The web-enabled
Ocean Genomics Horizon Scan
can be accessed at:

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Chapter 1: Introduction

ABOUT THIS DOCUMENT

This document is a first-of-its-kind assessment highlighting the opportunities to bring genomic insight and biotechnology innovations to complement current and future marine conservation. The health of the planet and marine ecosystems are under severe strain. Wildlife biodiversity and bioabundance are experiencing dramatic declines. Over the next 30 years, global human population will grow by 3 billion people, increasing the urgency to identify radically new interventions to maintain Earth's ecological health. Given the level of serious threats to ocean health, immediate engagement on the development of thoughtful and carefully considered innovative solutions is necessary to address significant marine conservation challenges.

A focused initiative to utilize state-of-the-art genomic technologies has the potential to help save not only specific species but entire marine ecosystems, such as coral reefs. However, these novel technologies are at different stages of utility and development, and some innovations can be controversial and risky. Many disciplines (ethics, conservation, regulatory, and policy) across civil society must play a role in evaluating genomic solutions for ocean conservation.

Philanthropy, non-governmental organizations, and public agencies have the ability to help catalyze this field and to evaluate the risks and address the controversies. These sectors can guide the technological development, convene conversations, commission studies, identify priorities, and connect the emerging technology with civil society to identify safeguards.

To support the development of this emerging "Genetic Rescue Toolkit" this document highlights: the current use of genomic technologies and synthetic biology and potential applications, the risks and challenges involved, and the innovative scientists and institutions forging new ground.

WHY GENOMIC SOLUTIONS NOW?

Ocean threats such as pollution and over-exploitation from a rapidly increasing human footprint are impairing the integrity of marine ecosystems. Climate change exacerbates nearly all of these threats and reaches parts of the ocean still relatively untouched from other anthropogenic effects. As a synergistic stressor, climate change-induced physical and chemical changes exacerbate more obvious threats. Globally, oceans have absorbed more than 93 percent of heat and over 26 percent of carbon dioxide produced by anthropogenic sources, contributing to rising sea levels, more frequent disease outbreaks, acidification of seawater, increased mortality and decreased productivity of key species, and changes in the geographic distribution of many important fish stocks (Laffoley and Baxter 2016; Weatherdon et al. 2016). Well-documented threats to kelp forests and coral reefs, two critically important ecosystems that provide a broad range of valuable ecosystem services, are examples of the potentially catastrophic combined effects of climate change with other stressors.

The World Economic Forum identified biodiversity loss and ecosystem collapse as one of the major drivers of global risk that will lead to further cascading and causal or correlated risks such as the spread of infectious diseases, food and water crises, and man-made environmental disasters. The Stockholm Resilience Center has identified strong correlations and linkages between biodiversity loss and poverty and human suffering. For example, with over 35 percent of global food production dependent on animal pollination, the loss of pollinating species could mean lower crop yields and less food around the world, thus presenting a major food security challenge.

Conventional conservation measures are still critically important and continue to provide resilience to a myriad of threats. However, these strategies cannot completely stem the tide of environmental threats. As economic growth accelerates in non-OECD countries (which are also often hotspots of biodiversity), and global demand and trade in goods continues to increase, the need to develop new conservation tools that can cope with the pace of this growth also increases. Current conservation strategies are not sufficient to address all environmental threats, and no one sector can address the multi-faceted nature of this challenge in isolation.

Since the first human genome was sequenced in 2003, genomic research and clinical applications have been revolutionizing medicine (Ginsburg et al. 2001; Roukos et al. 2010; Hood et al. 2012; Cardon et al. 2016). In that same time period, the integration of genomics into agriculture has created new crop lines that require less land use, lower feed inputs, and reduced pesticide use (Henry et al. 2016). By looking to the health and agricultural sectors, one can get a sense of how quickly genomics can transform practices in a field. Yet, the adaptation of genomic tools for addressing threats to global ecological health is only just beginning. Exploration of genomics as an innovative strategy to complement and support ocean conservation may provide wholly new strategies for consideration in combating the threats to ocean health. Genomics and biotechnology has been increasingly embraced by both human medicine and agriculture. Will the field of conservation follow a similar trajectory or lag behind?

THE GENOMICS REVOLUTION IN HUMAN HEALTH

Genomic medicine is the use of information from genomes and their derivatives (RNA, proteins, and metabolites) to guide medical decision making. The Human Genome Project to sequence every nucleotide of one individual laid the foundation for all of these developments. Today it is possible for patients to get their DNA sequenced, see it matched against known genetically related conditions, and have their medications checked for genetic suitability. It is now possible to understand how individual tumors will react to drugs and direct the choice of 'targeted' anticancer treatment for individual patients. Genomic and personalized medicine is now a regular part of patient care and includes diagnostics and targeted therapeutics applied primarily to oncology, cardiovascular, and infectious disease treatment and diagnostics. A February 2016 research report indicates that the core personalized medicine market will be worth over $149 billion by 2020, growing at a rate of 8.7 percent per year (Kelly Scientific Publications 2016).

As researchers and clinicians developed the technical abilities related to genomic and personalized medicine, skepticism, opposition, and confusion constrained the adoption of these

new tools by the health care system. The establishment of the Personalized Medicine Coalition in 2004 provided a framework to support the developing field (Abrahams et al. 2005). The coalition included all interested parties (biotech, pharmaceutical, healthcare providers and patients) in the healthcare ecosystem. Today this coalition includes more than 250 institutions that increase the body of knowledge and support investments in order to improve clinical care for patients. With support from the coalition, the medical field has largely overcome the valid considerations that once limited the social readiness of advanced reproductive techniques and the application of other genomic technologies to human medicine.

GENOMIC TOOLS TO IMPROVE AGRICULTURE

The application of genomics in agriculture has improved the productivity and sustainability of livestock and crop production. In the animal husbandry sector, cloning is now widely used in the production of bulls for cattle breeding and for top performing equine athletes in polo, dressage, show jumping, and western reining (Keefer 20016; Maserati et al. 2016). Transgenic cattle, born without horns, have been developed to avoid the suffering of de-horning (Carlson et al. 2016).

Genetic diversity is the greatest resource for plant breeders to select lines that could potentially enhance food quality and quantity. Until now, conventional breeding in agriculture is based exclusively on phenotypic selection. Agriculture genomics now makes it possible to identify genetic markers for specific traits. Marker assisted breeding has created genetic maps of cowpea (Boukar et al. 2016), one of the most important legumes in the world, cold tolerant tomatoes (Shah et al. 2016), and soft wheat that improves cookie quality (Jing et al. 2016). Gene editing is being used to improve fruit size and plant architecture in tomatoes and to create plant-based and synthetic alternatives to meat and fish products.

GENOMICS FOR CONSERVATION

The pace and scale of biotech advances, as well as significant cost reductions, in the medical and agricultural sectors indicate significant potential to apply genomic tools to conservation challenges. However, not unsurprisingly, there is a far stronger concentration in commercial opportunities such as livestock, crops, and medicines, and an absence of focused efforts on genomics for species that are priorities for biodiversity conservation perspective.

Conservation initiatives featuring genomics in the marine environment are nascent but show tremendous promise. The lag is largely due to the lack of foundational research and the limited molecular tools available to ocean ecologists. In addition, while sequencing costs continue to plummet, it is expensive and difficult to mimic marine systems in laboratory settings. Therefore, there is both a great need and a great opportunity to make investments in and contributions to building the genetic libraries and molecular tool kits used to understand microevolutionary processes driving durability and sustainability of ocean life.

Advances in genomics can transform ocean conservation by providing genomic insight, but to do so requires foundational work— biobanking, sequencing, and investigating the genetic origins of traits. Any solution set deploying genetic interventions for facilitated adaptation, controlling

problem species, or engineering resilience will rely on the deeper understanding of the functional genomics of the target species.

Chapter Two reviews the genomic tools that are useful and in development for conservation purposes. These can be applied to ocean conservation and management to better understand taxonomic relationships to inform management decisions; to identify the potential genomic vulnerability and or resilience to changing ocean conditions; and, to drive innovation (such as synthetic replacement of ocean products) to directly abate threats to ocean organisms and ecosystems. The goal of using these tools should not be to document the loss and decline of ocean species, but to “turn the tide” on loss and extinction. The primary opportunity of genomics is to develop tools that address threats with a much higher level of precision than conventional tools will ever be able to achieve. The most current genomic applications to address marine conservation are presented in Chapter Three.

At scale implementation of a Marine Genetic Rescue Toolkit will require a new era of interdisciplinary collaboration and coordination within the research, management, and conservation communities. The goal of genetic rescue is to build a platform for 21st century ocean conservation. Now we have the opportunity to apply biotechnology tools to address some of the most intractable problems in ocean conservation: overfishing, invasive species, biodiversity loss, habitat destruction, and climate change. Chapter 5 highlights some “Big Ideas” that would address a pressing conservation need and that would significantly advance conservation genomics in a marine setting.

Fundamental to the implementation of genomic solutions is a careful consideration and engagement on the ethical, social and regulatory elements required. These considerations are addressed in Chapter Three of this report.

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Chapter 2: Genetic Rescue for 21st-Century Conservation

Genomic technology has the potential to advance and complement conventional conservation management with precise genetic rescue. The tools of genetic rescue are adaptable and may be appropriate to address specific threats to marine ecosystems as well as provide powerful new monitoring insights. Different threats and ecological settings will likely be more amenable to intervention than others. Also, the extent to which different genomic tools will be appropriate, or even transformative, will vary across taxa and ecosystems. Factors for consideration include the nature of the conservation threat, the availability of genomic resources and technologies, the social and ethical implications of intervention, and the likelihood of success.

Most conservation focused genetic approaches have been limited to assessing very basic evolutionary processes such as gene flow, neutral genetic diversity, and effective population size, with little meaningful understanding of how genetic variation relates specifically to adaptation. Given the affordability of sequencing and the increasing prevalence of conservation threats potentially suited to genetic approaches, there is an opportunity to advance a suite of innovative new tools.

The Genetic Rescue Toolkit

Genetic rescue spans a continuum from “insight to intervention,” and can provide critical understanding of evolutionary processes that can better inform species conservation in a rapidly changing world. Genomics can document biodiversity, inform conventional management practices, and in some cases, enable genomic interventions.

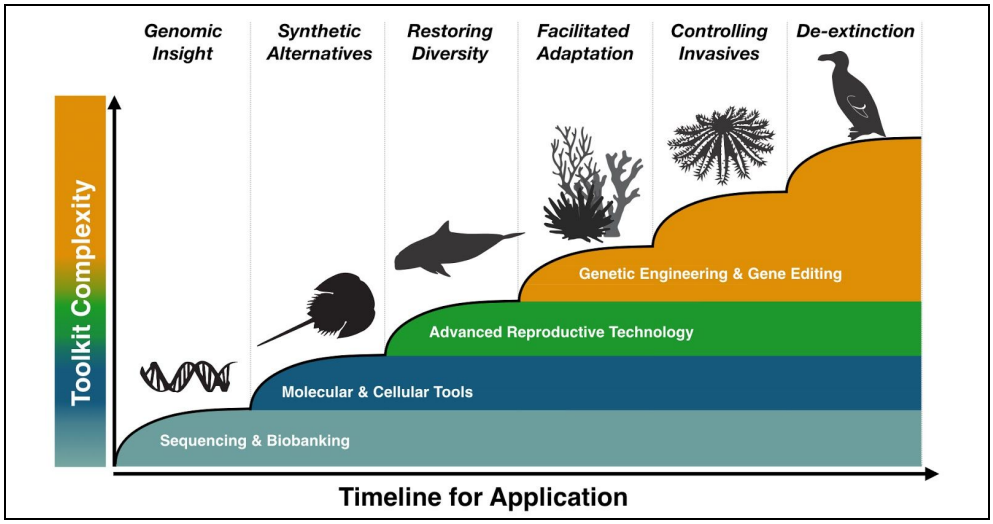

FIGURE 1 — The Genetic Rescue Toolkit.

INSIGHT TO INTERVENTION: THE GENETIC RESCUE CONTINUUM

Genetic rescue spans a continuum from “insight to intervention,” and can provide critical understanding of evolutionary processes that can better inform species conservation in a rapidly changing world. Genomics can document biodiversity, inform conventional management practices, and in some cases, enable genomic interventions (Figure 1).

Genetic insight has a relatively long history in conservation research; its use vital in providing information delineating species and species hybridization, evaluating loss of genetic diversity through population bottlenecks, and estimating the genetic diversity of healthy individuals to be translocated to a struggling population in order to reverse inbreeding depression.

However, traditional genetic markers enabled only a very peripheral understanding of the relationship between genetic diversity and population fitness. Recent advances in our ability to rapidly generate high volumes of genomic sequence data at a low cost is revolutionizing the study and use of whole genomes for non-model organisms like threatened wildlife. This capacity is vastly improving our understanding of both evolutionary processes and the genetic basis for adaptation and resilience, and carries the potential to inform management of imperiled species.

Documenting and Preserving Extant Biodiversity

Preserving biodiversity through bio-banking is an important strategy to both document and preserve genetic diversity. Bio-banking is the collection and preservation of living and frozen samples of biological materials. Somatic and germ line cells can be cryopreserved and potentially used in research and cloning programs. A variety of tissue and cell types can be preserved through culture and cryogenic methods, and as advanced reproductive technologies develop, bio-banked cells may be used for direct interventions, such as augmenting diversity to rescue shrinking populations. A growing number of organizations are growing collections of

cryopreserved tissue and cell line samples, including the San Diego Frozen Zoo with over 1,000 taxa preserved, the American Museum of Natural History, the Frozen Ark, Northeastern University's Ocean Genomics Legacy, and the for-profit company Cryogenetics (which has a special focus on commercial fisheries). However, cell culture techniques and reproductive technologies capable of harnessing these preserved tissues to rescue species requires extensive laboratory validation that is highly taxon-specific and underdeveloped for most oceanic species.

Bio-banking is a companion practice to comprehensive and targeted sequencing initiatives. As can be seen in the glossary, there are many genomics sequencing techniques that can be used for conservation purposes. The field of genomics forms the foundation for genetic insight and intervention applications; however, any use of genomics research for conservation begins with one building block: a reference genome. While there are many genome initiatives, they have accumulated primarily the genomes of non-endangered species, meaning there is a lack of available reference genomes for conservation purposes. Without high quality reference genomes, genetic studies for conservation are limited to older, much less informative sequencing techniques, and therefore have fewer conservation applications. A number of challenges need to be overcome to generate reference genomes for managed and threatened species, but with more sequencing, the full potential of genetic rescue innovations can be developed.

Environmental DNA (eDNA), provides marine conservation with an extraordinary new tool to document life and diversity in the ocean. Simply by sampling the environment, researchers can rapidly assess species diversity in a variety of habitats, as well as document the presence of focal species. This is a potentially transformative development in enhancing our ability to document marine biodiversity. In marine settings, rare or elusive species can be difficult and costly to monitor using conventional methods, challenging our ability to understand habitat requirements and estimate population size.

Metabarcoding approaches enable the characterization of entire ecological communities without prior knowledge of species likely to be present. This application of eDNA monitoring contributes a powerful genomic tool for conservation that will continue to improve as the technologies and bioinformatic abilities are developed. A large-scale program centered around cataloguing biodiversity in different habitats and locations (*Highlight 1a* can provide critical baseline information and identify critically imperiled habitats and may eventually allow for population level inferences of target taxa without the need for invasive or labor-intensive sampling. Notably, as invasive species expand into new marine environments, early detection can be difficult. eDNA enables a rapid assessment of the expanding presence of an invasive species which can greatly aid in controlling its spread (*Highlight 1b*).

The level of resolution provided by eDNA data is only as good as the database of reference genes and genomes used to sort and allocate sequence identities. The application of eDNA monitoring to specific conservation goals will require genomic references for target species. The processing of eDNA data comprehensively demands significant computational power, especially with highly diverse and large data sets. Narrowing the focus of questions to be addressed with

eDNA can decrease the references needed for an effective database and reduce the processing burden.

COMPARATIVE “OMICS”

Localized populations or groups can be compared across the genome, transcriptome, and proteome to determine the genetic basis of adaptation to local environments and responses to environmental stimuli. Such analyses can guide the formulation, execution, and monitoring of species management planning through enhanced understanding of adaptive and demographic processes related to landscapes- or “Landscape Genomics” (*Highlight 2a*).

These insights have been useful in guiding connectivity planning for fragmented populations, and identifying instances where populations have extremely low genetic diversity and require genetic rescue. These insights are also extremely important in conservation planning in response to climate change (*Highlight 2b*) and for understanding organismal response to disease and stress (*Highlight 2c*). Collectively, genomics tools applied appropriately can uncover insights on populations with various levels of suitable adaptations providing an opportunity to manage populations with greater precision and intent. Such information can ultimately transform conservation practice from a “one size fits all” to more targeted restoration and management.

GENETIC ENGINEERING

Genomic “intervention,” in contrast to “insights,” involves the direct manipulation of individual organisms’ and populations’ genes or genetic variants to enhance population fitness and improve viability. Such manipulations include introducing/deleting beneficial/harmful genetic variation either through genome-editing techniques (*Highlight 3a*) or the physical movement of individuals between populations, altering the reproductive success of particular species or variants, and harnessing gene editing and reproductive cloning technologies to rescue critically endangered species (*Highlight 3b*). In the case of genetic engineering and gene editing, such approaches rely upon advanced reproductive technologies, which are still poorly known for many taxa. Ultimately, genomic interventions will require not only adequate and robust genomic resources, but also stringent planning and program development that is specific to the target and thorough engagement with a multitude of technological and ethical challenges.

SYNTHETIC ALTERNATIVES

Biotech and synthetic molecular biology can also provide synthetic alternatives to harvesting wildlife for products in high demand. Alternatives include the “green agriculturalization” of wildlife food species, exemplified by AquaBounty’s terrestrial aquaculture of genetically engineered Atlantic salmon (*Salmo salar*) (*Highlight 4a*). Also in development are synthesized bioproducts for plant-based seafood products that mimic the consumer experience of seafood (plant-based) or genuine seafood products manufactured through cell culture rather than from whole animals (clean seafood). These strategies present a great opportunity to address the ecological and economic crisis associated with current seafood production systems (both wild-caught and farmed) in a highly scalable and sustainable manner. Many over-exploited species are harvested

for reasons other than direct human consumption, including biomedical applications. Synthetic alternatives for such products have been developed but not widely adopted (*Highlight 4b*), demonstrating that social forces can contribute strongly to the adoption and widespread application of genomic and biotech intervention.

HIGHLIGHTED CASES

Cataloguing Biodiversity through eDNA (*Highlight 1A*): CALeDNA is a program run by the University of California Conservation Genomics Consortium. Its mission is to catalogue and archive biodiversity across California to generate baseline data and to prioritize areas for conservation. It is a collaborative program to understand how biodiversity in California relates to habitat and land use. A rapid and highly accurate pipeline for species identification from eDNA sequence data has been developed and participants have direct access to the data (Curd et al., 2018). A similar program directed at cataloguing marine biodiversity across ocean ecosystems and documenting changes as a result of human activity over time could be pivotal to marine conservation science.

Invasive Species Mapping (*Highlight 1B*): The U.S. Geological Survey has invested substantially in developing eDNA-based surveillance methods to enhance management of invasive species in freshwater and terrestrial systems. One program that highlights the advantages, progress, and potential of eDNA as an invasive species monitoring tool is in the detection of Burmese pythons (*Python bivittatus*) and other non-native snakes that threaten native species such as wading birds, the endangered Florida Panther (*Puma concolor coryi*), and the endangered Florida Key deer (*Odocoileus virginianus clavium*). Traditional survey methods have proved inefficient or inadequate. Determining the true range limits for the invasive species will guide management efforts targeted at surveilling the invasion front, modeling habitat suitability, measuring impact and predicting risk to critical habitat and affected species, and targeting sites for removal efforts prior to ecological and economic impacts.

Less research has been directed at applying these techniques to invasive species monitoring in ocean environments. Implementing similar strategies for ocean ecosystems will depend on targeted research to address empirical elements of study design like detection rates and persistence of eDNA in the ocean environment.

Landscape Genomics of Endangered Tortoise to inform Development Strategies (*Highlight 2A*): Researchers at the University of California, Los Angeles conducted a landscape genomics study of the endangered Mojave Desert tortoise (*Gopherus agassizii*) in response to the Desert Renewable Energy Conservation Plan (DRECP). Tissue samples from across the species range were consolidated and used to generate a DNA dataset consisting of full genomes of 270 tortoises. Analysis enabled researchers to determine how the environment has determined modern patterns of relatedness and genetic diversity across the landscape (Schafer et al., 2017). This work strongly indicates that several well-defined genetic groups exist within the species. Combined with desert tortoise habitat modeling data, researchers predicted the relative impacts of five proposed development alternatives within the DRECP. Each scenario was ranked with respect to the likely effects on gene flow and connectivity. This study highlights the superior

insights that can be drawn from whole genome data to characterize population units and develop strategic plans to maintain population connectivity amidst rapid development.

Climate-Related Genomic Variation Can Predict Survival Of Migrating Songbirds (*Highlight 2B*): As local climate regimes shift, previously well-adapted populations can find themselves suddenly out of place, and in the absence of adequate genetic variation, populations will decline. For example, Bay et al. (2018) identified genetic variation correlated with climate factors in yellow warblers (*Setophaga petechia*). The study showed the greatest declines in populations needing the greatest shift in allele frequencies to be adaptive to new climate regimes. The study demonstrates how combining genomic, environmental, and demographic data can help predict population dynamics under climate change. Similar research in ocean taxa that are amenable to translocation, such as coral, may provide a strong basis for guiding translocation or genome editing approaches.

Transcriptome Analyses Reveal Link Between Rodenticide and Increased Disease (*Highlight 2C*): Transcriptome sequencing enables complete characterization of genomic pathways that are regulated in response to a specific stressor, such as toxicants or disease. Globally, carnivores and other predatory species are chronically exposed to anticoagulant rodenticides (AR) through the consumption of exposed rodents. Secondary poisoning can be lethal, but can also lead to reduced fitness from sublethal effects. Bobcats (*Lynx rufus*) in the Los Angeles area have suffered severe population declines from a type of mange that has never before been documented as causing widespread mortality in a wild cat. Extensive toxicant screening discovered a strong link between AR exposure and mange. New regulations implemented in 2014 still allowed for the use of first-generation rodenticides by consumers and for use of second-generation compounds by licensed professionals.

Recent analysis of the transcriptional changes in exposed versus unexposed bobcats provided the first plausible link between AR exposure and increased susceptibility to mange disease (Fraser et al., 2018). This research is being presented as evidence in a legal dispute to accelerate a ban of these substances from use entirely in California. Further transcriptomics research may disentangle impacts and could provide the concrete evidence needed to ban this family of pesticides.

Facilitated Adaptation to Disease in the American Chestnut (*Highlight 3A*): The American chestnut (*Castanea dentata*) is perhaps the best developed example of successful genomic intervention in a wild species. The American chestnut, once the most abundant tree along the Appalachian Mountain chain, was decimated by fungal blight introduced in contaminated ornamental Asian Chestnut trees. Scientists at the College of Environmental Science and Forestry (ESF) in Syracuse, New York have produced genetically engineered American chestnut trees that resist the blight infections that drove the tree to functional extinction (Zhang et al., 2013). The team discovered that the blight infection spreads toxic oxalic acid under the tree's bark, which eventually causes death. Immunity was achieved by inserting a single gene from wheat, oxalate oxidase, which neutralizes the oxalic acid and allows the tree to live with the blight, into a new strain of American chestnut trees (Zhang et al. 2013). This sort of intervention

may hold promise to facilitate adaptation to other diseases and to potentially create resilience to the effects of climate change.

Genetic Rescue and Advanced Breeding Strategies (*Highlight 3B*): Advanced reproductive technologies are an important tool for genetic rescue. Increasingly, scientists are able to transfer genomic material from one species directly into a surrogate, enabling the production of individuals that otherwise could not be born. Such technologies promise to improve endangered species conservation outcomes, particularly when paired with gene-editing techniques, but will require substantial investment and planning for each target and species.

More conventional technologies include *in vitro* fertilization (IVF) and somatic cell nuclear transfer (aka cloning), which require gametes (spermatozoa and oocytes) from the target species. Two newer methods exist in the absence of living oocyte donors for mammals: interspecies somatic cell nuclear transfer (iSCNT) and stem cell embryogenesis (SCE). iSCNT has been demonstrated to successfully produce surviving offspring of several wild felids (Gómez et al., 2009) and endangered gray wolves (Kim et al., 2007). There must be cellular and reproductive compatibility between the donor and surrogate species to generate viable embryos and pregnancies. Comparative genomics and epigenomics should be used to determine the best surrogate. The black-footed ferret is the subject of a restoration strategy centered around this technology (Wisely et al., 2015).

The revolutionary potential of SCE technology is that it can reduce the reproductive resources needed to yield offspring. A skin cell is first transformed into a stem cell (called an induced pluripotent stem cell, or iPSC). Once a stem cell, it can be reprogrammed to develop into sperm or egg cells. Once sperm and egg cells are made scientists can apply IVF to generate an embryo, implant the embryo into a surrogate mother, and give birth to a new unique individual. This process is currently being pioneered to save the Northern White Rhinoceros (*Ceratotherium simum cottoni*) from extinction. Currently, SCE has only been achieved in mice and stem cell technologies overall are most advanced in mammals.

iSCNT and SCE are not yet viable options for reproducing endangered birds. The only technology that allows the cryopreservation and reproduction of cells in birds is germline transfer/transmission (GTT) using primordial germ cells (PGCs), which was developed in 2006 (van de Lavoir et al. 2006). GTT requires the collection of cells from the target species. Like iSCNT, once donor cells are collected they can be transferred and transmitted through a surrogate species reproductive system. Research teams in 2012 transmitted domestic chicken PGCs through a male guinea fowl using this technique (van de Lavoir et al. 2012). While the technique of GTT could be universal for all birds, PGCs have only been successfully cultured for the domestic chicken, which currently prevents the technique from being applied for conservation of wild species.

The major complication for reproductive technologies is that every species' reproductive biology is unique, even among very closely related species. Substantial investment in genomic resource generation, cell culture conditions, and gamete/embryonic transfer techniques is required as these must be adapted for surrogate and donor species. Additionally, such endeavors require

extensive planning to specify short- and long-term goals, and ways by which success will be measured.

Genetically Enhanced Aquaculture in Atlantic Salmon *(Highlight 4A)***:** Land-based aquaculture offers an alternative production pathway to wild harvesting. However, aquaculture is limited to species suitable for captive breeding and rearing and is resource intensive. Salmon is one of the most consumed fish in the world, and the second most consumed fish in the U.S., making it an attractive species for aquaculture. Atlantic salmon is a docile species that has proven well suited to aquaculture, while larger Pacific salmon have not. In 1989, scientists inserted Pacific salmon growth hormone gene into Atlantic salmon, alongside an expression regulating gene from ocean pout (Zoarces americanus), to improve Atlantic salmon growth (Fletcher et al. 2004). The result was both increased growth rate and food conversion, producing salmon that reach market size in half the time of their wild counterparts and requiring less food intake per kilogram of meat produced. This dual improvement greatly reduces the resources needed for aquaculture. Put into production by the company AquaBounty, this strain, branded AquaAdvantage® Salmon, is the most sustainable and environmentally friendly consumer fish meat. But the benefits of AquaAdvantage® Salmon will be slow to reduce wild harvests due to consumer politics. While the product was approved for public sale and consumption by the U.S. and Canada in 2015, it did not reach markets until 2017 when the first batch was sold in Canada – making it the first genetically engineered animal food product to reach market in the world (Waltz 2017). Due to socio-political obstacles, including several grocery store chains' refusal to sell GMO fish products, the food has yet to reach U.S. markets; though, it is expected to do so in 2019 (Martin 2018).

Reducing Harvest of Horseshoe Crabs With A Synthetic Alternative *(Highlight 4B)***:** Horseshoe crabs have been integral to the safe production of injectable vaccines, drugs, and certain medical devices. In the United States, hundreds of thousands of the American horseshoe crab (*Limulus polyphemus*) are captured and bled alive every year for this purpose. Compounds in horseshoe crab blood effectively and efficiently detect bacterial contamination; the reaction between these compounds and contaminants is the basis of the biomedical test, Limulus Amebocyte Lysate (LAL). A synthetic alternative – recombinant factor C (rFC) – was developed approximately 15 years ago by two scientists at the Nation University of Singapore, Ling Ding Jeak and Bow Ho. But the test has not achieved sufficient market penetration to decrease the industry's reliance on the LAL by any significant amount. This highlight demonstrates that even with technologies in place, social and economic forces can hinder the value of technologies to conservation.

HOW TO ACCELERATE THE USE OF GENOMICS IN CONSERVATION?

Access to a reference genome of the target species: The cost of sequencing has dropped so radically that development of reference genomes should be a priority. Furthermore, conservation priorities should be a leading factor in the generation of reference genomes. However, conservation genomics implicates potential conflicts with international treaties like the Convention on International Trade in Endangered Species (CITES) and the Nagoya Protocol to

the Convention on Biological Diversity (CBD). Concerted efforts to standardize policies and ease scientific access to specimens are necessary to make real progress.

Create more alignment between academic agenda and conservation: There is a lag in the publication of genomic resources because researchers are incentivized in academia to guard their results until the broader study can be published. A genome sequence is no longer a publishable finding, so academic interests aren't rewarded for making sequence data publicly available in a timely way. An excellent example of this is in avian genomics: the B10K initiative reported the sequencing and assembly of 300 bird species on July 5, 2017, but only 130 species were publicly available on GenBank at the end of 2018, most of which are not part of the B10k initiative.

Address the bioinformatic bottleneck: Data generation is outpacing our ability to analyze the data and draw meaningful inferences. Private funding to hire experts specifically charged with developing software/pipelines to answer specific questions toward conservation is needed. Ecologists are not computer scientists – more interdisciplinary efforts are needed to turn genomic data into conservation application.

Social and regulatory concerns: Guiding conventional conservation measures with new insights from genomics presents few to no social or regulatory barriers. However, there are many valid concerns regarding genetic interventions that require authentic engagement. Endangered plants and animals are regulated and managed by the U.S. Fish and Wildlife Service, meaning that with current Food and Drug Administration rulings on gene editing, all gene editing interventions for conservation within U.S. territories will require coordinated oversight by both agencies. There are considerations to streamline genetic engineering/editing policies, offering hope for clear regulatory pathways in the near future. Systematic engagement between conservationists, scientists, politicians, and public stakeholders is necessary for both shaping future policies and following through with the deployment of genetic interventions.

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Chapter 3: Who Decides

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INTRODUCTION

Other sections of this Horizon Scan focus on what *could* be possible if genomic technologies were applied to the myriad of challenges facing marine conservation. Here the focus is on "who decides" if, when, and how these technologies might be developed and used.

The spectrum of biotechnologies that could be developed for conservation applications present a diverse spectrum of ethical, social, and legal challenges and opportunities. For instance, genomic insight for the management of fisheries is a precise and relatively uncontroversial tool to understand the population dynamics of a fishery. In contrast, genome engineering that limits the reproductive potential of an invasive species is controversial because of the intervention in a natural evolutionary process and may have far-reaching and unintended consequences. No matter the level of intervention, responsible innovation will require that potential benefits and risks be weighed with objectivity alongside thoughtful consideration of ethical concerns.

It is an essential question to ask: when should humankind intervene in a natural evolutionary process. Oceans are shared environments, and technological interventions should be developed in a manner that reflects the values and needs of the communities that depend upon and are in relationship with shared marine ecosystems.

Biotechnology developed for genetic interventions should be applied only with widespread multi-stakeholder engagement. This outreach process should take place early enough to allow the project design to be modified with the benefit of feedback from regulators, insurers, industry, and civil society. Questions pertaining to the purposes, methods, and potential implications of any potential intervention should be asked at the very earliest stages of research, with the intention to integrate the environmental, economic, health, safety, security, ethical, and governance aspects of biotechnologies into the design of the project.

Genetic engineering solutions for wildlife are both scientifically and ethically different in many ways from agricultural and medical applications, making it difficult to transfer conversations from the USDA and FDA to wildlife applications. Fundamentally, nature and natural systems are held in the public trust as a shared asset. This represents a wholly different ethical context from which to consider the ramifications of genetic interventions. Being cognizant of these long-standing regulatory frameworks is necessary as new paradigms are evolving in parallel with potential genomic applications in conservation. Therefore, there is an opportunity to be deliberate and systematic in how we develop these systems to serve both the public and ecosystems.

Ideally, any genomic intervention in marine settings will be shaped by a multi-tiered international regulatory system that attempts to systematically balance the potential benefits of innovation against associated risks. Therefore, early engagement of diverse disciplines (ethicists, scientists, conservationists, regulators and policy makers) and stakeholders across civil society must play a role in evaluating genomics as a solution set for ocean conservation. With objective and inclusive engagement and stringent risk assessment, it should be possible to develop safe and appropriate genetic solutions that turn the tide on our damaged oceans.

CHALLENGES OF GENETIC INTERVENTIONS FOR WILDLIFE CONSERVATION

Genetic engineering solutions for wildlife are both scientifically and ethically different in many ways from agricultural and medical applications, making it difficult to transfer conversations from the USDA and FDA to wildlife applications. Current regulatory policies aim to minimize the risk of environmental harm from the release or human consumption of a GMO. In contrast, for conservation efforts, the goal will be to have a large persistent beneficial impact on the environment, while also minimizing any negative impacts. This should be obvious, but the difference in desired outcome will necessarily require changes to the regulatory approval framework, as well as new methods for assessing long-term environmental impacts.

The specter of “unintended consequences” is a valid reaction to the abundant evidence of non-native invasive species that when released (intentionally or unintentionally) took over whole ecosystems, disrupted food chains and other ecological functions, or completely displaced critically important native species. However, modern conservation practice has developed norms and practices of planned biocontrol, re-introductions and translocations. It is necessary to distinguish between well-planned and intentional releases and those of the past that were poorly thought out. Also, genetic interventions may require additional inquiry into the ecological fitness of the modified organism in a specific ecological setting.

The release of any organism into the environment, whether to suppress, restore, or transform a wild species population, will likely create ripple effects through an ecosystem. For example, use of a genetically engineered mosquito to suppress its counterparts in the wild population could have negative impacts on the bird and fish species that depend on either mosquito adult or larvae, respectively, as a food source. Ecological risk assessments (ERAs) can be used to estimate the probability that an introduced organism would have adverse effects on non-targeted components (this includes other species) in a specific environment. To be useful for conservation applications, they will need to estimate positive environmental impacts, as well. The methods used to construct ERAs will also need to be updated so they can accurately predict ecological effects over increased spatial and temporal scales. The expertise of ecologists will be integral to successful genetic rescue projects; impacted food networks and the relational nature of species-species interactions and dependencies within those networks must be fully resolved to ensure a genetic technology fulfills its function to improve ecosystem health.

Another consideration unique to environmental applications of GMOs is the assessment of alternative approaches. Current policy compares new GMO technologies to existing equivalents to determine if they will be substantially equivalent or better. These considerations are usually part of the “alternatives analysis” required during the permitting process. In conservation applications for wild release, the evaluation of viable alternative methods should occur before the pursuit of a genetic intervention is even considered. In particular, more conventional conservation strategies should be thoroughly evaluated prior to the pursuit of genetic interventions.

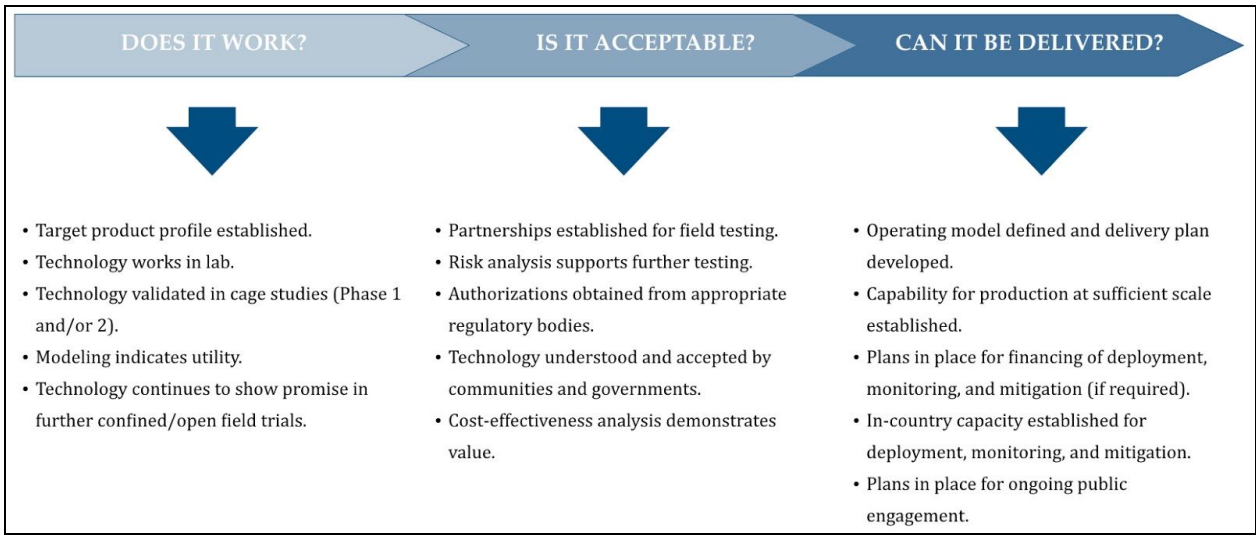

FIGURE 9 — The elements of the critical path for developing and deploying genetically modified organisms (World Health Organization 2014).

A number of U.S. government agencies and conservation NGOs should be involved in the drafting of our national policy and regulation, including the USFWS, EPA, and the Council on Environmental Quality. These agencies currently lack a clear framework for considering new genetic rescue tools. Overly specific regulations may undermine the customization necessary for implementing genetic solutions for diverse conservation applications, while overly vague policies will leave projects in limbo. A systematic framework for evaluating a wide variety of new genetic technologies for environmental applications will be needed to measure the safety and efficacy of these potentially impactful technologies (see Figure 9).

Planners should carefully consider which stakeholders to include and how they could influence the progress of a project.

Broad Stakeholder Engagement: An opportunity to integrate values into project design from the beginning.

Building public trust and engaging feedback will be essential elements of conservation efforts involving genomic technologies. These processes will depend upon relationship-building and should be built on procedures that earn the respect and trust of the public. Authentically incorporating stakeholders into the decision-making process requires the design of mechanisms for input, which in turn may necessitate transparency in decision-making. Responsible

development of genetic rescue strategies will therefore depend upon an exploration of the underlying values that are motivating the project and shaping its design. For example, how decision-makers relate to their natural environment will form the ethical justification for many of these interventions. Whether the ecosystem under consideration is perceived to have utilitarian value (i.e. valued for its benefits to humans) versus intrinsic value (value is independent of human benefit) could impact the resources and care available to save that ecosystem (Batavia and Nelson 2017).

Similarly, the value that decision-makers place on technology will have an enormous impact on outcomes. Some will see technology as something separate from nature and thus want to limit human technological intervention in “natural” processes. Others will be more open to using genetic strategies to conserve and restore nature. Therefore, it will also be important to explore alternative approaches and their respective ethical responses. Comparing and contrasting alternative approaches may allow decision-makers and the public to understand that deciding *not* to use a genetic technology may have equally, if not larger, negative consequences for marine ecosystems. During multi-stakeholder engagement activities, it will be important to openly explore these values and provide neutral space that allows for listening and reflection. Engaging diverse perspectives and value-systems may help create a path forward that is both fair and effective.

GMO foods and labeling provide a cautionary example of why transparent open dialogue with the public is so important. Despite the near universal acceptance of GMO foods as safe by the scientific community, most people in the general public consider them to be less healthy, or even dangerous to consume than “organic” or “natural” foods. Common GMO misconceptions are that they contain chemicals, that they are made with pesticides, or that they will cause allergic reactions or even mutations. Many scientists believe that a better-informed public and more genetic education would alleviate public pushback. But in fact, new research on cultural cognition suggests that distrust of technology or even scientific consensus on issues like climate change can correlate with high degrees of scientific literacy (Kahan 2014). Other ethical and social dimensions beyond safety may also inform public opinion, including anti-corporate sentiment and social justice issues.

Thus, it will be important to inform and engage the public, not only so the technology is clearly understood, but also so that the public may have to opportunity to convey the value systems that shape their perceptions of a proposed technology. Each of the marine conservation issues described in the following sections provides a potential opportunity to plan, develop and test mechanisms for coupling the science and technology with ethical and social perspectives and regulatory frameworks.

Suggested Engagement Principles for Considering Genomic intervention for Conservation:

1. Weighing Risks and Benefits: Decision makers should conduct a thorough review of both the intended outcomes and the potential benefits of a genomic intervention and the

potential harm and downstream risks. Questions to consider include: Have other, more established interventions been tried and failed? Do other interventions, such as the application of antibiotics or pesticides, have potentially worse environmental harms? Is it the most efficacious, lasting, *and* least risky solution to an environmental problem, not merely an equivalent or novel approach?

2. Transparency: Decision makers should proactively identify and inform key stakeholders in the early stages of technology development. This includes partners from the private sector (particularly biotech firms), social science experts, public sector, international organizations, research organizations, religious and ethical organizations, NGOs, and local communities. This should involve objective discussions with the public about the risks and potential impacts of proposed environmental solutions and also subjective discussions about values.
3. Procedures: Decision makers should conduct systematic and data-driven reviews of recommended best practices. These should include surveys of recent intentional environmental releases of organisms (biocontrol, re-introductions and translocations) into natural environments and studies of the resulting long-term effects.
4. Tests: Decision makers should first field-test genomic technologies in contained environments to minimize unintended environmental harm. These should be simple enough to be cost-effective, yet complex enough to sufficiently mimic natural ecosystems to yield useful data on the efficacy of developing technologies. Suitable test environments will be especially important for marine environments.
5. Predictions: Decision makers should employ computational models, particularly as they grow more sophisticated with time, to predict the long-term effects on ecosystems and highlight potential failures of specific applications of genetic technologies.
6. Measures: Decision makers should employ standard metrics for measuring safety and efficacy at appropriate environmental scales, so that direct comparisons of data sets can be made and the universal analytic tools be developed.
7. Protocols: Decision makers should understand the regulatory approval processes and jurisdictions that currently guide the transition of new technologies from the lab to field trials, and should be prepared to help guide new policies when needed.
8. Public-Private Partnerships: Decision makers should collaborate with philanthropists, NGOs, agencies, and community groups in the implementation of these technologies to evaluate risks, to address the controversies, and to gain critical stakeholder support. These sectors can convene conversations, commission studies, identify priorities, and connect the emerging technology with civil society to identify safeguards.
9. Remediation: Decision makers should prepare for the possibility of failure. In the event of unintended consequences, will it be possible to regain control of the organism? Studied possibilities for this include the creation of gene drives with “off switches.”
10. Learnings: Decision makers should continuously monitor the introduced organism and its environment once the technology has been deployed. Because these technologies remain new, decision makers should proactively share their findings and invite

researchers to learn from their successes or failures. Ultimately, this transparency will allow successful intervention techniques to be more rapidly adopted globally.

REGULATION OF BIOTECHNOLOGY

Background: The invention of recombinant DNA technology in the 1970s led the United States (U.S) and other countries to take an active role in regulating the research, development, and release of genetically-modified biological products (Berg et al. 1975). Determining a biotechnology's risk and whether it's safe for the public and the environment currently is primarily the task of federal regulatory agencies. The U.S., Australia, and the European Union (E.U.), all have regulatory frameworks largely centered on methods of containment, that address genetically modified organisms. Therefore, applications that aim for the intentional release of genetically altered organisms into uncontained, wild ecosystems place strain on existing frameworks.

In the U.S., no single agency is responsible for overseeing genetically engineered organisms. The Coordinated Framework for Regulation of Biotechnology governs regulatory policy; approval falls to either the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), or the Department of Agricultural (USDA), depending on the intended function of the GMO (U.S. Office of Science and Technology Policy 1986). In contrast, Australia has attempted to centralize GMO oversight, in part through the establishment of its Office of the Gene Technology Regulator, which alongside appropriate government agencies and under the Gene Technology Act 2000 would oversee contained field trial studies, as well as eventual intended release of any genetically modified organism (Australian Academy of Science 2017). Regulation procedures and outcomes of novel biotechnologies intended for marine conservation are likely to vary significantly between nations. We discuss the international regulatory climate later on in this chapter.

Challenge / Opportunity: Current U.S. policy focuses on the products under review, rather than on the methods that were used to create the product. Therefore, regulation of new gene editing techniques and gene drives intended for environmental conservation will fall under existing U.S. biotechnology policy.

However, recent attempts by British technology company Oxitec to gain U.S. regulatory approval for field trial assessment of their proprietary genetically engineered mosquito to suppress wild vectors of dengue fever and Zika underscores a need for better interagency coordination between U.S. regulatory agencies (Meghani and Kuzma 2018), as well as more robust public engagement procedures (Neuhaus and Caplan 2017). With significant omissions in both the draft risk assessment and preliminary "Findings of No Significant Impact," the FDA should make significant changes to its risk evaluation protocols to allow for a broader study of possible ecological impacts and to survey public constituencies to determine which values should shape the risk assessment of genetically engineered organisms (Meghani and Kuzma 2018). By mandating that the agency must engage the normative concerns of the public, future risk assessments will be less likely than current protocols to advance the biotechnology industry's

interests without the public's scrutiny and consent. This transparency would create a more democratic risk assessment process that controls for unacknowledged and unchallenged biases, which would result in more rigorous risk assessments. Attempts are currently underway to modernize U.S. agency procedures to address novel genetic technologies.

Traditional genetic engineering and the new techniques of precise gene editing may end up following different regulatory paths. For example, it looks as though the U.S. may not even regulate organisms that have been subjected to gene editing if the resultant genomic changes are indistinguishable from mutations that could have occurred through traditional breeding strategies. In contrast, the E.U. has decided to regulate gene edited organisms, no matter the level of changes made, as genetically modified organisms. Thus, any proposed genetically engineered organism will face an evolving regulatory path depending on the nation, the organism being altered, the alterations made, and its intended function. Such uncertainty makes planned engagement and comprehensive consideration more difficult. Additional foresight is needed to avoid ambiguity and administer relevant regulatory frameworks for conservation applications.

Control and Regulation of Research Involving Genetically Modified Organisms (GMOs)

Most universities and research institutes have special committees that are responsible for approving experiments involving genetic engineering. Some experiments, like contained field trials also need permission from national regulators. The development of genetic technologies specifically involving endangered species could also fall under the jurisdiction of the USFWS and the Endangered Species Act. It remains to be seen if the USFWS will develop policy that can specifically address novel genetic technologies for species conservation, invasive species control and/or restoration of threatened species.

To facilitate the rapid pace of research and innovation, most countries now have administrative exemptions for GMOs that only pose a low risk, including standard laboratory model organisms and organisms with no pathogenic impacts on humans. Work is underway to assess whether existing research regulations are appropriate for emerging conservation biotechnologies. The U.S. National Academies of Science recently reported that current laboratory containment protocols and biosafety procedures for contained field trials are adequate (with some modifications) to safeguard gene drive research (National Academies of Sciences, Engineering, and Medicine 2016).

However, containment in marine settings are objectively more challenging since creating impenetrable, fully sealed environs for field trial studies will be difficult to achieve. This "shared" nature of the marine realm raises the bar on innovation to accommodate these biosafety concerns. Risk mitigation will be critical to ensure safe research. For example, gene drive researchers are developing reversible gene drives and genetic "off-switches" that could be employed in the case of unintended release or escape. Remediation protocols—whether for gene drive expressing rodents or genetically engineered microbes for carbon-sequestration—should be an integral component of any project's design.

INTERNATIONAL REGULATORY COORDINATION

International regulation of genetically modified organisms varies widely from country to country. The ocean is a shared global resource without clear boundaries, which will make coordinated international regulation critical. For the synergistic global threats that are impacting these shared marine habitats, genetic interventions may provide innovative targeted responses. Therefore, it is worth the time and effort to forge new alliances to consider these tools.

Several international treaties and policies exist for the regulation of migratory wildlife and marine environments that are likely to impact global governance of novel marine biotechnologies:

- The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), 1973, is an international agreement to ensure that the trade in wild animals and plants does not threaten their survival.
- The objectives of the 1992 Convention on the Conservation of Biological Diversity (CBD) were the conservation of biological diversity, sustainable use, and the fair and equitable sharing of the benefits arising out of the use of genetic resources, particularly those of marine environments. The Cartagena Protocol, discussed in more detail below, is a particularly relevant portion of this agreement.
- The Nagoya Protocol was a supplemental agreement to the CBD that aimed to implement the fair and equitable sharing of benefits of genetic resources. These and other existing relationships can serve as a foundation or model for the international collaborations that will need to be pursued before genetic rescue efforts are attempted in ocean environments.

There have been recent proposals for improved global oversight of genetically engineered organisms designed to spread in the wild (Kofler et al. 2018). The International Union for the Conservation of Nature (IUCN) is in the process of developing policy that can address genetic engineering of wild species, including genetic rescue of marine ecosystems. However, at this point in time, global governance would largely fall to the UN Convention of Biological Diversity (CBD), which under its Cartagena Protocol oversees transboundary transport of living modified organisms. The 198 signatory parties to the CBD are in the process of updating the Cartagena protocol to specifically address genetic strategies for environmental conservation, including gene drives. Given that genetic interventions in marine settings present a specific set of potential risks, there is merit in building dedicated teams to explore these possibilities. Negotiations are likely to take considerable time, on the scale of 2-6 years, due to the polarized debate surrounding gene drive technology. In the meantime, soft governance proposals that call for responsible innovation (Stilgo 2013), self-imposed ethical frameworks for technologists (Emerson et al. 2017), engagement of impacted communities (Najjar et al. 2017), and multi-stakeholder inputs (Jasanoff and Hurlbut 2018) are likely to be the main structures that serve to safeguard genetic conservation technologies at the international level and hopefully ensure benefits are realized.

CONCLUSION

The innovative field of genomic technologies for ocean conservation has huge potential with diverse and innovative strategies that provide targeted responses to difficult and urgent needs. With these technologies still developing, responsible development necessitates engaging diverse viewpoints and expertise to steer the course. Policy developments often lag behind technology innovation. However, it's not too soon to anticipate high potential applications for genomic technologies in ocean conservation and begin the necessary preliminary discourse now. The goal should be to iteratively craft a set of guidelines that clearly communicate the social and ethical priorities of all affected societies, and articulate the full range of standards that genetic interventions must meet to be considered for environmental release. Such a framework would open the door to many new, powerful, and potentially less intrusive approaches to long-term environmental restoration and health. However, functional and effective guidelines need to be created through an iterative process. Therefore, regulatory and governance frameworks will be developed and tested in concert with the technologies through projects and case studies.

The hopes and concerns of scientists, the public, and regulators must always be taken into consideration. Balanced deliberation that engages multiple stakeholders will help to ensure genomic interventions develop with thoughtful safeguards and monitoring.

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Chapter 4: Threats & Solutions

The Horizon Scan identifies promising applications of genomics for addressing marine conservation challenges, current technologies and leaders in the field, and gaps in our knowledge. Conservation initiatives using genomics to address these issues are nascent, but show tremendous potential to compliment more conventional conservation strategies. Revive & Restore organized the Horizon Scan according to the threats to marine systems in keeping with the conventional deployment of conservation strategies.

Modern genomics can be applied to ocean conservation and management in order to better inform management decisions; to identify the potential genomic vulnerability and or resilience to changing ocean conditions; and to directly abate threats to ocean organisms and ecosystems. Despite the potential for these innovations, there is a lag due to a lack of foundational research in biobanking, sequencing, and investigating the genetic origins of traits, as well as the limited molecular tools available to ocean ecologists. Numerous researchers interviewed also lamented the disconnect between academia and conservation. Therefore, there is a great need and a great opportunity to make investments in and contributions to building the genetic libraries and molecular tool kits used to understand evolutionary processes driving ocean life.

Climate Change

Climate-related changes in ocean conditions are already having profound impacts on marine ecosystems. The warming ocean and melting polar ice is already causing sea levels to rise and may result in profound shifts in marine currents, bringing widespread impacts. Harmful algal blooms and other marine diseases appear to worsen significantly in warmer water. Meanwhile, the sea sequesters global carbon dioxide, making the ocean more acidic. Already, acidification effects have been documented; cracked and dissolving shells have been observed in pteropods, the tiny free-floating mollusks that represent the base of many marine food chains. Many of the physical and chemical changes in ocean conditions appear to exacerbate other anthropomorphic impacts to the integrity of marine ecosystems. Two critically important marine ecosystems, coral reefs and kelp beds, have been especially hard hit. Kelp beds and coral reefs provide essential ecosystem services for coastal communities. Since these habitats rely on living organisms to create the structures that provide diverse niches that drive biodiversity, they were focal ecosystems in the Horizon Scan.

CORAL REEFS

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EXPERT INTERVIEWS:

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Corals are the foundation of subtropical shallow water reefs, which provide habitat for 25 percent of all marine species. In favorable conditions, coral reefs expand as net accretion lays down new substrate for more organisms to thrive. Today there are up to 1 million square kilometers of coral reefs protecting 150,000 kilometers of coastline (Burk et al. 2011), servicing 500 million people who rely on them for food, coastal protection, and jobs in fisheries, tourism and marine recreation (Wilkinson 2004).

Coral reefs provide an estimated $10 trillion of ecosystem services annually (Costanza et al. 2014), but the impact of human development and increased ocean temperatures are causing frequent and widespread global coral bleaching events and die-offs. Reefs are declining at an average rate of 1 - 2.5 percent per year, and in some areas the rate of decline is considerably greater (Heron et al. 2017).

Fifty percent of the planet's corals have been lost already, according to the most recent estimates, and around 90 percent will be gone by 2050 (Rohwer and Youle 2010; Burke et al. 2011). While there is no global plan to deal with the failure of biotic reef infrastructure and maintain coral reefs beyond 2050, coral reef restoration and management techniques are in ever-increasing demand. This section reviews two broad categories of coral restoration and management techniques that have the potential to help conserve and repopulate coral reefs: coral production and assisted breeding techniques, and coral resilience and augmentation techniques.

Primary goals for coral conservation and restoration are ensuring that the unique coral genotypes remaining on reefs (otherwise referred to as genets) are preserved *ex situ*, conserved *in situ*, and restored at degraded reef sites. The techniques described in the following pages highlight seven of the most important technological approaches for achieving these goals through combinations of functional genomics, assisted migration, assisted reproduction, genetic engineering, epigenetic engineering, stress conditioning and microbial manipulation. A number of scientists interviewed for this Ocean Genomic Horizon Scan are more open-minded about the use of genomic and biotechnology-oriented solutions for coral bleaching than for any other marine threat, primarily because of the severity, scope, and immediacy of the problem.

THREAT

As mature coral populations continue to die-off due to a combination of stressors impacting reefs around the world, coral reproductive capabilities are diminishing, as documented by ongoing reports of declining sperm motility on bleached and bleaching reefs. The first reports of declining sperm motility surfaced many years ago and appear to have worsened with time. Simply put, not only are there fewer corals today, there are fewer healthy sperm and eggs to sample and preserve, and there will be even fewer in the years to come. With dying populations and declining fertility, affected corals do not have a chance to adapt and therefore are on the road to extinction.

As corals become further endangered or even extinct in the wild, cryopreserved coral samples will serve as enormously valuable resources for both scientific discovery and restoration efforts. It is critical that the genotypes that currently remain on reefs are preserved in long-term and redundant storage to ensure that they may be used as parental stock in future coral breeding and recovery programs. Moreover, these samples must be taken as soon as possible to maximize the chance of obtaining the coral germplasm in what is likely to be its healthiest state. However, at the present time, coral cryopreservation activities are enormously limited in scope: only a tiny fraction of reefs around the world have been sampled, and there is very little experience or knowledge in the field outside of the few laboratories that developed coral cryopreservation techniques.

INNOVATION

Cryopreservation serves as an extremely reliable insurance policy for coral biotechnology because it allows coral cells to be put on ice until later breakthroughs can be applied to corals for research or restoration purposes. While egg cryopreservation remains challenging, significant advances have made freezing coral sperm and larvae. Coral sperm cryopreservation techniques are now ready for low-throughput operation (Hagedorn et al. 2007), and near-ready for scale up and high-throughput operation in the field.

The Coral Restoration Consortium Genetics Working Group has concluded that capturing only four unique coral genotypes per reef type, along an environmental gradient, will generally be enough to capture more than 50 percent of the common alleles in a population (where 10 genotypes = 90 percent of the alleles, and 20 genotypes = ~100 percent) (Baums et al. 2018). Given that current methods allow for sperm to be reasonably captured from 10 to 20 individuals per night, such genotype capture rates are feasible and effective, even if they are low-throughput. Additionally, mechanical systems are being prototyped to collect more material, thereby boosting the potential for effective scaling of cryopreservation activities.

Coral larvae cryopreservation is a particularly innovative method that allows larvae to be preserved shortly after the point of conception. The availability of frozen larvae is a step forward for coral researchers because it frees them from relying on annual natural spawning. With frozen larvae, researchers can work with coral larvae on a weekly basis, which has speed up research

to grow and achieve coral settlement by a factor of fifty or more (Hagedorn et al. 2018a). Larval cryopreservation is currently being augmented with microfluidics to create a high-throughput method for preservation, capable of producing hundreds of thousands of larvae in a few hours. This method creates a pathway for using these conservation practices for large-scale restoration.

Significant innovations in coral fragment cryopreservation are likely to emerge in the coming months or years. These breakthroughs will provide the means for genotypes on reefs to be rapidly sampled and banked in a simpler manner, without the need to capture sperm and eggs. They could also potentially capture gravid colonies for rapid use in later breeding programs, as they could potentially be revived and begin producing eggs. Most critically, teams would be able to cryopreserve coral fragments all year round, instead of relying on annual spawning events.

Finally, cryopreservation can prevent species extinctions. It is the only science today that can provide this type of reef biosecurity.

LEADERS

The Hagedorn group at the Smithsonian Institution has established genetic banks for coral throughout the world, successfully freezing coral sperm and coral larvae. The banks have been used for assisted gene flow and have demonstrated that cryopreserved sperm can be used for selected breeding, hybridization, and other applications. Together with Dr. Kristen Marhaver at Caribbean Research and Management of Biodiversity (CARMABI), Dr. Mary Hagedorn's assisted gene follow experiments have demonstrated the successful use of cryopreserved sperm to fertilize and develop 4,700 juvenile Elkhorn corals (*Acropora palmate*), the largest living wildlife population ever created with cryopreserved material. (Hagedorn et al. 2018b).

The largest collection of frozen coral cells is at Taronga Conservation Society's "Frozen Zoo", which has enough frozen material to generate approximately 200 million coral colonies (Mary Hagedorn, personal communication). This program will expand enormously for the Great Barrier Reef in the next few years. Biobanking will play an important role within the large coral restoration and adaptation program that is about to be launched in Australia this year, and proposals for scaling up these facilities are currently being put forward for funding. MingDao University has also demonstrated successful sperm cryopreservation in China (Viakam et al. 2018), but it is not clear to what extent cryopreservation efforts are proceeding at scale in the region at this time.

RISKS AND CHALLENGES

A key technical challenge facing cryopreservation groups is the inability to preserve coral eggs. At this time, only coral sperm and larvae have been successfully frozen by the groups mentioned above. Frozen sperm still represents a highly valuable resource that may be used to bank alleles and genotypes on a reef at the present time. But without a supply of frozen ova, cryopreserved sperm must be paired with fresh ova, sourced from a living reef, live bank (aquaria), or nursery – or potentially from thawed gravid fragments. The challenge for the preservation of coral eggs is relatively steep and not inline with any mammalian species. Moreover, there are only a few

mammalian species, such as humans, where egg cryopreservation is successful. Oocyte cryopreservation is very difficult. The challenges for coral eggs is that they are very large, filled with lipids, and only viable for a few days each year for a few hours. These technical difficulties will require advanced techniques, such as laser warming.

There is also a need to improve methods for freezing coral sperm. Current methods require highly concentrated sperm (not easy to obtain in open water), and are limited in throughput. Novel antifreeze proteins with low toxicity to coral cells are required. Experiments are necessary to identify appropriate freezing concentrations, fertilization concentrations, and sperm-egg ratios for each coral species. To put it another way, there is no one-size-freezes-all method for sperm cryopreservation. These challenges are being tackled one-by-one by a handful of scientists, and a concerted effort by the broader coral scientific community appears to be forming. However, the collaborations are largely ad hoc thus far. The cryopreservation community would benefit from administrative and operational support as it grows.

Cryopreservation capabilities and expertise is very limited among the current generation of reef biologists. Experts posit that training programs should be conducted as part of regional cryopreservation sub-programs. Such training programs would create local capacity for cryopreservation techniques for local species and conservation program staffers and would bank viable material during training.

Despite advances in throughput offered by mechanical cryopreservation systems, there is a major need for more practitioners of such methods because many corals spawn only once a year. This is the rate-limiting step. As such, it is not practical to have a single team operating year-round at many sites; instead, many teams should operate simultaneously at many sites. To sample many reefs, a “coral army” needs to be recruited, trained, managed, and provided with equipment and funds.

The dynamics of cryopreservation activities could change significantly once coral tissue fragments are shown to be reliably frozen and revived. Cryopreservation could then be conducted year-round, rather than only during annual spawning events. Currently, there are significant burdens on cryopreservation practitioners who must stay on station at reefs for up to 16 consecutive days, over multiple consecutive months, to sample germplasm and rapidly conduct larval crosses. Improving and scaling up methods for parallel larval crosses would be useful as well, as there is presently no established system for executing dozens of parallel gamete crosses and rinsing all material quickly to prevent polyspermy. Improved fertilization systems and methods are needed to produce larvae for cryopreservation on a parent-by-parent basis rather than in a single mixed-batch format and to increase capacity for replicated experiments on the fertilization process itself. A significant advance would be the identification of optimal fertilization and rinsing methods for as many species as possible and the development of larger-scale crossing and rearing systems. These improvements will considerable time and coordination as they must currently be conducted during the period when corals are spawning in the wild.

It is not clear if existing storage locations at Taronga and the USDA's National Animal Germplasm Program will be able to scale their storage to meet the needs of the coral community in general. At this time it appears that there is no single dedicated coral cryopreservation facility available, nor is there a network that can coordinate the redundant sampling and banking required to provide long-term confidence that corals are banked in perpetuity. However, there are proposals around this within the reef restoration and adaptation program in Australia. The exact quantum of funding and resources is yet to be determined, and additional funding to match the government funds will be necessary. Regardless, this will likely be the single largest investment towards biobanking of coral material to date. Resolving the need for additional resources will require an understanding of the willingness among different stakeholders to engage and invest in cryopreservation capabilities at the present time, and to understand what the decision-gates are for unlocking funds.

Regulatory challenges complicate coral cryopreservation. The Convention on the International Trade of Endangered Species (CITES) and the Nagoya Protocol require extensive licensing and permitting to ensure corals can be legally sampled, transported across national borders, and legitimately used in research. These treaties have mandated all signatories to erect complex legislative barriers, which translate to significant legal and administrative overhead. Given that cryopreservation groups are likely to be operating across international borders from only a handful of source countries in the near term, additional support to cryopreservation groups must be provisioned in order to deal with the regulatory complexity associated with this activity.

A final challenge is that some States may even be reluctant to support public-facing cryopreservation programs for fear of sending a message that the responsible government is admitting failure in their efforts of conservation and environmental stewardship. This issue has been largely unreported in the literature, but poses a serious challenge to global conservation of coral genetic diversity.

STEM CELL TECHNOLOGY DEVELOPMENT TOWARDS CELL THERAPY

Stem cells are specialist progenitor cells that can give rise to a wide variety of other cells and are fundamental to natural selection and evolution. Stem cells have two important properties: self-renewal, the ability to undergo numerous cycles of cell division without aging, and potency, the capacity to differentiate into specialized cells and give rise to individual tissues or organisms. Stem cells derived from adult tissues can be kept in culture and used for experimentation, or be used to clone and propagate an individual organism without the use of sexual reproduction, thereby serving as a useful and versatile new tool for restoration programs and R&D.

The availability of stem cell lineages in mammals has provided a foundation for research into the mechanisms by which cells differentiate and specialize, and has also supported the development of cell therapies and regenerative applications involving stem cell transplantation. At a recent Coral Experts Meeting the isolation and growth of coral stem cells was identified as the number-one priority for achieving genetic resilience in corals. Presently, no such stem cell capability exists in coral biology, and no stem cell lineages (or cell lines) are available.

INNOVATION

Putative stem cells have recently been isolated from corals, using mechanical dissociation of soft tissues from their skeletons, and subsequent fluorescent activated cell sorting (FACS) separated cell populations using different fluorescent dyes and tags (Rosental et al. 2017).

Recent work has demonstrated the value of “coral tissue plugs” in which a recipient coral from one reef will receive a donor plug from another reef and subsequently exhibited improved and health (Glynn et al. 2006). The convergence of these two areas of research are likely to give rise to a stem cell therapy approach for corals, for which there is technical precedent in the human medical sciences. The ability to manipulate coral stem cells and reconstitute colonies also opens up the possibility of cryopreservation of coral genotypes from adult tissues, a powerful complement to gamete cryopreservation (given the complexity of sourcing gametes) and a particularly important tool for coral species whose gametes cannot be collected. An incoming generation of coral stem cell experiments and the development of protocols to isolate stem cells from wild coral tissues could lay the groundwork for coral stem cell applications to rescue dying corals both in the lab and on the reef.

Given the outcomes of coral tissue plug transplantation, coral stem cells derived from novel strains may be propagated in a continuous manner in bioproduction facilities to support industrial-scale cell culture and subsequent transplantation into living corals, or otherwise used to farm stem cells into larvae and colonies using more traditional methods. The availability of coral stem cells would also open the door to more robust genome engineering projects by providing a source of otherwise-scarce individual coral cells. Researchers would then be able to edit corals at the single cell stage and more reliably avoid the issue of somatic mosaicism associated with later-stage genome editing, where some cells in the organism are edited and others remain unedited, typically confounding experiments.

The scalability of coral stem cell division could be leveraged to provide a continuous source of cells for larval rearing and outplanting, and thus serve as a critical biotechnology tool for extending and speeding assisted migration of particular genotypes. By pairing stem cells with genome engineering (already established in corals), stem cells could be modified to serve as a cellular vehicle to deliver genetic elements for heat resilience into coral reefs. Standard techniques for stem cell isolation from corals are likely to enable many other essential technology developments that can be leveraged.

LEADERS

Dr. Nikki Traylor-Knowles at the University of Miami and Dr. Ben Rosental at Ben Gurion University are currently proposing research to further characterize and purify putative stem cells refine methods for their isolation; investigate their functionality and location in the holobiont using already developed cell tracking and transplantation methods; develop methods for in vitro stem cell propagation; and demonstrate the feasibility of stem cell transplantation as a therapeutic approach for saving corals.

Funding proposals are emerging from other consortia (Ventura et al. 2018; Shapiro et al. 2017), citing the potential to develop coral stem cell technologies alongside mechanical and microfluidic systems that would allow researchers to study the effects of thousands of diverse signaling factor combinations on cell viability, differentiation, and regeneration in a high-throughput fashion. The current proposals represent critical next steps for understanding and manipulating corals at molecular and ecological scales.

RISKS AND CHALLENGES

Identifying totipotency – the ability of a cell to become any other – in coral stem cells may prove challenging. This risk is compounded by a divergence of cell surface markers between coral species, meaning that the successful identification of stem cells could require challenging experimentation and fine-tuning of protocols on a species-by-species basis.

It is also unclear how effective the use of stem cell therapy for restoration will be. Success will generally depend on the precise way in which stem cells are to be used in each application, as well as the desired scale and production capacity of each group. It is unclear how amenable coral stem cells are to large-scale bioproduction, which may impact their longer term prospects. Regardless of the scale, high volume generation of coral stem cells would be subject to the constant challenges of bioproduction, including the need for round-the-clock management, disease control, and continuous optimization of growth conditions with changing scale and cell density per unit. Moreover, industrial-scale production is a long way off for a community only just beginning to evaluate the potential for coral stem cells.

There remain unanswered questions about the actual deployment of stem cells on a wild reef. Permission for stem cell transplantation, even without the use of genetic engineering, will likely require lengthy examination by public institutions, as there is no precedent for this in conservation science or biotechnology enterprise to date. On the one hand, the use of stem cells could be acceptable if genetic engineering is not used, as the cells would be from wild stock with beneficial and “natural” traits. On the other hand, stem cells or products containing them may be considered to be products of clinical relevance and be subject to a complex set of laws and regulations concerning production process, product characterisation, and dosing. Legal and regulatory perspectives will vary between States.

It appears that the more immediate scope for change emerging from coral stem cell technology is relegated to the lab, rather than on the reef. In the short term, the most salient challenge is to demonstrate the basics of coral stem cell isolation. In the medium term, the challenge is to assemble operating stem cell hardware-software-wetware systems that can meet real experimental objectives – in other words, the living and automated tools needed to manipulate coral stem cells with precision. This will be a great challenge for the coral community itself, requiring assistance from or field entry by engineers and biomedical experts. At the present time no one has yet put all of these components together into a working platform that fully ties together stem cells, microfluidics and FACS protocols for corals. While there have been promising innovations that partially bring elements of this platform together, such as the coral-on-a-chip system (Shapiro et al. 2017), these have not been used for significant R&D

beyond the originator lab. While the benefits of stem cells as laboratory tools is clear, the long-term challenge will be to demonstrate that stem cell therapies are an effective and scalable tool for coral restoration, before addressing any relevant regulatory systems to govern their use in the wild.

INDUCIBLE CAPTIVE SPAWNING

THREAT

To date, corals are almost entirely sourced using three approaches: directly collecting adult corals from the wild; collecting eggs, sperm, or larvae from remote reefs during seasonal broadcast spawning events; or by fragmenting adult corals already held in captivity (Craggs et al. 2016). These three approaches cannot be universally applied to all corals and are unsustainable in the face of the overwhelming loss of planet's reefs. Compounding these issues is the problem that corals cannot be easily bred in laboratory or aquarium settings without highly specialized equipment and training. Ultimately these issues translate to a major problem for coral researchers and conservationists – there is no truly reliable or convenient way to source corals or their larvae.

INNOVATION

Emerging inducible spawning methods allow operators to source new corals in a laboratory environment. The technique makes use of an accurate simulation of corals' natural environments in aquaria, modelling the photoperiod, seasonal insolation, lunar cycles and seasonal sea surface temperatures found in nature. This is coupled with attention to traditional coral husbandry techniques developed and honed in the aquarium and hobbyist trade.

The methods have been successful in inducing the captive broadcast spawning of at least 18 Acroporid species in a completely closed environment. Twenty-nine to 100 percent of colonies were shown to produce sperm and oocytes (egg cells) (Craggs et al. 2016).

Coral eggs generated from inducible spawning have since been fertilized with sperm of corals of the same species via IVF procedures, demonstrating that the technique can be used to generate offspring corals with parents from different locations that would not meet in nature. Inducible spawning thus provides a reliable source of eggs that could be paired with a coral sperm banking program to support a large breeding program focused on developing novel diversity.

Inducible spawning is a promising technique that can play a key role in assisted gene flow work, providing opportunities for coral farms or institutions distant from coral reefs to produce large numbers of coral larvae. Larvae and corals reared in captive spawning programs could be used to nurture juvenile corals for deployment in restoration programs and culturing experiments, to generate genetically diverse coral variants not found in nature, and to provide a source of germplasm that could be used for functional genomics experiments about the basis of coral biology. In particular, it is possible that the technique could be applied to corals that have

survived stressor events in the wild, allowing new strains of thermally-tolerant corals to be produced independent of natural and precarious reef ecosystems.

The inducible spawning approach is also highly amenable to parallelized batch production of coral larvae. One vision for an inducible spawning facility is to support 365 different coral tanks for a particular coral species, such that the facility could provide regional larvae for experimentation and restoration on a daily basis (Jamie Craggs, personal communication).

LEADERS

The Project Coral team headed by Jamie Craggs at the Horniman Museum Aquarium in London is the leading group in inducible spawning and has pioneered the technique over the last decade. The group has established collaborations with Florida Aquarium and the California Academy of Sciences, both of which are now scaling up the approach for use in additional species. The team in Florida, led by Keri O'Neil, is focused on scaling up coral production for restoration activities in the Gulf, Atlantic and Caribbean. The Cal Academy team led by Dr. Rebecca Albright is focused on ocean acidification research.

The Australian Institute of Marine Science is also developing inducible spawning techniques at their SeaSIM facilities, and beginning in mid-2019, the Reef Restoration and Adaptation Program will be working on this challenge as well.

RISKS AND CHALLENGES

The Project Coral team has noted the challenge in training new recruits and collaborators on their methodology, which can take at least as long as a single reproductive cycle (at least 1 year). To date there are little to no public training resources, and the technique must be learned tacitly, on location, or over great distances using 1-on-1 support. Inducible spawning remains a niche technique for the time being, and its nascent state and limited momentum is severely debilitating the propagation of skills to achieve critical mass. At the present time, there are very few people in the world who are adept at this technique, even in the laboratories that have developed or adapted the technology, of which there are three.

There is substantial interest from those working at the interface of coral physiology and genetic engineering, as well as from restoration practitioners. Meeting that demand is a major challenge.

There is an opportunity to expand the number of facilities operating programs like Project Coral, especially for species where inducible spawning protocols have yet to be established, and traditional propagation has failed to yield results. One vision would see regional associations of stakeholders such as public aquariums, commercial coral suppliers and wholesalers, and restoration facilities, collaborating to develop protocols for local species, and also to exchange germplasm for outbreeding programs in the longer term. However, such an association would require careful planning, funding and even political management, each of which will present their own complications.

Finally there is some question of the true scalability of an inducible spawning program. The technique is understood to take at least one year to perfect with a novel coral, even for an experienced person, and there are substantial material and time costs to operating a specialized inducible spawning system. Moreover, larger aquaria are needed, putting it out of reach of hobbyists and those without such facilities. It also remains to be seen if aquariums, wholesalers, and researchers will be able to form a critical mass of inducible spawning operators that is sufficient to meet the need for coral restoration.

FUNCTIONAL GENOMICS OF CORAL BLEACHING AND ASSISTED EVOLUTION

THREAT

Coral bleaching is a phenomenon involving the expulsion of photosynthetic *Symbiodinium* from coral polyps. *Symbiodinium* species differentially provide host corals with unique ratios of essential services. It is thought that the departure of one or more dominant or co-dominant *Symbiodinium* species during a period of stress leads to the invasion of another more appropriate species that can maintain photosynthesis function and resolve the stress response (Baker et al. 2001). In this way bleaching is typically considered to be a temporary event that lasts only a few days or weeks, and is a healthy coral's reaction to changing conditions.

When the period of stress becomes protracted, bleaching can become chronic and the loss of the algal source of nourishment can cause the polyps to starve and die. The precise mechanism of coral bleaching remains unknown, and is complicated in that the breakdown of the endosymbiotic relationship between corals and *Symbiodinium* can be triggered by a large number of stressors including: high or low temperature, UV radiation, reduced salinity, microbial infection, marine pollutants, and even an absence of light. Taken together, this implies a complex set of genetic circuits involved in the maintenance of this critical relationship between coral and *Symbiodinium,* one that remains largely unknown to this day. The lack of knowledge about basic functional genomics in coral symbiont homeostasis is a major impedance to the development of safe and effective coral reef intervention and restoration strategies.

INNOVATION

While the precise set of genomic mechanisms underlying bleaching is not yet understood, promising hypotheses provide numerous clues. The photo-inhibition model (Jones 2004) posits that high temperature leads to alterations in light capture, light utilization, heat dissipation, and a build-up of reactive oxygen species (ROS), or free radicals. ROS-scavenging enzymes become over-encumbered and denature, and the cellular outcomes are analogous to the mammalian inflammatory response - positive in short bursts, but harmful in chronic conditions as they inappropriately destroy healthy tissues (Palmer et al. 2008). Multiple groups are working on the characterisation of these protein responses in corals, and given the overlap with better-known mammalian responses to physiological stress, a list of gene targets putatively involved in the positive or negative resolution of coral bleaching is being developed (Hetz and Papa 2018; Ruiz-Jones and Palumbit 2017; Barshis et al. 2013).

The availability of lower-cost genome and transcriptome sequencing and analysis techniques has opened the door to conducting population-based assessments, for instance Genome Wide Association Studies (GWAS) on corals that have survived or died in bleaching events. Such work would support the identification of adaptive (and maladaptive) mutations in wild stocks and inform novel hypotheses about genotype-phenotype interactions and bleaching resolution. Moreover, the recent demonstration of the CRISPR genome engineering technique in corals allows researchers to investigate those hypotheses by way of targeted genetic engineering at low-throughput (Cleves et al. 2018) and potentially even high-throughput scales, such that functional and comparative genomics of mounting complexity are possible. Such techniques are crucial to advancing coral genomics to the level already attained with human cells and mouse models, and the therapeutic interventions enabled by advanced genomic knowledge.

LEADERS

A unifying factor to labs engaged in functional genomics investigations is their search for adaptive traits using comparative methods to develop actionable insight into natural selection in corals. Many of these labs employ widely used genomics tools and techniques from human biomedicine and functional genomics. However, the availability and maturity of analogous tools in coral-specific formats has only recently emerged, or has yet to emerge. The coral field must therefore catch up to the more mature human and mammalian genomics field and appropriately adapt its toolkit. Nevertheless, promising work is already emerging.

The Baums lab at the Pennsylvania State University has demonstrated the first steps toward genome-wide association studies (GWAS) by conducting a genome-wide SNP analysis for signatures of natural selection in the threatened Caribbean Elkhorn coral, Acropora palmata (Baums et al. 2017. The team revealed fine-scale population structure and inferred a major physical barrier to gene flow between populations. Scans detected 13 candidate genomic loci under positive selection, however there was no correlation between available environmental parameters and genetic distance. To this end, the team notes a critical barrier facing all coral functional genomics actors - the absence of fine-scale environmental and coral life history data, with which the putative adaptations may be correlated.

The van Oppen lab at Australian Institute of Marine Sciences, and the Gates lab at Hawai'i Institute of Marine Biology are both engaging in functional genomics experiments on corals and their symbionts, as part of more involved projects pioneering assisted evolution and assisted gene flow (van Oppen et al. 2015). The groups have advocated for the potential and eventual use of synthetic biology for use in corals, reflecting a position that promotes the direct genetic engineering of corals and their symbionts. Both groups are primarily concerned with an initial discovery phase focused on exploring the natural diversity of coral functional responses to environmental stimuli. These groups have recognized that functional genomics insight will likely come from nature, rather than from engineering de novo mutations. Indeed, by investigating adaptations in the Indo-Pacific from Australia to Hawaii, the groups are likely to identify a large set of potentially adaptive mutations across a broad geographic range in many coral species and environment types, fueling a promising second phase of engineering.

The Palumbi lab at Stanford uses molecular and physical approaches to understand the ways in which natural environmental parameters can modify coral physiology under changing conditions. In particular, this group is concerned with understanding how complex traits, such as heat resilience, are driven by hundreds of mutations in hundreds of genes, each with small effects that combine to have large outcomes. The group makes use of transcriptomics in corals and their model organisms, exposing wild corals from different environments to varied conditions over short timescales and measuring gene expression to characterize how each gene in each coral phenotype responds to these changes at the gene expression level. To this end, the Palumbi group uses an experimental stress system co-developed with the Barshis group at Old Dominion University. Indeed, the standardization of functional genomics tools across coral research groups could prove particularly useful by making relevant datasets directly comparable. One project underway by the Palumbi group seeks to functionally block gene expression by applying pharmaceutical agents known to disrupt particular genes and pathways, so as to determine the role of each gene. However, in the near term such an approach is likely to provide resolution of genes or pathways involved in adaptation, rather than more desirable knowledge about which mutations and nucleotide sequences are responsible. The latter could however be achieved by pairing this work with rational genome engineering experimentation to parallelize the “testing” of particular mutations in the genes identified.

The Pringle lab at Stanford University, in collaboration with the Bay lab at AIMS, have together demonstrated the use of CRISPR/Cas9 genome editing to functionally disrupt a single gene family in a coral species, marking the promise of an era in which any individual mutations identified as being adaptive (or maladaptive) in wild species could be edited into a coral genome for genetic rescue. Such a capability is in many ways critical to proving that a mutation exerts a causative effect, rather than a more simple correlative association with a trait. This capability may be regarded as the final step in bringing the “reverse genetics toolbox” online in coral, as well as the first step toward synthetic biology and forward genetics approaches, as advocated by the Gates and van Oppen groups. One key challenge is to demonstrate that a larger set of targeted genome editing outcomes can be achieved in more than one coral species such that CRISPR-based insertion, activation and interference options are available. These are all plausible and likely to be easily achieved. Another challenge is to automate the process used to deliver genome editing reagents into individual coral cells, which would support a scalable and high-throughput genome perturbation platform, something which is now common and often considered essential in functional genomics projects. Moreover there is a major need for a stable and productive source of single-celled corals, so that these can be made available to support wider use of genome engineering in coral genomics. These developments may come either from advances in coral stem cell propagation or otherwise from coral spawning technologies.

The Reef Genomics group, funded by the Great Barrier Reef Foundation, coordinated the sequencing and publication of the genomes of nine reef species found across the world, which will form foundations for functional genomics projects and will likely yield other important follow-on benefits as they are published. These genomes are available open access. Indeed, without the availability of coral reference genomes, functional genomics projects must rely on the

use of genomes from closely-related model organisms which do not reflect the complexity of a coral genome (Shinzato et al. 2011). The Reef Genomics collaboration has provided critically-needed funds and direction to these efforts, as well as a repository and focal point for groups to collate data and publish their work. There is an obvious path of convergence between the projects contained within the Reef Genomics group, and trait description projects such as ReefBase and the Coral Traits database.

Note: Several of the labs mentioned above also conduct work related to other topics highlighted in this section, including epigenetics and holobiont manipulation.

RISKS AND CHALLENGES

There are ongoing efforts to establish reference genome sequences for coral species, a key element of any functional genomics project, but the work is slow and often produced using data coming from only a handful of individual organisms via pooled sequencing, or redundant sequencing of a single individual, and is not yet suitable for GWAS to be practical.

Unfortunately there are few completed efforts to obtain whole genome data from individuals of a species living in extreme or marginal environments, which would provide gold-standard insights into how particular genes and intergenic regions are enriched for certain mutations in those environments. Rather, genome sequencing and assembly projects tend to pool sequencing data from multiple individuals from the same species. Reference genomes are typically composites of multiple individuals, and may not be useful for assessing local adaptive variations. However, as pooled data is published and reference genomes become available, this will likely encourage the re-sequencing of multiple individuals without pool-seq, as the reference data structure will serve as a scaffold around which additional genomes can be assembled. Thereafter, the challenge will be to coordinate programs to obtain data from individual coral colonies on a large scale, and to log this data alongside abiotic data, so that correlation and causation patterns can be more easily identified and investigated.

Functional genomics groups often make use of knowledge derived from one species to assess the function of a gene in another. However, in corals the high degree of variability between individual coral species genome structure, as well as a significant number of genes present in corals that do not occur elsewhere in nature, complicates matters. This challenge is further compounded by the fact that a coral is a meta-organism composed of many species, to such an extent that the host coral genome in of itself may not be the only piece of the puzzle – functional genomics work may need to be extended to the bacterial, archaeal, and eukaryotic microbes that inhabit the coral, which will bring challenges of its own (detailed in subsequent sections).

There is currently no standard to measure coral physiological response, and there are few tools or parameters to assess coral health or other biological functions in a reliable manner. Some metrics do exist: wound healing, skeletal growth, bleaching resilience, infectious disease resilience, and reproductive output (Baums et al. 2018), but they are all rudimentary. Additional molecular assay development would be prudent as it would support direct measurements of

particular traits across the coral research community, and dispense with the need to measure indirect indicators of physiological change.

With regard to the bioinformatics challenges in coral research, it is the scale of computational and intellectual work that requires attention. Numerous bioinformaticians will be needed to effectively sequence, assemble and annotate coral genomes, but there are few with the requisite skills to execute these projects in a timely manner within the coral research community itself. A more reasonable approach may see the involvement of dedicated genomics institutes or companies supporting the efforts to speed publication of the reference genomes that are fundamental to so many other elements of functional genomics programs.

Coral genetic engineers will need to be aware of translational synthetic biology, and must ideally practice a philosophy of public engagement as they publish their findings and take the next step toward engineered corals.

EPIGENETIC ADAPTATION AND CONDITIONING

THREAT

Phenotypic plasticity is a ubiquitous phenomenon that is increasingly gaining scientific attention. Corals from environmentally variable locations are generally better able to withstand stress through phenotypic plasticity and have greater adaptive potential compared to those that live in more stable environments (Torda et al. 2017). Most interestingly, there is mounting evidence that corals can adapt to different conditions, such that two genetically identical corals can exhibit different responses to the same conditions, with these adaptations being “learned” over time, as a coral is exposed to a novel environment.

The study of epigenetics is now the dominant research subject in this area, investigating all mechanisms that regulate gene expression. Understanding the epigenetic mechanisms by which corals are able to adapt to their environments by modifying gene expression (rather than by modifying their genomes), could provide a reliable means to intervene in the coral crisis without the need for breeding or genetic engineering. Indeed, the ability to generate “pre-adapted” coral colonies and larvae via epigenetic conditioning could allow the creation of seeding populations that repopulate reefs naturally, without the potential reduction of genetic diversity caused by artificially selecting corals, or the monumental effort required to restore reefs through continuous transplantation of single colonies. Such plasticity of phenotype is giving the coral research and restoration communities hope, but the precise epigenetic mechanisms of adaptation in corals remain poorly understood.

INNOVATION

Evidence of phenotypic plasticity across a range of coral life-history stages and traits is mounting, highlighting significant capacity for corals to respond to altered environmental conditions. It has been shown that some corals can modulate their growth form to optimize light environments for photosynthesizing symbionts (Willis 1985), physiologically acclimate to resist

elevated temperatures (Putnam et al 2016; Palumbi et al 2015), and show signs of acclimation under pH stress (Moya et al 2015).

Corals in the Persian Gulf survive temperatures that are too extreme for coral species elsewhere, while certain coral species naturally tolerate water with lower pH. On the Great Barrier Reef, temperatures vary by several degrees from North to South and large temperature differences also exist within the majority of reefs between habitats and depths. The thermal tolerance of corals generally reflects their local temperature environment, meaning that corals in warmer waters bleach at higher temperatures than those to the cooler waters.

In such cases, the genotype remained the same, but its expression was altered due to changes in epigenetic structure. These examples suggest that corals retain phenotypic plasticity in their adult life stage. The revolution in -omics approaches, in particular ChIP-seq and other means of detecting and measuring epigenetic modifications, provide innovative opportunities for exploring different epigenetic components in coral adaptive responses. Moreover, CRISPR has recently been adapted to artificially modify epigenetic states in a predictable manner, opening the door to meaningful epigenetic experimentation (Enriquez 2016).

LEADERS

The Putnam lab at the University of Rhode Island is a leader in the application of epigenetics to coral acclimatization, and is striving to understand how the abiotic environment and biotic interactions drive organism phenotype, ecological patterning, and evolutionary processes through the interaction of symbiosis, genetics, and epigenetics. The group is presently investigating whether phenotypic variation in coral larvae among different physiographic reef zones is related to genetics over and above epigenetics.

In Australia, researchers at AIMS are currently engaged in a number of experiments to measure epigenetic changes and genetic adaptations, and the conditions under which they can be promoted. Multiple groups are taking advantage of long-term “stress-conditioning” experiments occurring at the National Sea Simulator, which seek to acclimate corals to future thermal and pH conditions, using directed and assisted evolution methods in conjunction with epigenetics approaches. The van Oppen group is using these findings as part of a larger interest in deploying synthetic biology techniques to engineer or breed coral resilience (van Oppen et al. 2015). The AIMS project in general examines how conditioning occurs in corals, under what conditions it can be optimized, and whether it can prepare offspring for further ocean warming and acidification. Projects of this type are notable in that they will provide insight into the potential for coral larvae to be pre-conditioned to an environment, prior to their actual deployment in a reef restoration project. If successful, it could add an additional dimension for improving the resilience of coral reefs in the face of climate change, and potentially other stressors

The Aranda Group at King Abdullah University of Science and Technology (KAUST) in Saudi Arabia has discovered extensive DNA methylation in the genome of a Red Sea Coral, and identified specific patterns of epigenetic marks which emerge when corals are stressed for prolonged periods (Liew et al. 2018). Their research currently focuses on understanding the

exact ways these mechanisms work and to what extent they allow corals to adapt, alongside the impact of microbial changes on these systems.

Note: There are a great many other groups interested in epigenetics of the coral holobiont, rather than the coral host, and the subject is deliberately discussed in more detail in the following section.

RISKS AND CHALLENGES

The mechanisms and impacts of epigenetic adaptation in corals are challenging to segregate from genetic adaptations and other adaptations or parameters in an organism's genome or general life history. This will prove especially challenging given the limited experimental capacity and epigenetic toolkits available to marine biologists. Epigenetic tools will need to be adapted from other areas of the biological sciences, and potentially require input from specialists on a case-by-case basis. Furthermore, it is a distinct possibility that epigenetics will not be widely conserved between coral species.

Epigenetic changes in corals themselves are only one piece of a more complex puzzle, with extreme complexity emerging from the multitude of epigenetic states that may exist across the entire coral holobiont, which may consist of many thousands of distinct organisms (discussed below). Understanding the epigenetic and adaptive capacity of each organism, and understanding the interplay between them requires the application of -omics and molecular approaches within a rigorous experimental framework. Adaptive capacity might be highly variable across different coral species, and a model organism approach may be far more tractable in the short-term, but present additional challenges, namely to do with the extrapolation of one model to all corals.

CHARACTERIZING AND MANIPULATING THE HOLOBIONT

THREAT

Corals transcend our definitions of neatly-packaged species, and are better referred to as "holobionts" - metaorganism communities in which a single "individual" is composed of: the specific host coral animal, one or more species of Symbiodinium, and a collection of bacteria, viruses, fungi, archaea, protists and other microorganisms. The holobiont has been shown to serve as a metabolically complete system, where genes from multiple organisms act together to cycle essential nutrients from one organism to another, in mutually beneficial cycles (Rowher and Youle 2010), that adapt to survive adverse conditions (Abrego et al. 2008). Each coral species plays host to more than 100 unique bacterial species, which occupies a different spatial niche on the coral colony (Rowher and Youle 2010). More than 100 million microbes occupy each square centimeter of coral surface, and they are distinct from the microbes that occupy the water column. Further, the holobiont composition shifts in response to changing environmental conditions (Rowher and Youle 2003). The general states in which coral holobionts exist, form

metabolically complete circuits, undergo changes, and impact an individual coral's ability to resolve stress or injury, remain almost entirely unknown.

INNOVATION

It has been demonstrated that local natural adaptation to distinct reef habitats has occurred in the wild, through natural selection on at least three members of the coral holobiont: the coral host, its Symbiodinium and the tissue-associated bacteria. Omics tools have, in particular, supported the emergence of an innovative field of functional metagenomics in coral holobionts. Engineering the microbiome has been proposed by several groups, but has not yet been widely reported experimentally. Nevertheless, studies have already ranged from examining the preference of corals with particular microbiomes for a particular substrate to guide initial larval settlement, to documenting the ability of coral-associated viruses to augment the coral immune system, and for various surface microorganisms species to engage in nutrient cycling. It is posited that information about the reef microbiome can be used to drive assessments of the suitability of a location for a particular restoration approach and unlock new directions in engineering the coral holobiont itself (*Rowher, personal communication; Peixoto et al. 2017*). Symbiodinium species have in particular, received great attention as they are one of the ubiquitous constituents of the coral microbiome, and could potentially be manipulated to be resilient to disturbed conditions.

LEADERS

The Rohwer-Wegley Kelly group at San Diego State University is a pioneering developer of fundamental microbial reef genomics and metagenomics and was an early contributor to the field. The group is currently using metabolomics to gain insights into the relatedness of unknown coral holobiont molecules based on their molecular fingerprints, and is illuminating how and why specific molecules are created by coral holobionts in response to particular stimuli. Additionally the group has pioneered investigations into the mechanisms by which corals are able to assimilate and express viruses in their external mucous, as part of an immune system that protects them from marine pathogens, and has also identified other immune-relevant interactions between corals and their microbes (Rowher and Youle 2010). The group has further documented significant variation between coral species' microbial communities (Rowher and Youle 2010).

The Marine Microbial Symbiosis (MMS) group, a joint group headed by Dr. Madeleine van Oppen and Dr. Linda Blackall of the University of Melbourne is investigating the fundamentals of coral-microbe symbiosis using experimental evolution, genome editing, and epigenetic alterations via stress conditioning. Symbiodinium physiology, engineering and metagenomics projects are underway by the MMS, as is the application of these methods to other microbial species in the holobiont. There remains a need to identify and characterize organism-specific functions within the holobiont. To this end the team is using metagenomic approaches to determine the microbial community structure and its properties.

The Coral Symbiomics group, run by Dr. Aranda at KAUST, is determining the evolutionary history of the relationships between coral, microbe and symbiont, and characterizing the mechanisms that make them evolutionarily stable by using whole genome sequencing and

comparative multi-omic approaches. The group is analyzing the genes and associated functions and proteins used in the regulation of all aspects of symbiosis. In particular, the group has demonstrated that corals and related organisms share a common core set of thermal stress response pathways for dealing with protein-(mis)folding and reactive oxygen species observed in bleaching and known to be important to the homeostasis of symbionts (Cziesielski et al. 2018). The group has observed enhanced resilience in Red Sea coral holobiont organisms, likely through association with specific strains of Red Sea symbiont not found anywhere else.

The Voolstra Reef Genomics group at KAUST is further exploring the north-south environmental gradients of the Red Sea to gain a greater understanding of why the majority of Red Sea coral is healthy and more heat resilient to stresses versus reefs found in other parts of the world. The group uses a broad range of tools (genetics, genomics, bioinformatics, ecology, microbiology) to study marine ecosystems, specifically how they are structured, how their members interact, and how they are influenced by physical processes. The group uses a true eco-systems biology and environmental genomics approach that explores many scales of organism and environment type. Voolstra's team has shown that bacterial community dynamics are closely associated with, and may even drive, coral heat tolerance (Ziegler et al. 2017).

Dr. Peixoto, currently based at UC Davis, has advanced with her colleagues at UFRJ, Brazil, a concept of system-wide probiotic manipulations as part of a "Beneficial Coral Microorganisms" (BCMs) hypothesis (Peixoto et al. 2017). The BCMs concept is analogous to the Plant Growth Promoting Rhizosphere (PGPR) concept, which has been widely explored in the agricultural industry. PGPR concerns the microorganisms that inhabit the rhizosphere (root-associated niches) of plants, and the ways in which they directly or indirectly promote plant growth and development through the production of regulatory signals, antibiotics and nutrients. Peixoto and her colleagues have proposed similar potential mechanisms of the effects of BCMs on coral holobiont health, and have suggested strategies for the use of this knowledge to manipulate the microbiome, reverse dysbiosis (bleaching and other effects of a breakdown of the symbioses), and restore and protect coral reefs. Currently Dr. Peioxoto's work is directed toward development of BCM consortia as environmental "probiotics" to improve coral resistance or recovery before and after bleaching events, and to develop BCMs as a tool in human-assisted adaption to shifting environmental conditions (Peixoto et al. 2017). The project recently received support from the Great Barrier Reef Foundation (GBRF) under its Out of The Blue Box challenge, and the next step in the research will explore a range of delivery methods to identify the best way to introduce putative BCMs into a coral system. A key challenge will be to develop "broad spectrum" BCMs that can enhance health in many corals, without negatively impacting other marine organisms and corals.

RISKS AND CHALLENGES

The scale of work involved in decoding coral microbiomes is impressive. With over 1000 species of soft and hard coral, there may be as many as 100,000 unique bacterial species directly partnered with Earth's corals, not to mention the non-specialist bacteria and other microorganisms that may be more casually associated with corals. To this end, the concept of

generalist BCMs suitable for multiple coral species has been met with skepticism by some in the coral research community, but is not necessarily infeasible – at least given the current state of research, which is in promising first stages. Indeed, restoration without the manipulation or use of BCMs or microbial manipulations may prove to be a naive approach in the longer term, but this remains speculation. Tension between these two extremes may resolve as more research interest is cultivated in this subject, and findings are published. In other words, it is too early to tell if BCMs will be effective.

More generally, many microorganisms cannot be cultured directly, so researchers must rely on the use of -omics tools and measurement approaches to assess their presence and function. In most cases, coral researchers are generally unfamiliar with microbiome tools, as they are not yet widely available among the coral research community, and have only recently emerged in other branches of biology. Regardless of one's ability to measure the presence of a particular organism, the culture issue could prove problematic – especially if an organism is found to be essential to holobiont health and performance, but cannot be cultured under laboratory conditions or industrially scaled. Other, eukaryotic microorganisms that may be suitable for culture could be very challenging to genetically engineer; Symbiodinium, thought to be the keystone for microbial manipulation, is particularly difficult to "genetically transform," an achievement which has only been reported once.

Evidence is accumulating that although some host-associated microbes might facilitate adaptive responses in corals, there are fitness tradeoffs between each adaptation, which remain unclear at the present time and will prove difficult to characterize in detail. Furthermore, it is not clear whether microbial/holobiont manipulations could prove to harm corals by stimulating, rather than resolving, dysbiosis or how these manipulations may vary in different coral species. Such questions will need to be categorically answered before large-scale use of BCMs and holobiont manipulation is to receive informed consent, which may prove challenging. To this extent there are likely to be considerable regulatory challenges on the road to deploying these techniques in the wild.

ACTIVE CORAL RESTORATION AND TRANSLOCATION

THREAT

It is well-documented that corals genetically adapt to their local conditions (Polato et al. 2010; Barshis et al. 2013; Dixon et al. 2015), and each species is effectively made of sub-populations adapted to diverse environments. Despite the hopeful tone for genetic engineering applications, these technologies are not yet ready for field use in corals, and cannot readily make use of genetic adaptations in a meaningful way (National Academies Consensus Report 2018). Currently, the only practical approach to improving reef resilience is to harness and foster the adaptive genetic diversity that is already present in coral populations by harnessing the power of natural admixture and breeding of coral subspecies and by applying these tools in active reef restoration contexts.

Natural recombination and breeding have given rise to resilient corals in the past, and could give rise to new resilient corals in the future. However declining environmental conditions across most reef sites has destroyed reefs and is reducing connectivity between reef meta-populations. This will likely grow worse over the coming years, minimizing the ability of corals to naturally encounter one another, fertilize one another during breeding events, and subsequently create more resilient subspecies in the wild. As such it falls to conservation and restoration practitioners to actively restore reefs made up of appropriate coral genotypes, so that connectivity gaps between distant reefs may be filled with newly-restored, genetically-robust reefs, and restored reefs can be further invigorated with corals that have been specifically chosen for beneficial adaptive traits and genotypes.

Essentially, the goal of coral translocation, genotype selection, and active reef restoration is to establish self-sustaining populations of sexually reproducing corals that have sufficient genetic variation to adapt to changing environments over time. Such a goal requires integration and application of knowledge concerning adaptive genetic and epigenetic traits in the coral host and larger holobiont, alongside a program focused on physically relocating corals over large distances.

INNOVATION

Coral relocation has already been established as an appropriate restoration technique in select cases, particularly where corals are scheduled for destruction due to human development. However, severe and frequent mortality events have already exerted strong selection pressure on coral populations and have selected for wild alleles that are more likely to spawn a new generation of better-adapted corals (Libro and Vollmer 2016; Muller et al. 2018). Guidelines have been presented to support and standardize human-assisted migration with the aim of re-establishing or bolstering coral populations that are capable of sexual recruitment and genetic exchange of these adaptive wild alleles (Baums et al. 2018).

Current proposals contemplate much-needed enhancements to restoration practices such as routine genotyping of propagated stock, trait-based assessment of genotype performance, jump-starting genetic admixture by producing first-generation offspring in the lab for out-planting, and the deliberate facilitation of longer-range genetic exchange through assisted migration and assisted gene flow. The integration of these advanced technologies to guide translocation is a promising step away from “simply moving” coral reefs, and a step toward long-term ecosystem restoration and revitalization, which makes use of the cutting-edge tools of coral biotechnology and conservation practice.

Physical means of relocating corals en masse is likely to benefit greatly from advances in the use of cryopreservation, as well as the use of larval rearing and freezing programs, which will significantly reduce the volume and complexity of coral relocation efforts.

At the present time there are even early stage proposals to develop large scale “coral arks” that consist of modular sub-arks that can each be submerged to capture the broad range of coral reef diversity onto a fixed structure over an extended period. After an appropriate amount of time the

modules could be re-assembled into a larger ark super-structure which can be towed to submarine sea mounts in cooler waters, with the goal of translocating or seeding entirely novel ecosystems (at the expense of a pre-existing local ecosystem).

LEADERS

The Coral Restoration Consortium (CRC) is the leading community comprised of scientists, managers, coral restoration practitioners, and educators dedicated to enabling coral reef ecosystems to survive the 21st century and beyond. The group collaborates and shares technology and best practices between participants, and facilitates scientific and practical ingenuity needed to demonstrate that restoration can achieve meaningful results at scales relevant to reefs in their roles of protecting coastlines, supporting fisheries, and serving as economic engines for coastal communities. Organizers and advisory board members include experts from the NOAA Coral Reef Restoration Program, NOAA Fisheries Office of Science and Technology, Mote Marine Laboratories, UN Environment-Caribbean Environment Programme, SECORE, The Nature Conservancy, and Seascape Caribbean. The CRC is comprised of seven active CRC Working Groups focused on: Land-Based Propagation, Field-Based Propagation, Larval Propagation, Demonstration Projects, Genetics, Monitoring, and Management. Each working group is chaired by one to four active practitioners and experts in the subfields. The CRC recently established the Reef Futures conference, which will bring together restoration experts, planners, and practitioners on a regular basis. CRC was founded during a 2016 workshop focused on coral restoration specifically in the Caribbean Sea, but since that time has expanded in scope to encompass global coral propagation and restoration.

Coral translocation has proceeded even prior to the formation of the CRC. In Jordan in 2017, authorities proposed a plan to relocate an existing port to a new area towards the southern end of the Gulf of Aqaba (Kotb 2016). As a mitigation measure, a group funded by the United Nations Development Program (UNDP) and the Global Environment Facility (GEV) translocated 7000 endangered coral colonies from Al-Dirreh and transplanted them into degraded reef sites in Aqaba Marine Park. Overall survival rate for transplanted colonies was 87 percent. High survival and linear growth rates indicate that the transplantation site selection and the techniques employed were successful (Kotb 2016), and demonstrate powerful proof-of-principle for large-scale mature coral translocation efforts.

In Australia's Heron Island, a translocation and reproduction trial was conducted in which a large amount of coral spawn and eggs were collected, grown into larvae off-site, and then transplanted onto areas of damaged reef (Dela Cruz and Harrison 2017). After an eight month period, and with the assistance of underwater mesh tanks to reduce predation and ocean current interference, juvenile corals were successfully grown. The Australian experiment made use of high-density coral larvae enclosures, a significant step away from "coral gardening" which requires the use of a dedicated coral nursery site. This has promising implications for large scale translocation of juvenile corals, rather than bulkier mature corals.

RISKS AND CHALLENGES

There are promising precedents for coral translocation, with a good deal of work having been conducted over many decades. As yet, these uses of genetic insight to guide translocation efforts remain untested and unclear, putting the idea of a self-sustaining super-reef into doubt in the short term. Presently our understanding of genotype-phenotype relationships is rudimentary (described above), making it difficult to rationally select corals for use in translocation programs. These current approaches, based on phenotypic traits, are not comprehensive, and at present we do not know the sequence profiles of potentially adaptive alleles in order to successfully (and more easily) identify relocation candidates using DNA sequencing. Further, given the nascent state of coral functional genomics and genome engineering, it is unlikely that the community will have actionable results until functional genomics yield widely agreed upon conclusions. Finally, there are very few reliable biomarkers that can be used to facilitate the identification of candidate corals for translocation or breeding. To this end, coral translocation may be operating in the dark, without reliable molecular guidance to light the path forward for the foreseeable future. Such a dependency is likely to seriously impact the successful outcome of coral translocation in both the near and long term.

The CRC Genetics Working Group has concluded that capturing only 4 unique coral genotypes per reef type, along an environmental gradient, will generally be enough to capture >50 percent of the common alleles in a population (where 10 genotypes = 90 percent of the alleles, and 20 genotypes = ~100 percent) (Baums et al. 2018). However, without a targeted identification and collection system that is capable of easily identifying the most suitable corals, at this time only the most common genotypes are likely to be well-represented by a coral capture and translocation program. As such, it may prove challenging to collect corals that have suitable adaptive traits, until such identification tools or molecular assays are widely available.

Additionally, short-term adaptive responses, which are based on the genetic profile of a first-generation translocated coral, may only be sustained by future generations if the out-planted coral genotypes are able to successfully reproduce and generate novel allele combinations with sustained hybrid vigor over subsequent generations. As such, the long term success of translocation efforts may require ongoing interventions though continued sexual propagation and breeding programmes that continuously augment designer reefs, a prospect that presents major challenges. Furthermore, while it has been proposed that large populations of corals with varied genetic backgrounds could be strategically translocated in patterns to enable the largest amount of admixture and guarantee sexual recombination, there are many environmental parameters (temperature, current, predators etc.) that could easily interfere with successful reproduction.

While the translocation examples provided above were considered successful in their own right, these particular projects did not assess the impact of translocation on shorter term fecundity, nor did they assess long-term reproductive fitness of the translocated corals in historical or new environments. Such documentation has often been neglected due to legislative requirements, or a lack of funding, or site accessibility (Precht 2016).

Building on the above, reliable means to assess a reef’s suitability for restoration and translocation are much needed. There is a particular need for establishing a more

comprehensive and widely used system of biotic and abiotic measures and orders of coral provenance, such that a coral's life history and traits can be more easily logged and communicated. Elements established in the ReefBase global information system for coral reefs, as well as NCBI genomics standards, are useful places to start in this regard.

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KELP

THREAT

Recent dramatic declines in kelp forests globally have been linked to warming oceans caused by a changing climate.

Kelps (Order Laminariales) are brown algae that occupy 43 percent of the world's marine ecoregions living along the coastlines of all continents except Antarctica (Spalding et al. 2012). They are among the highest primary producers in any environment with extraordinarily high rates of growth and productivity. Kelps are vital to a bio-abundant marine ecosystem due to their role as 'foundation' or habitat-forming species, maintaining ecologically diverse communities commonly termed kelp forests. Kelps, and the kelp forest communities that they support, provide a range of critical ecosystem services, including nutrient cycling, biodiversity support, shoreline protection, and fisheries, collectively valued in billions of dollars annually across the globe (Bennet *et al*. 2016). Kelps are also a source of alginates (thickeners used in many products) and are increasingly being viewed as food sources for humans.

Warming ocean temperatures, caused by global climate change, is causing widespread declines in kelp abundance around the world, in locations as diverse as Norway, New Zealand, southern Australia, and more recently, along the west coast of the United States (Krumhansl et al. 2016). As a result, biodiversity and ecosystem services that kelp forests support are diminishing as well.

It is unknown exactly why kelps are unable to recover and rebound from disturbances, specifically during extended periods of unusually warm waters (Reed et al. 2016). What is known is that kelp forest die-offs are not ubiquitous. An analysis of global patterns of kelp forest change conducted by Krumhansl et al in 2016 found significant regional variations, with some populations declining, some increasing, and some maintaining kelp biomass. Specifically, a few locations in Fjordland, New Zealand, Southern California Bight, and Gulf of Alaska showed increases in kelp abundance over the past several decades, although no explanation of this trend can be found in the existing scientific literature. Importantly, this analysis concluded that more monitoring of kelp forests is essential for understanding the scale of the problem and discovering how unique regional characteristics drive kelp forest abundance.

Beyond the impacts of climate change, the near shore and shallow waters in which kelps grow exposes them to a diverse array of human activities that impact the coastal zone, including harvesting, pollution, sedimentation, and recreation. These localized stressors have documented effects altering fish and invertebrate community composition and causing localized declines in kelp abundance. However, these stressors have not, to date, produced the large geographical scale and rapid complete loss of kelp forests that have recently been observed as an ecological effect of climate-induced changing ocean conditions (Steneck et al. 2002).

California Kelp Context

Since 2014, more than 90 percent of the bull kelp canopy area north of San Francisco to the Oregon border has been lost due to a combination of oceanographic and ecological stressors. Concurrently, populations of sea urchins have exploded due to the widespread loss of their primary predator, the sunflower star due to sea star wasting syndrome (SSWS). A global analysis of trends has shown a decline in the aerial extent of kelp in this region (and extending north) since the mid-1970's (Krumhansl et al. 2016). Central California kelp forests have also been depleted from overgrazing by urchins but, not to the same extent. Recent studies of kelp forests in central California also show a pattern of large losses of kelp every winter, due to high wave disturbance, (Reed et al. 2011). Giant kelp (*Macrocystis pyrifera*) in Southern California faces a different suite of potential drivers of change than in northern and central California kelp forests. Historically, poor water quality and sedimentation also have negatively affected kelp in this region although long term trends of kelp biomass have remained stable or shown slight increases (Krumhansl et al. 2016).

PROGRESS TO DATE

Traditional conservation measures (harvest control and protected areas) have had limited, local success in reversing kelp forest declines.

Similar to coral reefs, kelp forest conservation and restoration efforts have been focused on maintaining not just the kelps themselves, but also the complex and diverse communities that comprise kelp forest ecosystems. These ecosystems support a vast array of commercial fisheries as well as important ecosystem services. All of these contribute to the high prioritization of conservation and restoration of kelp systems. While conventional conservation measures, including limiting pollution inputs from land, establishing protected areas, or altering fishery regulations have had some reported localized success, the regional and global outcomes for kelp conservation are not as hopeful (Strain et al. 2015).

Regions such as Western Australia and the west coast of the U.S. have experienced almost a complete extirpation of kelp in a single summer season. The predominant outcome of these events is the transition to what is often referred to as an 'alternative stable state' or "phase change" to sea urchin barrens and/or turf dominated reefs (Filbee-Dexter et al. 2018; Ling et al. 2014; Filbee-Dexter et al. 2014). In these instances, conventional efforts to restore sea urchin barrens back to kelp forest ecosystems have reported very limited and localized success. In locations including Tasmania, Western Australia, and Southern California, more restrictive fishery protections for urchin predators such as lobster, in combination with protected area designations, have resulted in some local kelp forest regrowth (Strain et al. 2015; Kriegisch et al. 2016; Bates et al. 2017). While very limited in geographic scope, traditional restoration approaches (such as kelp re-planting) have also seen some success in encouraging the re-establishment of associated fish and invertebrate communities. However, locally restored kelp forests remain threatened by warming ocean temperatures.

In summary, the approaches dominating kelp forest conservation include classic restoration and resource management/population control measures, implemented almost entirely by local and regional governing agencies. Private sector engagement, where it exists, is led almost entirely by local fishery/harvest interests, and in California, has been motivated by the recognition of ocean acidification as a worrisome stressor on commercial shellfish. New research suggests that primary producers such as kelp and seagrasses may play an important localized role in mitigating acidification effects on nearby shellfish beds.

Driven initially by commercial rather than traditional conservation goals, new research suggest that on both local and global scales kelps and seagrasses may ameliorate the impacts of ocean acidification and further may play a role in mitigating the impacts of greenhouse gas emissions by sequestering and storing carbon (Krause-Jensen et al. 2016; Chung et al. 2011; Duarte et al. 2013). Research in these fields is nascent and frequently limited by the current short duration of observational time series data. Evidence is fast accumulating for the role of seagrasses in ameliorating the impacts of ocean change and this warrants further research to illuminate biotechnology potential. By comparison, as discussed below, kelp contributions to climate change mitigation are most often considered in the context of aquaculture and biofuels production, and thus divorced from their role as foundational species of complex and important ecosystems. (Please see our section on market alternatives for more information on aquaculture and biofuels.)

INNOVATION

Increasing evidence suggests a role for kelps and seagrasses in climate mitigation and in ameliorating impacts of changing ocean conditions. Genomic insights and pilot projects may accelerate progress in this area.

Genomic understanding of kelps is hampered by the sheer size and inherent complexity of kelp genomes. Only a single kelp species has been sequenced to date, but this study, in combination with the transcriptome and metabolome, is providing new insights into the genetic regulation of growth and physiology as well as the impacts of changing temperature or ocean chemistry parameters (Ye et al. 2015; Konotchick et al. 2013).

Currently, genomics is being applied and used in kelp forest conservation primarily as an informational input to evaluate and prioritize among traditional conservation or management options. There is an accumulating body of knowledge revealing genetic differences through the geographic range of giant kelp that indicates adaptation to local environmental conditions (Wernberg et al. 2018). Similarly, genetic data has been used to inform the structure and characteristics of fishery regulations but not to promote production, unlike terrestrial agriculture (Bernatchez et al. 2017).

Similar to approaches used in terrestrial forest conservation, there may be opportunities to develop genetic interventions for other species that maintain the kelp community. For example, a tiered strategy could be implemented that mixes relatively labor intensive manual control approaches (such as the urchin harvest days sponsored by the California Department of Fish

and Wildlife Marine Region) with a “repressible lethal” genetic approach to diminish the reproductive capacity of urchins. Another approach would be to facilitate adaptation through genetic intervention for sea star wasting disease. This would have the benefit of maintaining sea star populations at levels that naturally control the prevalence of urchins.

Note: The genetic control of invasive and/or irruptive species like urchin is described in more detail in a subsequent section of this report.

RISKS AND CHALLENGES

For kelp forest ecosystems, there are very few examples of research collaborations between marine conservation ecologists and the ‘-omics’ fields of study; at least in so far as evidenced in the literature. This lack of transdisciplinary research presents a barrier to rapid progress, but creative convening of thought-leaders in the respective fields of study could provide a path forward.

Lack of genetic and genomic data inherently limits the insights that become possible through comparative genomics applications or derivative research trajectories (e.g., transcriptomics, metabolomics) that begin to link genetic information to functional consequences.

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THREAT

There is broad consensus of an immediate global threat from overfishing the ocean. Roughly 90 percent of fish stocks are fully or over-exploited, with more than 1/3 fished at unsustainable levels (http://www.fao.org/state-of-fisheries-aquaculture/en/). Overfishing threatens marine ecological integrity but also puts at risk whole economies and vital sources of protein for communities worldwide. Fisheries provide an estimated 15 percent or more of worldwide dietary protein (Bene et. al. 2015), as well as millions of vital livelihoods. Global fisheries represent the most valuable food commodities traded internationally, and are a particularly important source of export earnings in developing nations (Smith et al., 2010). In 2016, fishery exports were worth an estimated $148 billion (FAO 2016).

Compounding threats from climate change are worsening fears of massive perturbations in the availability of wild-caught fish. In the United States, climate-related impacts to marine ecosystems threaten the biological, social, and economic resilience of the United States' $208 billion fishing industry and the 1.6 million jobs it supports (NMFS, 2017). These include range shifts of mobile species, timing shifts of migratory species, fishery closures due to increased frequency and severity of harmful algal blooms, and potentially pervasive effects (such as disrupted food chains) from increased acidification (Pinsky and Mantua, 2014).

A fishery generally refers to the wild harvesting of fish or shellfish resources for commercial or recreational purposes. Fisheries are typically defined by the targeted fish stocks, where a stock represents both biologically and a jurisdictionally defined population. Fisheries have historically been managed using a "single-species" framework to achieve the highest amount of sustainable catch within the target stock. This management typically entails regular assessments of stock status (underexploited, fully exploited, or overexploited) used to set catch limits (NOAA Fisheries 2017). However, the expansive ocean environment makes assessing stocks extremely challenging and fisheries management is chronically data limited.

Unfortunately, in addition to managed and reported fisheries, illegal, unreported, and unregulated fishing threatens to undermine the sustainability of fishing even in the most conservation-oriented countries. Experts estimate the global economy loses $10-23 billion in revenue due to illegal, unreported, and unregulated fishing (Agnew et al 2009). In some cases, fish are deliberately mislabeled to disguise catch of forbidden species such as shark fin. In other cases, a high value catch such as bluefin tuna will be labeled as skipjack tuna, a lower value catch.

Explosive growth in the aquaculture sector has created new pressure on global forage fish stocks. In particular, the global demand for smaller fast-growing forage fish (that are pelletized into fish food) has skyrocketed in recent decades. Forage fish species such as sardine, capelin, herring and anchovy represent critical food chain linkages that support large commercial fish, marine mammals and marine birds. The top harvested forage fish species is the Peruvian anchovy followed by Atlantic herring. It is estimated that forage fisheries are valued at $5.6 billion and these forage species support the fisheries of larger species, valued at $11.3 billion. Today, the global aquaculture industry continues to grow at an annual rate of 5.8 percent and forage fisheries are looking deeper into the ocean for fish to convert into feed (*Sylvia Earle, pers. comm.*).

PROGRESS TO DATE

The combined global importance of fish as vital to a bio-abundant marine ecosystem and as a vital source of protein for human consumption compels immediate attention to improving the conservation and sustainability of global fisheries. However, transformative innovations in the control and management of fisheries have been few and far between. Synergistic climate change threats to marine ecosystems compound the issues and the need for innovation. To date, efforts have focused on spatial closures in the form of marine protected area designations and temporal restrictions on fishing effort. Advances in telecommunications and other electronic technologies present potentially powerful new tools that, when coupled with new fishery management paradigms, could prove to be transformative. Modern genomic technologies offer additional new approaches to enhance the sustainability of fisheries management.

A recent study used genetics to correct a pervasive misidentification of sardine populations, enabling improved management of a highly valuable fishery. Since the 1900s, the most commonly landed sardine species in the Philippines was identified as the Indian oil sardine (Sardinella longiceps). However, a study that combined morphological and molecular data to discovered that sardines caught at sites in the Philippines were Bali sardinella (Sardinella lemuru) (Willette & Santos 2013). These data informed an update of sardine management plans to enable accurate stock monitoring at a local and international level (Willette & Santos 2013). However, as of 2017, there were only 22 published, scaffold-level genome sequences for finfish (Yue and Wang, 2017). Broadening the base of genomic knowledge of important fisheries would enable new ecological and management insights.

As noted above, illegal, unreported, and unregulated fishing threatens the sustainability of fisheries worldwide, and modern genomics offers cost-effective new tools to transform the monitoring of fish products from the sea to the shelf. When coupled with modern electronic and

computing capabilities, genetic and genomic resources provide powerful tools, with high reproducibility and reliability, for tracing and identifying marine products. Genomic information can be combined and compared with reference materials (need reference) to determine authenticity, and to verify labelling information such as higher value eco-certifications. Further improvements in the ability to identify fish species and trace those species to their origins, can give seafood buyers, sellers, and consumers verification of the geographic and biological origins of seafood. In general, species and their origin may be identified by external traits until the fish is processed. Genomic tools offer new ways to monitor processed fish products.

Genomic insights that inform stock delineations have the potential to transform the management of certain fisheries. The best-known example of how genetic insights have improved management are in Pacific salmon stocks. For decades, scientists have used genetics to examine salmon population genetics and stock delineations. Genetic data are used to map stock structure, population structure, standing diversity, effective population size, and the demographic history of natural populations (Ward 2000). Using genetic markers, salmon caught at sea can be assigned to their "stock of origin," or natal river basin. When combined with fishery landings data, these insights can reveal how fishing pressure is distributed across the known stocks (Clemento et al., 2014). These tools are becoming increasingly powerful as technologies advance (Wilmot et al., 1996; Ramstad et al., 2004; Palsbøll, Bérubé & Allendorf 2006; Hess et al., 2011).

Genetic-based population data can also reveal particularly vulnerable stocks and provide evidence critical to justifying immediate mitigation action, such as closures or endangered species designations of "distinct population segments." A recent example of genetic insight applied to management concerns two species of anadromous river herring in New England, alewife (Alosa pseudoharengus) and blueback herring (Alosa aestivalis). These are important migratory fishes in coastal freshwater and marine food webs, but have experienced dramatic declines in the abundance of spawning adults. Twenty-five years of restoration efforts (principally restoring access to spawning streams) in Southern New England yielded few consistent signs of recovery. This raised concerns that bycatch of anadromous herring in the Atlantic herring fishery has been negating these restoration efforts.

In order to investigate this threat, researchers sampled several thousand fish in the offshore fishery and used genetic identification markers (microsatellites) to calculate how bycatch was partitioned among previously identified regional genetic stocks. The genetics identified the majority of bycatch in the Atlantic herring fishery as belonging to severely depleted genetic stocks (alewife of the southern New England stock—70 percent of sampled alewife bycatch; blueback herring of the mid-Atlantic stock—78 percent of sampled blueback herring bycatch).

The southern New England and mid-Atlantic genetic stocks overlap in the waters surrounding Long Island Sound, indicating that bycatch taken from this area is negatively impacting recovery efforts. These genetic insights compelled the State of Connecticut to request closures of the Atlantic herring fishery on the southern New England fishing grounds in order to reduce or eliminate bycatch. In September 2018, the New England Fisheries Management Council implemented a closure of the Atlantic herring fishery over 12 nautical miles off the New England

coast from Canada to the eastern end of Long Island Sound. The closure closely resembles what river herring conservationists had advocated based on the genetic studies.

With plunging sequencing costs, genomic data has become increasingly applicable to commercially important fish species as a means to guide conservation and management practices. These insights can increase the resolution of population structure at finer spatial scales and identify adaptive responses to a changing and impacted environment.

INNOVATION

The text below describes opportunities (eDNA, close kin mark and recapture, population analysis, traceability and outlier loci) to apply genomics as a tool to advance stock assessments in a manner that could transform fishery management and conservation.

eDNA

eDNA holds promise for vastly improving the tracking of temporal and spatial changes in species distributions. eDNA can inform model predictions for fishery stock distributions, improving the ability to accurately map the spatial extent of a fishery. This is of particular interest in the context of changing ocean conditions and climate change. A number of studies have demonstrated the utility of using eDNA metabarcoding to measure fish diversity (e.g., Thomsen et al. 2012; Miya et al. 2015; Evans et al. 2016). In various studies, eDNA sampling was comparable or superior to traditional and costly techniques like trawling, line fishing, and diver observations for characterizing fish diversity (Thomsen et al. 2012; Shaw et al. 2016, Thomsen et al. 2016, Port et al. 2016).

eDNA sampling has also been proposed as a cost-effective means to check existing species ranges both year-to-year and for those species that have intra-annual migrations such as inshore-offshore movements. These movements are correlated with water temperature, and thus eDNA could be used as an early warning indicator of when such movements occur each year.

The U.S. National Oceanic and Atmospheric Administration (NOAA) and Norwegian Institute of Marine Research (IMR) have created a joint working group for the application of eDNA to fisheries stock assessments. As noted, stock assessments are a quantitative metric. However, methods that correlate eDNA data to abundance metrics are underdeveloped. The working group is thus interested in furthering eDNA as a quantitative metric, with the ultimate goal of making stock assessments more cost effective. To do this, these agencies have begun building time series datasets. They will collect water samples and preserve them alongside “standard” trawls to create an index of relative abundance. This partnership represents a notable investment by two key government agencies that would benefit from such improvements.

Close-Kin Mark and Recapture

A recently optimized and insightful application of genetic data within fisheries management is the close-kin mark-recapture (CKMR) method. CKMR method uses non-invasive and inexpensive tissue samples to reconstruct pedigrees and estimate stock abundance independent of fishery-derived data. The basic premise of mark and recapture is that individuals can be distinctly

marked or “tagged,” and those marks will then be recognized if the individual is recaptured in a subsequent sample. CMKR uses DNA markers to reveal information about relatedness, and since most fish are highly fecund, the volume of “tagged” individuals represented by their offspring is huge – orders of magnitude more than would be possible in conventional mark and recapture programs. The recapture of marked individuals can provide estimates of species abundance and survival rate. The average time taken to “recapture” a parent after sampling its offspring can indicate species abundance and adult survival rate (Bravington, 2016 Grewe & Davies 2016). Bravington (2016) developed an estimated abundance of adult Southern bluefin tuna based on the detection of 45 parent-offspring pairs in 13,000 samples, constituting the first large scale application of CKMR. The CKMR procedure could be applied to commercially important stocks worldwide to estimate abundances instead of using often unreliable or biased fishery catch or effort data to derive estimates.

Genetic analyses, including close-kin mark-recapture (CKMR), are also revolutionizing our ability to estimate demographic connectivity of marine fishes over the small spatial scales relevant to the design and ecological benefits of marine protected areas (MPAs). When CKMR is applied to parent-offspring relationships, estimates of larval dispersal – the distance between a sedentary adult and its settled progeny – are obtained. CKMR also reveals the variety of dispersal trajectories from a single parent when genetic markers can identify full-sibling relationships.

In a recent study (Baetscher DS, et. al., in press), researchers sampled kelp rockfish (Sebastes atrovirens) along approximately 25 kilometers of coastline in Monterey Bay and applied CKMR to identify dispersal events. Large sample sizes and intensive sampling are critical for increasing the likelihood of detecting parent-offspring matches in such systems. The researchers genotyped more than 6,000 kelp rockfish and identified eight parent-offspring pairs, which included two juvenile fish that were born inside MPAs and dispersed to areas outside MPAs. Four fish born in MPAs dispersed to other nearby MPAs. Additionally, the research identified 25 full-sibling pairs, which occurred throughout the sampling area and included all possible combinations of inferred dispersal trajectories. This study provides the first direct observation of larval dispersal events in a current-dominated ecosystem and direct evidence that larvae produced within MPAs are exported both to neighboring MPAs and proximate areas where commercial and recreational harvest is allowed.

Further CKMR work on high-value and highly migratory commercial fisheries could revolutionize our understanding of population dynamics. Also, CKMR work on the ecological function of MPAs fulfills a long-standing data need concerning the benefits of the protection strategy to the sustainability of fisheries.

Traceability Innovations

Many instances of fish mislabeling have been detected via a technique known as DNA barcoding, but these have mostly taken the form of after-the-fact randomized checks in retail locations. It is possible, and indeed desirable, to integrate genetic traceability into an enforcement or supply chain framework. Recent technological advances, such as the DNA Barcode Scanner by Conservation X Labs, promise to shrink DNA barcoding into portable and

cheap packages that can produce real-time results that could conceivably be adopted at various checkpoints along seafood supply chains.

Even without such checkpoints, genetic markers with enough power to resolve the geographical origin of the traded seafood on their own, combined with existing traceability or enforcement programs, could radically transform supply chain transparency and facilitate penalization of entities fishing illegally. For example, by targeting genetic markers with higher levels of differentiation – which are suggestive of ongoing selection and, perhaps, local adaptation – fine-scale population structure can be identified that would otherwise not be captured by neutral markers. This can then be utilized within the existing frameworks such as FishPopTrace – a collaborative project involving 15 research groups from the EU, Norway and Russia – to expand the program and include more commercially important species. This would require investment in infrastructure to robustly sample individuals throughout a species' known geographic range to capture enough genetic variation to create a comprehensive marker panel. These panels could then be widely shared with entities already engaged in seafood authentication schemes to help identify offending fishers and enforce regulations.

DNA barcoding is less well developed in shellfish because species identification often requires the development and application of mitochondrial and nuclear molecular markers, as well as SNP panels, depending on taxon and these are largely undeveloped for shellfish. These small panels of informative SNPs usually perform better than microsatellite markers when allocating individuals to geographic origin. However, work to date has demonstrated the identification of Mediterranean mussel, common blue mussel, Baltic mussel, and Chilean mussel with high accuracy using a panel of 49 SNPs (Larraín et al. in preparation) and the separation of Chilean and Mediterranean mussels with a subpanel of 19 SNPs (Araneda and Larraín, S1). In Chilean mussel, it is possible to differentiate populations from three different environments, two of which were affected by the red tide in 2016. (Thomsen PF, Kielgast J, Iversen LL, Moller PR, Rasmussen M, Willerslev E.)

Fishery-Induced Evolution

Genomic tools are providing remarkable insights into the evolutionary pressures of fishing on heavily exploited species and effective management should ideally account for these pressures (Laugen et al., 2012). While the techniques used to assess evolutionary consequences are still developing, experimental investigations have identified significant genetic changes associated with notable phenotypic changes, often in traits that will affect long term productivity, such as size-at-age (Swain, Sinclair & Hanson 2007; Conover & Munch 2002). Thus, high quality, annotated genome data for many commercially important species will allow for a more reactive, eco-evolutionary approach to stock management, as well as long-term predictions of stock sustainability that could transform fisheries management.

"Outlier loci" are loci with elevated levels of differentiation, and therefore may be associated with selective pressures. Genome scans for outlier loci amongst kokanee salmon found these loci to be particularly effective in revealing differentiation at the ecotype-level (Russello et al., 2012). This finding suggests outlier loci would be far more powerful than current methods for identifying

differentiation amongst recently diverged populations, potentially producing more accurate stock delineations for fisheries management. An analysis of Atlantic cod revealed dynamic temporal and spatial patterns of selection over the last century (Therkildsen et al., 2013). These signatures of selection correlate with environmental variation and life history changes at certain loci, suggesting potentially adaptive responses to fishing pressure and expanding our understanding of fisheries-induced evolution (Therkildsen et al., 2013). Advanced investigations of local adaptation or evolutionary responses to selective forces are limited to species (such as Atlantic cod, rainbow trout, and salmon) with substantial genomic data resources.

RISKS AND CHALLENGES

The innovations outlined above will rely upon underlying genetic databases for species of concern. The availability of real-time data for any of these use cases is contingent upon sequencing with a high enough level of coverage to identify unique species or from a sample size large enough to identify genetic variation within a species.

Unfortunately, to date, genetic data for many species of concern is inadequate, with sequencing conducted at a level of coverage too low or from a sample size too small to provide these important insights.

The promise of eDNA is subject to significant technical challenges stemming from the validation and indexing of data from eDNA surveys. Several factors currently confound the ability to interpret eDNA data beyond simple presence/absence questions. This is inherently an empirical question that will likely be ameliorated by increased eDNA studies.

Emerging genomic technologies can only live up to the promise of more sensitive and precise management of fisheries if the management entities have the capacity and willingness to utilize new data. The management of fisheries is notoriously data-limited and fisheries management agencies are often under-funded, usually with a conflicting mandate to both protect the resource and the economic use of the fishery. Limited resources for monitoring and enforcement restricts the ability for management entities to incorporate new data or perspectives on management. Disparate management priorities in regional fisheries management entities and a lack of willingness to innovate within fisheries can compound these challenges.

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POLLUTION

LEAD CONSULTANT: Elif Demir-Hilton & Kevin Webb (MS Sustainability Science Candidate, Columbia)

Pollution is a major threat to the health of our oceans, and one of the greatest challenges for mitigation efforts due to the scale and diversity of sources that are tightly coupled to human activity and the global economy. It is essential that we develop ways to eliminate ocean pollution at the source, through new technologies that replace polluting industrial practices and products. In addition, to prevent a major loss of biodiversity, we must make methods of cleaning up the existing contamination of marine environments a priority. Two major types of pollution discussed in this chapter, industrial waste streams, as exemplified by crude oil spills, and solid plastic waste, could benefit from the application of biotechnology and genomics.

INDUSTRIAL WASTE STREAMS

THREAT

Marine pollution comes from a variety of sources and can have widespread and long-lasting effects on the ecosystem. Most marine pollution starts outside the ocean environment, as agricultural, industrial, urban runoff, wastewater, or even air pollution. Most human waste streams find their way to the ocean eventually. Once there, pollutants have a variety of effects, including acidification, eutrophication, and the accumulation of toxins in oceanic food webs (that then can make their way back to the terrestrial food webs). A comprehensive plan to cleanse the ocean environment must include efforts to reduce contamination of the land, fresh water, and air.

Petroleum pollution in marine environments is a particularly serious environmental concern, caused almost exclusively by human activity. Oil spills are among the most well publicized and dramatic pollution events. However, most of the oil pollution in the ocean is due to continuous small leaks from oil tanker ballasts, leaky oil pipelines, and improperly disposed of engine oil. The components of crude oil, such as polycyclic aromatic hydrocarbons, are toxic to marine life and are difficult to clean up. The restoration of ecological balance requires the development of effective ways to remediate oil-polluted environments.

Modern wastewater treatment can have a profound impact on the condition of discharges of pollutants and water quality in coastal waters. Unfortunately, sophisticated wastewater treatment facilities are costly to build and maintain. Also, contaminants of emerging concern are increasingly recognized as having profound impacts on the ecology of marine organisms. These contaminants include endocrine disruptors and other pharmaceuticals for which there are few recognized removal methods.

Nonpoint pollution, derived from many different sources and transferred as runoff from snowmelt or rain, is a particularly challenging management concern. Nonpoint pollution can contain toxic industrial chemicals, agricultural products like pesticides and fertilizers, pharmaceuticals from

human wastewater, petroleum, and solid debris like plastic and dust. Plastics are largely a diffuse "non-point" problem. Notable, fishing nets are a significant source of plastic pollution. Conventional and highly targeted point source wastewater control methods are not effective. Instead, managers rely on retrofitting infrastructure to capture and passively "treat" non-point source pollution sources.

The increased levels of carbon in the atmosphere due to the burning of fossil fuels is leading to the acidification of marine environments as carbon dioxide is dissolved into ocean waters. This effect is expected to continue along with overall climate change and will particularly affect marine wildlife. If the ocean becomes too acidic, the calcified shells of bivalves and corals with may begin to dissolve (Feely et al. 2009). If these key species are lost, dramatic degradation of ocean ecosystems will quickly follow. Biotechnologies that lead to a reduction in carbon dioxide production or that can capture carbon from the atmosphere are actively being sought to reverse the effect of air pollution on our oceans. Studies have documented a localized ameliorating effect on acidification from submerged aquatic vegetation.

PROGRESS TO DATE

The effectiveness of control and remediation of pollution discharges are wildly disparate around the world. This largely depends on the level of development and standard of living in a country. Even for the most developed countries, new biologically-based remediation efforts hold promise as particularly beneficial innovations to pursue. These advantages stem from the ability for biological methods to remediate pollutants for which remediation methods have proved challenging for more conventional treatment systems.

Intrinsic processes of pollution bio-remediation generally involve the metabolic breakdown of introduced waste chemicals by organisms native to the environment. While this natural process of bioremediation is effective, it is slow and toxins can persist in the food web for a long time. Recent biotechnological advances, particularly in the area of synthetic biology, now offer the potential to improve the efficiency of bioremediation and pollution sensing, both in the ocean and at the pollution sources. However, these technologies are only beginning to be explored in complex environments outside of the lab. Safety concerns and regulatory hurdles will need to be overcome before these new solutions can be fully deployed. These technologies are also being investigated as part of efforts to drastically reduce the amount of pollution produced by human activities, which is ultimately the only way that we will save the oceans if the human population and economy continue to expand.

Biostimulation

The rate of bioremediation by microbes in a contaminated environment can be limited by the availability of nutrients and oxygen. Biostimulation is a method to encourage faster bioremediation by supplementing the contaminated site with the needed nutrients, similar to providing fertilizer to improve the growth of plants. The advantages of this method are that they make use of the native ecosystem, avoiding the introduction of foreign organisms. However, the ability to spread biostimulants over large areas, especially in marine environments where dilution

can be a problem, is a major limitation. Furthermore, the added nutrients can also stimulate the growth of competitors or harmful algae that either do not help the bioremediation process or harm native wildlife, resulting in a net neutral or even a net negative effect.

Bioaugmentation

Bioaugmentation is a more aggressive approach that involves seeding a contaminated environment with microbes that can mediate the breakdown of the pollution. For this to be successful, the introduced organisms must be able to break down all the components of the pollution, which is a particular challenge in the case of crude oil spills. Furthermore, the introduced bioremediator must thrive in the potentially hostile contaminated environment alongside the native organisms. Therefore, this technique requires the use of microbial strains or microbial consortia that have been well-adapted to growth in the environment of interest. They are typically isolated from sites that have frequent contamination events, like near leaky oil pipes, or the sites of past oil spills.

Pathway Mining and Metabolic Engineering

As mentioned above, oil-degrading microbes are often first identified in contaminated environments, or by virtue of particular genes discovered through genome sequencing efforts. However, few strains can degrade all constituents of crude oil, or thrive in a range of natural environments that may require remediation, such as sand, soil, and fresh and marine water. This has led industry and academics to the idea of using genetic engineering to create new strains of oil-degraders, sometimes called “superbugs”, that possess metabolic pathways for multiple hydrocarbon substrates. In fact, the first patent ever filed for an engineered microbe was in 1971, for a strain of Pseudomonas engineered with four oil-degrading genes from other strains (Environment: oil-eating bug. Time Magazine 1975. Time.). This first example had a 10-100-fold faster oil remediation rate relative to naturally occurring soil bacteria.

Over the last four decades of molecular biology, a large catalog of metabolic parts has been generated from many organisms spanning many domains of life. The universality of genetics allows any one of these parts to be used by any organism that encodes it. Bioinformatic tools have been created to enable metabolic engineers to systematically explore the huge catalog of parts available to generate new metabolic pathways that will transform pollutant-X into safe product-Y. Because of the modularity of these pathways and the conservation of the enzymes for each intermediate reaction type, synthetic metabolism is just a matter of plug-and-play. Likewise, the pathway mining approach used to identify oil-degrading pathways is dependent on the number of sequenced genomes and the ability of bioinformatic algorithms to spot relevant patterns in the gene sequences or genomic structures. Similar efforts to uncover natural biodegradation pathways of other industrial pollutants based on genomic sequences and structure could enable the discovery of new bioremediation strains or communities from metagenomic data. The major challenges to these synthetic biology approaches are in scaling the manufacturing of these engineered microbes, and in gaining approval for environmental release.

INNOVATION

To date, most engineered microbes have been designed for use in closed environments, such as large fermenters. The controlled environment protects the modified microbes from harsh environmental insults that might inhibit function and also prevents the release of these GMOs into the natural environment. In order to adapt engineered microbes to in situ bioremediation applications, several innovations will be required. First, the host strain will need to be able to live in the natural environment of interest, which will be polluted with one or more contaminants, but will also contain competitors and potentially predators. In the case of marine environments, dilution of the pollutants will also be a challenge. The engineered strain will need to perform optimally under these environmental constraints. Conversely, the safe release of engineered microbes into natural environments will be a concern, especially if they are designed to thrive in those conditions. Engineered microbes will need to include safety mechanisms that control their spread beyond what is intended for their bioremediation purposes. Several techniques now exist that could prepare engineered microbes for *in situ* remediation in the near future.

Designer Pollution-Eating Microbes

The new tools for metabolic engineering described above have renewed the commercial potential for bioremediation strategies involving engineered microbes. It is now possible to rapidly screen thousands of potential pathways, both natural and synthetic, for a given reactant and product. Today, there are many aspiring companies focused on upcycling industrial waste streams, some relying on designer microbes to convert trash to treasure. For example, a startup called Lanzatech is developing technology to use carbon-rich gas waste streams from steel manufacturing, agricultural processes, and oil refining, as a carbon source for the biosynthesis of valuable commodities, like chemical precursors, and biofuels. Their process relies on engineered microbes that can ferment waste molecules, which are otherwise destined to become a source of pollution, much the way microbes can convert sugar into ethanol in a brewery. Lanzatech estimates that by feeding a factory waste stream into its bioreactors, they can reduce pollution emissions by up to 85%, and produce a valuable product in the process.

Many types of pollution from agricultural, industrial, or military waste streams are made of xenobiotic compounds, which are compounds not found in nature. Although these molecules can be substrates for biodegradation, it is rare for the complete biodegradation pathway to be encoded in the genome of a single microbe. Metabolic engineering has been used to create strains of the soil microbe, *Pseudomonas putida*, that can simultaneously degrade multiple pesticides that are commonly used together, such as methyl parathion and c-Hexachlorocyclohexane, and carbofuran and chlorpyrifos (Gong et al. 2016).

Adaptive Laboratory Evolution (ALE)

One pragmatic approach to engineering strains that can thrive and function in sub-optimal environments is to take advantage of yet another universal feature of biology: the ability to adapt to new conditions through evolution. In nature, the adaptive evolution of a population of microbes can take decades; however, the small size and short generation times of microbes allow

researchers to shorten evolutionary time dramatically in the lab by introducing increasing amounts of selective pressure with frequent artificial selection events. This approach has been successfully applied to enzymes numerous times in the creation of commercially-viable production strains for valuable molecules, like pharmaceuticals. The same approach is readily adapted at genome scale for bioremediation applications. Furthermore, researchers have begun to apply ALE to multi-strain consortia of microbes, which may be able to collectively degrade complex mixtures of contaminants, such as crude oil. The major challenge will be in the ability to simulate contaminated natural environments in a closed laboratory setting in order to select for genotypes that can thrive in those environmental conditions. This will be particularly challenging for marine environments.

Whole-Cell Bioreporters

The ability to reliably sense pollution in the environment at low levels is valuable for protecting ecosystems and for monitoring bioremediation of contaminated sites. Living cells naturally have acute sensory systems for detecting chemicals in their immediate surroundings. These natural abilities can be harnessed to create highly sensitive and specific environmental sensors for industrial toxins and pollutants, including hydrocarbons, heavy metals, and organic compounds (Belkin 2016).

Engineered microbial bioreporters are inexpensive, easy to use, and can be more sensitive than electrochemical alternatives. These reporters are already widely used for medicine, biology, toxicology, drug screening, and water quality testing, and several field tests have demonstrated their ability to detect pollutants in natural environments. Multiple bioreporters can be used in parallel in environments that are contaminated with complex mixtures, like crude oil. For example, Tecon et al demonstrated the use of a suite of five bacterial bioreporters to monitor the levels of hydrocarbon mixtures in marine environments over periods of 7-10 days. The results from the bioreporters were comparable to those obtained by chemical analytic methods, both in the concentration and timing of analyte detection after the oil spill. The authors concluded that this suite of bioreporters could constitute a simple analytical tool to measure the time scale of oil spills.

RISKS AND CHALLENGES

Low Anticipated Return on Investment

As described above, the ability to engineer microbes with desired metabolic and life-style properties has been greatly increased by affordable genomic sequencing and genetic engineering tools. It is now possible to create a microbe that can both thrive in an environment of interest and possess the metabolic genes necessary to degrade complex mixtures of toxins, like crude oil. However, these engineered biological systems are not being commercialized for bioremediation applications as fast as they are for other industries, such as fermentative biosynthesis. This may be for economic reasons; there are simply more commercial opportunities in the bioproduction of commodity chemicals.

Regulatory Hurdles

Additionally, steep regulatory hurdles exist for the environmental release of engineered organisms, which is a requirement for in situ bioremediation applications (Technology in Society 32 (2010) 331–335). The same public concern, policy, and governance that will regulate the release of GMOs for control of invasive species will also apply to bioremediation technology.

Unintended Consequences

Several advances in the way that engineered oil-remediating strains are designed and tested prior to release could alleviate concerns and speed the time to practical use. First, redundant genetically encoded safety circuits should be a standard design feature of any strain that could be considered for release to ensure that growth can be limited to the time and place where the activity is needed. Several genetic systems for growth limitation currently exist, such as toxin/anti-toxin pairs, cell division counters, addiction pathways that make a microbe dependent on a nutrient additive (Wright et al. 2013), and environmental sensors that restrict growth to a particular location or context (Simon and Ellington 2016). Second, genetically engineered strains can be designed with one or more non-standard codons to prevent them from either picking up or donating genetic material to the natural environment (Rovner et al. 2015). Finally, experimental platforms that allow the safety and efficacy of engineered strains to be rigorously tested in relevant conditions need to be developed. This is particularly challenging for marine environments because of the complexity of the ecosystems, but perhaps even more necessary because of that.

Safety and Control

The solution to the safety concerns associated with genetically engineered organisms may actually be even more engineering to produce strains that contain internal safety mechanisms that prevent uncontrolled growth and genetic transfer with organisms in the surrounding environment. Precision genomic tools have progressed to the point that worrisome features of the early engineered strains, such as antibiotic resistance markers, are no longer necessary. Furthermore, techniques like multiplex-assisted genetic engineering (MAGE) make it now feasible to design microbes that use unique genetic codes and are therefore genetically isolated from the native organisms in the environment (Lajoie 2013). Finally, the field of synthetic biology has demonstrated the ability to design and build programmable gene circuits that behave more like natural genetic pathways with regulatory inputs and logic-based functions. These programmable cells can be designed to behave according to environmental signals and will have a lower risk of behaving inappropriately in a natural setting. It is expected that the potential for highly engineered microbes will open the door to fieldable bioremediation agents capable of rapidly degrading crude oil contamination in a range of environments. In addition, regulatory requirements may need to be updated to allow for field tests of engineered microbes that have built-in safety features.

SOLID WASTE: PLASTICS

THREAT

Coastal communities are inundated with plastic waste and discarded fishing nets are a major source of plastic pollution globally. Currently there are five different known 'floating islands' of plastic circulating the Earth's oceans, reaching up to several square kilometers in size. Plastic is extremely slow to degrade, and different types of plastics have different degradation rates and degradation pathways. Unfortunately, plastic degradation tends to just make it ever smaller into microplastics that then enter aquatic food chains where, like many pollutants, it tends to bioaccumulate up the food chain. Plastic affects marine life through direct consumption, as evidenced by beached whales and other wildlife frequently found with high volumes of plastic in their digestive tracts (Brate et al., 2016; Schuyler et al., 2016; Avery-Gomm et al., 2018). It is also having ecological cascading effects through impacts on food webs and the introduction of ecotoxicants that can affect the endocrine and reproductive systems (Avio et al., 2017).

A promising approach for the treatment of marine pollution is microbial remediation. Microorganisms, particularly bacteria, can be bioengineered to optimally sense or break down and metabolize contaminants in the environment. Genomics, transcriptomics, proteomics and metabolomics can be applied in a systems biology approach to advance microbial remediation technology through synthetic biology.

PROGRESS TO DATE

Several technological innovations are being tested for efficacy in removing plastic waste from marine environments. Source reduction is an important strategy and several initiatives that target the reduction of the use of single use plastics are making strides. Other initiatives and start-up companies are seeking to reduce or replace the use of plastics in packaging.

In 2018, the launching of The Ocean Cleanup System 01 marked the first large-scale attempt to use a floating catchment system to localize and physically remove plastic waste from the environment. Despite some immediate setbacks, the company expects to move forward with testing system on the Great Pacific Garbage Patch. Smaller scale systems, such as the Seabin, have also been developed to filter plastic waste from calm marine environments such as ports and marinas. While these technologies hold potential to vastly reduce the amount of plastic waste accumulating in the ocean, neither offers a viable solution for permanent resolution of the plastic waste problem. However, if properly coupled around a bioremediation platform, technology and synthetic biology may be a viable and lasting solution to protect marine environments from plastic waste.

The most promising microbes for effective biodegradation of plastic are the *Pseudomonas* spp. (Table 1) although recent research has identified *Ideonella sakaiensis* 201-F6 as an efficient biodegrader of PET plastics (Yoshida et al., 2016). Additionally, a recent screen of microbial

communities sampled in the Arctic and screened for plastic degradative abilities showed promise for *Rhodococcus* sp. of bacteria and several fungal species (Urbanek et al., 2017). However, rates of biodegradation are slow and not currently efficient to solve the ocean plastic problem. Various bottlenecks in the metabolic pathways, and influence of the environment and microbial metabolism have precluded the development of robust solutions. A systems biology and metabolic engineering approach is a key innovation strategy that needs to be explored.

INNOVATION

Synthetic biology coupled with metagenomic, comparative 'omic' and metabolic engineering approaches should be explored and developed to address the surmounting issue of plastic waste in oceans. It is important to note that, while this approach holds promise, the full realization of a bioremediation-based strategy to remove plastic waste from oceans remains distant. A major advantage of a synthetic biology approach to solid waste is that the pollution can be physically removed from the natural environment prior to treatment with engineered microbes. This eliminates the need for releasing engineered microbes into the natural environment, and reduces the safety and regulatory concerns that go along with that. Progress toward this goal will require 3 steps according to Dvorak et al. (2017) as highlighted below and represented in Fig 2:

1. Model the appropriate degradation pathway for primary plastics in oceans using predictive software. This will require optimization for extracellular degradation of polymers into smaller chemicals, followed by transport into the cell and finally optimization of intracellular metabolism of plastic products.

2. Identify the best host. Ocean plastics should be sampled to characterize microbial communities on biofilms. Newly developed sequencing techniques that take advantage of the three-dimensional structure of DNA (Hi-C Phase Genomics) allows researchers to generate, assemble and identify novel whole genomes from a metagenomic sample (Heger. 2014). That is, not only can they identify which bacteria are in a sample, but they determine, better enabling understanding of how metabolic pathways are constructed within a single host, such that these can be targeted and engineered in highly specific ways, and combined into microbial consortia capable of overriding metabolic bottlenecks particular to any single microbial organism.

3. Apply computational and experimental tools to optimize metabolic pathways in the context of the host(s). Informed by the combination of various 'omic' analysis to identify the most suitable microbial host, whole genome and kinetic models can be coupled with genetic engineering techniques to tailor gene expression, reduce metabolic burden and optimize metabolic pathways (Whilkes and Aristilde 2017).

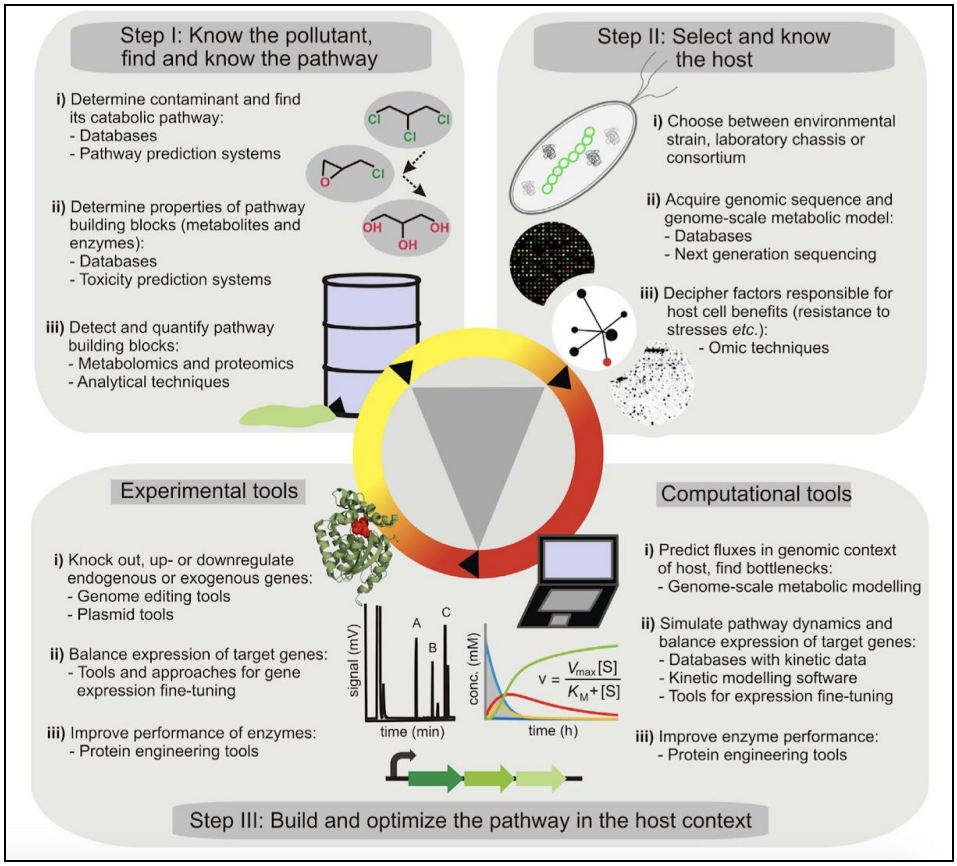

FIGURE 2 —Proposed workflow for engineering biodegradation pathways using systemic biology approaches and tools. (Dvorak et al. 2017)

RISKS AND CHALLENGES

Developing adequate bioremediation for plastic will be costly and requires highly specialized facilities. Further, advanced computational tools will need to be developed to integrate whole genome, transcriptome, and proteome data into metabolic pathway models. Once plastic is collected from the ocean, it may require manual processing or infrared sensors sort the plastic into subtypes for handling by different microbes or microbial consortia. Collected plastic may also require pre-treatment to optimize microbial attachment. Substantial time and resources will be required to fully realize bioremediation and synthetic biology as a strategy to combat the ocean plastic problem. However, with well-directed funds, such a strategy could immensely improve conservation outcomes in marine environments.

LEADERS

- The Ocean Cleanup- launched the Ocean Cleanup System 001 in September 2018. Despite initial functional failures, the system could provide a means to collect the waste which can then be collected into mobile bioremediation facilities onboard. This would alleviate environmental concerns over releasing bioengineered microbes into the natural environment, allowing containment and control of the engineering process.

- Enoveo- ENOVEO was founded in 2008 and specializes in environmental microbiology, chemistry and biotechnologies. The result of collaboration between researchers at the University of Lyon and contaminated site management professionals, this company provides a range of services to facilitate the research and development of specific bioremediation challenges. They provide feasibility tests for custom projects, which to date include Petroleum hydrocarbons, PAHs, chlorinated solvents, polar solvents, PCBs, heterocyclic compounds, pesticides, chromium VI, BTEX, nitronaphthalene, explosives (TNT, RDX, HMX), perchlorates, and anilines.

- Several investigators may be viable collaborators or investment targets including:

    - Shosuke Yoshida- Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology

    - Aneta Urbanek- Department of Biotechnology and Food Microbiology Wroclaw University of Environmental and Life Sciences

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MARINE INVASIVES

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EXPERTS INTERVIEWED:

John Teem (*International Life Sciences Institute*), **Owen Edwards** (*CSIRO*)

THREAT

Invasive species are non-native organisms that have been introduced into a new ecosystem (Richardson et al. 2000). A species irruption is a sudden change in the population density of an organism, typically characterized by population explosions followed by subsequent crashes (Roughgarden 1996). Both invasives and native-species irruptions can cause harmful impacts to natural resources and/or human use of the resource (Bax et al., 2003; Molnar et al., 2008). The eradication and control of such species can be particularly challenging in marine environments, and managers often rely on time- and labor-intensive manual removal (Sutherland et al., 2017).

Invasions and irruptions can displace native species (e.g. Huxel 1999), cause the loss of native genotypes (e.g. Gurevitch & Padilla 2004), lead to a reduction in biodiversity, modify habitats, change community structure, affect food-web properties and ecosystem processes (Byrnes et al. 2007; D'eath et al. 2014), impede the provision of ecosystem services (e.g. Katsanevakis et al., 2014), impact human health (Pyšek & Richardson 2010), and cause substantial economic harm (Marbuagh et al. 2014). Pimentel and Colleagues (2005) estimate invasive species in the United States cause major environmental damage, as well as economic losses totaling $120 billion per year.

Rapid globalization and increasing trends of trade, travel, and transport in recent decades have accelerated marine biological invasions by increasing rates of new introductions through various pathways, such as shipping, navigational canals, aquaculture, and the aquarium trade (Molnar et al. 2008; Riccardi et al. 2017). At any given moment an estimated 10,000 different species are being transported in ballast tanks alone (Carlton 2001). Furthermore, climate change is predicted to cause significant changes in species ranges and habitats, altering pathways for migration, and increasing flooding, thus also increasing the frequency and extent of species invasions (Rahel & Olden 2008).

Harmful invasive/irruptive species that damage ocean ecosystems include:

- The crown of thorns starfish (*Acanthaster planci*) is native in the Indo-Pacific region and is not harmful at low population densities. However, irruptive crown of thorns starfish (COTS) population booms are considered as a significant threat to coral reefs and are responsible for approximately 42 percent of observed cover loss in the Great Barrier Reef during an irruption (D'eath et al. 2014). Localized nutrient pollution can lead to an increased abundance of

COTS and increased sea temperatures is expected to also increase both abundance and range of COTS (Uthicke et al. 2015).

- The Northern Pacific Sea Star (*Asterias amurensis*) is native to the North Pacific along the coasts of Russia, China, and the Korean peninsula. This sea star is a problematic invasive along the south of Australia. During large population booms, the sea star voraciously consumes endangered native species (Ross et al. 2004).
- The Purple sea urchin (*Strongylocentortus purpuratus*) is native along the Pacific Coast of the United States. Populations of the purple sea urchin's natural predators, such as sea otters and sea stars, have dwindled (Pearse 2006). The resulting purple sea urchin population explosion has decimated kelp forests which are critical habitats for many marine species (Pearse 2006).
- Lionfish (*Pterois volitans and P. Miles*) are native to the South Pacific and Indian Oceans. Along the Eastern Coast of the United States and in the Caribbean, non-native lionfish are voracious fish predators and cause damage to coral ecosystems and native fish populations (Ballew et al. 2016).
- The European Green Crab (*Carcinus maenas*) is native to Europe and North Africa. Invasive in the Americas, Australia, and South Africa, the crab preys on a wide variety of organisms, but is particularly harmful to bivalves (Colnar & Landis 2007).
- The American Blue Crab (*Callinectes sapidus*) is native to the American Atlantic. As an invasive species carried east by transatlantic trade, it has regularly been observed on the Atlantic coast of Europe from Denmark to Portugal, as well as along the western and southwestern shores of the Mediterranean. An omnivore with high trophic flexibility, the American Blue Crab is also aggressive, a fast grower, and has a short reproductive cycle. As a result, invasive populations have expanded exponentially, wiping out native species (Fuentes et al. 2019).

PROGRESS TO DATE

Detection and Prevention

eDNA provides information on species presence without the need for capture or direct observation. eDNA is being used to study invasions in freshwater environments. eDNA was used for the first time to confirm the presence of a freshwater aquatic invasive species, the American bullfrog (*Rana catesbiana*), in 2008 (Ficetola et al. 2008), and has subsequently been used to detect other freshwater invasive species in a variety of environments, such as Mozambique tilapia (*Oreochromis mossambicus*) in the tropics (Robson et al. 2013) and Asian carp in the American Great Lakes (Jerde et al. 2011). Scientists and managers predict that in short order, rapid growth, widespread deployment, and automation of eDNA techniques will transform the sensitivity, speed, and scale with which we detect alien species (Riccardi et al. 2017). Detection of invasive species in the ballast water of incoming vessels at ports could be a strategic focal point of monitoring efforts (Zaiko et al. 2015).

Biocontrol

Current underwater control efforts of marine invasive and irruptive species primarily involve killing or capture by divers. These methods have been relatively successful for small geographic areas. For example, COTS are removed by divers or injected with chemicals (e.g. acetic acid). Targeted trapping has shown some promise for green crab population control (Duncombe & Theriault 2017). Generating a market to drive harvest of invasive such as the edible European Green Crab are underway. Lionfish is also edible and has an emerging commercial harvest, despite the fact that they can be toxic to humans (Morris et al. 2012). While these efforts have had some success, they are expensive and labor-intensive, and are difficult to scale.

There is a varying degree of progress to date with respect to understanding the genomics of the most significant invasive and irruptive species in the ocean. Table 1 presents a summary of the current state of the art including progress of breeding and genomics knowledge as well as special considerations such as the potential for social acceptance of genetic biocontrol. Based on this summary information, COTS and Northern Pacific Sea Stars (*Asterias amurens*) seem the most likely potential candidates for genetic biocontrol.

- COTS will spawn and breed in captivity and reach sexual maturity in two years, (Kessing et al. 1997). These factors are conducive to genetic manipulation. The genome has been sequenced and potential aggregating and alarm pheromones identified have been identified, which opens the door to genomic approaches for disrupting mating behavior (Hall et al. 2017). The primary technical hurdles to genetic manipulation efforts are establishing transgenesis protocols, and developing molecular components such as promoters and terminators.
- Northern Pacific Sea Stars breed in lab environments where it is possible to artificially induce spawning (Kanthani 1969). As with COTS, the primary technical hurdles to genetic manipulation are establishing transgenesis protocols and developing molecular components.
- Purple Sea Urchins are a model species for embryology with a long history of lab breeding, making genetic manipulation very feasible. The genome has been sequenced (Sodergren et al., 2006), spawning can be induced (Stepicheva & Song 2014), and transgenesis (Rast, 2000) and genome editing (Lin & Su 2016) demonstrated. The primary technical hurdles are developing molecular components such as promoters and terminators. Another potential major drawback to genetic biocontrol of sea urchins is the market for their use in sushi which may lead to public resistance to transgenic animals in the wild.
- For Lionfish, genetic manipulation is not currently feasible because they have not yet been bred under laboratory conditions, despite the efforts of Florida Tropical Aquaculture Lab (John Teem, personal communication). Further, consumer fear of eating transgenic lionfish may provoke resistance to genetic biocontrol and undermine complementary control efforts via commercial harvest.
- European Green Crab genetic manipulation is not currently feasible. Despite the fact that embryos can be collected from wild-caught crabs, they have not yet been bred in captivity.

Considerations for Genetic Manipulation

Acceptable
Minor Concern
Major Concern

	CAPTIVE BREEDING	DEMONSTRATE TRANSGENESIS	ACCESS TO GERM CELLS	AGE OF SEXUAL MATURITY	REPRODUCTIVE MODE	GENOME SEQUENCED	DEVELOPMENTAL TRANSCRIPTOME	OTHER CONCERNS
Crown of Thorns Starfish	Yes	No (but success likely)	Yes (Inducible)	2 Years	Dioecious Broadcast Spawning	Yes	No (limited transcriptomic data)	Pest in native range. Population management is goal.
Northern Pacific Seastar	Yes	No (but success likely)	Yes (Inducible)	1 Year	Dioecious Broadcast Spawning	No	No (limited transcriptomic data)	Edible, but no commercial harvest.
Purple Sea Urchin	Yes	Yes	Yes (Inducible)	2 Years	Dioecious Broadcast Spawning	Yes	Yes	Pest in native range. Consumed in sushi.
European Green Crab	No	No	No	1 to 3 Years	Dioecious Paired Mating	No	No (limited transcriptomic data)	None Identified
Lionfish	No	No	No	1 to 2 Years	Dioecious Paired Mating	No	No (limited transcriptomic data)	Major aquarium fish. Small, but growing seafood market

TABLE 1 — Considerations for genetic biocontrol.

INNOVATION

Chemical and physical methods as described above may be effective in reducing large problem species populations in a small area. However, they rarely eliminate the last few individuals which can subsequently repopulate the region. Genetic biocontrol methods have greater potential to control this residual population and contain unwanted and harmful species.

Genetic biocontrol is the release of organisms that have been genetically engineered for the purposes of controlling a pest species (Thresher et al. 2014). The engineered individual encounters and mates with the wild members of the species and introduces a genetic system that reduces the population size through a variety of mechanisms. An important benefit of genetic biocontrol is the potential to reduce pest species populations while minimizing the off-target effects. Other potential benefits include reduced toxicant use, more humane (non-lethal) approaches, and expanded application in locations where there may be human conflicts with other less specific methods (Campbell et al. 2015). Genetic biocontrol represents a potentially transformative advance for harmful ocean invasives that is not readily achievable with current technology (IUCN Synbio Assessment).

Repressible Lethal Systems

The Crown of Thorns Starfish and Northern Pacific Sea Star are feasible targets for genetic biocontrol through the use of repressible lethal disruption systems. Repressible lethal systems involve genetic circuits where immature life stages require the presence of a “repressor” molecule (often tetracycline) that prevents a toxic gene from getting expressed. Mature organisms no longer need the repressor and can be released. Offspring resulting from crosses between wild and engineered organisms die in the absence of the repressor (Thomas et al., 2000). The Commonwealth Scientific and Industrial Research Organisation (CSIRO) has performed population modelling which suggests that repressible-lethal systems may be effective against Northern Pacific Sea Stars (Bax et al. 2006). Oxitec has developed commercially approved mosquitoes with a repressible lethal system that has been effective in commercial field trials in Florida, India, Panama and Brazil(Carvalho et al., 2015).

Sex-specific repressible lethal systems are variants of repressible lethal systems where only males/females die in the absence of the repressor and the surviving sex passes copies of the gene to subsequent generations to skew sex ratios. Ideally, the engineered organisms carry multiple copies of the construct to mitigate the effects of the gene being diluted out over a few generations. A major advantage of this system over non-sex selective repressible lethality is that larval stages can be released into the wild which eliminates the costs of rearing engineered organisms to maturity.

Female lethal systems have been engineered into several insect species and fish (Fu et al., 2010; Concha et al., 2016; Thresher et al. 2014). However, field trials in mosquitoes have shown that males can also be affected by low levels of gene expression from the female lethal construct so that they are insufficiently competitive with wild males (Facchinelli et al., 2013). It should be possible to address this problem but it will still impose some limit on the number of copies of the genetic circuit males can carry. CSIRO’s modelling has shown that male-lethal repressible systems may outperform other repressible lethal approaches (Bax et al., 2006). Engineering the sex-specificity into these systems can be somewhat challenging and will require knowledge about gene expression differences between sexes.

Pheromone Disruption

Starfish communicate with each other using chemical signals dissolved in seawater. These protein pheromones can communicate the presence of danger or help to co-ordinate spawning aggregations. Recent work by Bernard Degnan’s group (Hall et al. 2017) identified genes encoding some of these pheromones. It might be possible to engineer starfish to misexpress these pheromones and induce mal-adaptive behavior in others. For example, starfish constitutively producing alarm pheromones may disperse mating aggregations or be used as repellants to protect sensitive areas. Misexpression of aggregation signals may be exploited to draw starfish *away* from sensitive areas and into traps. This remains an entirely theoretical approach for starfish at this time, with no empirical data. However, pheromone disruption is an active control method for some insect pests, used to either trap males or to flood the system so that becomes impossible for males to find females.

Synthetic Species

Synthetic Genetic Incompatibility (SGI) is a method to engineer species-like barriers to sexual reproduction where the SGI 'synthetic species' population cannot form viable/fertile offspring with wild type organisms (Maselko et al., 2017). Combining SGI with a sex-specific lethal system (SSIMS) would result in a limited *in situ* amplification of the engineered population in the wild. When an SSIMS organism mates with wild organisms, no offspring survive. However, when SSIMS mate with other SSIMS, only SSIMS males are generated. Modeling data presented at conferences has shown that SSIMS has the potential to be more effective than repressible-lethal alone for invasive carp (Siba Das, Personal Communication), however, it is not known if this would extend to starfish.

Gene Drives

Threshold independent gene drives can be used to spread genes through a population in a way that alters the standard model of inheritance (Burt 2003). Normally, releasing an organism with a single copy of a recessive-lethal gene (lethal when two copies present) will result in the gene's dilution as it only gets passed on to half of the offspring per reproductive cycle. If two carriers mate, 25 percent of them will be non-viable homozygotes, 25 percent will not carry the gene and 50 percent will be heterozygotes. If the recessive lethal gene is part of a gene-drive system, all offspring receive a copy of the recessive gene and it can spread through all populations connected by gene-flow. Any mating between carriers results in 100 percent non-viable offspring. Gene drive methods are in advanced development in mosquitoes as a mechanism to combat malaria (Hammond et al. 2016) and testing is ongoing in rodents (Leitschuh et al. 2018). However, the technique is controversial due to the concerns over uncontrollable negative effects on ecosystems or from a release of gene drive to unintended areas. Other research outlines large technical hurdles facing gene drives, such as inbreeding, poor homing, and development of resistance mutations (Unckless et al. 2018). Overcoming these challenges will need to be coupled with the development of gene-drive systems with population percentage thresholds and/or mechanisms for spatial/temporal control.

RISKS AND CHALLENGES

Improvement of detection and monitoring of invasives is an urgent priority. Innovation and further development of eDNA techniques could transform current detection efforts. However, while eDNA offers considerable promise for increasing the timeliness and ease in detecting alien species, its application to support quarantine or large-scale invasive species management requires significant development and standardization. Current eDNA methods can suffer from uncertainties in species identification (especially in marine environments), presents a risk of false positives, and could have weak statistical power (leading to overconfidence when no detections are recorded). Nonetheless, the power of eDNA technologies and their adoption at scale will likely become a major focus of invasion science (Riccardi et al. 2017).

The technical challenges to develop genomic biocontrol of marine invasive and irruptive species are significant but not impossible. A significant amount of foundational research and proof of concept will be required prior to field implementation. Specifically, ecological and population

genetic research on the target species is essential to determine the feasibility and effectiveness of the various biocontrol approaches. The genetic approaches for eradicating or reducing the impact of invasive rodents are still in their infancy, and biocontrol methods are even less advanced for ocean species; the timeline to develop a comprehensive field trial proposal is estimated to be at least five years. To accelerate this timeline, proof-of-concept marine biocontrol projects should target systems in which foundational ecological and genomic research has already been completed. For example, COTS biologists are able to predict with a high level of certainty where and when irruptions are likely to begin, making this invasive species a strong candidate for genomic biocontrol (Russ Babcock, *personal comment*).

Intervening in nature using genetic biocontrol methods is controversial largely due to the nascent state of the field. Because these are still unproven technologies, it is essential that the field moves forward cautiously and obtains social license from the public and policy makers. Any unintended harm that may result from premature implementation of these technologies would result in a more restrictive regulatory and funding environment and further delay the potential benefits of applying synthetic biology to environmental challenges. In Australia several biocontrol agents have been delayed through the approval process, possibly because of high risk-aversion and limited expertise within the regulatory agencies. Marine applications pose an even higher burden of proof due to the shared nature of marine ecosystems.

Many countries require risk assessments to be carried out before research and pilots with biocontrol agents as a means to predict environmental risk. Post-release studies to validate decisions are rarely required by regulators, but where these studies are conducted, provide valuable information for future decision support.

For some species like the Purple Sea Urchin with a long lifespan, it could take a long time for genetic methods to suppress populations. However, combining genetic controls with manual removal may still help to dampen population explosions in the near term. Species such as sea stars, and many other marine species, represent a hypothetically risky option for implementation of genetic engineering, since their young are planktonic and spread worldwide through cargo ship ballast. This uncontrolled movement may have legal ramifications that must be examined, however, the approaches discussed here would have a limited ability to persist for multiple generations (with the exception of gene-drives) and are not likely to impact distant populations. Examining the legal framework regarding the inadvertent movement of transgenic plant pollen and seeds may be illuminating. Ultimately, it is still unclear what impact genetically modified ocean species will have if released into the wild, accordingly ecological studies and careful testing will be required prior to implementation in natural systems.

The timelines and financial investment required to get from our current level of knowledge to implementing genetic control methods in the wild could be 5-10 years. However, this risk is mitigated by the knowledge that successful implementation of genetic biocontrol programs will provide a forerunner for other efforts with other species, and that foundational research will provide potential benefits for non-genetic biocontrol efforts. Such research will also provide great

benefits to other fields such as marine biology, conservation science, and perhaps even human health.

LEADERS

- As relatively isolated islands, where invasive species have an oversized impact, the governments of Australia and New Zealand have significant funding programs for national and international research of invasive and irruptive species.
- These efforts include developing innovative technologies and tactics for control and eradication of invasive species (NZ Ministry of Business, Innovation and Employment: Catalyst Fund investing in non-transgenic technology) and continuous marine pest biosecurity research and development and the development and validation of assays for detecting marine pests (Australian Marine Pest Sectoral Committee).
- The Bill and Melinda Gates and Tata foundations have invested in genetic biocontrol technology for disease vectors. The US Defense Advanced Research Projects Agency (DARPA) has invested in numerous genetic control projects including providing early funding to the Genetic Biocontrol of Rodents (GBIRd) Program, which is investigating the feasibility of, and assessing the social, ethical, and biological risks of, gene-drive modified organisms for eradication of island invasive species. This is a collaborative effort between governments, NGOs, and research universities including: CSIRO, Island Conservation, Landcare Research, North Carolina State University, Texas A&M University, University of Adelaide, and the United States Department of Agriculture (USDA).

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WILDLIFE DISEASE

Consultant: Devaughn Fraser

THREAT

Emerging infectious diseases in wildlife are increasing in frequency and severity, largely due to human impacts on natural environments. Human activities can compound the effects of disease by altering the biotic and abiotic environment, disseminating pathogens to new localities and populations, increasing domestic host densities, and introducing chemical pollutants that cumulatively compromise immune function in wild species.

Systematic reviews of the scientific literature show increases in marine disease outbreaks in several taxa since the 1970's including sea turtles, mammals, corals, echinoderms, and molluscs (Ward and Lafferty 2004; Simeone et al 2015) and several groups of aquatic plants (Egan et al. 2014). Major diseases in wildlife can have profound ecological consequences when foundational species (e.g. sea stars) are affected, and in some cases, can even drive extinction. Most documented marine disease outbreaks are associated with compounding factors including climate change, pollutants (nutrients, toxics and noise), aquaculture, invasive species, and habitat loss. Diseases in marine ecosystems pose a particular challenge due to several factors: lack of adequate monitoring for early detection and management, a gap in baseline data for current biodiversity and microbial assemblages in healthy states, the extent of exotic species introductions, and the sheer vastness of the ocean, which makes it particularly subject to both localized and global threats.

Aquatic Plants and Macroalgae: Disease in aquatic plants and algae are of great concern given the habitat-forming role of such organisms in marine ecosystems. Changing ocean conditions due to climate appears to be a major driver of marine disease, as changing climatic conditions can induce higher physiological stress, thereby increasing host susceptibility or altering pathogen dynamics such as growth rates and virulence. Identifying pathogens and linking pathogen presence and growth to environmental variables should be a priority in research. eDNA-based monitoring and surveillance should be established for a variety of marine aquatic plants and macroalgae.

Kelps: Macroalgae are the primary habitat-forming plants along temperate coastlines and play critical roles in food webs. Kelp forests are disappearing from major sections of temperate coastline around the world, and climate induced disease is increasingly recognized as one potential driver of population declines (Egan et al., 2013). Bleaching is a common phenotype of potentially diseased states that leads to reduced fitness. A single-strand DNA virus has been identified as a potential causative pathogen for bleaching in *Ecklonia radiata* along the Australian coast (Beattie et al., 2018), although in several instances, disease appears to be the outcome of not a single pathogen but of distinct combinations of microbial organisms (Egan et al., 2013;

Wahl et al., 2015; Kumar et al., 2016). In general, however, disease conditions in kelp are associated with climatic variables (Campbell et al., 2011), suggesting that climate change will continue to exacerbate disease issues in kelp.

Seagrasses and Eelgrasses: Similar to kelp forests, seagrasses have important roles in coastal ecosystems that are increasingly compromised by anthropogenic threats (Orth et al., 2006). Wasting disease, caused by a slime mold in the genus *Labyrinthula*, is an increasing threat to seagrass species worldwide that may operate in tandem with other major threats to drive species extinction (Orth et al., 2006). For example, *Labyrinthula zosterae* has been shown to be more highly virulent at warmer temperatures (Eisenlord et al., 2017).

Marine Invertebrates: Disease is an increasing threat to corals, molluscs, sponges, and echinoderms. Many affected species play critical roles in ecosystem function which can have ecological and economic consequences following disease outbreaks. Climate change is again likely causing increased disease occurrence in marine invertebrates, suggesting that disease impacts will likely continue to increase without intervention. Genomic research should aim to uncover mechanisms of high thermal and low pH tolerance, with the potential for gene editing or pharmaceutical approaches to conserve vulnerable populations.

Corals: As discussed at length earlier in this chapter, coral disease is a pervasive conservation issue, with several studies supporting the hypothesis that coral disease outbreaks are increasing in frequency and prevalence (Sutherland et al., 2004; Ward and Lafferty 2004; Willis et al., 2004). A large body of research is dedicated to characterizing microbial communities and pathogens associated with diseased states using molecular techniques (Littman et al., 2011; Hadaidi et al., 2018). Coral bleaching is associated with a variety of anthropogenic stressors including thermal stress due to climate change (Maynard et al., 2015), sedimentation plumes caused by dredging (Pollock et al., 2014), nutrient enrichment (Vega et al., 2014), and pollution (Lamb et al., 2018). Coral colonies exposed to temperature stress showed variable transcriptomic responses in a subset of genes associated with resistance to bleaching, which largely operate within the unfolded protein response (Traylor-Knowles et al 2017).

Sea Stars: Sea star wasting disease has caused mass mortality events in a top predator of intertidal environments across the Pacific Northwest and California coast. These top predators play a critical role in intertidal ecosystems by controlling foraging pressure on kelp (Schielbelhut et al., 2018). Higher temperature has been demonstrated in laboratory settings to increase susceptibility to this disease (Bates et al., 2009; Kohl et al., 2016). While the disease has been shown to be infectious, a causative pathogen has yet to be identified, although a densovirus is a strong candidate (Hewson et al., 2014). Genomic approaches targeting transcriptome profiling of diseased and resistant individuals are underway (Dawson Lab, UC Merced-personal communication). Further work is needed to identify the causative pathogen(s) and environmental causes before intervention can be considered.

Molluscs: Molluscs provide critical ecosystem services through filter feeding, essentially clearing the water of biotic and abiotic contaminants. As such, they can accumulate very high pathogen and toxin loads and are susceptible to a wide range of diseases. Several such diseases,

including Dermo, MSX, Marteliliosis, and OsHV-1 herpes virus, are associated with outbreaks with warmer temperature (Guo and Ford, 2016), suggesting that thermal stress due to climate change may increase mollusc disease occurrence in the future.

Sponges: Disease in sponges are being increasingly recognized and are geographically widespread, with high rates of mortality affecting both ecological and economic value of sponges (Luter and Webster 2017). Specific pathogens vary widely but disease is most frequently associated with bacterial agents, fungal species and general shifts in the cyanobacterial communities (Luter and Webster 2017). Elevated ocean temperatures may play an important role in disease outbreaks in sponges (Cerrano et al., 2000; Luter and Webster, 2017) but research is limited.

Marine Vertebrates: Sea turtles and sea mammals are highly vulnerable to demographic impacts of disease, as they are generally very long lived and have slow reproductive rates. Conserved evolution of vertebrate immune systems makes marine vertebrates an ideal system to develop precision medicine techniques for wildlife, as immune proteins/pathways are well characterized for humans and mice, and bioinformatics can be used to identify potential antigen and host immune cell receptor compatibility.

Fishes: Although reports of disease occurrence in fishes have not increased in the literature (Ward and Lafferty, 2014), this is possibly due to poor monitoring and insufficient baseline data. Aquaculture potentially intensifies disease occurrence and transmission in wild populations, as well as introduces novel pathogens into wild environments. In a list of 67 marine diseases that have direct economic impact, 49 percent were diseases of fishes, including viral (22 percent), bacterial (45 percent), protist (9 percent), and metazoan (24 percent) pathogens and parasites (Lafferty et al., 2015).

Sea turtles: Of the seven species of sea turtle, five are currently listed on the US Endangered Species Act and the remaining two are known to be declining in numbers across at least some part of their range. In addition, five of these species are known to be affected by fibropapillomatosis, a tumor-forming disease potentially associated with a herpes virus chHV5 (Lawrance et al., 2018). Transcriptome analysis showed shared genomic drivers with certain human cancers, which has led to advances in treatments and novel therapies following surgery on turtles afflicted by orbital tumors (Duffy et al., 2018).

Mammals: A systematic review of disease-related publications in marine mammals (Simeone et al. 2015) showed 65 percent of published cases from 1972-2012 occurred in California, with the peak number of cases occurring between 1998-2004. While this may reflect a significant reporting bias due to the presence of two major Marine Mammal rescue centers in the state that serve as major sources of disease data, such patterns may also reflect heavy coastal burden due to high human population densities across the California coast. The majority of pathogens were bacterial or caused by biotoxicosis associated with algal blooms. Fungal, viral, and protozoal disease made up a smaller proportion of reported cases, but all are increasing in frequency. Interestingly, there are broad scale geographic patterns in marine mammal disease cases. For example, protozoal diseases appear to be increasing, particularly in otter populations in

California and the Pacific Northwest; while viral infections appear to be increasingly more common on the Atlantic coast (e.g. morbillivirus in bottlenose dolphins).

Pollution is thought to be a major driver of disease in marine mammals due to negative impacts on immune function (Ross, 2002; Ylitalo et al., 2005; Desforges et al 2016; Penin et al., 2018). Marine mammals are at the top of the food chain so they bioaccumulate high levels of toxins over their lifetimes, and consequently are increasingly immunocompromised over time (Desforges et al., 2016). Genetic factors can also be critical predictors of disease manifestation (Bately et al., 2014; Browning et al., 2014), which can be exacerbated by low genetic diversity in small populations, and which may interact with environmental factors such as pollution. For example, urogenital carcinoma in California sea lions has been linked to homozygosity at a single microsatellite marker which maps to heparinase 2 gene, a gene associated with multiple carcinomas in humans (Browning et al. 2014). However, mortality in sea lions that develop cancer occurs with higher probability in animals that test positive for exposure to organochlorine residues (Ylitalo et al., 2005).

Viral infections, especially morbillivirus, in marine mammals are particularly concerning given the frequency of cross-species transmission among aquatic species and between terrestrial and aquatic species, including humans (Jo et al., 2017). Marine mammals are also increasingly contracting diseases likely to have been transmitted from terrestrial species such as toxoplasmosis (Burgess et al., 2018).

PROGRESS TO DATE

The technology era has introduced a wealth of tools to enable monitoring of wildlife through citizen science efforts (i.e. iNaturalist; CALeDNA) and centralized reporting. Some examples exist of citizen-based monitoring in oceans. For example, Ocean Sanctuaries allows non-researchers to upload photos to document wild species, enhancing baseline measures to monitor changing ocean conditions. Similarly, the NOAA sponsored Marine Debris Monitoring and Assessment Project is a citizen science initiative that engages partner organizations and volunteers nationwide in completing shoreline marine debris surveys. Cumulative effects and impacts to marine ecosystems, fish and wildlife are hard to track and monitor but these new efforts are a good step in attempting to track marine disease.

Centralized databases have been established in a variety of countries such as Canada and Australia to monitor and report disease incidence in wildlife. In the United States, the USGS has collated data for all wildlife health incidence reporting and established a framework to report and query wildlife health incidences. However, these are focused predominantly on terrestrial and freshwater ecosystems. Additionally, a major fallback of government sponsored projects is that they are subject to political events such as government shutdowns and administration changes, which can impair their efficacy and long-term viability. In marine environments, efforts have been established to establish a centralized database to monitor marine mammal health, but the project lacks sufficient support and is not updated regularly. Further, disease is a widespread conservation challenge, and given the complexity of ecosystem interactions, a more powerful

strategy would be to monitor and report disease across taxa in order to track changes with respect to environmental variables over time.

The use of eDNA has proven successful in identifying pathogen presence in freshwater systems, aiding in the management of chytridiomycosis in amphibians, one of the most devastating wildlife diseases known (Schmidt et al., 2013), as well as parasite presence in amphibians (Huver et al., 2015). However, no such efforts specific to disease have been reported in marine environments, although eDNA is a demonstrated tool for biodiversity monitoring in oceans (Stat et al., 2017).

Genomics has revolutionized medicine in humans. Gene expression and genome wide association studies have provided suites of genes that are useful in the early detection, prognosis, and therapy design for cancers (Kuderer et al., 2011) while several drug therapies have been developed to treat autoimmune disease based on genomic insights (Leiding et al., 2018). To date, genomics has aided in disease ecology primarily through a focus on the pathogen, resulting in the identification of putative virulence factors, geographic origin, and transmission dynamics (Ren et al., 2003; Picardeau et al., 2008; den Baaker et al., 2011; Rosenblum et al., 2012; Valdazo-González et al., 2012; Kamath et al., 2016). Increasingly, however, precision medicine is being explored as a viable option to manage emerging infectious disease in wildlife and to conserve species (Whilde et al., 2017; Duffy et al., 2018). For example, transcriptome sequencing of fibropapilloma tumors in sea turtles revealed similarities with human cancers and therefore informed a viable treatment option to prevent tumor regrowth following surgery (Duffy et al., 2018). Additionally, comparative genomics of cetacean morbillivirus (CeMV) strains have yielded insights into transmission and host-variability (Rima et al. 2005), and several candidate genes underlying resistance and susceptibility have been identified in bottlenose dolphins (Batley et al., 2018).

Vaccines in marine wildlife are relatively underexplored. Preliminary trials for CeMV targeting the fusion (F) and hemagglutinin (H) genes have been conducted in US Naval trained dolphins with some success (Vaughan et al., 2007). However, these vaccines are DNA vaccines, which carry several risks such as inducing antibody production against DNA or affecting genes controlling cell growth. These risks are minimal compared to using live attenuated viruses (where the virus has been mutated to inactivate virulence), indicating the potential of a mutation in the vaccine virus to reinstate virulence and cause an inadvertent disease crisis (Duignan et al., 2014). Such an outcome could be catastrophic in a wild species, particularly in an ocean ecosystem where transmission could occur rapidly over broad geographic scales and across species. Trial vaccinations against morbillivirus in Hawaiian monk seals have been implemented in recent years using a ferret recombinant vaccine (Robinson et al., 2017). However, manufacturer availability is a challenge, and the efficacy of the vaccine in this species remains uncertain.

INNOVATION

There are three key opportunities to improve conservation efforts directed at marine disease using technology and genomic tools. These include the 1) establishment of a centralized database for marine disease reporting across taxa; 2) establishment of 5 mobile laboratories equipped with real time sequencing technologies to rapidly characterize and monitor microbial

diversity and associations with disease in kelp; and 3) development of a precision medicine model for wildlife species through comparative “omics” research to inform vaccine and therapy design.

Genomic technologies hold great potential to improve conservation measures for marine wildlife threatened by infectious disease (Whilde et al., 2017; DeCandia et al., 2018). Specifically, pathogen sequencing initiatives can vastly advance the development of vaccines or enable discovery of exploitable weaknesses using a methodology termed “reverse vaccinology” (Del Tordelo et al., 2017), while large-scale whole genome resequencing of host populations can help to identify resistant populations and genetic variation associated with resistance that can potentially be engineered to protect vulnerable populations. Understanding genetic variation linked to resistance can aid in disease modelling, particularly where such variation can be spatially mapped against probable transmission corridors. It can also accelerate the development of disease-resistant stocks through marker-assisted selection (Guo and Ford 2016), which may be critical for coral conservation in the face of coral bleaching disease, for example. Finally, whole transcriptome sequencing studies in both controlled experimental settings and in wild populations can help to understand genomic pathways involved in the host response to stress and disease (Fraser et al., 2018). In summary, we need better monitoring and genomic resources for hosts, pathogens, and the environment to identify drivers of disease outbreaks.

Centralized Reporting and Monitoring Database

What is needed is the establishment of a centralized reporting database designed to enable observations from multiple stakeholders, researchers, and public and to link key research and sequencing data generated to understand drivers of emerging disease in marine wildlife.

In 2011, the Ecology of Marine Infectious Disease workshop identified the following priorities for data sharing:

- Improve baseline data collection by standardization of protocols and reporting methods.
- Support a coordinated expansion of existing data sources (e.g., USGS Wildlife disease information network database, wildlife disease.nbii.gov, environmental health tracking networks, coral disease registry).
- Share and archive all data from EID funded projects in appropriate data center(s) with a web portal created for purposes of disseminating information.
- Authorize resources for EID projects in collaboration with existing studies (e.g., NSF Long-Term Ecological Research (LTER) Network and the National Ecological Observatory Network (NEON)) to add pathogen and disease-relevant data.

Seemingly, none of these priorities have been implemented, and there remains no centralized repository for information and reporting of diseases in oceans. Such a database could link research efforts globally, help identify important drivers of disease based on common patterns or conditions during outbreaks, and can facilitate early action and response to a disease outbreak, all of which will greatly improve conservation outcomes. Further, NSF funding for the EMID

research coordination network is set to expire in 2019, suggesting opportunity for alternate funding sources to help the program meet its original objectives.

In partnership with the EMID Research Coordination Network, a centralized reporting database specific to marine diseases should be designed and implemented. This database should be public domain and provide opportunity for scientists, citizen scientists and marine rehabilitation centers to report eDNA studies, monitoring efforts and data, disease outbreaks, and anomalous mortality and morbidity events in sea life. The database would be linked to a genomics repository, where eDNA monitoring results, pathogen sequencing and phylogeography data, and genomic variants associated with resistance will be identified and mapped.

The initial investment will be into coordinating a working group meeting to identify key reporting priorities, establish a framework for the database, and determine roles. Subsequent investment will be in purchasing domain space or cloud storage as a repository for the data and development of the database infrastructure.

eDNA Mobile Monitoring Laboratories to Support Global Kelp Forest Health

Establishing an eDNA monitoring and surveillance program designed specifically for characterizing microbial communities across space and time in kelp forests.

A major priority for managing wildlife disease in oceans is early detection. Thus, there is a compelling need to develop tools that will better characterize microbial environments in stressed vs. non-stressed systems and potentially identify novel pathogens based on molecular and phylogenetic signatures of pathogenicity (Stobbe et al., 2014; Bass et al., 2015). We have discussed the power of eDNA to be used as a monitoring and surveillance tool. Establishing an eDNA program specific to assessing health in marine systems, where samples are collected longitudinally in strategic locations and in a standardized manner could vastly improve disease management efforts. We propose establishing several mobile marine laboratories equipped with on-site sequencing tools (such as the Oxford Nanopore MinION©) designated at key functional sites where disease outbreaks are likely to occur or would have the greatest impact. We propose to focus on foundational species such as kelp, as autogenic ecosystems, but increasingly recognized as major conservation priorities that would benefit from increased monitoring programs (Krumhansl et al., 2016). These laboratories will also enable direct sequencing of kelp on-site for rapid and coordinated studies to identify adaptive resistance variation. Such laboratories could also facilitate targeted PCR assessment of pathogen presence in focal species such as cetaceans, turtles, and pinnipeds sampled opportunistically or in coordinated efforts with nearby rehabilitation facilities to better characterize the ecology of other important wildlife diseases.

We propose establishing five mobile laboratories to facilitate long term eDNA-based monitoring of kelp forests in five ecoregions identified as most threatened (Krumhansl et al., 2016) in collaboration with global researchers studying kelp forest dynamics and microbial assemblages.

Precision Medicine and Reverse Vaccinology for Disease in Marine Vertebrates

For species of high conservation concern, develop a model for precision medicine in marine vertebrates informed by comparative genomic studies, pathogen sequencing, and recombinant protein technology. The selection of species and pathogens would need to be carefully considered with objective factors like the ecological role of the species and the source or cause of the pathogen. A model for precision medicine targeted at focal taxa based on the conservation genomics toolkit will greatly advance the treatment and prevention of priority wildlife disease. Two systems for evaluating this application of genomics in wildlife disease are currently ready for development.

California Sea Lions: Several diseases are affecting California sea lions. Understanding the ecology of these diseases will benefit greatly from a better understanding of genetic processes influencing susceptibility. For example, urogenital carcinoma in California sea lions is highly associated with homozygosity at a single microsatellite locus which maps to intron 9 of heparanase 2 gene [HPSE2] (Browning et al., 2014). Additionally, disease outcome to leptospirosis infection appears to be related to the number of MHC II DRB genes, but with surprising results (Acevedo-Whitehouse et al., 2018).

Until recently, there was no reference genome for California sea lions. Having a good quality, high coverage genome is an important first step. However, this should be immediately followed by comparative transcriptome and whole genome resequencing of diseased and healthy individuals. Then, identification of specific sequence variants that can recognize pathogen epitopes can be used to guide vaccine development.

California Sea Lions would make an ideal model for several reasons. First, they are not endangered, and in fact, populations appear stable or increasing, therefore providing ample opportunity for sampling and therapy testing without demographic impacts. Ultimately, treatments developed for California sea lion may be readily adapted to rarer species of concern. Second, since California sea lions are frequently treated for a variety of maladies, including disease, at marine rehabilitation centers. A coordinated sampling effort involving collection of RNA-stabilized blood samples could help evaluate transcriptional changes in response to infection.

Morbillivirus in marine mammals: Several morbillivirus' have emerged as conservation challenges in recent decades, such as phocine distemper (PDV) and cetacean morbillivirus (CeMV). These viruses are easily transmitted across different host species and thus may directly impact endangered populations. Endangered species, such as the Hawaiian monk seal, could be driven to extinction in the event of a morbillivirus outbreak. Research into recombinant protein vaccines (only proteins are used to elicit immunologic memory as opposed to live virus), guided by comparative genomics and bioinformatic analyses, could pave the way for vaccine development that is safe and effective for wild species.

Reverse Vaccinology and Bioinformatic Therapy Design in wild species would involve 4 components:

1. Comparative genomics and transcriptomics of host (requires sequenced and annotated reference genome). Whole genome/transcriptome sequencing susceptible vs. resistant hosts can identify key immune sequences involved in the response to infection and can direct bioinformatic and simulation modeling for antigen recognition and vaccine design.
2. Comparative genomics of the pathogen. Pathogenic vs. non-pathogenic strains can be compared to identify virulence factors that can be targeted by drug therapy. Bioinformatic analyses of the pathogen can also be used to scan for probable antigens.
3. Antigen epitopes (pathogen) and antigen receptors (host) can be modeled in silico to predict binding efficiency and antigenic response in hosts.
4. Recombinant protein research to produce a viable vaccine.

RISKS AND CHALLENGES

There are few risks associated with establishing a marine disease reporting database. This would provide an invaluable resource for documenting and identifying marine disease outbreaks, thereby identifying environmental and ecological associations with disease and providing researchers and managers with predictive power and early warning signs to improve disease management outcomes. Designing and implementing such a global database will be a challenge. The initial investment will be into coordinating a working group meeting to identify key reporting priorities, establish a framework for the database, and determine roles. Subsequent investment will be in purchasing domain space or cloud storage as a repository for the data and development of the database infrastructure. Another major challenge will be coordinating and training the global research community to utilize the database.

While there may be few risks associated with establishing an eDNA program for oceans, there are several challenges. First, ocean chemistries can significantly shorten the longevity of DNA in the marine environment. Sampling would therefore need to occur with high regularity depending on the purpose of the monitoring. This creates a significant data management challenge. Second, sample storage and processing will require adequate space and technical expertise and a dedicated lab space. Third, access to on-site technology capable of collecting and generating eDNA sequence data would greatly improve eDNA monitoring. Investment into mobile laboratories incorporating portable sequencing tools such as the nanopore ion would greatly improve program outcomes. Finally, the potential benefits of such a program will not be immediate. To maximize conservation potential, the program would need to be developed with long-term goals in mind, as one of the primary objectives would be to establish baseline measures of diversity. However, with coverage across a broad geographic scope (i.e. global), patterns may emerge rapidly that will provide important conservation insights.

Developing precision medicine for wildlife poses the greatest challenges for several reasons. First, a substantial initial investment will be necessary to generate the genomic resources necessary. While a genome can be sequenced, assembled and annotated relatively quickly and at low cost, it may take substantially more time to facilitate enough samples for gene expression studies or comparative genomic analysis. Second, insights from any comparative genomics would then need to be vetted extensively and verified in the lab using an accessible and logical

model system. Third, disseminating and monitoring vaccine efficacy would be the greatest challenge, as these are wild species and typically only opportunistically available for treatment. Vaccine often require boosters for long term protective immunity to be established. Therefore, tracking devices would be necessary at the launch of any vaccine evaluation, such that individuals can be relocated as necessary. In some instances, captive populations may be available for initial testing, however, this raises ethical challenges against experimentally infecting captive animals with a dangerous pathogen. Precision medicine has yet to be fully realized even in human disease, and the development of such a model will take time and substantial investment. Selecting the appropriate model is key, and such endeavors or only appropriate for species that are of high conservation concern.

LEADERS

- **Smithsonian Environmental Research Center**, Dr. Katrina Lohan

  Effects of anthropogenic activities on parasite dispersal and invasion; functional genomics of disease, parasite diversity; and relationship between biodiversity and disease.

- **Ecology of Marine Infectious Disease Research Coordination Network**

  NSF-funded science network that includes many universities and PIs to apply science to management of marine infectious disease.

- **Marine Science Institute, University of California, Santa Barbara,** Dr. Kevin Lafferty

- **Marine Mammal Center, Sausalito, CA**

  Several researchers are directly involved in research directed at disease in marine mammals, specifically leptospirosis and urogenital carcinoma in sea lions and morbillivirus in cetaceans.

  Dr. Frances Gulland (Research Associate, Wildlife Health Center, UC Davis; former lead scientist at the Marine Mammal Center is Sausalito, CA). Specializes in the study of disease in marine mammal populations. Conceptualized and initiated the Marine Mammal Health Map.
  http://sccoos.ucsd.edu/projects/mmhealth/

- **Dr. Torsten Thomas** (Professor, University of New South Wales) Specializes in microbial community dynamics, using high-throughput DNA sequencing and bioinformatics to make predictions about functional and ecological properties of bacterial communities.

- **Dr. Andrew Rassweiler** (Project Scientist, Marine Science Institute) Specializes in modeling kelp forest dynamics during transition states using long term ecological monitoring data.
- **Dr. J. A. Vásquez** (Departamento de Biología Marina, Universidad Católica del Norte) Specializes in kelp monitoring and restoration in Chile.

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WILDLIFE TRADE

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EXPERT INTERVIEWS

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BACKGROUND

Wildlife trade is one of the biggest drivers of biodiversity loss and leads to the direct death of millions of individual animals across tens of thousands of species worldwide (Challender et al., 2015). Biodiversity loss degrades ecological integrity, from food chain dynamics and ecosystem functions to mutualistic relationships. The harm inflicted by the wildlife trade is multiplied since species with an oversized ecological influence are often targeted: apex predators, keystone species, pollinators, dispersers, browsers, and ecosystem engineers (McKlennan et al., 2016; Ripple et al., 2016). The World Economic Forum's Risks' Report identified biodiversity loss and ecosystem collapse as one of the major drivers of global risk that may lead to the spread of infectious diseases, food crises, water crises, and man-made environmental disasters (Gascon, 2015).

Legal wildlife trade, including fisheries and timber, is worth an estimated $300 billion globally (TRAFFIC, 2018). Comparably, illegal wildlife trade is a USD $20 billion industry (UNODC, 2016; Global Financial Integrity, 2017). The primary marine organisms (non-fisheries) illegally traded include large-bodied, high-value species traded for food (i.e. sharks, rays, sturgeon, and whales) or for the entertainment industry (i.e. whales and dolphins) and smaller-bodied species traded for food (i.e. european eel) traditional Chinese medicine (i.e. seahorses) or for ornamental purposes (i.e. coral reef products, aquarium fish, and shells).

The scale of killing for the trade in oceanic species is massive.

- Between 63 and 270 million sharks and untold numbers of rays are killed each year, primarily for their fins, with a commercial value of USD $540 million to $1.2 billion.
- Despite the International Whaling Commission (IWC) enacting a moratorium on commercial whaling, more than 2,000 whales and 90,000 dolphins are killed annually for meat or for fishing bait (IWC; Fisher & Reeves, 2008), and this number is set to rise with Japan's recent decision to restart commercial whaling in 2019. The other major threat to cetaceans is marine parks, which contain more than 3,000 individuals, including more than 2,000 dolphins, 200 beluga whales, 60 orcas, and 30 porpoises taken from the wild (Lott & Williamson, 2017).

- All 27 species of sturgeon are listed by the International Union for the Conservation of Nature (IUCN), and 16 are Critically Endangered, due to the trade and consumption of caviar (i.e. sturgeon eggs). Without intervention against illegal and/or unsustainable trade, sturgeon will go extinct (IUCN Sturgeon Specialist Group, 2018).
- Every year approximately 37 million seahorses (genus *Hippocampus*) are caught in the world's non-selective fishing gears, and most find their way into international trade for use as traditional medicine (Lawson et al., 2017), selling in Hong Kong for as much as $1,000-$1,200 USD per kilogram of dried individuals (S. Foster, *personal communication*).
- Corals and reef fish are popular in the United States, European Union, and Japanese aquarium trades, with 30 million fish and 1.5 million coral colonies traded per year. In the United States alone, more than 400,000 pieces of coral are traded annually (Rhyne et al., 2014).

Even with the moratoriums and trade limits imposed by CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora), enforcement and monitoring are the greatest challenge for both the legal and illegal trade of all marine species. Legal products are often indistinguishable from illegal counterparts once they have entered the supply chain and current methods are limited due to financial and technical considerations and capacity gaps. However, due to recent advances in the field, this Ocean Genomics Horizon Scan identified a number of potential opportunities to employ genomic tools for monitoring and reducing the illegal and/or unsustainable trade of marine wildlife.

PROGRESS TO DATE

Molecular biology, particularly the advances in polymerase chain reaction (PCR) methods, made its first contribution to the detection of illegally-caught marine species when two scientists set up a small sequencing laboratory in a hotel room to identify "fish" being sold in the Tokyo fish market (Baker and Palumbi 1994; Baker et al., 1996; Palumbi & Cipriano, 1998). Dr. C. Scott Baker and Dr. Steve Palumbi identified 28 species of cetaceans among the marine life for sale, including several protected species such as humpback, western gray, fin, Bryde's, and small-form Bryde's whales. This work provides the foundation for many current genomic interventions.

In 2010, Baker and colleagues established that whale sashimi sold in Los Angeles and Seoul was sourced from Japanese "scientific" whaling, by comparing mitochondrial sequences and microsatellites of the whale used for sashimi with validated reference genomes curated by DNA Surveillance, a web-based database for cetaceans (Ross et al., 2003). Following this study, molecular registries have been completed for Norwegian minke whales (Glover et al., 2012) and Japanese whales (data not publicly available). Baker and colleagues (2007) also combined these techniques with classic mark-recapture methods to estimate the number of whales entering the market. While these efforts required technical skills and the transportation of samples to the

United States and New Zealand for analyses, new technologies and assays are now available to identify whale and dolphin meat in the field.

Building upon these PCR-based methods, Diego Cardeñosa at Stony Brook University and Dr. Demian Chapman at Florida International University recently developed a rapid-tool for detecting CITES-listed sharks, funded by Paul G. Allen Philanthropies (Cardeñosa et al., 2018). The real-time PCR test for 9 CITES-listed shark species is rapid (approximately four hours), reliable (all 9 species are regularly identified from field samples), and cost-effective. After the initial purchase of the portable Chai Bio Open qPCR unit for $4,300, the per-sample cost of running the test is USD $0.94 in reagents. This tool is being championed at CITES meetings.

One of the most prevalent data gaps in fisheries management, including sharks and rays, is the lack of traceability of products. However, Genetic Stock Identification (GSI) methods could be used to assess the stock composition of a fishery or market that could have multiple sources, which would play an essential role in assessing population-specific exploitation levels. In one example, Chapman et al. (2010), reconstructed the natal source population of origin of 62 scalloped hammerhead shark fins sampled from the Hong Kong shark fin market using GSI methods to demonstrate mitochondrial DNA regions exhibited regionally distinct haplotypes.

Because of caviar's commercial value, moderate progress has been made on sturgeon genomics. Genidaqs, a Sacramento, California-based company, is sequencing the whole genome of white sturgeon (Scott Blankenship, personal communication). In the European Union, genomic techniques of tracking sturgeon have been prioritized as critical to maintaining sustainable trade practices. The SturSNiP program, led by Dr. Rob Ogden at the Tools and Resources for Applied Conservation and Enforcement (TRACE) Network, represents the first major step in the development of a comprehensive suite of new DNA markers for the forensic identification of caviar products traded within the European Union. SturSNiP aims to provide a standardized identification system for fish parts and derivatives and for supporting sustainable aquaculture practices. Researchers within the SturSNiP project are working to discover SNP markers in several sturgeon species: Russian (A. gueldenstaedtii), Persian (A. persicus), Siberian (A. baerii) and Adriatic (A. naccarii). The SNP discovery method was enriched for markers that are polymorphic among species and candidate SNPs were tested to confirm their ability to authenticate pedigree.

Two researchers from the SturSNiP consortium, Dr. Elisa Boscari at University of Padova and Dr. Milos Havelka at University of Hokkaido developed primers and simple PCR-gel and electrophoresis-based tools that can identify species of sturgeon and hybrids from their eggs. These researchers showed that many (though not all) sturgeon products can be identified to the species level by analysis of the mitochondrial cytochrome b gene (Boscari et al., 2014; Havelka, et al., 2017). Dr. Boscari also investigated genetic bases for sex-determination of sturgeon, which can be found on AnaccariiBase, while Chen and colleagues (2017) at the China Academy of Sciences recently completed exploratory CRISPR work that could enable future genetic manipulation of sturgeon. These studies demonstrated the potential to apply genomic techniques for selective breeding and genetic engineering of farmed sturgeon, which could increase yields of

farmed sturgeon and reduce the pressure from the trade on wild populations. Although promising, much more research is necessary before it can be applied in the field.

Seahorses are notably understudied, with fewer than thirty scientists working on the genus around the world (Sarah Foster, personal communication). Currently there are only two whole genomes published: the tigertail seahorse (Quiang Lin et al., 2017) and lined seahorse (Lin et al., 2016). Some efforts have been made to evaluate genetic structure and breeding studies based on microsatellite markers (Mobley et al., 2011 review). Limited studies have utilized DNA barcoding and PCR methods to identify traded species in California (Sanders et al., 2008) and Taiwan (Hou et al., 2018), but these techniques have not been operationalized for a conservation use case (i.e. enforcement) as with the shark fin tool.

Genomic techniques needed to monitor the coral reef trade are still rudimentary. However, a database of mitochondrial DNA genotypes across its geographic range, including data from dried corals, was used to characterize the origin of red coral, Corallium rubrum. Steinke et al (2009) developed genetic assays to identify ornamental reef fish, and genomes have been sequenced for popular reef species: Blacktail butterflyfish (Batista et al., 2018), orange clownfish (Marcionetti et al., 2018), and pygmy angelfish (Fernandez-Silva et al., 2017).

INNOVATION

The scale of the wildlife trade and its impacts on ecosystem integrity compound the importance of monitoring and regulation through the rapid identification of species and products at all stages of the wildlife trade supply chain. Until now, these methods have proven too time-consuming, expensive, and technically complex for implementation in the field. Genomics holds tremendous promise of providing portable, cost-effective, and accurate tools that can support monitoring and interdiction of wildlife trade. These tools would enable enforcement agents to act against shipments, illuminate trends in the illegal wildlife trade, and provide evidence for CITES regulation. With proper evidence and legislation, CITES will have the ability to implement international trade sanctions.

The promising mechanism for creating change is through the development and distribution of tools that can enable the rapid identification of illegal or regulated species by enforcement staff at ports and borders. Other potential opportunities include the use of population genetics to monitor the ecology of commonly traded marine wildlife. Longer-term, through the development of either recombinant-based or genetically-engineered tradeable animals or products, it may be possible to reduce or eliminate the demand for wild populations.

To be effective, genomics monitoring and enforcement methods must be portable and accessible to those who need them. The methods also need to be easily deployed in the field without requirements for a fully equipped lab and technical training. The tools must be affordable enough for regular use and capable of running a reasonable number of samples concurrently. Effective detection must identify multiple illegal samples in a shipment. For example, if one sample contains a single illegal fin, this is often considered insufficient to drive prosecution or confiscation, depending on country.

The current tools for species identification include: 1) a sequencing approach to identify DNA-barcodes for a given species 2) RT-PCR with species-specific primers, assays, and tools, developed from DNA barcodes. The highest priorities for immediate genomics work include developing primers and assays for the most vulnerable ocean species in wildlife trade, including whales, dolphins, seahorses, sturgeon, corals, and reef fish.

Where assay development is advanced, there is an opportunity to jump-start the conservation application of these tools through advance market commitments with the vendors that would supply discounted equipment and consumables, and developing training programs with leading researchers. NGOs in Peru have already purchased real-time PCR units, and Florida International University has developed a training plan that is being adopted by Peru and Hong Kong. Final implementation must make the tool available to the most critical nations for CITES regulation (Diego Cardeñosa, *personal communication*).

In order to successfully transfer and implement these technologies on a much larger scale, including routine inspections, collaborative testing initiatives must be developed involving the various stakeholders (e.g. governments, non-governmental organizations, industry, academic institutions, funders, etc.) to ensure the necessary investments in capacity and financing to enable these efforts. There is a widespread perception that genomic techniques are cost prohibitive for routine screening of products. Accordingly, engagement between nations successfully using these approaches, such as the United States or Hong Kong, and others that need to use them would be a substantial step forward to seeing broader uptake. With successful uptake, a PCR-based approach provides a model that has the potential to transform the monitoring and interdiction of the wildlife trade.

Currently on the market are three commercial products (Chai Bio Open qPCR, Biomeme, and ConservationXLabs Scanner advertising low-to-moderate-cost, portable units, and rapid RT-PCR analyses of DNA barcodes. A fourth company, Thermofisher Scientific, have a range of more expensive units, one of which is currently utilized in Hong Kong. These products are operational for species for which assays and primers have been developed, or for species whose variation is well characterized, and limited assay development is required. Each product is capable of running different numbers of samples in a run and each company offers different costs and services in terms of assay development, need for sample preparation, portability, web interfaces, and training requirements prior to use.

Longer read sequencing approaches would enable a deeper understanding and insight into genetic variation than the tools listed above. This would lower the risk of species misidentifications and provide insights into the extent of geographical variation in DNA sequences. Such insight could be critical for species with only limited samples available for study. Similar to the rest of the field, promising innovations are occurring in this technology. In particular, the Oxford Nanonpore: MinION, is a portable, field-ready device for real-time DNA and RNA sequencing. Each consumable flow cell can generate 10–30 Gb of DNA sequence data across ultra-long read lengths. While this is tremendous power in a miniaturized unit, only one sample can be run at a time, potentially limiting its utility for inspections. Furthermore, the length

of the reads is limited by the volume of DNA extracted from a sample, which is in turn limited by lab facilities and labor, again limiting its utility at point of inspection.

RISKS AND CHALLENGES

As we explore the opportunities, it is also important to consider the associated risks and challenges. It is unclear whether the policy and enforcement mechanisms in place, especially in the developing world, are stringent enough to achieve tangible results from detecting illegal or regulated species during the trade. Still, enforcement agents, such as the U.S. Fish and Wildlife Service, have used genomics-based tools to identify traded products at airports (i.e. Fields et al., 2015).

The implementation of these tools carries risk as well. Several of these genomics tools require significant expertise (i.e. MinION) or have high start-up costs (i.e. Biomeme), making them undesirable for governments or NGOs. These issues can be mitigated with comprehensive and repeated training courses and long-term sustainable funding. However, cost reductions are ongoing, and innovations in the design of rt-PCR units are improving the user-friendliness, compactness, and reliability of the units.

The most significant challenge is a general lack of data; identifying species relies on having a comprehensive database of DNA barcode sequences and variation from all possible target species. For example, seahorses are relatively understudied and difficult to encounter, meaning there is a lack of samples within the Barcode of Life Database (BOLD) library that can be used for barcoding (Dirk Steinke, *personal communication*). However, this also provides opportunity for researchers to identify DNA barcodes that can become part of the International Barcode of Life database and to develop assays for identifying species. Further, these efforts can be used to develop methods and datasets for eDNA and population genomics to fill in significant gaps in our knowledge of distributions and abundances within the trade.

LEADERS

- A range of funders provide support for leading researchers in genomics and the wildlife trade:

- Diego Cardeñosa and Dr. Demian Chapman's work on the rapid shark-fin tool was funded by Vulcan / Paul Allen Philanthropies. Dr. Chapman was a Pew Foundation Marine Fellow, and Pew Charitable Trusts funded some of the earlier work, which enabled the tool's development. Paul Allen Philanthropies has a history of funding similar projects tackling the wildlife trade including Dr. Sam Wasser's work determining the geographic origin of poached African elephant ivory (Wasser et al., 2015), which could provide a model for similar work with marine species.

- Dr. C. Scott Baker at Oregon State University is a Pew Marine Fellow and has previously been funded by National Geographic and the U.S. government. Dr. Steve Palumbi at Stanford University is funded by various sources, including Chan Zuckerberg's Biohub.

- The E.U. and the European Commission fund significant sturgeon genomics work through the SturSNiP program, a collaboration led by TRACE Network with the Russian and Iranian Fisheries Research Institutes, and Edinburgh and Padova Universities. Research outputs include the work of Dr. Elisa Boscari at University of Padova and Dr. Milos Havelka (now at the University of Hokkaido).

- Amanda Vincent and Dr. Sarah Foster at Project Seahorse are the world leaders in studying seahorses and the seahorse trade. Very few researchers study seahorses, and therefore there are significant knowledge gaps, including the use of genomic techniques. They are funded by a variety of sources, including their major donor Guylian Belgian Chocolates.

- Dr. Dirk Steinke and Dr. Paul Hebert at International Barcode of Life, iBOL project developed assays for barcoding commonly traded ornamental reef fish. Further, iBOL maintains the library of DNA barcodes (available on the BOLD database). They are largely funded by the Canadian government, but lean on additional international infrastructure and multilateral / bilateral funding. A similar project for African terrestrial species, *Barcode of Wildlife*, is funded by Google.

- The Gordon and Betty Moore Foundation is funding the development of the ConservationXLabs Barcode Scanner, and the USFWS's Combating Wildlife Trafficking Program provides approximately $2m in grants per year.

- The leading companies in this space include BioMeme, Oxford Nanopore, Thermofisher Scientific, and ChaiBio. Each company has proprietary technology that contribute to product selection for particular use cases. This competitive environment should continue to foster innovations in technology and design.

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THREAT

Invasive rodents on islands are one of the greatest threats facing global seabird bird populations. Recent research shows these rodents are altering near-shore marine environments through a cascading effect that links seabird guano to ocean health. In aggregate, this is a significant problem for the ocean because there are more than 400,000 oceanic islands across the globe that are critical for marine ecosystems and at-risk seabird populations.

Two studies show the ecological link between invasive rodents and healthy oceans.

In 2012, McCauley showed that when seabirds in the Palmyra Atoll roost on native trees and produce guano that fertilize soils, the resulting increase in coastal nutrients increases the abundance of plankton and subsequently manta rays along island coastlines that eat this primary food source.

Figure 3 below illustrates the difference between the two ecological states – one with higher and one with lower seabird populations, which produce more or less nutrient-rich guano, respectively. Bar graphs comparing processes in native (N) and palm (P) forests, indicate that reductions in native tree abundance (A) reduce seabird abundance (B), which diminishes the contribution of seabird-derived nutrient subsidies to terrestrial ecosystems (C,D), which severely impair the movement of nutrients to the marine environment (E), reducing zooplankton abundance (F), and ultimately eliminating manta ray (*Manta birostris*) utilization of native forest coastlines (G).

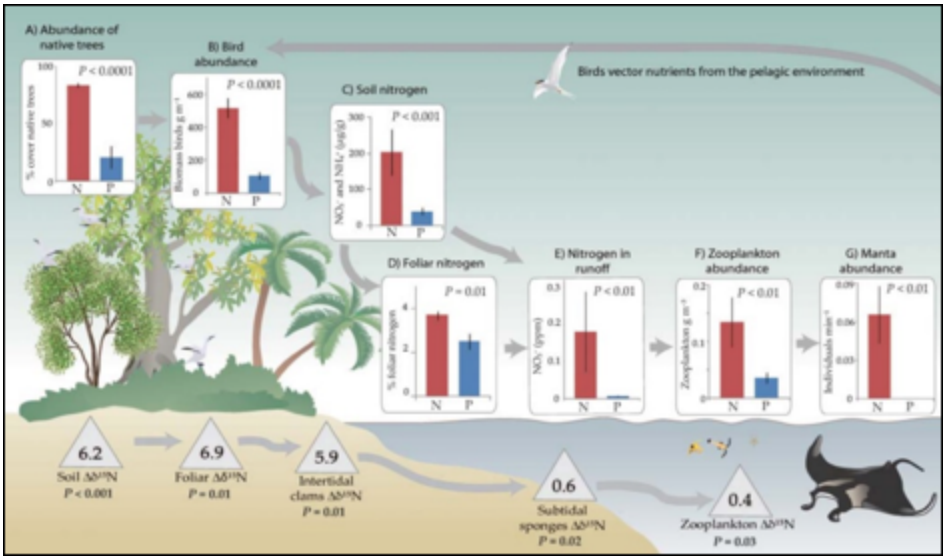

FIGURE 3 — Factors influencing island biodiversity.

The 2018 study conducted by Graham et al observed that invasive rodents can drive this reduction in seabird populations and nutrient-rich guano. The rodents prey on adult seabirds, their eggs, and chicks. They also eat seeds and saplings that create the trees the birds need for roosting and nesting. As a result, “seabird densities and nitrogen deposition rates are 760 and 251 times higher, respectively, on islands where humans have not introduced rats,” writes Graham et al. Fish biomass was 50 percent greater around the rat-free islands than the rat-infested islands. The paper concluded that “rat eradication on oceanic islands should be a high conservation priority as it is likely to benefit terrestrial ecosystems and enhance the functioning and productivity of coral reefs by restoring seabird-derived nutrient subsidies from large areas of ocean.”

PROGRESS TO DATE

There have been more than a thousand successful island invasive species eradications worldwide, benefiting thousands of threatened island ecosystems and species (Simberloff et al. 2018). Overall, the success rate for island rat eradications has been 84 percent, according to a study of 617 eradication events (Simberloff et al. 2018). Improvement in techniques has made it possible to remove rodents from larger territories. The removal of rats from 30,000 hectares on South Georgia Island near Antarctica represents the largest rat eradication to date (Simberloff et al. 2018).

Currently, the best method for rodent eradication is rodenticides, which kill existing residents. The first, non-trivial, step is to obtain both government and public approval for using toxicants on the landscape. Restorationists must plan to capture and care for existing domestic and wild species that could be vulnerable during the application of the rodenticide. To be effective, rodenticide must penetrate every rodent territory. In some cases, aerial distribution via helicopter is the most effective way to achieve widespread application.

The most effective toxins used today are anticoagulants that block the vitamin K cycle, causing an inability to produce essential blood-clotting factors (Broome et al., 2014). The rapid effect of anticoagulants is the main advantage over other toxins so that the rodents do not identify the poison as a dangerous food source. After the death of the rodents, workers return wild and domestic animals to the environment.

Rodent eradication is highly effective. Despite seeming expensive and time consuming, eradications turn long-term conservation expenses into a single campaign and end up being a much more ecologically sustainable solution than ongoing management of invasive species. For instance, rat eradication on South Georgia Island cost more than $13 million and the planning and implementation took nearly a decade. Poison bait was distributed by helicopter in three separate trips during three summers from 2010 to 2015. However, when compared to the chronic expense of mitigating the threat to seabirds from rats every year, the eradication was a notable success (Island Conservation pers. comm.).

Despite significant advances in the use of toxicants over recent decades, in many situations these eradication methods are extremely challenging or unfeasible. Challenges include islands

with significant human populations, stakeholder communities averse to the use of biocides, potential off-target harm to livestock and domestic animals, or potential negative impacts on native species (Campbell et al. 2015; IUCN Synbio Assessment).

Invasive rodents have been introduced to 80 percent of the world's island groups. That means a large number of the world's 400,000 islands are still struggling with invasive species today. The scale and scope of this problem far exceeds eradication efforts to date. To match the solution to the problem, new tools that are more effective, efficient, less toxic, and lower risk are essential.

INNOVATION

Invasive species removal is one of the most feasible and powerful conservation interventions available today for protecting island ecosystems above and below water. Removing invasive species is like pressing an island ecosystem's reset button: native animal populations rebound and plants flourish, often with little additional intervention. New species sometimes colonize the islands, and species feared to have gone extinct reemerge in the absence of invasive predators.

Genetic tools are some of the most promising potential tools to increase the scale, scope, and pace of eradications on islands. The Genetic Biocontrol of Invasive Rodents (GBIRd) program is a partnership of geneticists, biologists, social scientists, ethicists, and conservationists from research universities, government agencies, and other not-for-profit organizations. Its mission is to investigate the feasibility and suitability of using gene drives or other genetic tools to save island species by efficiently eliminating invasive species.

RNAi

Gene-silencing, or RNA interference (RNAi), uses a double-stranded RNA to block or destroy messenger RNA, thus blocking transcription. While technical challenges remain, successful eradications of agricultural insect pests and rodents using RNAi suggests the technique could be applied in wildlife settings with a high likelihood of success (Campbell et al. 2015). RNAi has also showed promise in other areas: the technology can help with problematic fungal pathogens; pilot projects on two beetle agricultural pests in enclosed settings have succeeded (Baum et al. 2007; San Miguel and Scott 2016); and the U.S. Environmental Protection Agency registered four genetically modified plants that produce RNAi against corn rootworm (EPA 2017).

Because the technique targets specific genes, gene silencing through RNAi promises to be far more effective than either biocides or heritable genetic technologies. Some research even suggests that RNAi could be engineered to differentiate between introduced and native genotypes.

Gene Drive

Gene drives are found in the genomes of many species and can override typical Mendelian inheritance. Gene drives bias inheritance of a particular gene to make it a dominant feature in a population. Scientists are learning how to capitalize on this by hitching specific genes to an engineered gene drive system to bias gene inheritance. In applications focused on eradication, scientists use gene drive to distribute fitness-reducing genes throughout a population to drive it to local extinction. Specifically, a self-limiting gene drive could distort the sex ratio to all male or all

female, for example (Webber, Raghu and Edwards, 2015). In such cases, the drive mechanism employed would need to be strong enough to overcome any selective disadvantage incurred by the individuals carrying the genetic manipulation for it to spread. The potential benefits of this approach include species specificity, reduced toxicant use, more humane (non-lethal) approaches, and expanded application on human inhabited islands (Campbell et al., 2015). This represents a potentially transformative advance for the island restoration field not readily achievable with current technology (International Union for the Conservation of Nature, Task Force on Synthetic Biology and Biodiversity Conservation 2018).
The project is beginning work with the house mouse (*Mus musculus*) because it is one of the most studied and understood mammalian genomes, and because a natural gene drive exists in the house mouse. However, any progress in the development of gene drives that control population levels in mice will lead directly to applications for invasive rat species, and other problematic invasive mammals frequently found on islands.

Obstacles related to social and regulatory acceptability are potentially more significant than technical factors, and these three components are prerequisites for any potential field trial or future release. Social acceptability will be strongly influenced by public perceptions of the need for action, potential efficacy of the technology, potential benefits and adverse effects, and how these relate to socio-economic and cultural factors. Regulatory acceptability will depend upon the specific country, state, local regulations, and case-by-case assessments (International Union for the Conservation of Nature, Task Force on Synthetic Biology and Biodiversity Conservation 2018).

RISKS AND CHALLENGES

Socio-economic and Cultural Considerations

Although the situation will differ depending on jurisdiction, some potential areas of impact on socio-economic and cultural considerations for rodent eradication using synthetic biology approaches can be identified:

- Perception of probably efficacy of the method.
- Acceptability of genetic modification as interpreted by cultures and belief systems at a particular site.
- Perceptions of, and likely positive and negative impacts to natural resources and culturally significant species.
- Perceptions of, and potential positive and negative impacts to income generating activities such as tourism, farming, agriculture, and exports
- Potential human health benefits due to the reduction of rodents that could vector diseases (Morand, Jittapalapong and Kosoy, 2015).
- The socio-economic and cultural effects of accidental transfer to non-target populations. (IUCN Synbio Assessment).

Technical Considerations

To date, the genetic constructs for gene drive for use in mammals have proven elusive. There have also been biological concerns raised concerning the ability of an organism to evolve “around” a gene drive that biases sex selection.

LEADERS

The GBIRd program represent the leaders of research and conversation about invasive rodent eradications and includes: Island Conservation, Texas A&M University, USDA, CSIRO, University of Adelaide, University of North Carolina, and Landcare Research (Figure 4). Many organizations have experts working toward the goals of rodent eradication technology development. Many organizations have missions dedicated to preserving biodiversity. GBIRd brings together organizations with both of these missions in an interdisciplinary manner to developing best practices technology and consider societal implications.

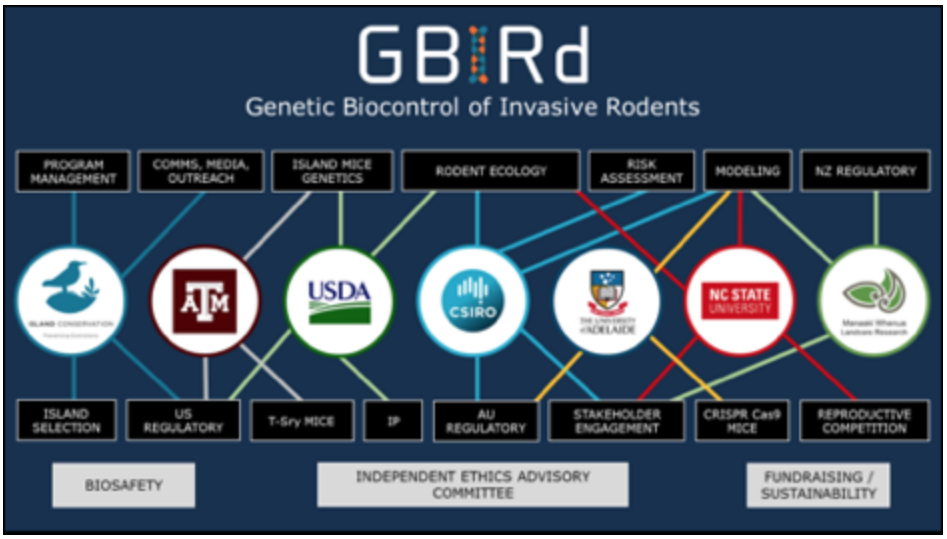

FIGURE 4 — Gbird partner organizations.

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HIGH SEAS EXPLOITATION

THREAT

The high seas – defined by the United Nations (1982) as open ocean waters that lie beyond the economic zones and jurisdiction of any one country – account for two-thirds of total ocean area. The world's largest ecosystem, the high seas are home to some of the least well-known and most unique biodiversity on Earth (Ramirez-Llodra et al., 2010). Over the past few decades, fishing and mineral exploitation has expanded into the high seas because of the overexploitation of coastal waters, increasing demand driven by growing populations, the availability of government subsidies, and technological innovation that has enabled access (Swartz et al., 2010; Sumaila et al., 2015).

Because there is no universal law protecting biodiversity, the high seas are very vulnerable to exploitation. Just one percent is closed to commercial use. Overfishing, deep sea mining projects, and climate-related effects of ocean warming and acidification threaten unique deep sea and benthic communities (Ramirez-Llodra et al., 2011; Clark et al., 2016). The depth of the high seas, with little light and food available, provides habitat for fishes that are long-lived and slow growing, characteristics that make them particularly vulnerable to overexploitation and extinction.

Since the 1990s, the most significant human-caused damage to deep sea ecosystems is associated with fishing (Rameriz-Llodra et al. 2010). Inadequate management of the high seas has led to the overfishing of many economically important fish stocks (Cullis-Suzuki & Pauly, 2010) and these fisheries are also responsible for the by-catch of threatened or vulnerable species, widespread habitat destruction by trawling, and ghost-fishing by discarded nets (Rameriz-Llodra et al., 2010). Heavy trawling reduces the diversity and biomass of critical habitat-forming species, such as corals, with limited recovery even decades after fishing has ceased (Althaus et al., 2009). Overfishing of a number of highly migratory, pelagic species such as tunas and billfishes are particularly worrying. Stocks of tunas and their relatives have declined on average by 60% during the last half century and the majority of these stocks are now either fully exploited or overexploited (Juan-Jorda et al., 2011).

Meanwhile, the mining of deep-sea minerals is a rapidly developing enterprise; the International Seabed Authority (ISA) has recently granted the first licenses to 29 mining contractors, with commercial exploitation expected to begin in the next five years. A single mining operation is projected to remove nodules and near-surface sediments from 300 to 700 km^2 of seafloor per year, causing near total mortality in the area directly mined. Re-deposition of sediments disturbed by mining activities will disturb seafloor communities over an area perhaps two to five times greater. Over a 15-year period, a single mining operation could severely damage abyssal

communities over an area of 50,000 km^2 and three mining operations might disturb a seafloor area half the size of Germany (Ramirez Llodra et al., 2010).

Despite the technological advances that enable fishing and mining at great depths, the vast majority of the deep ocean remains unexplored and poorly understood (Ramirez-Llodra et al., 2010). As a result, the overall ecological impacts of these threats and the large-scale changes to population dynamics are also largely unknown.

To fill the gap in governance, the United Nations is developing a legally binding instrument to protect marine biodiversity on the high seas: Biodiversity Beyond National Jurisdictions (BBNJ).

UN member states have agreed that the negotiations for the BBNJ will focus on four priority areas:

1. Marine genetic resources;
2. “Area-based management tools” such as marine protected areas (MPAs);
3. Environmental impact assessments; and
4. Scientific capacity building and the transfer of marine technology.

International collaboration, and the development and deployment of new technologies is crucial to build capacity, fill gaps in knowledge, and enable a science-based approach to the conservation and sustainable use of biodiversity under BBNJ. Genomic tools and techniques have the potential to make the UN treaty more affordable and effective, lowering both the burden of regulating UN treaty for states and the burden of compliance for commercial fishing and mining interests.

PROGRESS TO DATE

The United Nations began in 2018 to lay the foundation for a binding international legal instrument for governing the conservation and sustainable use of biological diversity in areas beyond national jurisdiction. (The second session of the intergovernmental conference was taking place at the time of this writing, and the treaty is slated to go into effect in 2020.) As described above, the sessions are focused on developing a multi-tiered treaty with which to protect and equitably share marine genetic resources by employing “area-based management tools” to conserve biological diversity and environmental impact assessments to determine the sustainability of fishing, mining, and other extractive commercial operations. Protecting and sustainably using the high seas is ultimately dependent on understanding the high seas ecosystem, and conserving and managing ocean resources requires incorporating scientific advice into policy-making decision. Deploying new technologies in the service of these goals will require transferring technology to developing countries. Therefore, building scientific capacity in all UN members states is an essential piece of this treaty.

1. Global Governance of Marine Genetic Resources:

Ocean health and ecosystem functions are dependent on genetic diversity. Marine biological processes provide a range of ecosystem services that include nutrient cycling, climate regulation,

fisheries production, and cultural significance (Armstrong et al., 2012). Genetic diversity provides systems with the potential to adapt to changing conditions (including climate change) by maintaining a greater range of possible responses (Lande & Shannon, 1996). The study of marine genetic resources can enable better understanding of how ecosystems function and the response to various stressors, with important implications for conservation research in the microbial world (Rodgers et al., 2012). These ecosystem services are essential, therefore a precautionary approach to managing marine genetic resources, particularly of rare or fragile organisms, is key.

Marine organisms have evolved to live in environments of extremes – extreme pressure, temperature, and darkness. These unique adaptations have made marine species the object of commercial interest, especially for biomedical and industrial applications. For example, raw extracts from marine organisms – including individual genes, proteins, and the chemicals they generate – have been engineered to increase crop resilience to disease, catalyze industrial reactions (Hadar et al., 2009) and develop new medicines and pharmaceuticals (Hunt & Vincent, 2006).

With the ocean an engine of economic growth, commercial interests have rushed to claim ocean space and resources (Blasiak et al 2018). The global market for marine biotechnology is estimated to reach $6.4 billion by 2025. This reality underscores the need to develop a proactive and consistent legal and regulatory framework for ocean waters beyond national jurisdiction that will manage marine genetic resource development and foster collaborative research to understand the ocean's underlying ecosystem services. The nascent BBNJ negotiations provide a timely opportunity to mobilize the scientific and private sectors in support of a clear legal framework that ensures policy keeps pace with rapid scientific developments (Wynberg & Lynn, 2018).

It is critical that the BBNJ facilitates the global benefit-sharing from marine genetic resources:

- Any benefit sharing regime must maintain open sharing of genetic data and samples from the high seas while building tools and protocols that ensure access for all countries.
- A system of best practices for data and sample management must be promoted and embedded into protocols.
- In order to advance knowledge, enable reproducible science, and support the conservation and sustainable use of BBNJ, sample and data repositories must be adequately maintained.
- Efforts should be made to develop international and global collaborations to collect, analyze, and share genetic resources from the high seas.

2. Establish Effective and Equitable Marine Protect Areas and Other Area-Based Management Tools

Marine protected areas are an important tool for the protection of biodiversity and the management of fisheries. Scientific knowledge, including the development of genetic tools and libraries, can inform the development of "Area Based Management Tools" and provide baselines against which to monitor success. Also, recent research used genetic insights from next

generation sequencing to confirm the ecosystem benefits of marine protection along the California coastline (Baetscher et al., 2018). Knowing which species live and breed in or use different habitats can enable the identification of biodiversity hotspots, vulnerable marine ecosystems, and ecologically and biologically significant areas that need protection. Well-connected marine protected areas and fisheries closures can support movement of wide-ranging species, supply of nutrients to areas of low productivity and sufficient recruitment to rebuild fish stocks. Predictive tools can support area-based management when knowledge is limited, as in the vast high seas. Lastly, identifying vulnerability in deep-sea ecosystems and their ability to recover from human impacts can help prioritize which areas to protect against what activities.

3. Environmental Impact Assessments

Assessing the impacts of different activities on ecosystems cannot be done in the absence of basic science knowledge. Only by knowing which individuals and species live in a given environment can we calculate changes in abundance, biomass, or composition. This basic knowledge can also be used to infer how proposed activities may disrupt ecosystems and reduce resilience. Given the complexity of marine ecosystems and the challenge of detecting marine species, new tools are needed to improve our ability to assess impacts. Baseline information is needed to even begin to understand ecosystem functions. The development and use of genomic tools (such as environmental DNA) can be used to cost effectively identify the presence of certain species or species assemblages, thus streamlining environmental impact assessments. Then, predictive tools can be used to develop indicators that provide simple metrics of ecological status. These types of tools can provide the basic assessment tools that are timely and appropriate for proposed mining and fishing activities in high seas ecosystems.

4. Capacity Building and the Transfer of Marine Technology

Scientific capacity development and technology transfer are vital for the conservation and sustainable use of biodiversity in the high seas. There is a key need to strengthen national and regional capabilities in marine science and technology to enable developing countries to share in marine scientific advances and absorb and apply technology and scientific knowledge (Harden-Davies, 2016). Greater focus on the development and transfer of marine technology would lay the foundations for equitable participation by all states in:

1. Efforts to explore, protect, and potentially use marine genetic resources;
2. Increase the scientific understanding and rationales for selecting and monitoring marine protected areas; and,
3. Enable sufficient environmental impact assessments to prevent ecological damage from extractive uses in the high seas.

As such, capacity building in genomic tools and techniques should be one of the cornerstones of this technology transfer in order to enable protection of the high seas.

INNOVATION

Genomic tools and techniques have the potential to make the UN treaty more affordable and effective.

Numerous organizations are developing a range of technological solutions including robotic platforms, underwater autonomous vehicles, benthic rovers, elevators, and landers for collecting genomic samples from the deepest oceans (Wynn et al., 2014). Genomic tools have the potential to complement these new technologies to provide much-needed data on biodiversity in the high seas. Genomics can distinguish species through genetic barcoding, provide information on linkages among populations of a given species through population genetics, and identify adaptations to specific environments through sequencing approaches.

A key question for conservation use cases is what species are present? The development of DNA barcoding using short fragments of DNA to identify species overcomes some of the limitations of traditional morphological taxonomy (Hebert et al. 2003). The combination of DNA barcoding with high throughput sequencing of environmental DNA (eDNA metabarcoding) represents a new potentially powerful tool for the study of biodiversity, including in remote locations (Taberlet et al. 2012, 2018; Pedersen et al. 2015). Environmental DNA (eDNA) combined with barcoding enables the rapid detection of species diversity and the presence of specific organisms or biological toxins from water samples.

In the context of the BBNJ, eDNA has the potential to provide:

- A rapid and cost-effective protocol to describe a comprehensive picture of presence or absence of existing biodiversity in the high seas, allowing the identification of marine genetic resources and baselines for prioritization and evaluation of protection strategies.
- Rapid, cost-effective environmental impact assessments, better informed spatial plans for the high-seas, and monitoring of the biological impacts of extractive uses in near real-time.

Marine research institutions are attempting to develop technology that can remotely sample eDNA and couples this sampling with edge machine-learning techniques and high-performance computing for near-real time analysis of samples taken from remote parts of the ocean.

eDNA for Environmental Impact Assessments of Mining to Protect the High Seas:

Deep sea mining threatens benthic and deep-water marine biodiversity, yet the high seas are a major potential source of mineral resources. The International Seabed Authority (ISA) has recently granted the first licenses for deep sea mining, with commercial exploitation expected to begin in the next five years. How to regulate international mining presents major difficulties; creating impact assessment methods for mining alone is a huge challenge. The ISA, the international regulator, works with a small annual budget of less than $9 million, and the cost of monitoring international mining efforts on the high seas is prohibitive. At present, the ISA is almost entirely dependent on the mining contractors it regulates for information regarding the

deep seas. Its limited budget prevents any more active role in collecting data or initiating a robust impact assessment methodology.

However, new genomic technology has the potential to make regulation far more affordable and effective. Such technology would lower the financial burden to enable a cost-effective assessment method under the BBNJ UN treaty for commercial mining interests. The development of eDNA technology would provide the ISA with a low-cost and updated monitoring tool capable of providing the raw material needed for robust enforcement of regulations and for strong and independent audits.

Several laboratories and marine research institutions are attempting to develop technologies that sample eDNA, coupling it with modern machine learning and data processing capabilities. The Monterey Bay Aquarium Research Institute (MBARI) is a leader with their Environmental Sample Processor, but other labs are also aspiring to develop similar technology. Furthermore, the development of this technology could allow regulators to monitor and evaluate the biological impacts of extractive uses in real-time for enforcement of regulations.

An eDNA-based monitoring tool could be uniquely suited to the ISA's regulatory needs for independent environmental assessments and enforcement of seabed mining permits. This technological advancement could provide regulators with the ability to assess biodiversity much more cost effectively than the current manned expeditions that rely on video capture. With this technology, ISA and by extension the BBNJ, would for the first time be empowered to establish baselines of biodiversity, audit, monitor, and enforce regulations such as impact assessments pertaining to the extraction of deep-sea minerals and thus avoid activities that unreasonably damage deep sea ecosystems. The continued development of eDNA technology could be transformative by increasing metrics of high seas biodiversity as well as the economic feasibility and practicality of assessment methods. However, it is important to note that there are many steps and milestones that need to occur for successful implementation.

RISKS AND CHALLENGES

A number of challenges will need to be overcome for the development and implementation of the BBNJ and for the use of genomic tools in supporting the treaty. As such, genomics should be seen as one small part of the solution, alongside policy mechanisms and traditional conservation methods like mapping, satellite monitoring, and patrols.

The high level of unknown biodiversity in many deep-sea environments will require baseline assessment in high priority areas.

Given the deep sea is severely under-sampled (Ramirez-Llodra et al., 2010), molecular databases (e.g. GenBank, Barcode of Life) required for assigning species and identifying ecosystem services to sequences will require development in order for genomic technologies to be functional. However, additional molecular tools (e.g. metagenomics, metatranscriptomics, metabolomics) promise to be helpful in addressing these challenges.

The large size and remoteness of the high seas will require careful prioritization and cooperation to maximize return on effort for implementing any data collection, area management programs, or environmental impact assessments.

The sparse and fragmentary governance frameworks currently in place will require significant negotiation and will likely evolve over time for particular sectors and regions, as such the BBNJ should be considered the first step on this road.

The high cost and technological challenge to collect these data will require international cooperation and technology transfer in the form of basic research, data-sharing, training, and enhanced access to novel tools.

LEADERS

- **Monterey Bay Aquarium Research Institute (MBARI)**

  The mission of MBARI is to achieve and maintain a position as a world center for advanced research and education in ocean science and technology, through the development of better instruments, systems, and methods for scientific research in the deep waters of the ocean.

- **Schmidt Ocean Institute**

  Schmidt Ocean Institute works to advance the frontiers of global marine research by providing state-of-the-art operational, technological, and informational support to the pioneering ocean science and technology development projects at sea.

- **Professor Marcel Jaspars,** University of Aberdeen

  Research in the Jaspars group focuses on the functions and applications of natural products, particularly from marine organisms. The goal is determining the biological role of selected natural products and the potential for their use as pharmaceuticals and tools for biomedical research. Professor Jaspars established the Marine Biodiscovery Centre, a £1.6 million ($2 million) investment bringing together scientists from different disciplines to investigate how marine resources can be used for novel pharmaceuticals and to investigate chemical ecology and biosynthesis.

- **Dr. Maria Baker**, Senior Research Fellow, University of Southampton.

  Dr. Baker is the project manager of INDEEP (International Network of Scientific Investigations of Deep-sea Ecosystems). This program aims to develop and synthesize our understanding of deep-sea global biodiversity and functioning across all habitats and provide a framework to bridge the gap between scientific results and society to aid in the formation of sustainable management strategies. She is also project manager of DOSI (Deep Ocean Stewardship Initiative). DOSI seeks to integrate science, technology, policy, law and economics to advise on ecosystem-based management of resource use in the deep ocean and strategies to maintain the integrity of deep-ocean ecosystems within and beyond national jurisdictions.

- **Professor Craig Smith,** University of Hawaii

  Professor Smith's research includes biodiversity, disturbance ecology, and human impacts in seafloor ecosystems. He has conducted research in Antarctica, mangroves, submarine canyons, organic-fall communities, cold seeps, continental slopes, and abyssal plains. He has led over 50 research expeditions and has conducted over 100 HOV, ROV, and AUV dives. He has also published over 140 papers on seafloor ecology, biodiversity, climate-change impacts, and the design of marine protected areas, including at proposed deep-sea mining sites. Professor Smith has used eDNA for new approaches to assess biodiversity and ecological functions of microbes and animals living in sediments, on manganese nodules, and in the waters above.

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EXTINCTION

AUTHOR: Ben Novak

THREAT

The threat of extinction looms over endangered species. But losing species (defaunation) is not the only problem contemporary ecosystems must contend with; historic extinction events continue to impair ecosystems to this day. Extinctions not only degrade an ecosystem's biodiversity, but can also degrade complex ecological interactions such as food chains, ecosystem engineering, and mutualistic relationships – leading to reductions in bioabundance and bioproductivity. The impacts that extinction may have on marine ecosystems have been characterized by several seminal experiments in marine ecosystems.

In the late 1960s, Robert Paine experimentally removed different species from several marine sites in the Pacific Northwest of the United States. Many of the "local extinctions" he experimentally created had little impact on biodiversity; however, when the ochre sea star (Pisaster ochraceus) was removed, the mussels on which it primarily preyed overpopulated and pushed out all the other species also inhabiting the rocky surfaces of the tidal zone (Paine 1966), resulting in a far less diverse system that was predominated by a single species. Paine labeled the sea star a "keystone species," a term he coined (Paine 1969) to describe the species' pivotal role in supporting biodiversity. Similarly, in its original architectural usage, a keystone refers to the central stone at the top of an archway that locks the arch together, without which the arch topples to the ground.

Biodiversity is more difficult to catalog and monitor for marine species than terrestrial life, due to the physical properties of the ocean environment, and those same conditions also mean that marine defaunation has occurred at a much slower rate than that for terrestrial fauna (McCauley 2015). But it appears that rate is rapidly increasing. The International Union for the Conservation of Nature records 15 extinctions of marine species documented over the past 500 years, including the Steller's sea cow, Caribbean monk seal, and a single species of fish, the New Zealand grayling (Vermeij 1993, Wolf 2007, Atkore 2011). The growing footprint of human society is increasingly impacting oceans and marine defaunation, a fact that is reflected in the acceleration of marine defaunation. The westward expansion of Europeans led to the extinction of the great auk and the Labrador duck in the mid-19th century. Sadly, it appears the Vaquita will be the next species to join this list, with only 22 individuals left. Although only a few extinctions have been recorded to date, defaunation of oceans likely has had many widespread impacts (McCauley 2015).

An important example of the negative effects of marine defaunation comes from the indirect impacts that historic whaling incurred upon kelp forests decades later (Estes 2004). The decimation of large whales in the 1800s forced orcas to prey on smaller marine mammals, wiping out Steller's sea lions, followed by harbor seals and fur seals, until the only suitable prey remaining were sea otters. This led to a crash in Alaskan sea otter populations in the 1990s, just

a decade after the otters had fully recovered from the 19th century fur trade. Without sea otters to keep sea urchin populations in check, the urchin populations boomed and overgrazed kelp forests – creating kelp barrens, which effectively eliminated entire habitats for hundreds of species.

At least one of the fifteen recorded extinctions was caused, in part, by a similar trophic downgrading, the extinction of the Steller's sea cow (Estes 2016). In addition to the direct pressure from human hunting, the decimation of sea otters during the fur trade set in motion the destruction of kelp forests upon which the sea cows grazed. It is significant that the sea cow's extinction happened less than three decades after the species was discovered by Europeans in 1741, emphasizing the importance of kelp forest loss had on the species. While sea otters eventually rebounded and kelp forests recovered by the 20th century, the sea cows were gone. Scientists can now only speculate how the loss of the sea cow has impacted these recovered kelp forests.

It is likely that temporary historic shifts in habitat around the world, similar to that which contributed to the extinction of the Steller's sea cow, have led to many extinctions of species that were never recorded. The full extent to which human caused extinctions have altered marine ecosystems may never be fully understood.

PROGRESS TO DATE

Restoring the roles of extinct species through reintroduction or translocation can have valuable benefits, and can reverse the ecological damage caused by an extinction. Conservationists have reintroduced individuals from a neighboring population; the reintroduction of wolves to Yellowstone National Park is an excellent example. Other times, a related species can replace an extinct species, such as the introduction of the Aldabra giant tortoise on Mauritius to replace the extinct endemic giant tortoise. In each case, unexpected widespread ecological benefits were observed as a result of the restoration.

Restoration of the ecological function of completely extinct species has been impossible. However, thanks to paleogenomics and gene-editing, the practice of "de-extinction" via precise-hybridization may recreate ecotypes of such species. This possibility has led to significant discussions of the ethical and ecological implications of de-extinction, from which a series of de-extinction criteria have been created. Each candidate for de-extinction should have a functional role in the ecology of an existing ecosystem; the cause of extinction must be removed; and, habitat must be available at a scale for the species to recover.

In the marine environment, two extinct species have been discussed as potential candidates for de-extinction: the Steller's sea cow, which went extinct in 1768, and the great auk, the only flightless seabird of the North Atlantic Ocean, which went extinct in the 1850s. Both species met the requirements listed above. While still poorly understood because of how long each species has been extinct, the specific food chain and ecosystem implications are expected to be significant. The severe hunting pressure led to each species' extinction is no longer a threat, and

extensive habitats are available for the recolonization of de-extinct Steller's sea cows and great auks.

Though little is known about the great auk's ecology, recent research into historic whaling records has illuminated the species' former range. This data suggests that the former breeding habitat of the great auk remains largely unchanged, and thanks to modern conservation measures, it is likely that the North Atlantic Ocean can support the reintroduction of a flightless marine bird ecotype. Sequencing of ancient DNA from great auk specimens, including the sequencing the whole genome, has revealed its closest living relative to be the razorbill and inspired population genetics work that will clarify the species' eco-evolutionary history.

While restoring the great auk ecotype to the North Atlantic Ocean would potentially initiate a cascade of beneficial trophic and habitat changes in that ecosystem, the most important benefit of de-extinction would be the development of biotechnologies that can be used for the genetic rescue of some endangered species of seabirds and shorebirds, which are among the most endangered groups of living birds (Figure 5).

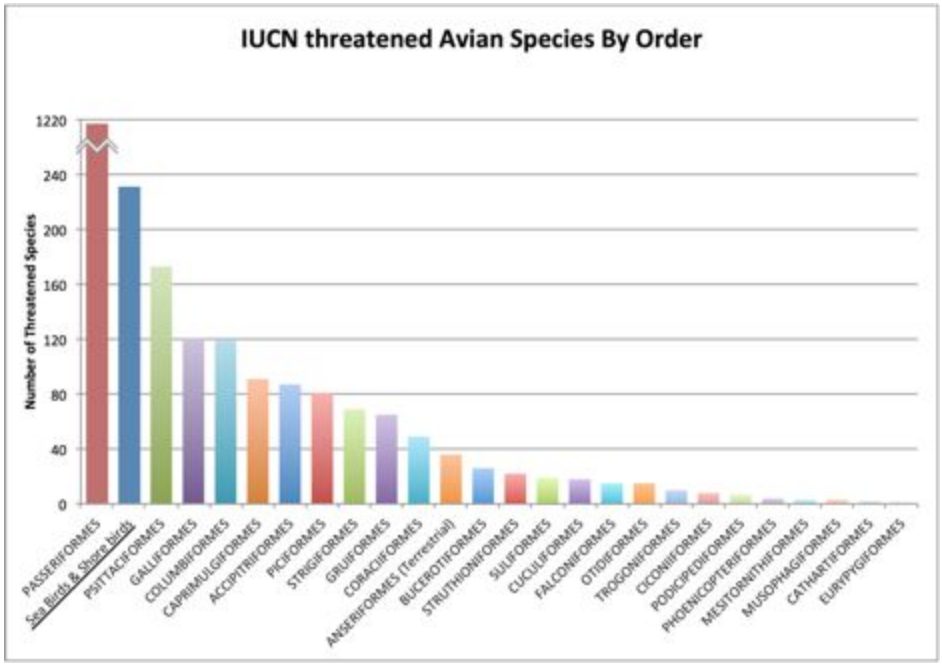

FIGURE 5 — According to avian species listed as threatened by the International Union for the Conservation of Nature (IUCN), seabirds and shorebirds comprise one of the largest groups of conservation concern among avian diversity. Seabirds and shorebirds comprise the orders Charadriiformes (100 species), Procellariiformes (83 species), Pelecaniformes (27 species), Sphenisciformes (13 species), Gaviiformes (1 species), and Anseriformes (7 species).

INNOVATION

The de-extinction process (graphically shown for the great auk in Figure 6) for any species has five phases:

1. *In Silico*: Genomic sequencing of the extinct species and the living template species.

2. *In Vitro*: Reproductively competent cells of the template species edited to possess the genetic traits of the extinct species.
3. *In Vivo*: Advanced reproductive technologies used to generate gametes and/or embryos from the gene-edited cells.
4. *Ex situ*: De-extinct species propagated in captivity for release to the wild.
5. *In Situ*: De-extinct species released to the wild with managed and monitored recovery.

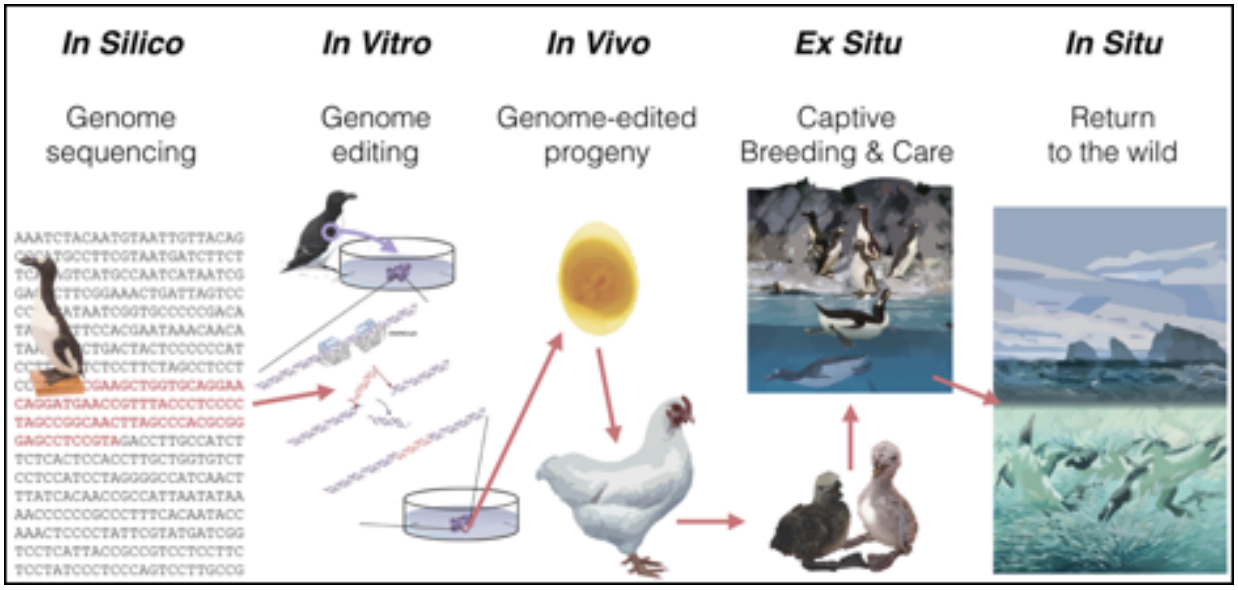

FIGURE 6 — The five stages of great auk genetic intervention and de-extinction via gene editing (known as precise hybridization).

DNA from the Steller's sea cow has been successfully sequenced, revealing that the dugong is the closest living relative. The DNA is of sufficient quality that an entire genome sequence is likely attainable. Therefore, in the first step of de-extinction research, comparative genomics will be used to discover the genes responsible for cold climate adaptation and gigantism.

For any genetic rescue project involving gene editing, the major bottleneck is implementing reproductive techniques to progress from in vitro to in vivo. For placental mammals, like the Steller's sea cow, somatic cell nuclear transfer (aka cloning), offers a proven method (increasingly common for livestock) for creating embryos from gene-edited cells. But to date, no attempts have been made to clone a marine mammal. Other reproductive technologies for mammals in development, mentioned in Chapter 2, include stem cell embryogenesis.

The only viable reproductive technology in birds useful to genetic rescue is the germ-line transfer/transmission of cultured primordial germ cells (PGCs), outlined in Figure 6. This technology has only been developed for the domestic chicken, and limited attempts at using these techniques with other species have yet to be successful, creating a technological barrier for the application of genetic technologies to avian conservation.

Germ-line Transmission works as follows:

1. PGCs are isolated from a donor embryo and then cultured in vitro. (Cultured PGCs can also be cryopreserved for biobanking or gene-edited for genetic intervention.)
2. Donor PGCs are then injected (transferred) into a developing recipient embryo, which can be the same or a different species. The example in Figure 6 uses a chicken. This creates what is known as a “germ-line chimera.” The germ-line chimera has the physical appearance of the recipient species, but carries the PGCs of the donor species in its reproductive system.
3. The germ-line chimeras are bred to produce offspring of the donor lineage.

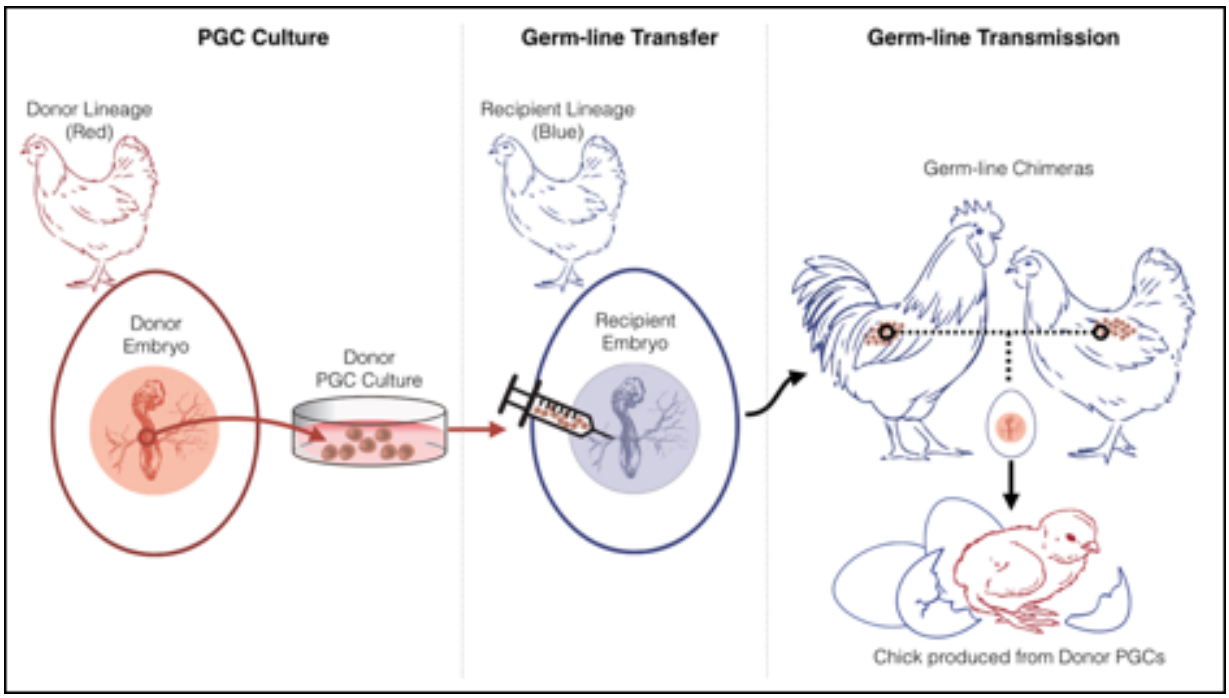

FIGURE 7 — The process of Germ-line Transmission with cultured PGCs. First PGCs are isolated from a donor embryo, then injected (transferred) into a recipient embryo, which matures to become a germ-line chimera that when bred reproduces offspring of the donor lineage.

Developing these techniques for great auk de-extinction would be a great benefit for avian conservation. But while perfecting germ-line transmission for wild bird species would be transformative, another model organism that is easily bred and worked with in captivity may be a more suitable starting point for developing PGC culture methods in seabirds. Button quail (genus Turnix) are small quail -like birds in the Charadriiformes, the same order as the razorbill and great auk.

Even the development of interspecies-germ-line chimeras would be an important step forward. Bred specifically for domestic settings and high egg production, the ideal recipient species is the domestic chicken – shown transmitting gene-edited razorbill PGCs in Figure 7. A single breeding pair can lay nearly 300 fertile eggs in a single year. If the PGCs of a wild bird could be transmitted through domestic chicken germ-line chimeras, it may also be possible to transfer

multiple donor lineages into a single male and female chicken chimera, allowing researchers to breed a genetically diverse flock of wild birds from a single breeding pair of domestic chicken chimeras. In combination with foster parenting and/or hand-rearing, this could make a major difference for rapid population recovery of rare birds.

RISKS AND CHALLENGES

Developing PGC culture and germ-line transmission techniques for birds is "high risk/high reward" tech development. The initial stages are resource-intensive, and there are still large knowledge gaps in PGC cellular biology and reproductive physiology of most avian species. To date, techniques that have worked in domestic chickens have failed with other birds, possibly due to the chicken's long history of selective breeding.

Exacerbating the complexities of PGC cellular biology and reproductive physiology, the "low tech" components of animal reproductive technologies – the animal husbandry and egg handling – can be even more challenging. For wild birds, animal husbandry, semen collection, artificial incubation, and embryological developmental stages are typically poorly characterized or lacking any foundational knowledge. These efforts will require careful selection of model species to build the foundational animal science knowledge necessary to enable PGC culture. It is for this reason that the button quail was highlighted as a possible model organism for the development of interspecies germ-line transmission techniques.

LEADERS

Leaders in avian genetic engineering and reproductive technologies are:

- Academic laboratories led by Michael McGrew and Helen Sang at the Roslin Institute
- A governmental laboratory group led by Tim Doran at the Commonwealth Scientific and
- Industry Research Organization's Australian Animal Health Laboratory (CSIRO AAHL).
- Revive & Restore's avian de-extinction program, led by Ben Novak who is currently working with the CSIRO AAHL to advance gene-editing research in pigeons.
- The Schusser lab at Technische Universität München, which is attempting to transmit cultured spermatogonial cells of the greater prairie chicken through domestic chicken interspecies germ-line chimeras.
- Texas A&M University (TAMU), a leader in exotic bird research, is developing a programmatic focal area on PGC culture and transmission for diverse wild bird species.

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Chapter 4: Market Alternatives for Ocean Products

The harvesting of resources from our oceans for industrial and commercial products, or as a food source for the ever-growing human population, places relentless stress on marine ecosystems. The need to reduce this demand for natural marine products can put conservation efforts at odds with economic growth, especially in developing countries. There is an urgent need to balance the health of ocean ecosystems with the demands of industrial human societies. The Market Alternatives section of our Key Findings explores some recently developed and emerging biotechnologies that offer alternatives to natural products derived from the ocean, allowing commercial interests and human needs to be met without adding stress to fragile marine ecosystems.

Marine organisms possess a greater degree of chemical and enzymatic diversity than do those of terrestrial environments. For this reason, the ocean represents a rich source of new chemical products for the pharmaceutical and cosmetic industries. Due to technical advances allowing further exploration of marine environments, approximately 20,000 unique bioactive natural products have been discovered from marine organisms in the past 50 years. Thanks to concurrent advances in metabolic engineering and genomics, many of these potential products will not be harvested from marine organisms, but instead will be made through synthetic biology. Alternative sources of life-saving natural products, like marine-derived drugs, are necessary to prevent potentially devastating consequences to the ecosystems in which they are discovered.

The demand for seafood by the global human population is high and is expected to increase by 30 percent in the next decade. Global fisheries and aquaculture are not in any position to meet this demand. Meat substitutes have been available for a decade; however, recent trends driven by advances in biotechnology are creating new alternatives to meat that are nearly indistinguishable from their natural counterparts. These new advances in plant-derived and cell culture-derived meat sources could provide urgently needed alternatives to seafood for the growing global population of hungry humans, without asking anyone to sacrifice their favorite foods. In this section, we describe several existing products coming to market, as well as many others that are expected to break out in the near future. These products all exemplify the idea of designing ecologically healthy products that match consumer preferences. However, many challenges still exist for this new industry with respect to government regulation, price of production and scaling, and product marketing to industrial and private consumers.

HORSESHOE CRAB

BACKGROUND

Four species of horseshoe crab, the Atlantic horseshoe crab (Limulus polyphemus) and three additional Asian species, have been integral to the safe manufacturing of injectable medications for the past 40 years. Horseshoe crab blood is extraordinarily sensitive to bacterial contaminants that can cause life-threatening fever and toxic shock if introduced to the bloodstream. A unique clotting protein in the crab's blood is extracted to create the Limulus amebocyte lysate (LAL) test, which is used to screen every injectable drug approved by the U.S. Food and Drug Administration. In fact, anything that might go inside the human body – every shot, IV drip, or implanted medical device – is tested with LAL for contamination.

An estimated 70 million LAL tests are performed each year, which in the United States alone, results in the bleeding of 500,000 horseshoe crabs annually. The horseshoe crabs are captured and drained of as much as a third of their blood before being returned to the ocean. Although some estimates are higher, at least 15 percent die from the bleeding procedure, with a similar percentage of bled crabs annually sold as bait in other fisheries. The released crabs often suffer sublethal effects of the bleeding process, such as injury and disorientation, increased incidence of disease, and unclear long-term effects possibly including lower spawning rates.

In addition to the bleeding for LAL, there is a fishery for horseshoe crab, which in the US, is used as bait in the whelk and eel fisheries. The Atlantic States Fisheries Commission now regulates the number of crabs harvested for bait, after overharvesting in the 1990s caused a population crash. Today, despite a decade of conservation management, the horseshoe crab population in Delaware Bay (the largest population in the U.S.) is still depleted to about a third of the Bay's carrying capacity. It is from this already depleted population that crabs are harvested for bait and for use in the pharmaceutical industry. The slow recovery of horseshoe crabs makes it clear that the current levels of harvest for bait and LAL manufacturing is ecologically unsustainable.

The current overexploitation of horseshoe crabs is dangerously similar to that of other mismanaged species that have been driven to extinction. The International Union for the Conservation of Nature declared in 2019 that one of the three Asian species of horseshoe crab is now endangered. Three years earlier, in 2016, IUCN assessed the mid-Atlantic populations of the American horseshoe crab as vulnerable to extinction its Red List.

Furthermore, in the mid-Atlantic region of North America, the overharvest of the horseshoe crab is causing significant ecosystem-level impacts. The six species of shorebirds that synchronize their spring migration along the Atlantic flyway to gorge on the eggs of spawning horseshoe crabs in Delaware Bay, a critical food stop on their journey north to Arctic nesting grounds, are some of the most rapidly declining shorebirds in North America. In 2014, the dwindling horseshoe crab population in North America prompted the classification of the red knot (Calidris canutus rufa), whose 9,500-mile migration from the tip of South America to the Arctic is among the longest of any bird in the world, as threatened under the US Endangered Species Act.

PROGRESS TO DATE

Developing the Synthetic Alternative: In 1997, scientists Ling Ding Jeak and Bow Ho of the University of Singapore developed a synthetic version of factor C, the key enzyme in horseshoe crab blood that coagulates in the presence of endotoxins. This reaction is the basis of the LAL test. This synthetic alternative, produced using recombinant DNA, is known as recombinant Factor C (rFC).

Multiple manufacturers subsequently developed their own products. Germany-based Hyglos developed its own version of rFC in 2013, and more recently, Japan-based Seikagaku developed a synthetic that mimics the "full cascade" of enzyme reactions that comprise the LAL test. This product can also replace horseshoe crab blood for a different set of bacterial endotoxin tests. In the United States, Lonza Group owns the patent for recombinant factor C, which it sells under its own label.

Demonstrating Efficacy: More than a decade of research has proven that for the detection of gram-negative bacterial endotoxin, rFC is just as effective as the LAL assay, if not more effective, in its ability to quantifiably measure endotoxin and in its ability to detect endotoxins across a range of concentrations. However, the pharmaceutical industry has been slow to adopt rFC due to concerns over efficacy, among other issues.

The California conservation nonprofit Revive & Restore set out to dispel lingering doubts over the efficacy of the rFC synthetic alternative. The results, which were published in PLOS Biology in 2018, demonstrated that a synthetically produced test for bacterial contaminants was just as effective as the LAL test produced with blood components of the horseshoe crab. Revive & Restore analyzed data from ten separate and independent studies, each showing that efficacy and reliability of the synthetic alternative was equal to or better than the product derived from horseshoe crab blood.

This review paper, “Saving the horseshoe crab: A synthetic alternative to horseshoe crab blood for endotoxin detection,” establishes a path forward for the pharmaceutical industry to eliminate the practice of bleeding horseshoe crabs for biomedical testing. The review paper is the basis of a broad stakeholder engagement effort to shine a light on the opportunity for industry to convert to rFC and contribute to the recovery and sustainability of the horseshoe crab and the birds that depend on them. Revive & Restore’s effort has galvanized a renewed sense of purpose on this issue within the conservation community.

Adoption by the Pharmaceutical Industry: Eli Lilly has become the first pharmaceutical manufacturer to adopt rFC. Three of its largest U.S. manufacturing facilities are now testing pharmaceutical water and other common manufacturing materials using the synthetic alternative, a step that reduces the number of LAL tests performed at each facility by 90 percent, according to endotoxin experts with decades of experience. The company took an even bigger step forward in its adoption of the synthetic alternative in 2018, when its migraine prevention drug (galcanezumab) that had been tested only using the rFC assay was approved by the FDA. Eli Lilly’s progressive pivot towards rFC is the result of years of research on the efficacy of the synthetic alternative by one of the company’s top biologists.

The existence of an effective synthetic alternative to the LAL test provides the biomedical and pharmaceutical industries the opportunity to modernize procedures and to significantly contribute to the conservation of horseshoe crabs and Delaware Bay shorebirds. In the late 1970s, the pharmaceutical industry transitioned from using live rabbits to detect fever-inducing contaminants to using crabs, now the industry to modernize its methods and use modern technology instead of the blood of an ancient species. Immediate conversion to rFC for the testing of water and other common manufacturing materials presents no risk of diminution in reliability or sensitivity in endotoxin detection and is enabled under current regulatory guidance.

Most importantly, converting to rFC for only the testing of common pharmaceutical manufacturing materials like water would decrease the demand for LAL by 90 percent, which means that mortality resulting from bleeding would decrease.

Thanks to the leadership of Eli Lilly and Company, as well as the recently published PLOS review paper, there is now a clear path forward for the pharmaceutical industry to begin converting to the synthetic alternative.

INNOVATION

Recombinant factor C has begun getting traction in the market with Eli Lilly's adoption of the synthetic alternative at several of its U.S. manufacturing facilities. Yet widespread uptake by the industry has been slow, despite the fact that the biomedical industry can switch immediately to the testing of water and other common manufacturing materials with virtually no regulatory oversight. Innovation leadership from within the pharmaceutical industry is needed to improve the sustainability of their manufacturing processes.

Continued technological innovation is also needed. Charles River Labs has developed a microfluidic device that reduces the use of horseshoe crab-derived LAL by as much as 80 percent. Until regulators fully adopt rFC as an equivalent test, there will still be demand for LAL. More widespread innovation is needed to limit the use of this product from wild caught sources.

Lastly, given the trend toward rapid product development in the pharmaceutical industry, new companies offering new variants of the synthetic alternative should be entering the market.

Innovation of a synthetic bait alternative may help mitigate the harvest of 500,000 crabs in the bait fishery. However, given the dire resource management constraints of the American eel and whelk fisheries, it is hard to rationalize a significant investment. Each species is suffering from its own form of mismanagement, or in the case of whelk, a lack of management.

RISKS AND CHALLENGES

Conservatism in the industry has slowed the conversion to rFC, but leadership from other manufacturers in the pharmaceutical industry is now essential. Without any change to the current drug manufacturing regulations in the United States, pharmaceutical companies can significantly reduce their unsustainable dependence on a wildlife product by converting to the equally safe and effective synthetic alternative for testing only common manufacturing materials.

To fully ensure the adoption of the synthetic alternative, rFC needs to be validated as an acceptable endotoxin detection method by regulatory bodies around the world. Because vaccines and drugs are manufactured and distributed worldwide, various regulatory bodies (e.g., FDA) rely on different compendia (e.g., US Pharmacopeia) and, where possible, a harmonization process to assure uniformity in endotoxin testing methods across all regulatory jurisdictions. Because the use of rFC detection methods has not been incorporated as an accepted method into the harmonized Pharmacopeias, for final-product testing manufacturers must take the extra step of validating the rFC assay, which is a more burdensome process than the streamlined method of verification used for accepted methods described in the harmonized Pharmacopoeias.

LEADERS

Recent scientific advancements only build upon decades of conservation efforts for both crabs and shorebirds spearheaded by conservation groups including National Audubon, New Jersey Audubon, the American Littoral Society, and the Western Hemisphere Shorebird Reserve Network, as well as government agencies like U.S. Fish & Wildlife Service. These groups have pushed the Atlantic States Fishery Commission to include the biomedical take of horseshoe crabs in its regional annual fishery catch limits; extensively studied the relationship between

migratory shorebirds and spawning horseshoe crabs; and advocated for the adoption of the synthetic alternative to LAL.

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MARKET ALTERNATIVES OCEAN PRODUCTS

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THREAT

As the human population grows from 7.7 billion today to 9.7 billion by 2050 and to 11 billion by 2100 (United Nations), the global consumption of seafood is likely to grow with it. Fish is a primary source of protein for roughly a billion people (World Health Organization), and in 2016, fish made up 17% of all animal protein consumed (UN FAO Report). It is unclear if the planet, has sufficient resources to support the demands of 11 billion seafood consumers. Since 1950, global seafood production has grown from approximately 20 million tons annually to roughly 170 million tons today. Wild-caught fish comprised most of this growth until the 1980s, when annual production topped out at 80-90 million tons. Nearly all growth since then has come from aquaculture ("farmed fish"), where farmers raise commercially viable, relatively hardy species such as carp and tilapia in controlled settings. In 2016, aquaculture accounted for 80 million tons of production, compared to 90.1 million tons of wild-caught "capture fisheries" (UN).

Fueling this growth are the twin forces of population growth, particularly in nations where seafood is a culturally important part of the diet, and changes in consumer preference. The United Nations projects the demand for seafood will increase by more than 47 million tons between the mid-2010s and the early-2020s, even though higher prices are expected (Cai 2017).

Compounding sustainability concerns in fisheries management is a simple biological fact. Since most popular consumed fish are predators, and sit higher on marine food chains, fish are fundamentally less efficient as a protein source than beef or chicken. Where a cow eats photosynthesizing plants, most commercial fish are predatory, eating smaller fish that consumed photosynthetic plankton. A general rule is that for every one of these linkages, or trophic levels, roughly 90 percent of consumed energy is lost (Bonhommeau 2013). In order to feed Earth's growing population most efficiently, lowering the trophic level of consumed food is critical.

Environmental challenges with capture production

Continual growth in wild-caught fisheries have had significant consequences for ocean health and biodiversity. One concerning impact is bycatch, or the accidental harvesting of non-target species. These can include marine mammals, turtles, or endangered fish that become ensnared in nets, and are easier to kill than to disentangle. By some estimates, as many as 43 billion pounds of bycatch are produced—and discarded—annually.

Additionally, the composition of fish capture has changed over time; as populations of slow-growing, top-predator species like bluefin tuna and marlin have shrunk due to overexploitation, fishers have caught and marketed smaller, previously unattractive species (Pauly 1997). While regulations have helped to stem overfishing, they are difficult in practice to

enforce, and the UN estimates that 33.1% of all fishing stocks in 2015 were harvested at biologically unsustainable levels (UN FAO).

Due to fish population shrinkages, fishing boats must travel further to reach productive fishing areas, increasing their CO_2 impacts. Another damaging practice is deep trawling, where long, typically plastic nets are cast and pulled against the ocean floor. Trawling damages the sensitive floor habitat and ensnares most life that comes in its path. Further, broken nets are a primary source of plastic pollution; in the infamous great Pacific garbage patch, a gyre of marine debris particles in the north central Pacific Ocean, 46% of all plastic waste came from broken or discarded nets (Lebreton 2018). In the ocean, plastics typically degrade into microplastics, which have been found in 100% of oceanic species studied, and which are likely to interfere with endocrine and reproductive systems (Thompson 2018).

Lastly, there is early evidence that fish may be more complex than previously thought, with examples of tool use, cross-species collaboration, and cultural transmission of knowledge (Bshary 2002). Through overfishing, we may risk permanently losing some of this complexity before we have the chance to fully understand it.

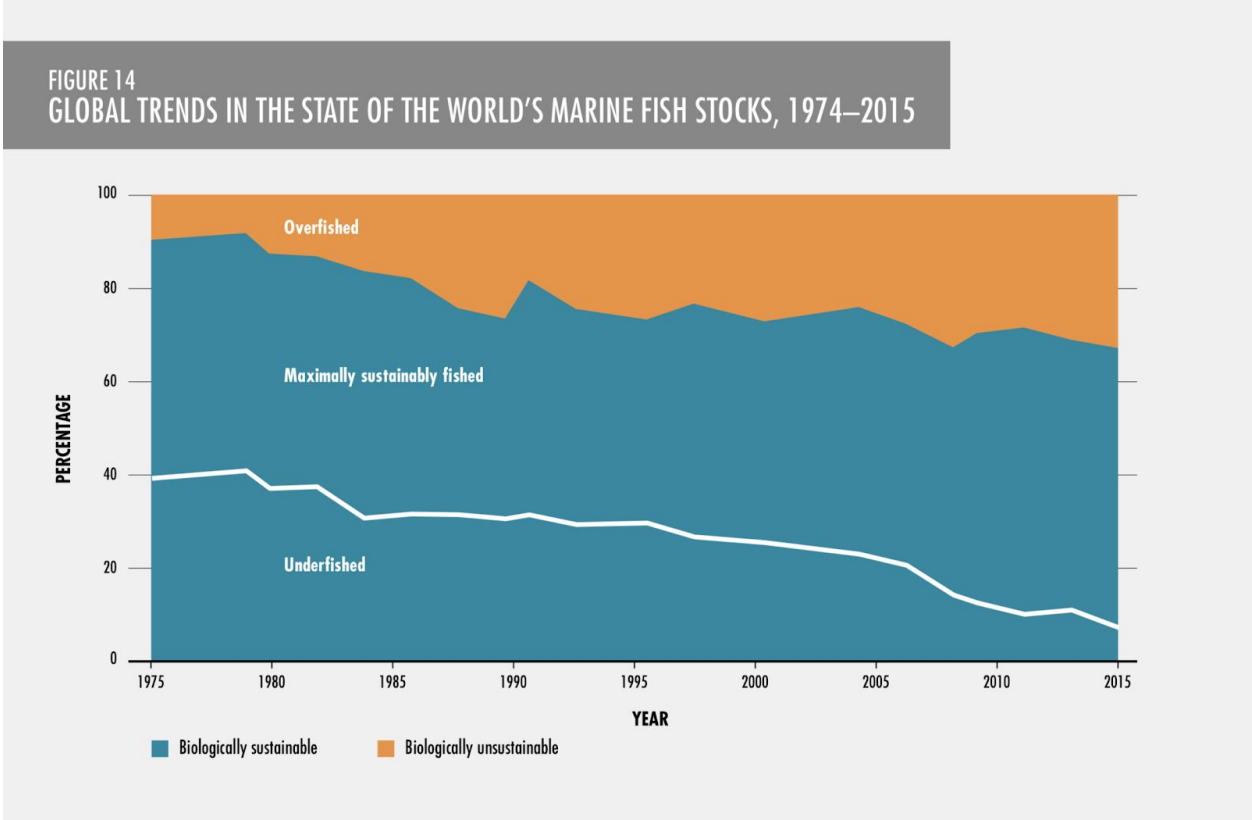

Figure 8 – Global Trends in the State of the World's Marine Fish Stocks

Environmental challenges with aquaculture

Unfortunately, as practiced today, aquaculture is not yet a solution for addressing the expanding market for seafood. Its sudden rise since the 1980s has led to the development of pens often placed in biologically important regions, such as lakes, estuaries, and mangrove forests, which crowds out native species and may reduce storm resilience of mangroves. As with many other commercially farmed animals, farmed fish are often more stressed than wild-caught equivalents, due to higher densities of fish and potentially a less varied or natural life. This stress may lead to less healthy and flavorful fish (Paynter 2017).

More damaging, the conditions of commercial fishing are perfect for breeding diseases. Because only a handful of fish lineages are used commercially, genetic diversity is typically low, which means that when one fish becomes sick, it's very likely its stressed, genetically similar neighbors will become sick as well. As these pens typically share water openly with their surrounding environments, any sufficiently powerful disease will likely spread beyond the pen to put wild populations at risk. The use of antibiotics or insecticides to treat fish stocks may impact unintended species or create antibiotic resistance to disease.

Because commercial farming pens are often low tech, the simple nets used are embedded in bodies of water where there is significant risk of farmed, non-native species escaping and contaminating their surrounding environments. This happened in 2017, when 300,000 Atlantic salmon escaped their pens in Washington state (NPR 2018), and were still found eight months later (Mapes 2018). Although caught individuals to date have not appeared healthy, the greater the number of released non-native species, the more likely it is some will adapt to their new settings. As farmed fishes have not been domesticated for very long, they may be well-equipped to survive in the wild, where complete recapture or population control is nearly impossible. Washington has since introduced laws to phase out the use of Atlantic salmon.

Another critical challenge facing aquaculture is the over-harvest of forage fish, the most common food source used for aquaculture. These are small, fast-growing species like anchovies and sardines that are a vital linkage in marine food webs. Although most of these fishes are suitable for human consumption, it is often more profitable to grind them into fishmeal and fish oil, then feed them to farmed fish instead. Approximately 20 million tons, or 12 percent of wild-caught fish, are forage species used annually in the aquaculture industry. This figure is anticipated to rise to 16 percent by 2030 (FAO 2018).

Despite its rapid rise, aquaculture production is still growing too slowly to supply the projected global demand for seafood and is only anticipated to keep pace with increased demand from about 17 countries, while around 170 countries will be left with substantial unmet demand for protein from the sea (Cai 2017). There is an urgent and substantial need for innovations to meet the ever-increasing global demand for seafood in a sustainable way.

PROGRESS TO DATE

To solve the growing demand for seafood, aquaculture has become the fastest growing food sector, and today produces nearly as much seafood as capture production (FAO 2018). Innovations have included the development of deep sea pens to reduce the risk of impacts on more productive coastal ecosystems (Gunther 2018); whole food chain aquaculture systems that

take a life cycle approach to the management of nutrients; the use of sensors and artificial intelligence to proactively monitor fish health and behavior (Spencer 2018); and perhaps most importantly, precision breeding of relatively hardy fish stocks that are now grown commercially around the world. Most notably, these include several types of freshwater carp, tilapia, Atlantic salmon, and rainbow trout (FAO 2013).

Aquaculture systems are actively innovating their design and approach. Specifically, aquaculturists are increasingly adopting a multi-trophic or whole systems approach that seeks to mitigate well-documented nutrient and disease issues in first generation, single species, or conventional aquaculture systems. These systems work to engineer nutrient budgets that utilize the waste stream from high trophic levels to feed kelp. Kelp byproducts have a number of beneficial uses and fundamentally help with carbon capture and cycling. One notable company, Primary Ocean Producers is significantly advancing the whole life cycle perspective for the aquaculture industry and takes a biotech approach to developing useful products from kelp grown in their farms.

Genetic engineering has been one approach taken to meet the growing market demand for seafood protein, with fish engineered for size, taste, and disease resilience. In 1989, scientists inserted a Pacific Chinook salmon growth hormone gene into Atlantic salmon, alongside an expression regulating gene from ocean pout, to improve Atlantic salmon growth (Fletcher et al. 2004). The result was both increased growth rate and food conversion, producing salmon that could reach market size in half the time of their wild counterparts and require less food intake per kilogram of meat produced. Applying this research methodology, AquaBounty started to commercially develop these genetically modified Atlantic salmon in 1991. After decades of battling regulatory hurdles, the FDA has cleared a path for the sale of their AquaAdvantage™ salmon eggs in the U.S. as of March 2019. The company intends to grow these fish only in controlled facilities on land and has taken measures to ensure the fish cannot reproduce if they somehow escape to the wild. Still, it remains to be seen whether the U.S. market will embrace a genetically engineered salmon.

Another application of biotechnology eliminates the need for fish altogether, by creating plant- or cell-based alternatives that look, feel, and taste like their equivalents. Plant-based substitutes use specific proteins and compounds from plants to approximate the texture and experience of animal products. Cell-based meats are an emerging category of alternative meat, produced through cellular agriculture, in which an animal's extracted stem cells are multiplied into muscle fibers until they form an entire piece of meat.

Demand for alternatives to meat has grown over the last few years, with global sales increasing eight percent annually since 2010 (Skerritt 2017). Between July 2017 and July 2018, sales of plant-based meat products grew by 24 percent in the U.S. alone (Nielsen Data Release 2018). Newer companies like Beyond Meat, Impossible Foods, and Good Catch (which focuses on salmon replacements) have produced plant-based meat products that they market as premium, nutritious, and environmentally conscious options. This contrasts with a previous generation of alternative meats, which often were relatively unpalatable and poorly marketed to consumers.

Significantly, Beyond Meat has filed to go public in 2019, demonstrating their confidence in significant market interest in this emerging sector.

Consumer sentiment is changing as products capitalize on new biotech technologies, ingredient sourcing, product structuring and manufacturing capabilities. The latest generation of alternatives exhibit greater mainstream appeal, especially for consumers seeking to diversify their protein intake without eschewing animal products altogether.

INNOVATION

There are three primary areas where innovation in biotechnology can reduce the environmental hazards of producing seafood and lead to a more bioabundant ocean. First, biotechnology is currently being used to improve the efficiency of aquaculture, which could help farmers keep pace with growing consumer demand. Second, there are several promising new approaches to reduce or eliminate the use of forage fish in aquaculture, which would prevent the capture of ecologically important smaller species. Finally, biotechnology is being used to create new alternative seafood products that do not rely on animals for producing seafood and fish meal.

Technologies to improve aquaculture

Similar to AquaBounty's genetically modified salmon, additional opportunities exist to apply biotechnology to improving fish stocks. With thoughtful controls to avoid contamination—such as producing sterile fish, utilizing land-based farms, or breeding fish that depend on engineered nutrients unavailable in the wild—genetic technologies could be used for several purposes:

- **Genetic Variation:** Farmed fishes are typically derived from genetically similar stocks, which may decrease population resilience to disease or other stressors. Genomic technologies could be used to monitor the genetic diversity of farmed fish over time or to introduce or promote alleles that exist in healthy wild populations.
- **Rapid growth:** As with AquaBounty, engineered fish capable of using more energy toward fueling their own growth could potentially grow significantly faster and require less input feed, which would decrease the environmental cost of their production.
- **Cold Tolerance:** By engineering in genes that are cold-adapted, farmers could raise a wider range of desirable fish in colder regions.
- **Disease Resistance:** Researchers and entrepreneurs could potentially mitigate the risk of serious infection by viral diseases or parasites by engineering in better resistance (Muir 2004). If effective, this could reduce the possibility of farmed fish becoming large disease reservoirs that then infect the outside environment, and it would prevent the use of antibiotics and pesticides that may lead to greater environmental harm.
- **Stress Reduction:** Survival in pens is different from survival in the wild, and genetic engineering could help fish better adapt to their artificial settings. For example, researchers could increase a fish species' stress tolerance, which would enable fish to more comfortably stay close to one another and make them less vulnerable to parasites and diseases (Devlin 2009). It is possible these techniques could be used in the future to

quickly produce domesticated varieties of regionally specific fish, which might prevent the farming of nonnative species.

Alternatives to forage fish for aquaculture

Today 20 million tons of forage fish are harvested from the ocean annually to support aquaculture. This demand is only likely to grow. To reduce aquaculture's environmental impacts, researchers, innovators, philanthropists, and industrial groups are turning to biotechnology for alternatives. Farmed fish require nutritious feedstock and various approaches can be used to harvest or synthesize these proteins, sugars, fats, and nutrients. A move to plant-based feed may one day eliminate the need for forage fish and could reduce the mean trophic level and energy costs of production of farmed fish.

Important efforts in this sector include:

- **Bacterial meal:** Feeding industrial waste gases like carbon dioxide and methane to hungry bacteria that can be processed into fish feed is an exciting new field of research. NovoNutrients uses a gas fermentation process to transform industrial waste carbon dioxide into useful, edible proteins, initially for animal feed. If commercially viable, that would reduce the need for forage fish, currently overfished yet essential for the growing aquaculture sector. It would also reduce net carbon dioxide emissions. Other companies like Calysta, Unibio, and KnipBio are transforming methane gas and ethanol from sources like wastewater treatment facilities or agricultural soils into bacterial meal. Microbial meals have been tested and approved by the FDA for use as an alternative protein in the aquaculture sector. Significant investments by Cargill on the supply side and Marine Harvest on the industry side are developing this space.
- **Algae:** Microalgae has great potential as a protein and essential fatty acid source for fish feed. Companies such as Corbion, Earthrise, and Cellena are actively producing algae products that can be used in fish feeds. Promising algae species that could work well in fish feeds include schizochytrium, spirulina, nannochloropsis, and desmodesmus. In order for these ingredients to be economically viable for commercial feeds, innovation is needed to improve the efficiency of producing algae biomass.
- **Plant Proteins:** Derived from agricultural plant species such as soybeans, corn, peas, and wheat, plant proteins can function as potential substitutes for fish meal. These products are not as rich in essential oils or protein as fish meal. If improperly balanced with other ingredients in feed design, they can result in digestive problems for fish. Fermented soy products are emerging as a new alternative.
- I**nsect Meal:** Considering that some fish naturally feed on zooplankton and insects, it may make sense to use insect meal in fish feed. For example, black soldier fly larvae can be fed waste streams such as spent brewers' grain and then be ground into meal that is high in protein and essential fatty acids. A number of other insect species such as crickets, locusts, mealworms, beetles, and silkworms have also been tested for fish feeds. Biomin, Enviroflight, Entocycle, Ÿnsect, and Ovipost are insect meal companies active in the global market.

Alternative seafood products for human consumption

Alternative ways of producing seafood through plant-based products or cell culture could help alleviate pressure on both wild fisheries and aquaculture systems. Currently, plant-based seafood products like Good Catch's tuna and Gardein's "Fishless Filets" and "Crabless Cakes" are commercially available, but have not yet seen market adoption like Beyond Meat or Impossible Foods, which provide alternatives for land-based meat (beef and pork).

Meanwhile, companies like Memphis Meat, Mosa Meat, Shiok Foods (shrimp), Wildtype (salmon), Blue Nalu, and Finless Foods are developing cell-based products that they hope will be completely indistinguishable from meat derived from an animal. Although they have attracted considerable investor interest, none of these companies has yet to release a product commercially.

While not yet as successful as terrestrial meat alternatives, plant- and cell-based seafood may ultimately grow faster as a category. Underpinning causes for this include the rapidly growing unmet demand for seafood globally, the potential collapse of important fisheries, consumer awareness around environmental challenges, consumer fears around pollutants like mercury or microplastics that bioaccumulate in wild fish, and significant investment interest from countries in Asia that view alternative meats as a means of ensuring better food stability (Roberts 2017). This transition will likely be facilitated by applying lessons from the development, commercialization, and rapid demand for plant-based substitutes for meat.

> For the industry, these products have the potential to increase efficiency and reduce losses throughout the seafood supply chain. Seafood products are highly perishable foods. Nearly half of the edible U.S. seafood supply was lost from 2009 to 2013 (Love 2015). Plant-based items have a longer shelf life and reduce the need for costly refrigerated transportation while providing a potential opportunity for local production in landlocked regions. Furthermore, the production process for both plant-based and clean seafood is more controllable and predictable, allowing for tighter responsiveness to demand and more customizable end products. These efficiencies should reduce food waste and make plant- and cell-based seafood more sustainable, more reliable, and eventually less expensive alternatives to conventional seafood.

To advance the field of cell-based meat, there are several biotechnologies that could have rapid and far-reaching benefits if successful:

- **Molecular Analysis of Seafood:** First and foremost, the entire plant-based and cell-based seafood industry would benefit from the broad availability of a detailed molecular characterization of seafood. This characterization should include comprehensive analyses to define the molecular composition of muscle tissue from a number of different species as well as biophysical analyses of the structural patterns and textural properties that define these products. These data will define the design requirements of both plant-based and clean meat products that both emulate the

consumer experience (taste, texture, mouthfeel, aroma) and provide a comparable or superior nutritional profile.

- **Fetal Bovine Serum:** Secondly, the cell-based industry is currently restricted by its dependence on fetal bovine serum, an expensive compound extracted from developing cow fetuses that contains many compounds that facilitate cellular growth. Currently, a liter of fetal bovine serum can cost $400 to $900, and producing a single cultured hamburger can take as many as fifty liters (Reynolds 2018). For cell-based meat to succeed economically, producing a biosynthetic alternative to fetal bovine serum which can be easily mass produced is critical. This may be analogous to Revive and Restore's initiative to promote the recombinant replacement to horseshoe crab blood (Zhang 2019).
- **Cellular Matrix Development:** Lastly, more work remains ahead for cell-based meat companies to replicate the complicated structures that give meats their texture and mouthfeel. Due to this, it is likely that the first products to be market-ready will be ground meats, such as cell-based hamburgers or tuna spread. Due to intellectual property concerns, cell-based companies appear to be pursuing proprietary technologies internally. Blue Nalu in San Diego plans to use 3D printing to precisely layer cells and intracellular matrices, but most have not disclosed their methodologies. There may be an opportunity for research to develop and share best practices for structuring muscle fibers to help companies get to market and scale faster. Such investments in fundamental science would specifically advance the development of higher-fidelity, lower-cost products.

RISKS AND CHALLENGES

Wild-caught seafood markets are significantly more fragmented in vertical integration and control than the poultry, meat, and dairy industries. This represents some strategic advantages (limited organized opposition – see below) but also presents some structural challenges. Wild-caught and farmed seafood supply chains are complicated and relatively opaque. To meaningfully penetrate markets, seafood alternatives will need new, vertically-integrated companies that can remove middlemen and compete on price.

Alternatives will need to be more attractive than existing products to overcome consumer reticence to change behavior. At first this will entail creating premium products; however, to make wild-caught fishing economically unviable, producers must reach price parity with traditional seafood. Until these emerging cell-based meat businesses dramatically reduce their production costs, their economic models will remain unviable. The same cost parameters will apply for any new technologies targeting forage fish.

Apart from economics, alternative meat product companies have been bracing for resistance from existing market players who understand they are at risk of disruption. Some, like Beyond Meat, have accepted capital from large meat producers like Tyson and Cargill in order to secure their support in the future. In contrast, advocacy groups such as the National Cattlemen's

Association have begun lobbying for regulations to label meat alternatives in ways that would be less appealing to consumers (Garfield 2018).

For the development and customer adoption of genetically modified seafood, public acceptance of genetically modified foods is a significant concern. For example, while AquaBounty salmon, the first genetically engineered animal food product to reach market in the world, was approved for public sale and consumption by Canada in 2015, the first batch was not sold until 2017 (Waltz 2017). Due to sociopolitical obstacles, including several grocery store chains' refusals to sell GMO fish products, AquaBounty continues to face marketing challenges. It was not until March 2019 that the FDA approved the product for sale in the U.S. market.

Farmers considering the use of genetically modified fish must take precautions to avoid unintentionally contaminating natural environments, as any breaches could damage ecosystems and would ultimately make future, responsible applications of genetic technologies harder to implement. Examples of precautions include farming only sterile fish, engineering dependence on a chemical provided by farmers, or setting up these aquaculture systems away from the ocean or river systems to preclude potential escape to natural settings.

LEADERS

Nonprofits:

- The Good Food Institute is a nonprofit organization that promotes plant-based meat, dairy, and eggs as well as clean meat, as alternatives to the products of conventional animal agriculture. The organization launched in February 2016 with the vision of creating a healthy, humane, and sustainable food supply, and works with scientists, investors, and entrepreneurs.
- The Anthropocene Institute's mission is to drive thought leadership and investment by accelerating the technological and community innovations necessary to address the needs of the planet.
- F3, a collaborative effort between NGOs, researchers, and private partnerships through the Aquaculture alliance, supports innovation and adoption of alternative ingredients to replace fish meal and fish oil in aquaculture feeds. The organization also collects and provides data and protocols for alternative feed ingredient companies to use.
- SynBioBeta is an innovation network for biological engineers, investors, innovators and entrepreneurs who share a passion for using biology to build a better, more sustainable world.

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Chapter 6: Big Ideas

The applied use of biotechnology to solve troubling ocean conservation challenges is still a nascent field —despite offering tremendous potential. With that in mind, the following “Big Idea” proposals were primarily evaluated on the potential for early investments to build transformative change and to be catalytic for this emerging field. Each of these game-changing proposals have:

- Attracted outstanding expertise and an engaged team of scientists passionate about their work.
- Address a significant conservation barrier or marine management issue.
- Demonstrate a clear technology development path with opportunities for early wins based on clear milestones.
- And with success, have high potential for scalability and follow-on funding and support.

If you are interested in learning more about these projects, please contact Ryan Phelan, ryan(at)reviverestore.org.

1. MARINE BANKING & SEQUENCING FUND

This initiative brings an innovative conservation focus to generating sequences and banking life for critical conservation needs and threatened wildlife by identifying novel applications that would benefit from the use of genomic data. An advisory committee and competitive grants program will ensure that funded projects have well defined benefits.

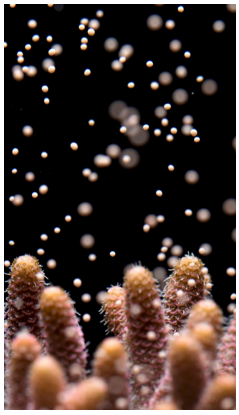
TECHNOLOGY: Cryopreservation; NextGen Sequencing

TIMELINE:

2 – 3 YEARS

BUDGET:

$2,025,000



2. ADVANCED CORAL TOOLKIT

The objective of this many faceted project is to develop tools needed for coral research and restoration to respond to the coral crisis. Research and development of techniques in the cryopreservation of coral eggs, micro-fragments as well as advancing techniques for inducible spawning will help in the short term. Longer term research of stem cell capabilities may open genetic engineering pathways.

TECHNOLOGY: Cryopreservation; Coral Micro-fragments; Inducible Spawning; Stem Cells

TIMELINE:

3 YEARS

BUDGET:

$1,694,000

3. GENOMICS GUIDING MPA'S

An innovative genomic-based tool known as "close-kin mark and recapture" will leverage a $1M study to monitor the effectiveness and ecosystem benefits of marine protection- a potential game-changer for planning and evaluating marine protection strategies.

TECHNOLOGY: Genotyping; Close-Kin Mark and Recapture

TIMELINE:

3 YEARS

BUDGET:

$600,000

4. DISRUPTING ILLEGAL TRADE

Enhanced monitoring using modern genomic tools could transform the interdiction of illegal wildlife trade. This project would couple advanced market commitments for hand-held sequencers with focused training programs to help officials monitor and stop the trade in illegal wildlife and fisheries.

TECHNOLOGY: Digital Quantitative Polymerase Chain Reaction (qPCR)

TIMELINE:

3 YEARS

BUDGET:

$415,800

5. COUPLING TECH FOR BIOSURVEILLANCE

This project will transform traceability in global marine fisheries by coupling emerging molecular genetic tools, such as environmental eDNA, with satellite-based fishing vessel tracking systems.

TECHNOLOGY: eDNA

TIMELINE:

2 YEARS

BUDGET:

$381,150

6. RESTORING ISLANDS TO RESTORE REEFS

Recent research has uncovered strong correlations between vibrant seabird colonies and more resilient nearshore ecosystems like coral reefs. This study would leverage significant co-funding to explore and confirm the causal benefits of restoring seabird colonies by eradicating invasive rats. The findings could transform our understanding of contributing factors of resilience in a changing climate.

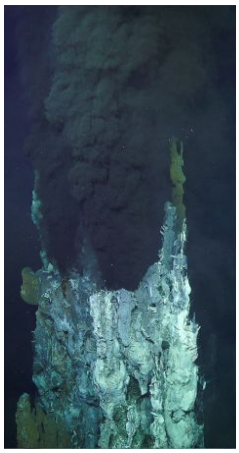
TECHNOLOGY: eDNA

TIMELINE:

3 YEARS

BUDGET:

$3,078,000



7. PROTECTING SEABEDS & THE HIGH SEAS

Exploitation of the high seas is increasing and exacerbating threats to biodiversity, which is both poorly known and difficult to study. In response, negotiations at the United Nations have been initiated to create a governance structure to assess, share, and protect these global resources. Environmental DNA technologies represent a potentially game-changing tool to assess and protect these resources. This competitive project would accelerate the development of critically needed eDNA tools.

TECHNOLOGY: eDNA

TIMELINE:

3 YEARS

BUDGET:

$3,000,000

8. TRANSFORMING POLLUTION DETECTION

TIMELINE:

2.5 YEARS

BUDGET:

$400,000

This big idea includes three areas for innovation: diagnostics, sampling, and data visualization. The diagnostics relies on deploying digital PCR machines to detect selected indicator bacteria. The project will reduce monitoring program costs by developing and deploying rapidly evolving remote aerial and aquatic drone platforms. Lastly, data visualization tools will engage the public to foster increased involvement in pollution abatement.

TECHNOLOGY: eDNA; Digital Quantitative Polymerase Chain Reaction

9. TARGETING MARINE INVASIVES

TIMELINE:

3 YEARS

BUDGET:

$450,000

This proof of concept project brings much needed innovation to the intractable problem of marine invasives. Genetic biocontrol could reduce pest species populations while minimizing the off-target effects of other control options. A partnership with CSIRO is central to the early foundation steps in developing these biocontrols.

TECHNOLOGY: Genetic Engineering of Repressible Lethal and Pheromone Distraction Traits

10. DE-EXTINCTION OF THE GREAT AUK

TIMELINE:

3 YEARS

BUDGET:

$675,000

This project would initiate the scientific research to bring back the great auk, a large flightless seabird of the North Atlantic. De-extinction efforts require the full suite of genetic rescue tools. In addition to potentially replacing a key component of North Atlantic ecosystems, the pursuit of de-extinction of the great auk would likely create new techniques to facilitate adaptation and enhance resilience in threatened marine birds.

TECHNOLOGY: Avian Germ-Line Transmission

Chapter 7: Genetic Rescue Primer

We have devised this report to highlight the potential for biotechnology and synthetic biology to revolutionize conservation practice in the world's oceans. We define these two important fields below:

BIOTECHNOLOGY:

Biotechnology broadly refers to the methods and processes by which living organisms are modified by humans for human purposes. This can include more historic processes such as animal and plant domestication and subsequent artificial selection to enhance particular traits. Currently, it now includes more advanced methods such as genetic engineering and cell and tissue culture techniques. Biotechnology is largely informed by a variety of fields ranging from molecular biology, to chemical engineering and computer science.

SYNTHETIC BIOLOGY:

Synthetic biology refers to the application of biotechnology toward the development of artificial biological systems for research, engineering, consumer, medical, and increasingly, conservation applications. Synthetic biology in conservation may include targeted engineering of DNA sequence to enhance species fitness (i.e. facilitated adaptation), creation of novel microbial organisms capable of degrading environmental pollutants (bioremediation), and the development of viable alternatives to animal products (i.e. clean-meat, rFC, polycarbonate or cellulose sponges). Advancements in synthetic biology are largely contingent on understanding how cells are naturally programmed to do what they inherently do, so that we may reprogram them to function in specific ways that are tailored to a particular outcome.

DATA PROCESSING AND ANALYSIS:

While advancements in sequencing technologies allow us to generate immense volumes of genomic data, processing and analyzing such data so that relevant inferences can be applied requires its own suite of tools. These are defined below:

Bioinformatics

Bioinformatics is an interdisciplinary field that incorporates biology, computer science, engineering, mathematics and statistics in order to understand biological data. Bioinformaticians produce software and software pipelines that enable efficient processing of genomic data, such that sequence reads can be assembled into genomes, genomes can be annotated, and genetic

variants can be identified, which can then be used in specific analyses such as population assignment or identification of adaptive variants, to name a few.

Genome Wide Association Studies

A genome-wide association study (GWAS), also known as whole genome association study (WGAS), is an observational study of a genome-wide set of genetic variants in different individuals to see if any variant is associated with a trait. GWASs typically focus on associations between single-nucleotide polymorphisms (SNPs) and traits like major human diseases, but can equally be applied to any other genetic variants of any organism. GWA studies investigate the entire genome, in contrast to methods that specifically test a small number of pre-specified genetic regions. Any two genomes differ in millions of different ways including small variations in the individual nucleotides of the genomes (SNPs) as well as many larger variations, such as deletions, insertions and copy number variations. Any of these may cause alterations in an individual's traits. Most GWA studies to date have been targeted at biomedical research, but are increasingly used to study wild species. For example, a genome-wide survey of SNPs in bottlenose dolphins allowed researchers to identify five candidate genes involved in host resistance to cetacean morbillivirus (Batley et al., 2014), providing potential targets for vaccine or therapy design.

Genoscapes and Landscape Genomics

A genoscape is a map of genetically distinct populations across geographical space. Genoscapes correlate genetic variants to dynamic population movements from breeding grounds to dispersal patterns. Genoscapes can be informative for delineating boundaries for protected areas, sustainable harvests, and restocking efforts. A genoscape also directly informs the field of landscape genomics, whereby the geographic distribution of genetic variation can be mapped and used to understand how gene flow between populations is related to specific features of the landscape, including anthropogenic barriers and habitat fragmentation. It may also inform the distribution of critical adaptive traits such as disease resistance or climate change tolerance.

Population Genomics

Population genetics is the study of genetic differences within and between populations. In theory, a population's genetic composition can be predicted over time if there is no gene flow, no selection, no genetic drift, and very large (infinite) population size. No natural population meets all these conditions, so allele frequencies change over time causing populations to develop differences. Genetic diversity can be lost within a population due to random sampling of alleles across generations, a process known as drift, and this occurs more rapidly in small populations. Many species exist as a network of several connected populations that are linked by dispersal, thereby replacing diversity that may be lost locally over time. Population genomics offers a higher resolution for estimating neutral genetic diversity, as well as the opportunity to understand how selection shapes genetic differences between populations.

TOOLS AND TECHNOLOGY

Several tools and technologies have proven critical in synthetic biology as they allow us to investigate and compare the entire complement of DNA and RNA sequences between organisms across the tree of life, and allow us to cultivate and preserve cells in very particular ways. These technologies are described below:

Ancient DNA

Ancient DNA is a term used to describe genetic material preserved in degraded post-mortem biological remains such as museum specimens, including that of extinct species. Ancient DNA is an incredibly useful means of describing a variety of historic ecological attributes on the scales of both individual species and communities. Samples can be used to reconstruct former communities and species interactions (Bellemain et al., 2013), clarify the demographic history of a population (Barnes, 2002), gain insight into genomic sequences of extinct species (Miller et al., 2008), and reveal historic haplotypes and alleles that may have been lost in a population over time (Campos et al., 2010).

Barcoding

DNA barcoding uses short genetic markers to identify specific organisms. It is possible to take an unknown sample tissue and query small fragments of its DNA sequence against reference databases of known genetic barcodes to identify the exact species from which the tissue was derived. The Barcode of Life Data Systems (BOLD) is the largest reference library available for annotating sequences of unknown origin. DNA barcoding can be broadly divided into two main approaches: a single-species approach (targeted barcoding) or a multispecies approach (metabarcoding).

Targeted barcoding is aimed at detecting a single species, through the use of a primer that selectively targets that species' DNA. It is useful for detecting the presence of species of interest, such as rare and endangered or invasive species by sampling environments (i.e. soil, water; see eDNA below) where they potentially occur.

Metabarcoding, by contrast, simultaneously identifies multiple taxa from a sample without the need for *a priori* knowledge of the species that are likely to be present. The difference between the two can be informally described as "see if a certain species is here" (single-species barcoding) versus "see what species are here" (metabarcoding). Metabarcoding thus allows for characterization of full communities, dietary diversity (fecal metagenomics), or to passively monitor for new/unexpected invasive species or pathogens.

Cryopreservation

Cryopreservation is the process of preserving cells, tissues or whole organisms by cooling them to temperatures between -80 °C and -196°C. At these temperatures, chemical and enzymatic activity is largely halted. Cryoprotectants are injected into or used to coat the sample to prevent the formation of ice crystals that would otherwise damage cell tissues. Cryopreservation has already acted as an effective insurance policy to maintain the genetic diversity of many wildlife and agriculture species (Rall 1993; Zhang 2011; Woelders and Hiemstra 2011). The hope is that in the future these cells such as egg, sperm, larvae, etc. can be returned to an active state to carry out their biological functions. Cryopreservation facilities currently harbor a very small number of marine taxonomic groups. Of these, commercial species, along with a few model species and species of special concern dominate banked collections (Martínez-Páramo et al., 2017). However, these focused efforts have led to major gaps across the marine tree of life, and there are currently large groups of taxa whose specimen are rarely or never preserved.

Environmental DNA (eDNA)

Environmental DNA (eDNA) is a non-invasive method for detecting and identifying species that were recently present in a specific location as indicated from cells detected in air, soil, and water samples taken from the environment. Small volumes of ocean water contain cells from all species that were recently in the vicinity of the collection area. Paired with DNA barcoding, eDNA samples can identify all cells within a sample often to the species level (Stat et al., 2017). Thus, eDNA can establish reliable distribution information on all present species, including rare, cryptic, nocturnal, microscopic, and even visibly indistinguishable species (Boussarie et al., 2018; Fukumoto et al., 2015; Kumar et al., 2009; Laramie et al., 2015) without sampling them directly. This technique has several conservation applications including determining the habitat preferences of species of special concern (Laramie et al., 2015), and for invasive species management (Deiner et al., 2015; Smart et al., 2015). Another benefit is that eDNA sequencing is much less costly to use than traditional biological surveys, which can require extensive resources to implement. Finally, eDNA can often be collected in tandem with other surveys, rather than requiring a dedicated sampling trip.

Transcriptomics and Proteomics

Genomic DNA sequences are fixed, but the rates of transcription into RNA and translation into proteins fluctuate widely and rapidly depending on environmental and physiological needs. Recent advances in molecular techniques have enabled researchers to quantify the precise amount of RNA molecules and proteins that are present in a sample at a particular time, which are major contributors to the appearance and behavior of an organism. Comparing samples from individuals under different circumstances such as environmental stressors, life history stages, and exhibiting different physical characteristics thus facilitates a new understanding of how genes and proteins are involved in the adaptive response to stimuli (Storey and Wu, 2013). Such knowledge has important conservation implications as it can help predict how species will

respond to environmental changes and can be used to inform selective breeding and genetic engineering.

Whole Genome Sequencing

DNA sequencing is simply determining the order of nucleotides in a particular string of DNA. Sequencing all of the DNA of an organism is called whole genome sequencing, which provides perhaps the most critical information for documenting and understanding processes of biodiversity. Sequences allow researchers to calculate relatedness both within and between species, identify physical traits and geographic associations with genetic sequences, and quantify genetic variation on multiple scales, from individuals, to populations, to entire communities. Knowing a genomic sequence can increase the speed and efficiency of species monitoring by allowing researchers to look for signals of selection (Therkildsen et al., 2013), estimate the nature and timing of demographic events (Excoffier et al., 2013), and identify the geographic origin for any given sample within a species (Helyar et al., 2011). Decreased costs of sequencing and technological innovations that take advantage of the 3-D structure of DNA enable entire genomes to be sequenced at low cost and assembled to chromosome-level resolution. It is now common and affordable to investigate many thousands of markers across hundreds of individuals using whole genome resequencing (WGR); targeted capture, or reduced representation library sequencing.

RECOMBINANT DNA/GENOMIC ENGINEERING/ SYNTHETIC TECHNIQUES:

An important component of synthetic biology involves the direct manipulation of genomes so that cells and organisms can be programmed to perform in a specific manner, as well as recapitulation of biologic environments for the production of cells and cellular products (i.e. cell culture). There are several important tools and mechanisms relevant to conservation. These are defined below:

Gene Editing

Genome editing refers to the process by which DNA is inserted, deleted, modified or replaced at a specific location in the genome of a living organism. Technologies that enable this process include TALENS, Zinc fingers, and most recently CRISPR. Genome editing makes it possible to study gene functions in plants and animals and advance the field of synthetic biology by modifying genes and genomes in very specific ways and toward very specific goals.

Cell Culture

Cell culture involves the growth of cells under controlled conditions, generally outside of their natural environment. The cells are provided a media containing nutrients and potentially specific chemical and mechanical stimuli which mimic true biological and physiochemical conditions that direct the development of the cells toward a desired end point or phenotype. Generally, primary cells harvested directly from a living organism have a finite lifespan. Immortalized cell lines have been established for a variety of species and tissue types. Cell culture directly supports recombinant DNA/gene editing technology, vaccine development, and tissue engineering for biomedical and commercial purposes.

Cellular Agriculture

Cellular agriculture refers to the use of molecular and cell-culture methods toward the production of agricultural products that are otherwise obtained through the harvest of whole organisms, and generally falls into two main categories of production. Tissue-engineering involves the harvesting and culturing of primordial cells (i.e. stem cells) which can be grown on a scaffold with the right chemical signals to stimulate differentiation and growth into consumable tissue (i.e. muscle). Acellular production, on the other hand, involves the use of recombinant DNA technology to engineer microbes to produce desirable organic molecules such as proteins and fats (i.e. Yamashita et al. 1987). The latter approach relies on commonly used industrial biotechnologies and so may be more immediately scalable, whereas tissue engineering requires a greater research investment to develop cell culturing protocols that properly mimic the chemical and biological environments of the target species or tissue (Stephens, et al. (2018)

CRISPR

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is the technology that is revolutionizing genomic engineering. CRISPRs are short sequences that originate from viral genomes and have been incorporated into the bacterial genome to protect against viruses. Specialized proteins (CRISPR associated proteins- Cas) process these sequences and cut matching viral DNA sequences. Plasmids containing Cas genes and specifically constructed CRISPRs can be introduced into eukaryotic cells so that the eukaryotic genome can be cut at any desired position. The resulting DNA cut can then be repaired by natural cellular processes using the sequence of a different allele, or even a gene from another species, to make a permanent change to the genome.

Gene Drive

A gene drive is a genetic engineering technology that manipulates sexually reproducing species, (excluding viruses and bacteria) to propagate a particular suite of genes throughout a population. The technique can be used to add, delete, disrupt, or modify genes. Gene drives have been proposed as a means to genetically modify specific populations and entire species, specifically

problematic species such as disease-vectoring insects, invasive species and pesticide-resistant pest species.

Given the opposition to genetically modified organisms, gene drive technology has yet to be tested in the wild. However, it has proven incredibly powerful in laboratory settings. For example, female fertility genes were identified and knocked out in malaria-carrying mosquitoes using a gene drive carrying a CRISPR/Cas9 system. This sterility genotype was then passed on to 91-99% of progeny, leading to significantly fewer offspring in the population and promising results for disease control (Hammond et al., 2016). Similarly, a rodent pest control study estimated that a single introduction of just 100 mice carrying a gene drive sequence could eradicate a population of 50,000 mice within 4 to 5 years (Prowse et al., 2017).

Researchers and environmentalists worry that gene drives could become invasive, spreading unintentionally far in nature with undesired effects on non-target species through hybridization. Despite their clear potential to improve ecosystem function, these cutting-edge techniques need to be fine-tuned and thoughtfully evaluated before gene-edited organisms are released into the wild (Mout et al., 2017; Spicer and Molnar, 2018).

Guidelines for research on gene drives have been spelled out in a June 2016 report from the National Academy of Sciences, titled “Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values.” The report concludes: “The potential benefits of gene drives for basic and applied research are significant and justify proceeding with laboratory research and highly controlled field trials.”

RNA Interference (RNAi)

RNA interference (RNAi) silences or suppresses the expression of specific genes of interest (often referred to as gene-silencing). Silencing of a single salivary gene, for instance, was shown to be lethal in pea aphids (Mutti et al., 2006). Knockdown of a sex determination gene led to the production of an entirely male progeny of red flour beetles (Shukla and Palli, 2012) and crustaceans exposed to heat stress exhibited much lower survival rates when their heat shock protein transcripts had been silenced compared to unaltered transcriptomes (Iryani et al., 2017).

REPRODUCTIVE TECHNOLOGIES

Technologies that enable the cloning and production of individuals to augment diversity and population size:

In Vitro Fertilization

In Vitro Fertilization (IVF) is currently the most universal pathway used to aid in the conservation of mammals, particularly for species that do not readily reproduce in captivity.

Interspecies Somatic Nuclear Transfer

Interspecies Somatic Nuclear Transfer (iSCNT) involves the transfer of a nucleus from any cell type into a donor oocyte of an appropriate surrogate. The power of this technology is that non-germ line tissues, which are often the only type preserved in biobanks, can be used to recapitulate genomes from individuals that cannot directly contribute to the gene pool.

Stem Cell Embryogenesis

Stem Cell Embryogenesis (SCE) has been proven to be feasible in laboratory mice and could overtake SCNT cloning as the leading mammalian reproductive technology in the coming decades. The revolutionary potential of the technology is that it can reduce the reproductive resources needed to yield offspring. In SCE, a skin cell is first transformed into a stem cell (called an induced pluripotent stem cell, or iPSC). Once in stem cell form, it can be reprogrammed to develop into sperm or egg cells. Once sperm and egg cells are made scientists can use IVF to generate a genetically diverse embryo, implant the embryo into a surrogate mother and give birth to a new unique individual. This process is currently being pioneered to attempt to save the Northern White Rhinoceros from extinction.

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Chapter 8: Expert Interviews

For this report, Revive & Restore interviewed almost 100 marine biologists, conservationists, and technologists representing over 60 institutions. Each was challenged to identify ways that rapid advances in genomics could be applied to address marine conservation needs.

Mike Allen, *Plymouth Marine Lab*

Jesse Ausubel, *Rockefeller University*

Diana Baetscher, *U.C. Santa Cruz*

Dan Barshis, *Old Dominion University*

Jim Birch, *Monterey Bay Aquarium Research Institute*

Jay Bolden, *Eli Lilly and Company*

Deron Burkepile, *U.C. Santa Barbara*

Jarred Callura, *Biotech Industry*

Kristina Cammen, *University of Maine*

Diego Cardeñosa, S*tony Brook University*

John Carlin, *Fincasters*

Mark Carr, *U.C. Santa Cruz*

Demian Chapman, *Florida International University*

Nathan Churches, *University of Southern California*

Philip Cleves, *Stanford University*

Ann Cohen,*Woods Hole Oceanographic Institution*

Kathy Coyne, *University of Delaware*

Jamie Craggs, *Horniman Museum*

Lee Crockett, *Shark Conservation Fund*

Sarah D'Adamo, *Wageningen University*

Bernie Degnan, *University of Queensland*

Nishan Degnarain, *London School of Economics*

Tom Dempsey, *The Nature Conservancy*

James Deutsch, *Vulcan*

Dan Distel, *Ocean Genome Legacy*

Craig Downs, *Haereticus Institute*

Sylvia Earle, *National Geographic*

Owain Edwards, *CSIRO*

Sarah Foster, *Project Seahorse*

J. Carlos Garza, *National Oceanic and Atmospheric Administration*

Ruth Gates (dec), *Hawaii Institute of Marine Biology*

Kelly Goodwin, *National Oceanic and Atmospheric Administration*

Frances Gulland, *U.S. Marine Mammal Commission*

Mary Hagedorn, *Smithsonian Conservation Biology Institute*

Gator Halpern, *Coral Vita*

Ove Hoegh-Guldberg, *University of Queensland*

Ling Ding Jeak, *National University of Singapore*

Robert Jones, *The Nature Conservancy*

Ryan Kelly, *University of Washington*

Natalie Kofler, *Yale University*

George Leonard, *Ocean Conservancy*

Petra Lundgren, *Great Barrier Reef Foundation*

Molly Lutcavage, *Large Pelagics Research Center*

Skyli MacAfee

Kristen Marhaver, *Caribbean Research and Management of Biodiversity*

Matthew Markus, *Pembient*

Mark McAnallen, *Biomeme*

John McCosker, *California Academy of Science*

Chris Meckley, *ACI Aquaculture*

Michael Melkonian, *University of Cologne*

Gary Molano, *University of Southern California*

Michael Moore, *Woods Hole Oceanographic Institution*

Phillip Morin, *National Oceanic and Atmospheric Administration*

Ned Mozier, *Pfizer*

Gavin Naylor, *University of Florida*

Ben Neely, *National Institute of Standards and Technology*

Rebecca Ng, *Vulcan*

Larry Niles, *Consulting Biologist*

Heidi Norton, *Biomeme*

Sergey Nuzhdin, *University of Southern California*

Heath Packard, *Island Conservation*

Barbara Page, *Anthropocene*

Caroline Palmer, *University of Plymouth*

Megan Palmer, *Stanford University*

Per Palsboll, *University of Groningen*

Stephen Palumbi, *Stanford University*

Sumi Paranjape, *Vulcan*

Devon Pearse, *National Oceanic and Atmospheric Administration*

Josh Perfetto, *ChaiBio*

Dan Pondella, *Occidental College*

Katie Prager, *University of California, Los Angeles*

Stephen Ranson, *Coral Vita*

Ciro Rivera, *Florida International University*

Forest Rohwer, *San Diego State University*

Dirk Rosen, *Marine Applied Research and Exploration*

Benyamin Rosental, *Ben-Gurion University*

Scotty Schmidt, *Primary Ocean Producers*

Chris Scholin, *Monterey Bay Aquarium Research Institute*

Jerry Schubel, *Aquarium of the Pacific*

Kim Selkoe, *University of California, Santa Barbara*

Bart Shepard, *Steinhart Aquarium*

Levi Simons, *University of Southern California*

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Dirke Steinke, *International Barcode of Life*

Eric Stiles, *New Jersey Audubon*

John Teem, *International Life Sciences Institute*

Sam Teicher, *Coral Vita*

Karsten Temme, *Pivot Bio*

Nina Therkildsen, *Cornell University*

Tierney Thys, *National Geographic*

Sasha Tozzi, *Microbiotech Solutions*

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Madeleine van Oppen, *Australian Institute of Marine Science*

Rebecca Vega-Thurber, *Oregon State University*

Amanda Vincent, *Project Seahorse*

Nate Walworth, *University of Southern California*

Tariq Warsi, *Syntheseas*

Luke Warwick, *Wildlife Conservation Society*

James West, *Catalina Sea Ranch*

Demian Willette, *Loyola Marymount University*

John Wise, *University of Louisville*

Alex Worden, *Monterey Bay Aquarium Research Institute*

Chapter 9: About This Report

This report was prepared by Revive & Restore – a nonprofit organization with a mission to enhance biodiversity through new techniques of genetic rescue for endangered and extinct species. We work with the world's leading molecular biologists, conservation biologists, and conservation organizations to develop pioneering, proof-of-concept genetic rescue projects using cutting-edge genomic technologies to solve problems posed by inbreeding, exotic diseases, climate change, and destructive invasive species.

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