered Reads (in millions)	Contigs exhibiting increased expression	Contigs exhibiting decreased expression
SC	52.05	4,563	3,887
NS	51.14	2,290	1,188
NJ	56.06	3,769	2,311
MA C1	47.16	1,558	914
MA C2	61.54	6,607	7,493
MA C4	50.45	3,054	2,228
MA C5	71.32	6,672	4,155

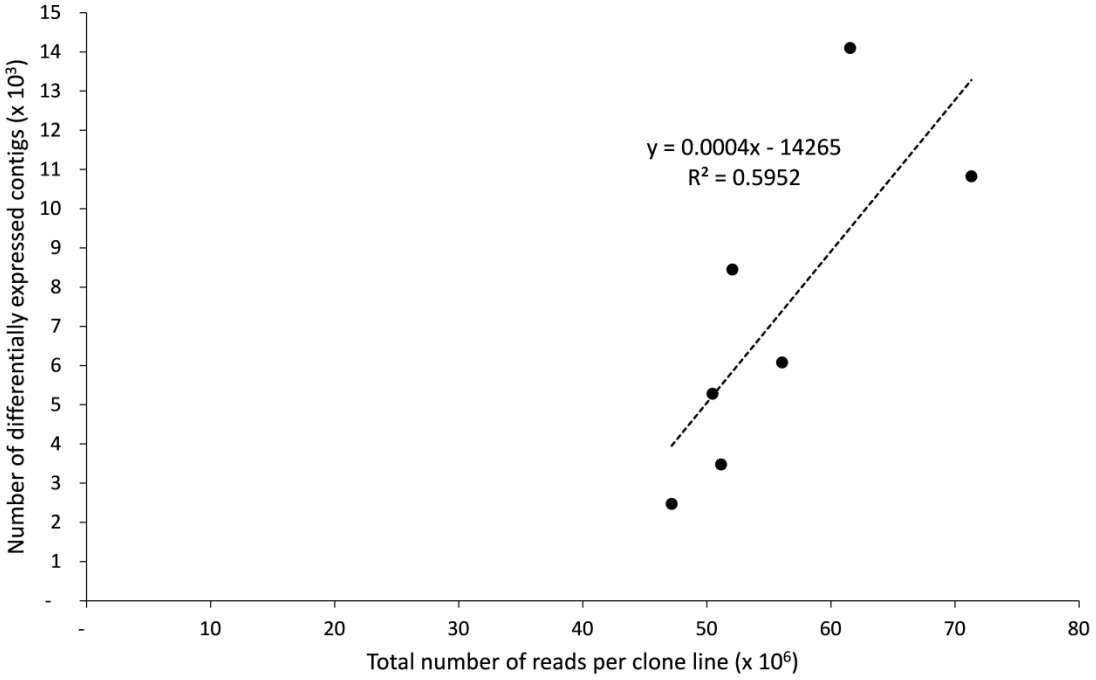


Figure 3.4. Total number of filtered reads per clone line (in millions) vs. number of differentially expressed contigs (in thousands) for each of seven sequenced clone lines.

Widely shared aspects of the transcriptional response to peroxide

Despite differences among clone lines in the number of differentially expressed genes, several aspects of the transcriptional response to peroxide exposure are widely shared between genets. A list of transcripts was compiled with significantly increased or decreased expression in all seven clone lines (Table 3.3). The majority of transcripts had no provisional ontology assigned. In all sequenced clone lines, there were five named transcripts with significantly increased expression including a cell surface protein, a tysosine kinase receptor, two von Willebrand factor D and EGF domain-containing proteins, and a tyrosinase and monooxygenase related protein. The transcripts with significantly decreased expression included a sequestome 1, gamma-glutamyltranspeptidase, cytochrome c, Arf-GAP with coiled-ankyrin repeat and PH domain-containing protein 2 (Table 3.3).

Table 3.3. Transcripts with significantly increased or decreased expression in response to peroxide exposure in all 7 clone lines (** $p < 0.01$; * $p < 0.05$)

Contig ID	Contig Name	Expression Change
Nv.T1.20400.1	no annotation available	up **
Nv.T1.80280.1	no annotation available	up **
Nv.T1.63571.1946	no annotation available	up **
Nv.T1.50161.1	no annotation available	up **
Nv.T1.63571.5071	no annotation available	up **
Nv.T1.63571.1951	no annotation available	up **
Nv.T1.63571.2341	no annotation available	up **
Nv.T1.63571.1958	no annotation available	up **
Nv.T1.63571.5052	no annotation available	up **
Nv.T1.63571.5051	no annotation available	up **
Nv.T1.45201.1	no annotation available	up **
Nv.T1.48842.1	no annotation available	up*
Nv.T1.8595.3	cell surface protein	up*
Nv.T1.27264.1	tyrosine kinase receptor	up*
Nv.T1.46148.1	No annotation available	up*
Nv.T1.75457.1	von Willebrand factor D and EGF domain-containing	up*
Nv.T1.38369.1	ame: full=tyrosinase ame: full= monophenol monooxygenase	up*
Nv.T1.42013.1	protein	up*
Nv.T1.26243.1	von Willebrand factor D and EGF domain-containing protein	up*
Nv.T1.63571.5157	no annotation available	up*
Nv.T1.55798.1	no annotation available	down **
Nv.T1.55797.1	no annotation available	down **
Nv.T1.883.1	no annotation available	down **
Nv.T1.20503.1	no annotation available	down **
Nv.T1.8106.2	sequestosome 1	down **
Nv.T1.65316.2	no annotation available	down **
Nv.T1.63571.5070	no annotation available	down **
Nv.T1.56385.1	no annotation available	down **
Nv.T1.69486.1	no annotation available	down **
Nv.T1.56386.1	no annotation available	down **
Nv.T1.30370.1	gamma-glutamyltranspeptidase	down **

Nv.T1.22734.1	no annotation available	down **
Nv.T1.29563.1	ame: full=cytochrome c	down **
Nv.T1.49554.1	predicted protein	down **
Nv.T1.33376.1	no annotation available	down **
Nv.T1.15771.2	no annotation available	down *
Nv.T1.49402.1	no annotation available	down *
Nv.T1.20190.1	no annotation available	down *
Nv.T1.38703.1	ARF-gap with coiled- ankyrin repeat and PH domain-containing protein 2-like	down *
Nv.T1.54415.1	protein	down *
Nv.T1.4991.1	protein	down *
Nv.T1.21522.1	no annotation available	down *

An analysis of gene ontology revealed that the same functional categories of genes are impacted by peroxide exposure in different genets (Figures 3.5, 3.6, 3.7). For example, if we rank the GO categories under "Biological Process" based on the number of genes in each category whose expression is significantly altered by peroxide exposure, the categories are ranked in almost the same relative order in all seven clone lines (Figure 3.5). The category with the greatest number of genes impacted by peroxide exposure in all clone lines is "oxidation reduction process." Other categories with high numbers of impacted contigs include processes related to translation and post-translational processing of proteins (GO categories: translation, protein phosphorylation, translational elongation, translational initiation, protein folding), membrane transport (GO categories: transmembrane transport, signal transduction, transport) and energy use (GTP and ATP catabolic processes). Similar analyses were performed for GO categories "Molecular Function" and "Cellular Component" (Figure 3.6 and Figure 3.7). Cellular Component GO categories with many differentially expressed transcripts included those related to membranes and the mitochondria. The Molecular Function categories with the most differentially expressed transcripts included protein binding, nucleic acid binding, ATP binding, and ion binding (including calcium, iron, zinc, and metal ions).

The GO analysis is useful for identifying broad functional categories of genes, but individual genes within a given functional category may respond differently in different clone lines, and the genes that respond to peroxide exposure may belong to multiple functional categories. Therefore, the broad similarity between clone lines that is apparent from the GO analysis may obscure key differences among clone lines in the expression of

particular genes. For this reason, I compared the expression changes that resulted from peroxide exposure in candidate genes of individual clone lines.

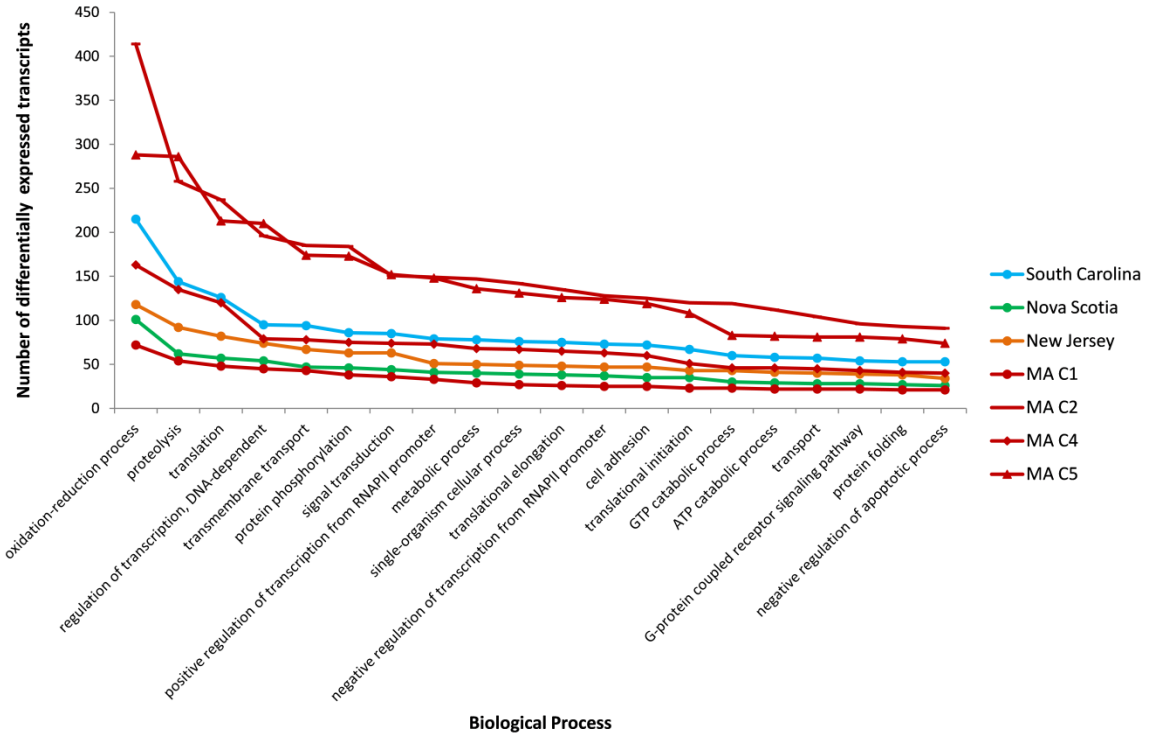


Figure 3.5 Top 20 classes of gene ontology terms in the biological process category that are associated with genes identified as differentially expressed.

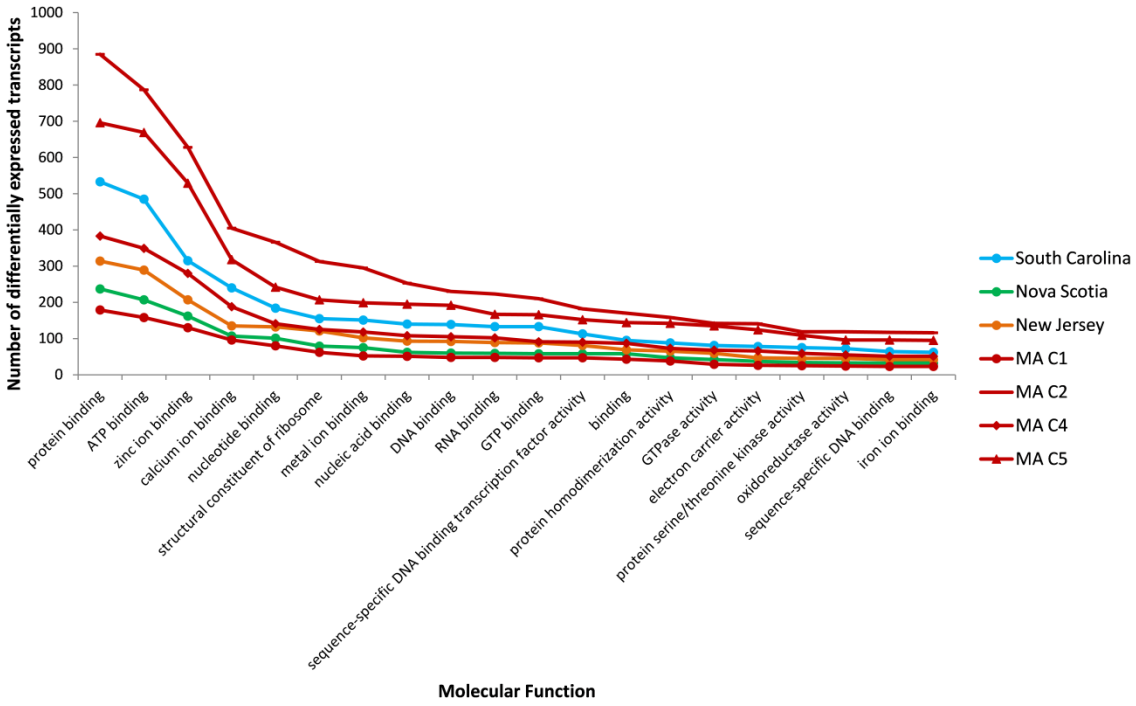


Figure 3.6. Top 20 classes of gene ontology terms in the molecular function category that are associated with genes identified as differentially expressed.

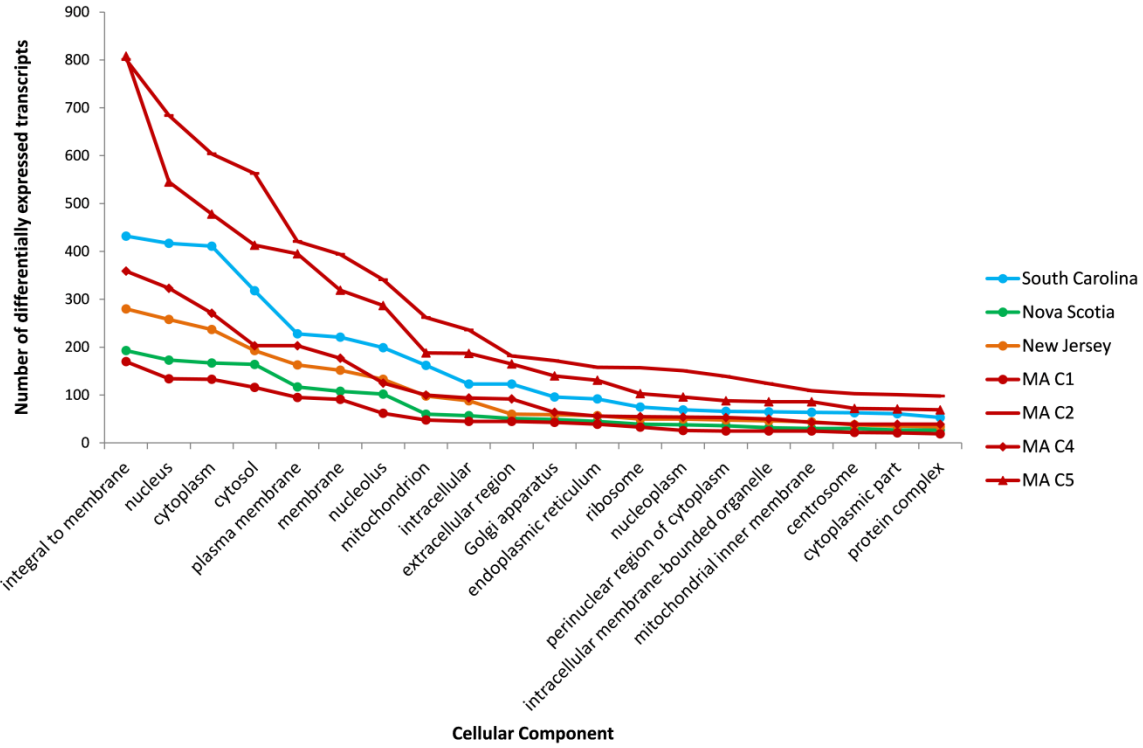


Figure 3.7. Top 20 classes of gene ontology terms in the cellular component category that are associated with genes identified as differentially expressed.

Transcriptional response of known oxidative stress response genes

A wide range of genes are known to exhibit altered expression under conditions of oxidative stress, including superoxide dismutases, heat shock proteins, catalases, peroxidases, glutaredoxins, oxidoreductases, disulfide isomerases, glutathionine cycling enzymes, ferritins, cytochromes, and oxidases. (Acharya et al., 2010; Blouin et al., 2013; Bubici et al., 2006; Carillon et al., 2013; Chen et al., 2013; Dash and Phillips, 2012; Downs et al., 2002; Edge et al., 2005; Enjalbert et al., 2003; Han et al., 2008; Miramon et al., 2014; Plantivaux et al., 2004; Raj et al., 2014; Richier et al., 2003; Richier et al., 2008; Saragosti et al., 2010; Schwarz et al., 2008; Shinzato et al., 2012; Sies, 2014). Using these gene names as search terms, I identified all differentially expressed transcripts in each clone line that appear homologous to these known oxidative stress response genes based on BLAST results (Table 3.4). It is important to note that different transcripts listed under each gene family may correspond to the same gene, because the reference transcriptome used in this study preserves allelic variation as well as splice variants.

For most clone lines, I was able to identify multiple differentially expressed transcripts for each gene family, and a majority of the transcripts exhibited increased expression under conditions of peroxide exposure. For example, in each of the seven clone lines, from 18 to 62 cytochrome transcripts were differentially expressed, and in all but two clone lines (MA C4 and MA C5), more of these cytochromes exhibited increased expression when the anemones were exposed to peroxide. Two gene families were exceptions to this general pattern: catalase and glutaredoxin. In all but two clone lines

(again, MA C4 and MA C5), there were no catalase transcripts and no glutaredoxin transcripts that exhibited differential expression. Ferritin transcripts showed variation among clone lines. A majority of identified ferritin transcripts exhibited increased expression in 4 clone lines (NJ, MA C1, MA C2, MA C5), while the majority exhibited decreased expression in the other clone lines (SC, NS, MA C5).

Table 3.4 Candidate differentially expressed transcripts for each clone line

	New Jersey		South Carolina		Nova Scotia		MA C2		MA C4		MA C5		MA C1	
Transcript Category	Transcripts Up Regulated	Transcripts Down Regulated	Transcripts Up Regulated	Transcripts Down Regulated	Transcripts Up Regulated	Transcripts Down Regulated	Transcripts Up Regulated	Transcripts Down Regulated	Transcripts Up Regulated	Transcripts Down Regulated	Transcripts Up Regulated	Transcripts Down Regulated	Transcripts Up Regulated	Transcripts Down Regulated
superoxide dismutase	3	0	3	0	4	0	4	2	2	0	4	0	0	0
heat shock protein	6	4	6	10	10	1	21	8	9	3	23	5	8	0
catalase	0	0	0	0	0	0	0	0	0	1	1	2	0	2
peroxidase	7	5	5	3	5	1	9	6	4	4	5	3	3	2
glutaredoxin	0	0	0	0	0	0	0	0	0	1	1	1	0	0
oxidoreductase	2	0	6	4	3	0	9	2	3	1	5	6	1	0
disulfide-isomerase	3	1	3	5	1	1	11	10	1	5	2	4	2	0
glutathione cycling	6	5	9	4	4	1	10	13	3	4	6	3	2	1
ferritin	7	2	9	14	2	4	23	10	7	9	10	7	6	2
cytochrome	18	4	37	8	16	0	39	23	6	12	14	16	12	1
oxidases	13	5	31	9	12	5	31	15	8	13	15	23	2	1

Expression of superoxide dismutases (SOD)

I performed a more detailed gene-by-gene analysis of the superoxide dismutases (SODs), which are known to constitute an important component of the oxidative stress response in a wide range of taxa. The *N. vectensis* genome had previously been shown to contain five distinct superoxide dismutase genes: CuZnSOD1, CuZnSOD2, CuZnSOD3, MnSOD1, and MnSOD2 (Tarrant et al., 2014). In addition, *N. vectensis* possesses a single CCS gene (copper chaperone of superoxide dismutase), which shares the SOD domain but lacks the dismutase activity of the other SOD proteins. The effect of peroxide exposure on the expression of these SOD and CCS genes can differ dramatically between clone lines. For example, peroxide exposure results in a pronounced and significant increase in CuZnSOD1 in the NS, NJ, and SC clone lines (Table 3.5). By contrast, CuZnSOD1 transcripts were not recovered in the peroxide or control data sets of the Sippewissett MA clone lines. CuZnSOD2 expression was elevated in all of the peroxide-resistant clone lines (NJ, SC, NS, MA C2 and MA C4) and depressed in the two sensitive clone lines (MA C1 and MA C5), although not all of these changes were statistically significant. CuZnSOD3 exhibited higher expression in response to peroxide exposure in all clone lines, and significantly higher expression in the majority of clone lines. The effect of peroxide on the expression of MnSOD1 was variable across clone lines, including a relatively slight but significant increase in the Nova Scotia clone and a slight but significant decrease in the MA C2 clone line. The expression of MnSOD2 was dramatically and significantly increased in response to peroxide in three clone lines (NJ, NS and MA C2), but it was depressed in other clone lines. Finally, peroxide exposure

resulted in a significant increase in the expression of copper chaperone (CCS) in the NJ clone line, but there were no significant changes in any other clone line.

Table 3.5 Log-2 fold change in expression of SOD genes under peroxide relative to control conditions. **Green text** = genes exhibiting increased expression in response to peroxide. **Red text** = genes exhibiting decreased expression in response to peroxide; **nd** = not detected; ****** = p<0.01; ***** = p<0.05.

	Moderately	**Resistant**	**Highly Resistant**			**Highly Sensitive**	
	NJ	**NS**	**SC**	**MA C2**	**MA C4**	**MA C1**	**MA C5**
CuZnSOD1	**7.91****	**11.06****	**6.31****	nd	nd	nd	nd
CuZnSOD2	**9.56**	**11.24****	**9.60**	**12.21****	**9.97**	**-1.25**	**-0.11**
CuZnSOD3	**0.74**	**1.19**	**1.29****	**2.77***	**3.81****	**3.62****	**1.18**
MnSOD1	**1.60**	**1.30****	**0.41**	**-1.12****	**-1.58**	**-4.02***	**5.27***
MnSOD2	**13.63****	**3.39***	**13.59****	**11.12****	**-0.70**	**-8.92***	**0.66**
CCS	**5.40****	nd	**-0.06**	**0.002**	**-7.00**	**0.63**	**5.46**

Discussion

Overview—the broader significance of this study

Oxidative stress is a challenge to all organisms, and it has been linked to more than a dozen major human diseases including cancer, heart disease, and degenerative neurological conditions. As a result, there is a tremendous amount of ongoing research into the molecular basis of oxidative stress response mechanisms. Whereas past research in this area tended to pursue a candidate gene approach, transcriptomic approaches are being applied to study the oxidative stress response at a systems level. Therefore, the study described in this chapter is part of a growing research trend. However, the study described here is unique in that we investigated intraspecific variation in oxidative stress responses. Given the connection between oxidative stress and other stressors that are posing an increasing threat to coastal ecosystems (e.g., coastal pollution, thermal stress, UV stress), variation in oxidative stress responses (both interspecific and intraspecific) may partially determine the fate of populations and the composition of communities in coastal marine habitats. In the discussion that follows, I summarize my results regarding known oxidative stress genes and compare my findings to other studies of gene expression in response to oxidative stress with a focus on marine invertebrates.

Increased expression of known oxidative stress response genes

In examining the transcriptomic data generated in this study, we observed that several known oxidative stress response genes exhibited elevated expression in response to peroxide, particularly SODs and ferritins. Superoxide dismutases (SODs) are

antioxidant enzymes that constitute an important component of the oxidative stress response in a wide range of taxa (Carillon et al., 2013). SODs eliminate superoxide anion radicals derived from either extracellular stimulants or produced in the mitochondrial matrix (Fridovich, 1995; Miao and St Clair, 2009). The SOD family enzymes have similar cellular functions but are regulated through differences in protein structures, chromosome locations, metal cofactors, gene distribution, and cellular compartmentalization (Miao and St Clair, 2009). Expression levels of SODs are regulated by numerous transcription factors, including NF-κB, AP-1, AP-2, Sp1, and C/EBP (Miao and St Clair, 2009).

Free iron is absorbed from the environment and plays a role in mitochondrial respiratory complexes and a variety of other iron-containing molecules, including enzymes needed for cell proliferation (Cammack et al., 1990; Eaton and Qian, 2002). However, iron is also toxic to cells and it acts as a catalyst in the formation of free radicals from ROS. Cells use protective mechanisms to bind iron in various tissue compartments and keep it from causing cellular damage (Kurz et al., 2011; Orino et al., 2001).

Ferritin is a globular protein complex that stores and transports iron in a non-toxic form regulating iron deficiency vs. overload in cell. It sequesters transition metals to reduce the possibility of these metals catalyzing formation for free radicals (Theil, 1987). It has been shown that upregulation of the ferritin is a rapid adaptive mechanism that counteracts lysosomal membrane permeabilization and apoptosis during oxidative stress (Kurz et al., 2011; Orino et al., 2001; Richier et al., 2008; Theil, 1987). By scavenging

iron, ferritin protects cells from oxidative damage by preventing iron-mediated generation of hydroxyl radicals. Ferritin may also interact with MnSOD to help prevent generation of superoxide radicals and promote the breakdown of hydrogen peroxide (Holley et al., 2011).

In human fibroblasts, ferritin has been shown to be up-regulated after stimulation by UVA radiation or H_2O_2 (Allen and Tresini, 2000). Ferritin was shown to be upregulated in the symbiotic cnidarian *Anthopleura elegantissima* subjected to increased temperatures (Richier et al., 2008). This upregulation has been shown in response to in oxidative conditions in the common periwinkle *Littorina littorea* with ferritin heavy chain transcript levels elevated twofold during anoxia exposure (Larade, 2004). Ferritin also has been shown to be highly regulated in the corals *A. palmata* and *A. millepora*, where is hypothesized to be undergoing adaptive evolution (Schwarz et al., 2008).

Altered expression of oxidative stress response genes in other animal models

The effects of oxidative stress on gene expression have been studied in a handful of animal models, mostly using a candidate gene approach. In zebrafish, oxidative stress caused by exposure to arsenic resulted in increased expression of both MnSOD and CuZnSOD, in addition to a number of other genes including glutathione peroxidase, catalase, and oxidase enzymes (Sarkar et al., 2014). In the intertidal polychaete *Heteromastus filiformis*, while both hypoxia and hyperoxia caused increases in catalase activity, neither had a pronounced effect on SOD activity (Abele et al., 1998b). In the freshwater hydra, *H. vulgaris*, a cnidarian distantly related to *Nematostella*, exposure to

0.03% H_2O_2 for 1 h significantly increased the expression of both an intracellular CuZnSOD and an extracellular CuZnSOD as judged by RT-PCR (Dash et al., 2007). The expression of both CuZnSODs was also increased by exposure to arsenic and zinc (Dash et al., 2007). In the sea anemone *Anemonia viridis,* exposure to elevated pCO_2, which is thought to reduce ROS production, led to a reduction in SOD activity (Borell et al., 2014).

One previous study has examined stress-induced expression of oxidative stress response genes in *N. vectensis* itself. Tarrant and co-workers exposed *N. vectensis* to a combination of 96 h of UV-light and varying concentrations of benzo[a]pyrene (B[a]P) and examined the expression of CuZnSOD2, CuZnSOD3, CCS, MnSOD1, MnSOD2, catalase, and HSP70 (Tarrant et al., 2014). The expression of four genes was found to be increased under UV exposure alone or by UV exposure in combination with one or more concentrations of B[a]P (CuZnSOD3, CCS, MnSOD1, catalase, and HSP70).

Relative to these previous studies, the study described here is an important advance for two reasons. First, in the prior studies on whole animals (as opposed to plants, yeast, or vertebrate cell cultures), the expression of only a few predetermined genes could be examined, whereas our study investigated the entire transcriptome. Using multiple oxidative stress biomarkers is important in studies to gain insight about the complete system (Costantini, 2008). Second, the prior studies did not characterize intraspecific variation, which our study suggests is substantial and important in determining the resilience of natural populations in the face of many factors that promote oxidative stress.

However, the study I performed did not address other sources of complexity, such as variations in the stress response caused by different levels of peroxide, different periods of exposure, and different combinations of other naturally co-occurring stressors. For example, the Tarrant *et al*. study (Tarrant et al., 2014) observed variation in stress response between different doses of B[a]P, between different periods of exposure, and between single versus combinatorial stressors.

Additionally, it will be important to incorporate field studies and different life-history stages to address realistic conditions of exposure to multiple environmental factors that can affect stress response. Intertidal pools experience lots of photosynthesis during the day and experience oxygen saturation, but these same pools can become suboxic or anoxic at night (Abele et al., 1998b). Additionally many of the enzymes identified in the stress response pathways may be especially important in embryos with complex signaling pathways related to development. Known inducers of oxidative stress include reproduction, immune responses to increased oxygen uptake or consumption and physical activity. For example, in albatross, a long-lived bird, breeding individuals (vs. non-breeding) have higher oxidative damage in plasma. Oxidative stress has been correlated with age, sex, and cost of reproduction. It would be important to extend these tolerance studies to embryos to determine the relation to developmental and structural pathways (Ambrosone and Tortiglione, 2013; Costantini, 2008; Costantini et al., 2014; Goldstone, 2008; Tarrant et al., 2014). *N. vectensis* has previously been suggested as an ecotoxicological model. Both field studies and additional exposure experiments will be

needed to look at the longer term effects of oxidative stress and other environmental contaminants.

CHAPTER FOUR: CONCLUSIONS AND FUTURE DIRECTIONS

Broader Significance of Major Findings

Intraspecific Variation in Stress Tolerance

Coastal habitats, such as the estuaries inhabited by *N. vectensis*, are under increasing stress from habitat alteration and loss, eutrophication, overfishing, freshwater diversion, contamination from industrial and agricultural processes, and the many effects of climate change including elevated temperatures changing precipitation patterns, extreme weather events, shifting sediment distribution, and changing salinity gradients resulting from changes to the geomorphology of estuaries, freshwater runoff, and patterns of water mixing (Fujii, 2012). An organism's ability to cope with environmental changes and specifically climate change are determined by its ability to deal with stress (Kassahn et al., 2009).

In this era of rapid environmental change, intraspecific variation in environmental stress responses will influence the fate of natural populations. Despite its importance, relatively few studies have demonstrated intraspecific variation in the stress tolerance of animals at either the organismal or transcriptomic levels. However, increasingly studies of coastal marine animals are focusing on naturally-occurring variation within a species. For example, a recent study comparing echinoderms found in rocky intertidal and subtidal habitats showed that these population assemblages correlated strongly with the abiotic factors present in their environments including inorganic pollution and salinity (Iken et al., 2010). In *N. vectensis*, three of the same genets used in this study (NJ, NS,

and SC) were found to vary in their growth and regeneration rates at higher temperatures, with only the South Carolina clone line exhibiting higher growth rates at 29° than at 21°C (Reitzel et al., 2013a). In reciprocal transplant experiments of the coral *Acropora hyacinthus*, genets from a relatively warm lagoonal pool proved more tolerant of higher temperatures, with higher chlorophyll retention rates than con-specific corals from moderate temperature pools (Palumbi et al., 2014)

Importance of Intraspecific Variation in Oxidative Stress Tolerance

A. Environmental sources of ROS

ROS from a variety of sources accumulate in marine habitats including reefs, intertidal pools, surface waters in the open-ocean, estuaries, and tidal marshes (Fan, 2008; Johnson et al., 2013). H_2O_2 has been shown to be relatively stable in sea water compared to other ROS (Lesser, 2006). Hydrothermal vents have also been shown to produce high levels of oxidative stress. In these environments, high levels of H_2S and O_2 are oxidized in the water which leads to the production of both oxygen and sulfur radicals (Lesser, 2006; Tapley et al., 1999). Accumulation of ROS has also been observed in coral reef habitats, where it directly contributes to coral bleaching (Downs et al., 2002; Lesser, 2006; Nii and Muscatine, 1997; Saragosti et al., 2010).

As a result of the fact that surface waters are more prone to processes that generate ROS, and many shallow water habitats are not subject to extensive mixing with deeper waters that are often lower in ROS, aquatic animals are more profoundly affected

by external sources of ROS and by extreme fluctuations in ROS if they live near surface waters or in tidal areas (Berghahn, 2001). For example, seasonal variation in H_2O_2 has been measured with higher concentrations found in the spring and summer in intertidal sandflats (Storch et al., 2001). Intertidal species have been shown to have a higher threshold for oxidative stress, which is likely related to suitability of these species for these variable environments. As mentioned in Chapter 1, ROS from abiotic and anthropogenic sources accumulate at particularly high levels in tidal marsh waters and sediments (Abele-Oeschger et al., 1997; Clark et al., 2008).

B. Possible indirect contributors to oxidative stress

Three environmental stressors in the marine environment that are currently on the rise can directly or indirectly contribute to oxidative stress. These stressors are elevated temperatures, UV radiation, and pCO_2 (Lesser, 2006).

1. Elevated temperatures.

The connection between thermal stress and oxidative stress has been studied in a number of marine model systems. Increased temperatures can destabilize cellular membranes, including lysosomal membranes. Lysosomal destabilization, and the consequent enzyme leakage, results in increased cellular catabolic activity, formation of ROS byproducts, and progressive cellular damages (Abele et al., 1998a; Winston et al., 1991). Temperature fluctuations also directly affect an organism's metabolic rates including mitochondrial function and energy production (Abele et al., 2002).

Mitochondrial oxygen consumption is directly correlated to production of cellular ROS as a metabolic byproduct. A study in the star coral *Montastraea annularis* showed that increased water temperature was correlated with increased levels of the two oxidative damage products tested (protein carbonyl and lipid peroxidation). It was shown that oxidative damage products increased as water temperature increased and preceded bleaching events (Downs et al., 2002). Corals and other marine are in peril due to ocean acidification and oxidative stress, so understanding cnidarians' response to these environmental effectors is increasingly important (Lewis et al., 2013; Shinzato et al., 2012; Shinzato et al., 2011; Wolenski et al., 2013).

2. UV radiation

Another environmental stress that is both on the rise and correlated with oxidative stress is UV-radiation. Climate change effects include increased CO_2 and other greenhouse gases and ozone depletion which both lead to increases in the amount of UV radiation reaching the earth's surface (Lister et al., 2010a). Increased UV stress can directly contribute to oxidative stress in marine species (Lister et al., 2010b; Shick and Dunlap, 2002), as well as photoproduction of oxidative stress in the chloroplasts of marine phytoplankton, algae, and coral symbionts (Marshall et al., 2005; Oda et al., 1997; Saragosti et al., 2010). UV radiation causes cellular damage when it is absorbed by DNA or proteins and by increasing the levels of ROS present in a cell (Lister et al., 2010a). Photosensitive molecules absorb UV energy, which leads to a more excited electron state. When the molecules return to their ground state, the added energy is transferred to

molecular oxygen molecules that produce ROS (Lister et al., 2010a). Additionally, UV-B can be absorbed and react with dissolved organic carbon to induce ROS formation (Lister et al., 2010b). For example, sea urchin embryos exposed to UV-B light showed elevated levels of DNA damage, higher concentrations of SOD and transcriptional activators (p53 and p21), and increased cellular apoptosis due to increased DNA damage (Lesser, 2003). In the Antarctic sea urchin *Sterechinus neumayeri* Meissner, UV-B exposure also lead to increased oxidative damage of lipids and proteins. The high UV exposure resulted in developmental abnormalities in embryos, which have a limited capacity to protect themselves from oxidative damage (Lister et al., 2010b). A similar study showed UV-B exposure lead to developmental abnormalities and oxidative damage to proteins in sea urchin embryos of the tropical sea urchin *Tripneustes gratilla* (Lister et al., 2010a). In the sea anemone *Anthopleura elegantissima*, production of the oxygen radicals OH^- and O_2 increased with exposure to sunlight enriched for UV wavelengths (Dykens et al., 1992).

3. Elevated pCO_2

Over the past two and a half centuries, atmospheric CO_2 has increased from 280 ppm to 387 ppm. The ocean absorbs CO_2 from the atmosphere including 30% of the total from human generated emissions. In the oceans, carbon dioxide is converted to carbonic acid, which decreases the pH in seawater and makes the water more acidic (Feely et al., 2010; Shamberger et al., 2014; Stumpp et al., 2013). This ongoing acidification of the oceans has led to concerns about the many marine organisms, such as diatoms, bivalve mollusks, and corals, whose survival depends upon the ability to produce calcium carbonate skeletons (Feely et

al., 2010; Reyes-Nivia et al., 2013; Wittmann and Pörtner, 2013). However, the possible effects of elevated pCO_2 on oxidative stress in the marine environment are complicated. It has been pointed out that elevated CO_2 can increase the efficiency of photosynthesis resulting in decreased generation of ROS (Asada, 1994; Suggett et al., 2008); for marine organisms that engage in photosynthesis, including the many animals that harbor photosynthetic endosymbionts, this effect could potentially counteract those environmental changes that would tend to increase the production of ROS (Borell et al., 2014). However, in a recent laboratory experiment on the coral *Acropora millepora*, high CO_2 was associated with a loss of photosynthetic symbionts, a decrease in photosynthesis, and an increased in the expression of genes involved in mitigating oxidative stress, specifically catalase, FAD-linked oxidase, and selenoprotein (Kaniewska et al., 2012).

C. Oxidative stress and coral bleaching

The work described here has direct implications for the resilience of coral reefs. Coral reefs are diverse ecological communities whose foundation consists of the mutualistic symbiosis between photosynthetic dinoflagellates of the genus *Symbiodinium* and cnidarian corals. Corals provide the *Symbiodinium* with a high light environment and protection from herbivory and nutrients including inorganic nitrogen, phosphorous, and carbon. In turn, the symbionts provide corals with photosynthetically fixed carbon. The symbionts supply as much as 95% of energy requirements for the coral (Weis, 2008).

Coral reefs have experienced substantial degradation over recent decades due in large part to coral die-offs attributable to bleaching. Coral bleaching is a host innate immune response that leads to breakdown in symbiosis between the host cnidarian and intracellular symbiont. Bleaching is reversible, but if not reversed bleaching decreases growth and reproduction while increasing the frequency of disease and ultimately causing the death of the coral animal. Coral bleaching can be triggered by a number of environmental stressors including high light levels, high levels of UV radiation, disease, pollution, altered salinity or sedimentation. Due to global climate change, the most pervasive environmental trigger is heat stress (Downs et al., 2002; Richier et al., 2008; Weis, 2008).

Whatever the initial environmental trigger, the cellular condition that leads to bleaching is thought to be oxidative stress. Elevated temperature, light, or UV-radiation can result in destabilization of photosystem II and the whole electron transport chain of oxidative phosphorylation. As a result, ROS production increases. Heat stress further exacerbates ROS induced oxidative stress through deactivation of ROS fighting/neutralizing pathways. The resulting ROS diffuses out of chloroplasts and into organelles of both the symbiont and cnidarian coral cytoplasm, which leads to oxidative damage in both the symbiont and coral. Corals respond by neutralizing ROS through enzymatic and non-enzymatic mechanisms. Below a threshold level of ROS, cells can effectively neutralize ROS. Above a threshold concentration, however, ROS cause damage and corals get rid of the source of ROS by expelling symbionts, which is the signature cause of coral bleaching (Baird et al., 2008; Downs et al., 2002; Weis, 2008).

This model of coral bleaching predicts that variation in stress response is related to molecular mechanisms that prevent production of ROS or protect against ROS damage. Responses include Cu/Zn SOD, antioxidant pathways, production of heat shock proteins, changes in lipid composition, and production of stress-stable components of enzymatic complexes for electron transport (Downs et al., 2002). In multiple coral species, bleaching has been shown to be related to antioxidant capacity and the cellular oxidative stress damage. Accumulation of oxidative damage is positively correlated with bleaching in corals. Conversely, high levels of antioxidants and small heat shock proteins were negatively correlated with oxidative damage present (Downs et al., 2002; Lesser, 1997). Furthermore, the addition of exogenous antioxidants has been shown to improve photosynthetic function and reduce the occurrence of bleaching (Lesser, 1997).

Corals and other cnidarians share a variety of conserved mechanisms for dealing with heat, light, UV, and oxidative stress including heat shock proteins, protective pigments, and expression of antioxidant enzymes (Weis, 2008). However, there is significant variation between and within coral species in resistance to temperature induced coral bleaching (Crook et al., 2013; Downs et al., 2002; Palumbi et al., 2014; Richier et al., 2008) . Many corals increase production of SODs and other enzymes to control the levels of superoxide and other ROS in cells (Lesser and Farrell, 2004; Saragosti et al., 2010; Yakovleva et al., 2004). It has been suggested that SODs are a significant component of antioxidant capacity and have a role in preventing bleaching (Downs et al., 2002; Lesser and Farrell, 2004). Overall, it has been shown that concentrations of specific oxidative damage products are correlated with bleaching and that severe oxidative damage

precedes coral bleaching. Additional components of cell defenses, including antioxidants and stress response proteins, play a role in protecting against bleaching (Downs et al., 2002; Saragosti et al., 2010).

Future Directions: Forward genetic analysis

Rationale for forward genetics experiment

My organismal studies have succeeded in identifying a pronounced difference in phenotype that appears to have a strong genetic component. Deep mRNA sequencing of peroxide resistant and sensitive lines, under peroxide exposed and control conditions, has provided clues to the genetic basis of this phenotypic difference. The transcriptomic data generated in this study make it possible to identify many differences among clones in the expression or the sequence of a given gene. However, the data do not address a critical question: which genotypic variation is responsible for the observed phenotypic variation in peroxide tolerance? To get at this question, I propose an approach combining forward genetics and experimental evolution using controlled crosses of peroxide resistant and peroxide sensitive clone lines. This allows us to evaluate the phenotypes and genotypes of the offspring over multiple generations of selection for peroxide resistance.

The generation time of *Nematostella* (~50 days from fertilization to sexual maturity) makes it feasible to cross animals that differ dramatically in their peroxide tolerance and to evaluate multiple generations of their progeny. Given the strong selection imposed by peroxide exposure, only a few generations should be necessary for peroxide resistant individuals to supplant peroxide sensitive individuals in experimental

mesocosms. If multiple peroxide-exposed experimental populations are compared to control populations after several generations, it should be possible to evaluate which genetic variants represented in the standing genetic variation of the Massachusetts population are important for peroxide tolerance. Evaluating the phenotype of the offspring should provide clues about how many loci are involved in this phenotypic difference. If a specific allele is universally advantageous under oxidative stress and detrimental under normal conditions, the experimentally evolved populations should diverge towards different alleles. Below, I outline a proposal to conduct this kind of study.

Forward genetics approach and evaluation

The approach I propose would begin by making multiple crosses, each using a resistant and sensitive clone line from the same population (as determined in chapter 2). *Nematostella* adult females can lay tens to hundreds of eggs approximately every 7 days, and offspring reach sexual maturity at 50 days. Clone lines made from a random subset of the F1 individuals from each of these crosses will be exposed to 0%, or 0.00025% H_2O_2 for 14 days. F1 individuals from each cross will be assayed for survival and monitored daily for (1) tentacle number, (2) ability to respond to mechanical stimulation, and (3) growth rate. Additional individuals from each clone line will be bisected on the transverse axis and scored on regeneration ability in 0% or 0.00025% H_2O_2. Regeneration ability will be scored daily on (1) presence/number of tentacles, (2) size (both body length and surface area), and (3) ability to feed after seven days.

The F1 individuals that have not been exposed to peroxide will be crossed, and clone lines will be made from a random subset of the F2 individuals. Survival and regeneration assays, as described above, will be performed on F2 clone lines to determine their levels of peroxide tolerance. Subsets of 20 F2 individuals will be divided into six pools of either 0.00025% peroxide or control conditions, and these populations will be allowed to grow and reproduce for a number of generations. Replicates from each of the six populations will be sequenced to evaluate genes selected for under the imposed oxidative stress. By forcing populations to survive and reproduce under prolonged peroxide exposure, we will be able to determine specific genes that are important in these environmental conditions.

The duration of the directed evolution study (number of generations) will be dependent on how many loci are likely involved in peroxide tolerance. If the F1 and F2 populations reproduce the parental phenotypes quickly, oxidative stress tolerance is likely controlled by a few genes that have large effects on the individual's phenotype. If few or none of the F2 individuals have the parental phenotype, then peroxide tolerance is likely dependent on many genes each playing a small role. By sequencing the genomes of multiple genets that were independently evolved from a common pool of genetic variation, it should be possible to determine which loci are critical to peroxide resistance.

Future Directions: Crosstalk between oxidative stress and epigenomic regulation

Oxidative DNA damage has been linked to aging and neurodegenerative diseases. Mitochondrial DNA damaged during replication has been shown to impair cellular

energy metabolism. Both mitochondrial abnormalities and damaged nuclear DNA have been found in patients with Alzheimer's, Parkinson's, and Huntington's diseases (Ventura et al., 2010). Specifically, the oxidized purine 8-oxo-dihydroguanine (8-oxo-dG) is a frequent DNA lesion. Guanine has a low reduction potential, and more than 20 oxidation products can be formed from guanine bases, including 8-oxo-dG. 8-oxo-dG base pairs with adenine (A) instead of cytosine (C) and can induce G/C to T/A transversions during replication. In mammals, 8-oxo-dG is excised from DNA by 8-oxo-dG DNA glycosylase (OGG1) during base excision repair. This repair mechanism removes 8-oxo-dG and leads to degradation, so 8-oxo-dG doesn't become incorporated into replicated DNA. Low levels of 8-oxo-dG may be important for transcription and cellular signaling, but high levels have been shown to accelerate the aging process, increase cancer susceptibility, and are associated with neurodegenerative diseases (Radak et al., 2011; Ventura et al., 2010). It is estimated in nuclear DNA that there are approximately 1 to 2 8-oxo-dG bases per 106 guanine residues and about 1 to 3 8-oxo-dG per 105 guanine residues in mitochondrial DNA (Radak et al., 2011).

A highly specific monoclonal affinity-purified antibody 1F7 was developed toward 8-oxo-dG for use in ELISAs (Litton Bionetics, Kensington, MD) (Yin et al., 1995). It has been shown that the 1F7 antibody can be used for detection of 8-oxo-dG in tissue sections, and the levels of DNA damage were consistent after chemical exposures. This immunochemical approach eliminates the need for DNA extraction, which reduces processing effects of these samples. It also requires only small amounts of frozen tissues

from fixed cells for detection and was shown to be able to discriminate DNA damage among various cell types (Machella et al., 2004).

Recently the 1F7 antibody toward 8-oxo-dG has been applied to tissues of marine organisms, specifically Mediterranean mussels (*Mytilus galloprovincialis*) and European eels (*Anguilla anguilla*) after exposure to benzo[*a*]pyrene, copper, or control conditions. It was shown that higher levels of oxidative DNA damage, as measured by relative staining intensities, were detected in the digestive glands of mussels and in the livers of eels (Machella et al., 2004).

Nematostella is a relatively transparent organism, which eliminates the need for sectioning tissues to visualize staining differences under a microscope. *Nematostella* individuals that have been exposed to peroxide or control conditions can be fixed and stained with the 1F7 antibody. The relative staining intensities can be visualized using cell image analysis software. The 1F7 antibody would presumably show variation in 8-oxo-dG levels in response to peroxide exposure, similar to the differences observed from copper and benzo[a]pyrene exposure.

REFERENCES

Abele-Oeschger, D., Tug, H., Rottgers, R., 1997. Dynamics of UV-driven hydrogen peroxide formation on an intertidal sandflat. Limnology and Oceanography 42, 1406-1415.

Abele, D., Burlando, B., Viarengo, A., Portner, H.O., 1998a. Exposure to elevated temperatures and hydrogen peroxide elicits oxidative stress and antioxidant response in the Antarctic intertidal limpet *Nacella concinna*. Comparative Biochemistry and Physiology Part B 1998, 425-435.

Abele, D., Ferreyra, G.A., Schloss, I., 1999. H_2O_2 accumulation from photochemical production and atmospheric wet deposition in Antarctic coastal and off-shore waters of Potter Cove, King George Island, South Shetland Islands. Antarctic Science 11, 131-139.

Abele, D., Grobpietsch, H., Portner, H.O., 1998b. Temporal fluctuations and spatial gradients of environmental PO_2, temperature, H_2O_2 and H_2S in its intertidal habitat trigger enzymatic antioxidant protection in the capitellid worm *Heteromastus filiformis*. Marine Ecology Progress Series 163, 179-191.

Abele, D., Heise, K., Pörtner, H.O., Puntarulo, S., 2002. Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. Journal of Experimental Biology 205, 1831–1841.

Acharya, A., Das, I., Chandhok, D., Saha, T., 2010. Redox regulation in cancer: a double-edged sword with therapeutic potential. Oxidative Medicine and Cellular Longevity 3, 23-34.

Allen, R.G., Tresini, M., 2000. Oxidative stress and gene regulation. Free Radical Biology & Medicine 28.

Ambrosone, A., Tortiglione, C., 2013. Methodological approaches for nanotoxicology using cnidarian models. Toxicology Mechanisms and Methods 23, 207-216.

Anjos, V.A., da Silva, F.M., Jr., Souza, M.M., 2014. Cell damage induced by copper: an explant model to study anemone cells. Toxicology in Vitro 28, 365-372.

Arthur, W., 1997. The Origin of Animal Body Plans. Cambridge University Press, Cambridge.

Asada, K., 1994. Production and action of active oxygen species in photosynthetic tissues., in: Foyer, C.H., Mullineaux, P.M. (Eds.), Causes of photooxidative stress and amelioration of defense systems in plants. CRC Press, London, pp. 77–105.

Aseervatham, G.S., Sivasudha, T., Jeyadevi, R., Arul Ananth, D., 2013. Environmental factors and unhealthy lifestyle influence oxidative stress in humans- an overview. Environmental Science and Pollution Research International 20, 4356-4369.

Baird, A.H., Bhagooli, R., Ralph, P.J., Takahashi, S., 2008. Coral bleaching: the role of the host. Trends in Ecology and Evolution 24, 16-20.

Berghahn, R., 2001. Indications of climate change in coastal areas: what may fish otoliths tell us? Climate Research 18, 113-118.

Blouin, M.S., Bonner, K.M., Cooper, B., Amarasinghe, V., O'Donnell, R.P., Bayne, C.J., 2013. Three genes involved in the oxidative burst are closely linked in the genome of the snail, *Biomphalaria glabrata*. International Journal for Parasitology 43, 51-55.

Borell, E.M., Steinke, M., Horwitz, R., Fine, M., 2014. Increasing pCO_2 correlates with low concentrations of intracellular dimethylsulfoniopropionate in the sea anemone *Anemonia viridis*. Ecology and Evolution 4, 441-449.

Bubici, C., Papa, S., Dean, K., Franzoso, G., 2006. Mutual cross-talk between reactive oxygen species and nuclear factor-kappa B: molecular basis and biological significance. Oncogene 25, 6731-6748.

Cammack, R., Wrigglesworth, J.M., Baum, H., 1990. Iron-dependent enzymes in mammalian systems, in: Ponka, P., Schulman, H.M., Woodworth, R.C. (Eds.), Iron Transport and Storage. CRC Press, Boca Raton, pp. 17–40.

Carillon, J., Rouanet, J.M., Cristol, J.P., Brion, R., 2013. Superoxide dismutase administration, a potential therapy against oxidative stress related diseases: several routes of supplementation and proposal of an original mechanism of action. Pharmacy Research 11, 2718-2728.

Chen, H., Osuna, D., Colville, L., Lorenzo, O., Graeber, K., Kuster, H., Leubner-Metzger, G., Kranner, I., 2013. Transcriptome-wide mapping of pea seed ageing reveals a pivotal role for genes related to oxidative stress and programmed cell death. PLOS ONE 8, e78471.

Clark, C.D., De Bruyn, W.J., Jakubowski, S.D., Grant, S.B., 2008. Hydrogen peroxide production in marine bathing waters: Implications for fecal indicator bacteria mortality. Marine Pollution Bulletin 56, 397-401.

Cooper, E.D., Bentlage, B., Gibbons, T.R., Bachvaroff, T.R., Delwiche, C.F., 2014. Metatranscriptome profiling of a harmful algal bloom. Harmful Algae 37, 75-83.

Cornet, V., Henry, J., Corre, E., Le Corguille, G., Zanuttini, B., Zatylny-Gaudin, C., 2014. Dual role of the cuttlefish salivary proteome in defense and predation. Journal of Proteomics 108, 209-22.

Costa, V., Angelini, C., De Feis, I., Ciccodicola, A., 2010. Uncovering the complexity of transcriptomes with RNA-Seq. Journal of Biomedicine & Biotechnology 2010, 853916.

Costantini, D., 2008. Oxidative stress in ecology and evolution: lessons from avian studies. Ecology Letters 11, 1238-1251.

Costantini, D., Meillère, A., Carravieri, A., Lecomte, V., Sorci, G., Faivre, B., Weimerskirch, H., Bustamante, P., Labadie, P., Budzinski, H., Chastel, O., 2014. Oxidative stress in relation to reproduction, contaminants, gender and age in a long-lived seabird. Oecologia 175, 1107-1116.

Crook, E.D., Cohen, A.L., Rebolledo-Vieyra, M., Hernandez, L., Paytana, A., 2013. Reduced calcification and lack of acclimatization by coral colonies growing in areas of persistent natural acidification Proceedings of the National Academy of Sciences 110.

Crowe, S.A., Dossing, L.N., Beukes, N.J., Bau, M., Kruger, S.J., Frei, R., Canfield, D.E., 2013. Atmospheric oxygenation three billion years ago. Nature 501, 535-538.

Darling, J.A., Kuenzi, A., Reitzel, A.M., 2009. Human-mediated transport determines the non-native distribution of the anemone *Nematostella vectensis*, a dispersal-limited estuarine invertebrate. Marine Ecology Progress Series 380, 137-146.

Darling, J.A., Reitzel, A.M., Burton, P.M., Mazza, M.E., Ryan, J.F., Sullivan, J.C., Finnerty, J.R., 2005. Rising starlet: the starlet sea anemone, *Nematostella vectensis*. BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology 27, 211-221.

Darling, J.A., Reitzel, A.M., Finnerty, J.R., 2004. Regional population structure of a widely introduced estuarine invertebrate: *Nematostella vectensis* Stephenson in New England. Molecular Ecology 13, 2969-2981.

Das, K.C., Lewis-Molock, Y., White, C.W., 1995. Activation of NF- kappa B and elevation of MnSOD gene expression by thiol reducing agents in lung adenocarcinoma (A549) cells. The American Journal of Physiology 269, L588-602.

Dash, B., Metz, R., Huebner, H.J., Porter, W., Phillips, T.D., 2007. Molecular characterization of two superoxide dismutases from *Hydra vulgaris*. Gene 387, 93-108.

Dash, B., Phillips, T.D., 2012. Molecular characterization of a catalase from *Hydra vulgaris*. Gene 501, 144-152.

Djavaheri-Mergny, M., Javelaud, D., Wietzerbin, J., Besancon, F., 2004. NF-κB activation prevents apoptotic oxidative stress via an increase of both thioredoxin and MnSOD levels in TNFa-treated Ewing sarcoma cells. FEBS Letters 578, 111-115.

Downs, C.A., Fauth, J.E., Halas, J.C., Dustan, P., Bemiss, J., Woodley, C.M., 2002. Oxidative stress and seasonal coral bleaching. Free Radical Biology & Medicine 33, 533-543.

Dykens, J.A., Shick, J.M., Benoit, C., Buettner, G.R., Winston, G.W., 1992. Oxygen radical production in the sea anemone *Anthopleura Elegantissima* and its endosymbiotic algae. Journal of Experimental Biology 168, 219-241.

Eaton, J.W., Qian, M., 2002. Molecular Bases of Cellular Iron Toxicity. Free Radical Biology & Medicine 32, 833-840.

Edge, S.E., Morgan, M.B., Gleason, D.F., Snell, T.W., 2005. Development of a coral cDNA array to examine gene expression profiles in *Montastraea faveolata* exposed to environmental stress. Marine Pollution Bulletin 51, 507-523.

Ekblom, R., Galindo, J., 2011. Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity 107, 1-15.

Enjalbert, B., Nantel, A., Whiteway, M., 2003. Stress-induced gene expression in *Candida albicans*: absence of a general stress response. Molecular Biology of the Cell 14, 1460-1467.

Fan, S.-M., 2008. Photochemical and biochemical controls on reactive oxygen and iron speciation in the pelagic surface ocean. Marine Chemistry 109, 152-164.

Feely, R.A., Alin, S.R., Newton, J., Sabine, C.L., Warner, M., Devol, A., Krembs, C., Maloy, C., 2010. The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. Estuarine, Coastal and Shelf Science 88, 442-449.

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239-47. .

Fridovich, I., 1995. Superoxide Radical and Superoxide Dismutases. Annual Review of Biochemistry 64, 97-112.

Fujii, T., 2012. Climate Change, Sea-Level Rise and Implications for Coastal and Estuarine Shoreline Management with Particular Reference to the Ecology of Intertidal Benthic Macrofauna in NW Europe. Biology 1, 597-616.

Gloire, G., Legrand-Poels, S., Piette, J., 2006. NF-kappaB activation by reactive oxygen species: fifteen years later. Biochemical Pharmacology 72, 1493-1505.

Goldstone, J.V., 2008. Environmental sensing and response genes in cnidaria: the chemical defensome in the sea anemone *Nematostella vectensis*. Cell Biology and Toxicology 24, 483-502.

Han, E.S., Muller, F.L., Perez, V.I., Qi, W., Liang, H., Xi, L., Fu, C., Doyle, E., Hickey, M., Cornell, J., Epstein, C.J., Roberts, L.J., Van Remmen, H., Richardson, A., 2008. The in vivo gene expression signature of oxidative stress. Physiological Genomics 34, 112-126.

Hand, C., Uhlinger, K.R., 1991. Culture, Reproduction, Development and Growth of the Sea-Anemone, *Nematostella vectensis* Stephenson (1935). American Zoologist 31, A138-A138.

Hand, C., Uhlinger, K.R., 1992. The Culture, Sexual and Asexual Reproduction, and Growth of the Sea Anemone *Nematostella vectensis*. Biological Bulletin 182, 169-176.

Hand, C., Uhlinger, K.R., 1994. The Unique, Widely Distributed, Estuarine Sea-Anemone, *Nematostella vectensis* Stephenson - a Review, New Facts, and Questions. Estuaries 17, 501-508.

Harter, V.L., Mattews, R.A., 2005. Acute and Chronic Toxicity Test Methods for *Nematostella vectensis* Stephenson. Bulletin of Environmental Contamination and Toxicology 74, 830-836.

Head, S.R., Komori, H.K., LaMere, S.A., Whisenant, T., Van Nieuwerburgh, F., Salomon, D.R., Ordoukhanian, P., 2014. Library construction for next-generation sequencing: overviews and challenges. BioTechniques 56, 61-64.

Heise, K., Puntarulo, S., Portner, H.O., Abele, D., 2003. Production of reactive oxygen species by isolated mitochondria of the Antarctic bivalve *Laternula elliptica* (King and Broderip) under heat stress. Comparative Biochemistry and Physiology Part C 134, 79-90.

Herman, J.R., 2010. Global increase in UV irradiance during the past 30 years (1979–2008) estimated from satellite data. Journal of Geophysical Research 115, D04203.

Hoffmann, A.A., Sgro, C.M., Lawler, S.H., 1995. Ecological Population Genetics: The Interface Between Genes and the Environment. Annual Review of Genetics 29, 349-370.

Holley, A.K., Bakthavatchalu, V., J.M., V.-R., St. Clair, D.K., 2011. Manganese Superoxide Dismutase: Guardian of the Powerhouse. International Journal of Molecular Sciences 12, 7114-7162.

Iken, K., Konar, B., Benedetti-Cecchi, L., Cruz-Motta, J.J., Knowlton, A., Pohle, G., Mead, A., Miloslavich, P., Wong, M., Trott, T., Mieszkowska, N., Riosmena-Rodriguez, R., Airoldi, L., Kimani, E., Shirayama, Y., Fraschetti, S., Ortiz-Touzet, M., Silva, A., 2010. Large-Scale spatial distribution patterns of echinoderms in nearshore rocky habitats. PLOS ONE 5, e13845.

Janbon, G., Ormerod, K.L., Paulet, D., Byrnes, E.J., 3rd, Yadav, V., Chatterjee, G., Mullapudi, N., Hon, C.C., Billmyre, R.B., Brunel, F., Bahn, Y.S., Chen, W., Chen, Y., Chow, E.W., Coppee, J.Y., Floyd-Averette, A., Gaillardin, C., Gerik, K.J., Goldberg, J., Gonzalez-Hilarion, S., Gujja, S., Hamlin, J.L., Hsueh, Y.P., Ianiri, G., Jones, S., Kodira, C.D., Kozubowski, L., Lam, W., Marra, M., Mesner, L.D., Mieczkowski, P.A., Moyrand, F., Nielsen, K., Proux, C., Rossignol, T., Schein, J.E., Sun, S., Wollschlaeger, C., Wood, I.A., Zeng, Q., Neuveglise, C., Newlon, C.S., Perfect, J.R., Lodge, J.K., Idnurm, A., Stajich, J.E., Kronstad, J.W., Sanyal, K., Heitman, J., Fraser, J.A., Cuomo, C.A., Dietrich, F.S., 2014. Analysis of the Genome and Transcriptome of *Cryptococcus neoformans* var. grubii Reveals Complex RNA Expression and Microevolution Leading to Virulence Attenuation. PLOS Genetics 10, e1004261.

Jiménez-Gómez, J.M., 2011. Next generation quantitative genetics in plants. Frontiers in Plant Science 2, 77.

Johnson, Z.I., Wheeler, B.J., Blinebry, S.K., Carlson, C.M., Ward, C.S., Hunt, D.E., 2013. Dramatic variability of the carbonate system at a temperate coastal ocean site (Beaufort, North Carolina, USA) is regulated by physical and biogeochemical processes on multiple timescales. PLOS ONE 8, e85117.

Kaniewska, P., Campbell, P.R., Kline, D.I., Rodriguez-Lanetty, M., Miller, D.J., Dove, S., Hoegh-Guldberg, O., 2012. Major cellular and physiological impacts of ocean acidification on a reef building coral. PLOS ONE 7, e34659.

Kassahn, K.S., Crozier, R.H., Portner, H.O., Caley, M.J., 2009. Animal performance and stress: responses and tolerance limits at different levels of biological organisation. Biological Reviews of the Cambridge Philosophical Society 84, 277-292.

Khan, A.H., Levac, E., Chmura, G.L., 2013. Future sea surface temperatures in Large Marine Ecosystems of the Northwest Atlantic. ICES Journal of Marine Science: Journal du Conseil 70, 915-921.

Kirwan, M.L., Megonigal, J.P., 2013. Tidal wetland stability in the face of human impacts and sea-level rise. Nature 504, 53-60.

Kurz, T., Gustafsson, B., Brunk, U.T., 2011. Cell sensitivity to oxidative stress is influenced by ferritin autophagy. Free Radical Biology & Medicine 50, 1647-1658.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9, 357-359.

Larade, K., 2004. Accumulation and translation of ferritin heavy chain transcripts following anoxia exposure in a marine invertebrate. Journal of Experimental Biology 207, 1353-1360.

Lee, F.J., Rusch, D.B., Stewart, F.J., Mattila, H.R., Newton, I.L., 2014. Saccharide breakdown and fermentation by the honey bee gut microbiome. Environmental Microbiology.

Lesser, M.P., 1997. Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16, 187-192.

Lesser, M.P., 2003. Exposure to ultraviolet radiation causes apoptosis in developing sea urchin embryos. Journal of Experimental Biology 206, 4097-4103.

Lesser, M.P., 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. Annual Review Physiology 68, 253-278.

Lesser, M.P., Farrell, J.H., 2004. Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. Coral Reefs 23, 367–377.

Lewis, C.N., Brown, K.A., Edwards, L.A., Cooper, G., H.S., F., 2013. Sensitivity to ocean acidification parallels natural pCO_2 gradients experienced by Arctic copepods under winter sea ice. Proceedings of the National Academy of Sciences 110, E4960-7.

Li, B., Dewey, C.N., 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12, 323.

Li, Y., Shiffman, D., Oberbauer, R., 2011. Analysis of Single Nucleotide Polymorphisms in Case–Control Studies. Methods in Molecular Biology 719, 219-234.

Lister, K.N., Lamare, M.D., Burritt, D.J., 2010a. Oxidative damage in response to natural levels of UV-B radiation in larvae of the tropical sea urchin *Tripneustes gratilla*. Photochemistry and Photobiology 86, 1091-1098.

Lister, K.N., Lamare, M.D., Burritt, D.J., 2010b. Sea ice protects the embryos of the Antarctic sea urchin *Sterechinus neumayeri* from oxidative damage due to naturally enhanced levels of UV-B radiation. Journal of Experimental Biology 213, 1967-1975.

Lubinski, T.J., Granger, B.R., Stefanik, D.J., Friedman, L.E., McAnulty, S.J., Schweiker, R.M., Bryd A., Gilmore, T.D., Finnerty, J.R., 2014a. New transcriptome assemblies for *Nematostella vectensis* incorporate data from multiple populations, life stages, and environmental conditions. PLOS ONE *In prep*.

Lubinski, T.J., Granger, B.R., Stefanik, D.J., Friedman, L.E., McAnulty, S.J., Schweiker, R.M., Byrd, A., Finnerty, J.R., 2014b. An overhaul of StellaBase enables comparative transcriptomic studies on multiple populations, life stages, and environmental conditions. Nucleic Acids Research *In prep*.

Machella, N., Regoli, F., Cambria, A., Santella, R.M., 2004. Application of an immunoperoxidase staining method for detection of 7,8-dihydro-8-oxodeoxyguanosine as a biomarker of chemical-induced oxidative stress in marine organisms. Aquatic Toxicology 67, 23-32.

Marshall, J.A., de Salas, M., Oda, T., G., H., 2005. Superoxide production by marine microalgae. Marine Biology 147, 533-540.

Martin, J.A., Wang, Z., 2011. Next-generation transcriptome assembly. Nature Review Genetics 12, 671-682.

Merle, P.L., Sabourault, C., Richier, S., Allemand, D., Furla, P., 2007. Catalase characterization and implication in bleaching of a symbiotic sea anemone. Free Radical Biology & Medicine 42, 236-246.

Miao, L., St Clair, D.K., 2009. Regulation of superoxide dismutase genes: implications in disease. Free Radical Biology & Medicine 47, 344-356.

Miramon, P., Dunker, C., Kasper, L., Jacobsen, I.D., Barz, D., Kurzai, O., Hube, B., 2014. A family of glutathione peroxidases contributes to oxidative stress resistance in *Candida albicans*. Medical Mycology 52, 223-239.

Mopper, K., Kieber, D.J., 2000. Marine photochemistry and its impact on carbon cycling, in: Mora, S.D., Demers, S., Vernet, M. (Eds.), The Effects of UV Radiation in the Marine Environment. Cambridge Univ. Press, Cambridge, UK, pp. 101–130.

Morgan, M.J., Liu, Z.G., 2011. Crosstalk of reactive oxygen species and NF-kappaB signaling. Cell Research 21, 103-115.

Mostertz, J., 2004. Transcriptome and proteome analysis of *Bacillus subtilis* gene expression in response to superoxide and peroxide stress. Microbiology 150, 497-512.

Nii, C.M., Muscatine, L., 1997. Oxidative Stress in the Symbiotic Sea Anemone Aiptasia pulchella (Carlgren, 1943): Contribution of the Animal to Superoxide Ion Production at Elevated Temperature. Biological Bulletin 192, 444-456.

Oda, T., Nakamura, A., Shikayam, M., Kawano, I., Ishimatsu, A., Muramatsu, T., 1997. Generation of reactive oxygen species by raphidophycean phytoplankton. Bioscience, Biotechnology, and Biochemistry 61, 1658-1662.

Orino, K., Lehman, L., Tsuji, Y., Ayaki, H., S.V., T., Torti, F.M., 2001. Ferritin and the response to oxidative stress. The Biochemical Journal 357, 241–247.

Paital, B., Chainy, G.B., 2014. Effects of temperature on complexes I and II mediated respiration, ROS generation and oxidative stress status in isolated gill mitochondria of the mud crab *Scylla serrata*. Journal Thermal Biology 41, 104-111.

Palumbi, S.R., Barshis, D.J., Nikki Traylor-Knowles, N., Bay, R.A., 2014. Mechanisms of reef coral resistance to future climate change. Science 344, 895-898.

Perkins, N.D., 2007. Integrating cell-signalling pathways with NF-kappaB and IKK function. Nature Reviews. Molecular Cell Biology 8, 49-62.

Peterson, K.J., Butterfield, N.J., 2005. Origin of the Eumetazoa: testing ecological predictions of molecular clocks against the Proterozoic fossil record. Proceedings of the National Academy of Sciences 102, 9547-9552.

Plantivaux, A., Furla, P., Zoccola, D., Garello, G., Forcioli, D., Richier, S., Merle, P.L., Tambutte, E., Tambutte, S., Allemand, D., 2004. Molecular characterization of two CuZn-superoxide dismutases in a sea anemone. Free Radical Biology & Medicine 37, 1170-1181.

Putnam, N.H., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J., Salamov, A., Terry, A., Shapiro, H., Lindquist, E., Kapitonov, V.V., Jurka, J., Genikhovich, G., Grigoriev, I.V., Lucas, S.M., Steele, R.E., Finnerty, J.R., Technau, U., Martindale, M.Q., Rokhsar, D.S., 2007. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. Science 317, 86-94.

Radak, Z., Zhongfu, Z., Sataro, G., Erika, K., 2011. Age-associated neurodegeneration and oxidative damage to lipids, proteins and DNA. Molecular Aspects of Medicine 32, 305-315.

Raj, D., Ghosh, E., Mukherjee, A.K., Nozaki, T., Ganguly, S., 2014. Differential gene expression in *Giardia lamblia* under oxidative stress: significance in eukaryotic evolution. Gene 535, 131-139.

Ramsköld, D., Kavak, E., Sandberg, R., 2012. How to analyze gene expression using RNA-sequencing data, Methods in Molecular Biology 802, 259-274.

Reitzel, A.M., Chu, T., Edquist, S., Genovese, C., Church, C., Tarrant, A.M., Finnerty, J.R., 2013a. Physiological and developmental responses to temperature by the sea anemone *Nematostella vectensis*. Marine Ecology Progress Series 484, 115-130.

Reitzel, A.M., Darling, J.A., Sullivan, J.C., Finnerty, J.R., 2008a. Global population genetic structure of the starlet anemone Nematostella vectensis: multiple introductions and implications for conservation policy. Biological Invasions 10, 1197-1213.

Reitzel, A.M., Herrera, S., Layden, M.J., Martindale, M.Q., Shank, T.M., 2013b. Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. Molecular Ecology 22, 2953-2970.

Reitzel, A.M., Sullivan, J.C., Finnerty, J.R., 2009. Discovering SNPs in Protein Coding Regions with StellaSNP: Illustrating the Characterization and Geographic Distribution of Polymorphisms in the Estuarine Anemone *Nematostella vectensis*. Estuaries and Coasts 33, 930-943.

Reitzel, A.M., Sullivan, J.C., Traylor-Knowles, N., Finnerty, J.R., 2008b. Genomic survey of candidate stress-response genes in the estuarine anemone *Nematostella vectensis*. Biological Bulletin 214, 233-254.

Reitzel, A.R., Darling, J., 2003. Global population structure of the invasive salt marsh anemone *Nematostella vectensis*. Integrative and Comparative Biology 43, 842-842.

Reyes-Nivia, C., Diaz-Pulido, G., Kline, D., Guldberg, O.H., Dove, S., 2013. Ocean acidification and warming scenarios increase microbioerosion of coral skeletons. Global Change Biology 19, 1919-1929.

Richier, S., Merle, P.-L., Furla, P., Pigozzi, D., Sola, F., Allemand, D., 2003. Characterization of superoxide dismutases in anoxia- and hyperoxia-tolerant symbiotic cnidarians. Biochimica et Biophysica Acta (BBA) - General Subjects 1621, 84-91.

Richier, S., Rodriguez-Lanetty, M., Schnitzler, C.E., Weis, V.M., 2008. Response of the symbiotic cnidarian *Anthopleura elegantissima* transcriptome to temperature and UV increase. Comparative Biochemistry and Physiology. Part D, Genomics & Proteomics 3, 283-289.

Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. EdgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139-140.

Röhrdanz, E., Kahl, R., 1998. Alterations of antioxidant enzyme expression in response to hydrogen peroxide. Free Radical Biology & Medicine 24, 27-38.

Ross, P.M., Adam, P., 2013. Climate Change and Intertidal Wetlands. Biology 2, 445-480.

Saragosti, E., Tchernov, D., Katsir, A., Shaked, Y., 2010. Extracellular production and degradation of superoxide in the coral *Stylophora pistillata* and cultured Symbiodinium. PLOS ONE 5, e12508.

Sarkar, S., Mukherjee, S., Chattopadhyay, A., Bhattacharya, S., 2014. Low dose of arsenic trioxide triggers oxidative stress in zebrafish brain: Expression of antioxidant genes. Ecotoxicology and Environmental Safety 107, 1-8.

Schwarz, J.A., Brokstein, P.B., Voolstra, C., Terry, A.Y., Manohar, C.F., Miller, D.J., Szmant, A.M., Coffroth, M.A., Medina, M., 2008. Coral life history and symbiosis: functional genomic resources for two reef building Caribbean corals, *Acropora palmata* and *Montastraea faveolata*. BMC Genomics 9.

Shamberger, K.E.F., Cohen, A.L., Golbuu, Y., McCorkle, D.C., Lentz, S.J., Barkley, H.C., 2014. Diverse coral communities in naturally acidified waters of a Western Pacific reef. Geophysical Research Letters 41, 499-504.

Sheader, M., Suwailem, A.M., Rowe, G.A., 1997. The anemone, *Nematostella vectensis*, in Britain: considerations for conservation management. Aquatic Conservation: Marine and Freshwater Ecosystems 7, 13-25.

Sheaves, M., Johnston, R., 2008. Influence of marine and freshwater connectivity on the dynamics of subtropical estuarine wetland fish metapopulations. Marine Ecology Progress Series 357, 225–243.

Shendure, J., Ji, H., 2008. Next-generation DNA sequencing. Nature Biotechnology 26, 1135-1145.

Shi, Q., Gibson, G.E., 2007. Oxidative stress and transcriptional regulation in Alzheimer disease. Alzheimer Disease and Associated Disorders 21, 276-291.

Shick, J.M., Dunlap, W.C., 2002. Mycosporine-like amino acids and related Gadusols: biosynthesis, acumulation, and UV-protective functions in aquatic organisms. Annual Review of Physiology 64, 223-262.

Shinzato, C., Hamada, M., Shoguchi, E., Kawashima, T., Satoh, N., 2012. The repertoire of chemical defense genes in the coral *Acropora digitifera* genome. Zoological Science 29, 510-517.

Shinzato, C., Shoguchi, E., Kawashima, T., Hamada, M., Hisata, K., Tanaka, M., Fujie, M., Fujiwara, M., Koyanagi, R., Ikuta, T., Fujiyama, A., Miller, D.J., Satoh, N., 2011. Using the *Acropora digitifera* genome to understand coral responses to environmental change. Nature 476, 320-323.

Sies, H., 2014. Role of metabolic H_2O_2 generation: redox signaling and oxidative stress. Journal of Biological Chemistry 289, 8735-8741.

Stefanik, D.J., Friedman, L.E., Finnerty, J.R., 2013a. Collecting, rearing, spawning and inducing regeneration of the starlet sea anemone, *Nematostella vectensis*. Nature Protocols 8, 916-923.

Stefanik, D.J., Lubinski, T.J., Granger, B.R., Byrd, A.L., Reitzel, A.M., DeFilippo, L., Lorenc, A., Finnerty, J.R., 2014. Production of a reference transcriptome and transcriptomic database (EdwardsiellaBase) for the lined sea anemone, *Edwardsiella lineata*, a parasitic cnidarian. BMC Genomics 15, 71.

Stefanik, D.J., Wolenski, F.S., Friedman, L.E., Gilmore, T.D., Finnerty, J.R., 2013b. Isolation of DNA, RNA and protein from the starlet sea anemone *Nematostella vectensis*. Nature Protocols 8, 892-899.

Storch, D., Abele, D., Portner, H.O., 2001. The effects of hydrogen peroxide on isolated body wall of the lugworm *Arenicola marina* (L.) at different extracellular pH levels. Comparative Biochemistry and Physiology Part C 128, 391-399.

Stumpp, M., Hu, M., Casties, I., Saborowski, R., Bleich, M., Melzner, F., Dupont, S., 2013. Digestion in sea urchin larvae impaired under ocean acidification. Nature Climate Change 3, 1044-1049.

Suggett, D.J., Warner, M.E., Smith, D.J., Davey, P., Hennige, S., Baker, N.R., 2008. Photosynthesis and production of hydrogen peroxide by *Symbiodinium* (Pyrrhophyta) phylotypes with different thermal tolerances. Journal of Phycology 44, 948–956.

Sullivan, J.C., Reitzel, A.M., Finnerty, J.R., 2008. Upgrades to StellaBase facilitate medical and genetic studies on the starlet sea anemone, *Nematostella vectensis*. Nucleic Acids Research 36, D607–D611.

Sullivan, J.C., Ryan, J.F., Watson, J.A., Webb, J., Mullikin, J.C., Rokhsar, D., Finnerty, J.R., 2006. StellaBase: The *Nematostella vectensis* Genomics Database. Nucleic Acids Research 43, D495–D499.

Sullivan, J.C., Wolenski, F.S., Reitzel, A.M., French, C.E., Traylor Knowles, N., Gilmore, T.D., Finnerty, J.R., 2009. Two alleles of NF-κB in the sea anemone *Nematostella vectensis* are widely dispersed in nature and encode proteins with distinct activities. PLOS ONE 4, e7311.

Tapley, D.W., Buettner, G.R., Shick, J.M., 1999. Free radicals and chemiluminescence as products of the spontaneous oxidation of sulfide in seawater, and their biological implications. Biological Bulletin 196, 52-56.

Tarrant, A.M., Reitzel, A.M., Kwok, C.K., Jenny, M.J., 2014. Activation of the cnidarian oxidative stress response by ultraviolet radiation, polycyclic aromatic hydrocarbons and crude oil. Journal of Experimental Biology 217, 1444-1453.

Technau, U., Steele, R.E., 2011. Evolutionary crossroads in developmental biology: Cnidaria. Development 138, 1447-1458.

Theil, E.C., 1987. Ferritin: structure, gene regulation, and cellular function in animals, plants, and microorganisms. Annual Review of Biochemistry 56, 289-315.

Vandenabeele, S., Van Der Kelen, K., Dat, J., Gadjev, I., Boonefaes, T., Morsa, S., Rottiers, P., Slooten, L., Van Montagu, M., Zabeau, M., Inze, D., Van Breusegem, F., 2003. A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco. Proceedings of the National Academy of Sciences 100, 16113-16118.

Velez, S., Feder, J.L., 2006. Integrating biogeographic and genetic approaches to investigate the history of bioluminescent colour alleles in the Jamaican click beetle, *Pyrophorus plagiophthalamus*. Molecular Ecology 15, 1393-1404.

Ventura, I., Russo, M.T., Luca, G.D., Bignami, M., 2010. Oxidized purine nucleotides, genome instability and neurodegeneration. Mutation Research 703, 59-65.

Wang, S., Zhang, L., Matz, M., 2009a. Microsatellite characterization and marker development from public EST and WGS databases in the reef-building coral *Acropora millepora* (Cnidaria, Anthozoa, Scleractinia). Journal of Heredity 100, 329-337.

Wang, S., Zhang, L., Meyer, E., Matz, M.V., 2009b. Construction of a high-resolution genetic linkage map and comparative genome analysis for the reef-building coral *Acropora millepora*. Genome Biology 10, R126.

Wang, Z., Gerstein, M., Snyder, M., 2009c. RNA-Seq: a revolutionary tool for transcriptomics. Nature Review Genetics 10, 57-63.

Weis, V.M., 2008. Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. Journal of Experimental Biology 211, 3059-3066.

Winston, G.W., Moore, M.N., Straatsburg, I., Kirchin, M.A., 1991. Decreased stability of digestive gland lysosomes from the common mussel *Mytilus edulis* L. by in vitro generation of oxygen-free radicals. Archives of Environmental Contamination and Toxicology 21, 401-408.

Wittmann, A.C., Pörtner, H.-O., 2013. Sensitivities of extant animal taxa to ocean acidification. Nature Climate Change 3, 995-1001.

Wolenski, F.S., Bradham, C.A., Finnerty, J.R., Gilmore, T.D., 2013. NF-kappaB is required for cnidocyte development in the sea anemone *Nematostella vectensis*. Developmental Biology 373, 205-215.

Wolenski, F.S., Chandani, S., Stefanik, D.J., Jiang, N., Chu, E., Finnerty, J.R., Gilmore, T.D., 2011a. Two polymorphic residues account for the differences in DNA binding and transcriptional activation by NF-kappaB proteins encoded by naturally occurring alleles in *Nematostella vectensis*. Journal of Molecular Evolution 73, 325-336.

Wolenski, F.S., Garbati, M.R., Lubinski, T.J., Traylor-Knowles, N., Dresselhaus, E., Stefanik, D.J., Goucher, H., Finnerty, J.R., Gilmore, T.D., 2011b. Characterization of the core elements of the NF-kappaB signaling pathway of the sea anemone *Nematostella vectensis*. Molecular and Cellular Biology 31, 1076-1087.

Wu, M., Bian, Q., Liu, Y., Fernandes, A.F., Taylor, A., Pereira, P., Shang, F., 2009. Sustained oxidative stress inhibits NF-kappaB activation partially via inactivating the proteasome. Free Radical Biology & Medicine 46, 62-69.

Yakovleva, I., Bhagooli, R., Takemura, A., Hidaka, M., 2004. Differential susceptibility to oxidative stress of two scleractinian corals: antioxidant functioning of mycosporine-glycine. Comparative biochemistry and physiology. Part B, Biochemistry & Molecular Biology 139, 721-730.

Yeaman, S., Hodgins, K.A., Suren, H., Nurkowski, K.A., Rieseberg, L.H., Holliday, J.A., Aitken, S.N., 2014. Conservation and divergence of gene expression plasticity following c. 140 million years of evolution in lodgepole pine (*Pinus contorta)* and interior spruce (*Picea glauca* x *Picea engelmannii*). New Phytologist 203, 578-591.

Yin, B., Whyatt, R.M., Perera, F.P., Randall, M.C., Cooper, T.B., Santella, R.M., 1995. Determinations of 8-hydroxydeoxyguanosine by an immunoaffinity chromatography-monoclonal antibody-based ELISA. Free Radical Biology & Medicine 18, 1023-1032.

Zhang, Y., Choksi, S., Chen, K., Pobezinskaya, Y., Linnoila, I., Liu, Z.G., 2013. ROS play a critical role in the differentiation of alternatively activated macrophages and the occurrence of tumor-associated macrophages. Cell Research 23, 898-914.

CURRICULUM VITAE

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EDUCATION

Boston University, Boston, Massachusetts August 2014
PhD, Cell and Molecular Biology
Dissertation: Microevolution of the oxidative stress response: organismal and transcriptomic effects of peroxide exposure in the starlet sea anemone *Nematostella vectensis*

Skidmore College, Saratoga Springs, New York May 2009
B.A., *cum laude,* Integrative Biology
Periclean Honor Society

PUBLICATIONS

1. **Friedman, L.E.**, Lubinski, T.J., & J.R. Finnerty. Microevolution of oxidative stress response in an estuarine invertebrate — transcriptomic effects of peroxide exposure in the starlet sea anemone, *Nematostella vectensis*. *In prep*.
2. **Friedman, L.E,** Gilmore T.D. & J.R. Finnerty. Intraspecific variation in peroxide sensitivity in the starlet sea anemone, *Nematostella vectensis,* an estuarine cnidarian. *In prep*.
3. Lubinski, T.J., Granger, B.R., Stefanik, D.J., **Friedman, L.E.**, McAnulty, S., Schweiker, R.M., Byrd, A., Gilmore, T.D. & J.R. Finnerty. New transcriptomic assemblies for *Nematostella vectensis* incorporate data from multiple populations, life stages, and environmental conditions. *In prep.*
4. Lubinski, T.J., Granger, B.R., Stefanik, D.J., **Friedman, L.E.**, McAnulty, S., Schweiker, R.M., Byrd, A., Klein, J., & J.R. Finnerty. StellaBase 2 — an overhaul of StellaBase enables comparative transcriptomic studies on multiple populations, life stages, and environmental conditions. *In prep*.
5. Stefanik, D.J., Wolenski, F.S., **Friedman, L.E.,** Gilmore, T.D., & J.R. Finnerty, 2013. Isolation of DNA, RNA and protein from the starlet sea anemone, *Nematostella vectensis*. Nature Methods, 8(5): 892-899.
6. Stefanik, D.J., **Friedman, L.E**., & J.R. Finnerty, 2013. Collecting, rearing, spawning, and inducing regeneration of the starlet sea anemone, *Nematostella vectensis* (phylum Cnidaria; class Anthozoa). Nature Methods, 8(5): 916-923.

RESEARCH EXPERIENCE

Boston University Boston, MA
Graduate Researcher August 2009 - August 2014
Advisor: Dr. John R Finnerty
Project focus: Microevolution of the oxidative stress response phenotype in the starlet sea anemone *Nematostella vectensis*

- Investigated stress response in marine species through organismal and transcriptomic assays
- Collaborated with 4 researchers to develop novel protocols for an emerging model organism
- Collaborated with 10 professors, graduate, and undergraduate researchers to produce 6 peer-reviewed publications
- Trained and mentored 12 high school, undergraduate, and graduate researchers in laboratory techniques
- Presented original research at symposia attended by 50+ scientists and to BU department of 30 scientists (4 times)
- Wrote originally researched dissertation consisting of 4 chapters drawn from 100+ sources

Skidmore College Saratoga Springs, NY
Independent Research May 2008 - May 2009
Advisor: Dr. Bernard P. Possidente
Project focus: A behavioral-genetic study of lead toxicity in a *Drosophila melanogaster* model of Alzheimer's disease

- Investigated effects of lead toxicity in a fruit fly model of Alzheimer's disease
- Acquired and analyzed circadian rhythm expression data
- Presented original research to conference of undergraduate researchers

PRESENTATIONS AND CONFERENCES

Boston University Boston, MA
Second Annual Nematostella Meeting, August 2012
Research Presentation: Microevolution of the oxidative stress response in the starlet sea anemone, *Nematostella vectensis*

Woods Hole Oceanographic Institute Woods Hole, MA
First Annual Nematostella Meeting July 2011

TEACHING AND LEADERSHIP EXPERIENCE

Boston University Boston, MA
Teaching Fellow, Department of Biology August 2009 - August 2014

- Taught scientific literature discussion and laboratory techniques to 250 graduate, undergraduate, and high school students in 8 classes
- Provided feedback and assessed students' scientific lab reports
- Facilitated review sessions and advised students during over 400 office hours

Boston University Boston, MA
Laboratory Safety Coordinator July 2013 - August 2014

- Ensured proper laboratory safety practices were implemented in daily operations

- Performed laboratory inspections to ensure compliance with university safety standards
- Served as primary contact with Environmental Health and Safety

Skidmore College Saratoga Springs, NY
Peer Advocate, Center for Sex and Gender Relations Sept 2006 - May 2008

- Counseled students on a variety of personal issues
- Created and presented relevant programs in residence halls on topics including relationships, sexual violence, and harassment.

Skidmore College Saratoga Springs, NY
Residential Assistant, Office of Residential Life August 2006 - May 2007

- Managed university residence hall of 60 diverse undergraduate students
- Counseled students on a variety of personal and academic issues
- Collaborated with colleagues to arbitrate disciplinary issues
- Enforced university regulations resulting in a safe and positive living environment

ACTIVITIES

Boston University Boston, MA
Grader January - May 2011

- Provided feedback and assessment on weekly assignments for 50+ students in core science class Biodiversity Causes and Consequences