



Applications and Methodologies of Human-Assisted Evolution in Sustaining Coral Reefs

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Abstract

Coral reefs play a crucial role in sustaining marine biodiversity by nurturing life that stabilizes the ecosystem. They have incalculable ecological and economic value, supporting 25% of marine life (NOAA 2019) as well as 450 million people from 109 countries that live in close proximity and depend on resources from coral reefs (Oppen et al., 2015). However, in the 21st century, corals have been exposed to various climate and anthropogenic threats which require immediate action to mitigate the effects. This paper will evaluate the many solutions posed to further enhance conservation efforts in response to these rapidly changing environmental conditions. Coral species and individuals can have varying tolerance levels in response to the impacts of climate change, including rising temperatures and ocean acidification. To combat this, geneticists and marine biologists pose a solution termed assisted evolution: the process of promoting evolution and adaptation via human assistance. Changes in environmental conditions are increasing in frequency and intensity, and only some populations may be able to adapt and evolve with the change. Uncertain of a species' evolution potential, scientists have been performing ways of assisted evolution such as preconditioning, genetic engineering, and selective breeding. Assisted evolution attempts to change the genetics of some species to enable them to tolerate the changing environmental conditions. Though the topic of genetic editing holds controversies, its purpose is to sustain marine ecosystems and ultimately, to ensure global sustainability.

Keywords: Evolvability, genetic, coral bleaching, environmental conditions.

Introduction

A crucial component to ensure the sustainability of the globe is ocean health and particularly the coral reef ecosystems. Coral reefs host immense biodiversity by supporting the surrounding communities, where each species plays an important role to sustain the greater ecosystem. Moreover, coral reefs also help human populations in several ways such as providing food and protecting shorelines (Elliff et al., 2017). Reefs are composed of corals; colonial marine invertebrates that belong to the Cnidarian family. Corals obtain energy in two ways; first, as carnivores, they catch zooplankton using nematocysts, cells that contain venomous barbs used for defense or to catch prey (Houlbrèque and Ferrier-Pagès 2009). Their other source of energy is obtained from its symbionts—zooxanthellae and associated microbiome—which forms the coral holobiont that ensures coral health. The symbionts will photosynthesize, generating sugars which are metabolized by the coral. The exchange of this symbiosis is the symbionts will provide energy, and the coral will provide a safe environment for the symbionts (Roth 2014). However, increasing ocean temperatures are causing a breakdown of this symbiotic relationship in a phenomenon called coral bleaching. The majority of widespread coral bleaching is induced by high temperatures, but can also be caused by other stressors like ocean acidification. Mass coral bleaching events are the most prevalent and detrimental threat to coral reefs today (Van Woesik et al., 2022).

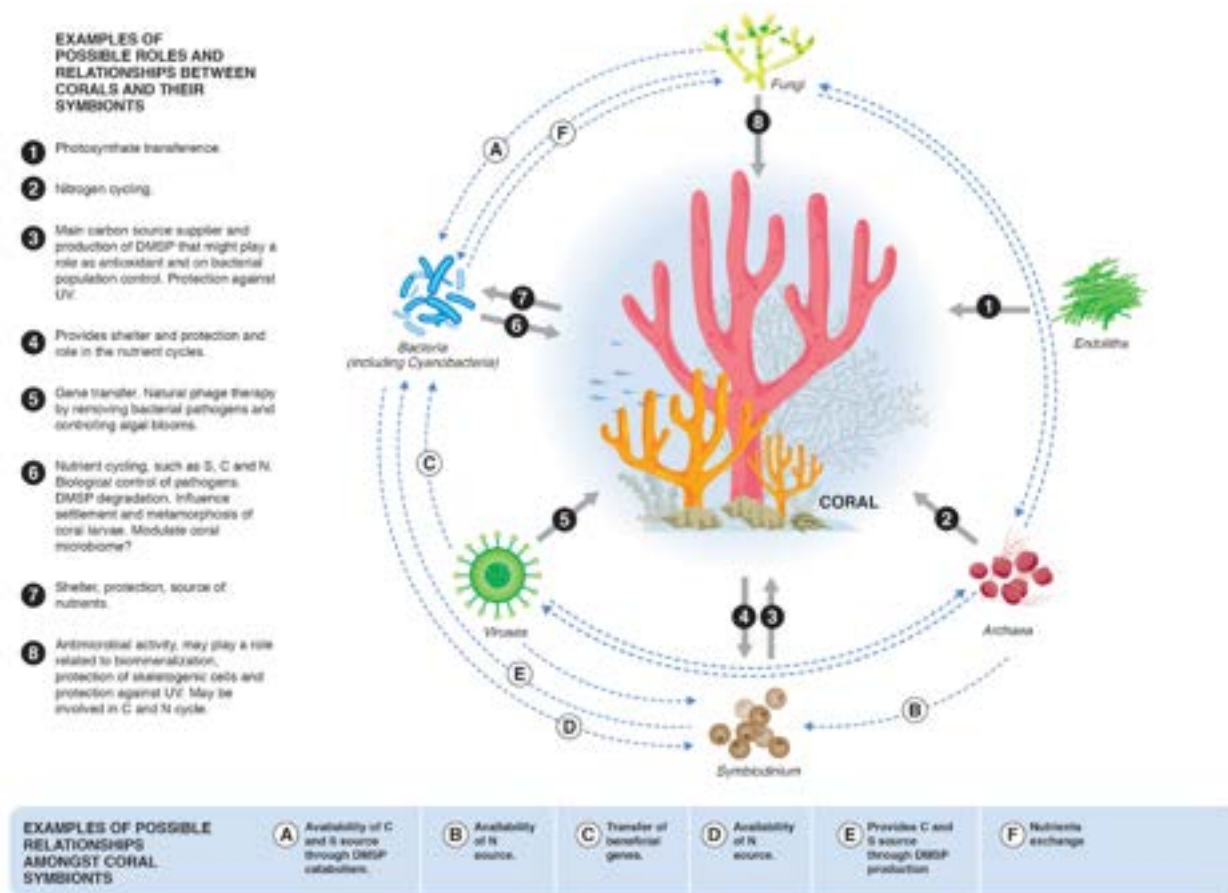


Figure 1. Symbiotic exchange between corals and their symbionts. The listed microorganisms are the principle that forms the coral holobiont which each have a specific beneficial exchange with the coral host (Peixoto et al., 2017).

Climate change leads to more intense and frequent temperature anomalies

Climate change is one of the major contributors to coral bleaching (Done et al., 2003; Hoegh-Guldberg 1999; Baker et al., 2008). The effects of these drastic environmental changes primarily fall under: sea surface temperature anomaly, prolonged heat waves, and ocean acidification. The primary cause of climate change is the greenhouse effect. Greenhouse gasses (mostly carbon dioxide and methane) traps heat near the Earth's surface and absorbs radiation from the sun that warms the Earth (Crowley 2000; Stern and Kaufmann 2014). This causes temperature anomalies where temperature departs from its typical occurrence. The ocean stores an approximate of 91% (0.64 to 0.83 watts per square meter) of the excess heat trapped in the atmosphere (IPCC 2021). The heat absorbed in the ocean leads to an increase in the ocean's surface temperature. This temperature anomaly led to several heatwaves, periods of unusually warm temperature in the water. Heatwaves are highly destructive for coral reef systems.

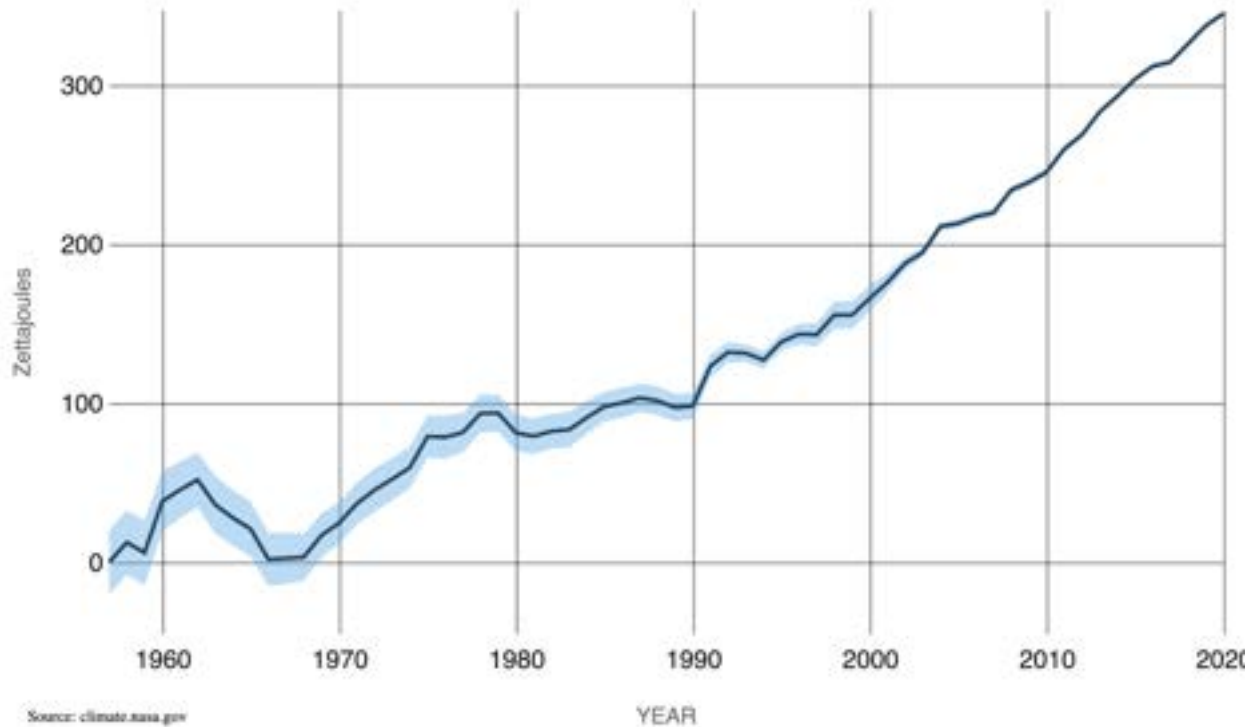


Figure 2. NASA has been regularly monitoring the ocean temperatures since 1955, and the heat content has been steadily increasing. It is predicted that the frequency of heatwaves are likely to continue the positive trend, leading to higher temperatures (Oliver et al., 2018).

Increased CO₂ intake in the ocean results in ocean acidification and oxidative stress in corals

Climate change is caused by excess amounts of greenhouse gasses in the atmosphere. The ocean not only absorbs most of the excess heat, but also absorbs about 31% of all the CO₂ emitted (NOAA 2022) This leads to ocean acidification, where the pH of the ocean is lowered, acidity is increased, due to excess CO₂ intake. When CO₂ is absorbed into the ocean, it reacts with H₂O and creates H₂CO₃ carbonic acid (H₂O + CO₂ → H₂CO₃). Which carbonic acid will create more H⁺ ions thus increasing ocean acidity. It will also produce more bicarbonate (HCO₃). With an increased acidity in the ocean, it induces oxidative stress in corals. Coral skeletons are made of CaCO₃ (calcium carbonate) and it will react with H₂CO₃. Where the carbonic acid will affect the development of calcium carbonate in the skeleton, thus impeding coral growth (Mollica et al., 2018; Guo et al., 2020). More bicarbonates being produced indicates lower pH of the ocean where before the Industrial Revolution, the average pH of the ocean was about 8.2 while today's average is about 8.1 (EPA 2024).

Coral bleaching: The physiological process and cause.

Coral bleaching is one of the most lethal events that harms coral (NOAA 2020). Coral bleaching is caused by high temperatures that lead to the expulsion of its symbionts. The symbionts are one of the most vital components of a healthy coral: providing energy and

pigmentation for the coral (Martinez et al., 2022). When the symbionts are no longer present, the coral loses its pigmentation and remains white. Coral bleaching is a stress response primarily caused by rising temperatures (Porter et al., 2001). This expulsion is a result of oxidative stress due to excessive amounts of reactive oxygen species (ROS) within the cell (Peleg-Grossman et al., 2012). ROS are highly reactive oxygen-containing compounds that are produced as a byproduct from cellular respiration and photosynthesis, a natural process that occurs in all living organisms (McClelland et al., 2024). ROS contribute to normal cell processes; however, it can become toxic in high concentrations that disrupts normal physiology with detrimental effects (Sies et al., 2022; Dove and Hoegh-Guldberg 2006). Increase in temperature accelerates cellular respiration, thus increasing the amount of ROS produced, especially in symbiotic relationships (Peleg-Grossman et al., 2012). The excess ROS from the symbionts will leak and accumulate into the coral cells that harms the coral by damaging tissues and DNA. The coral will remove the symbionts to reduce the amount of ROS in its cells (Lesser 2011; Helgoe et al., 2024). Corals are removing all its symbionts because of the excess ROS the symbionts are producing.

Coral bleaching has been happening prior to human influence, originally as a natural occurrence due to temperature changes throughout the year. However, as climate change accelerates, it leads to more intense global warming that prolongs the bleaching period. Long term bleaching is lethal (Loya et al., 2001; Brown and Ogden 1993). As in recent years, bleaching events have been occurring more frequently with the longest period recorded to be from 2014 to 2017 in the Great Barrier Reef, Australia (Guo et al., 2020). On April 15, 2024, the National Oceanic and Atmospheric Association (NOAA) confirmed that the world is currently experiencing the 4th global bleaching event (NOAA 2024). The scale of impact is very different from a local bleaching to a global bleaching. Bleaching can occur in different areas, but global bleaching is on a much severe scale where a large proportion of corals around the globe is experiencing some degree of bleaching (Leggat et al., 2022). This is a dire issue that has the potential to affect ecosystems beyond the ocean and eventually exacerbate to the point where humans can no longer mitigate.



Figure 3. The process and cause of coral bleaching; the expulsion of zooxanthellae and other symbionts due to various stress factors. With temperature as the most direct cause leading to large scale bleaching around the globe at an increased frequency (NOAA 2024).

Discussion

Inter- and intra-species stress response variability

Heat tolerance levels vary among different species and individuals of corals; also known as inter- and intra-species variation. Inter-species variations refer to differences between species, potentially due to foundational physiological differences. Research conducted by the Hawai'i Institute of Marine Biology presents evidence that biological response to temperature and $p\text{CO}_2$ is highly species-specific (Bahr et al., 2016). For example, *Leptastrea purpurea* and *Porites compressa* are different Hawaiian coral species that show a significant difference in response to high temperature and $p\text{CO}_2$ levels (Bahr et al., 2016). *L. purpurea* shows to be a very hardy coral that had little to no change under the treatment while *P. compressa* had reduced calcification rates (Bahr et al., 2016). This is inter-species variance. Intra-species variations are differences between individuals of the same species. This variance can be determined by the differences in the genetic sequence and/or physiology between individuals. Prime example is the heat tolerance variation within individuals from the same species (Strand et al., 2024).

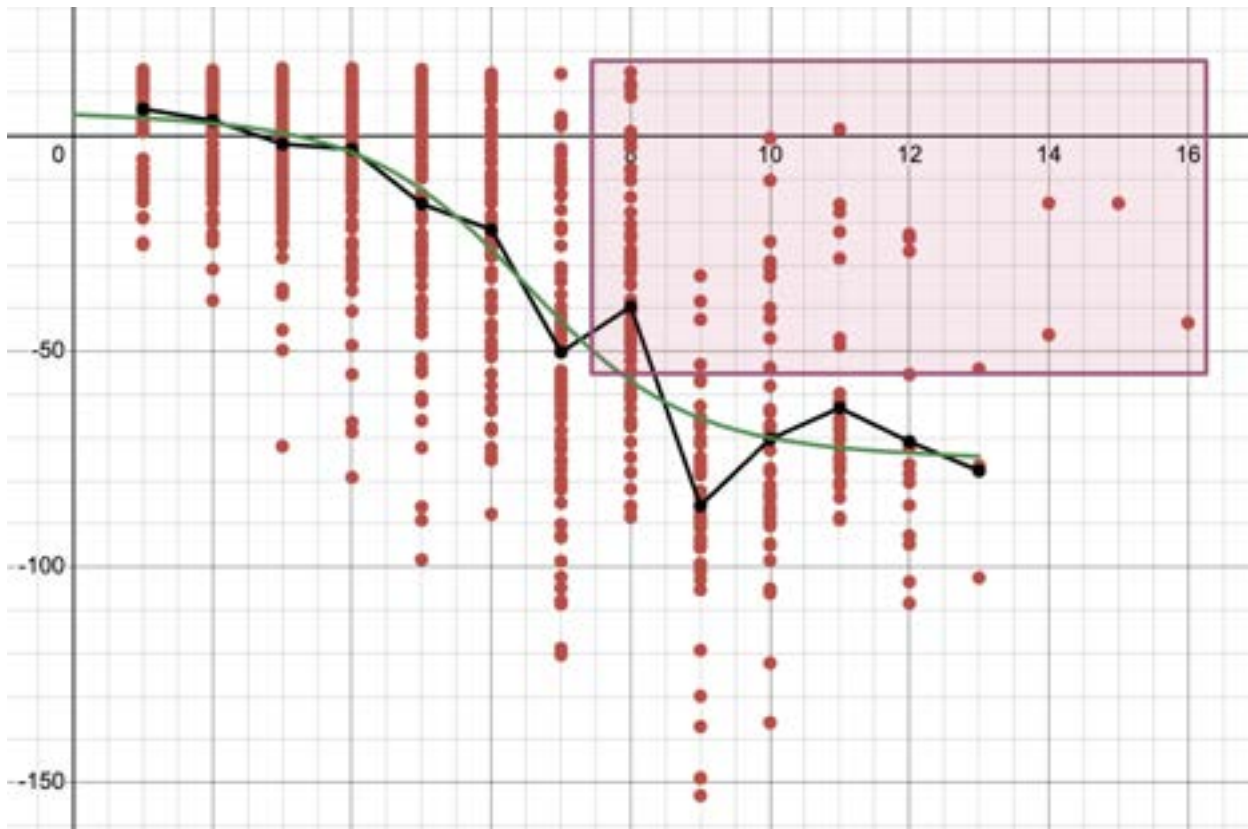


Figure 4. A population of *Pocillopora acuta* is exposed to high temperature (29.5 °C) followed by a two month return to ambient temperature (27.5 °C). Researchers assessed their bleaching intensity and physiological reaction. Where x-axis is time (weeks) over the course of 16 weeks, and the y-axis is the bleaching score. The bleaching score indicates the severity of bleaching in the coral that is determined by assessing its tissue color as a proxy for its cell density and pigmentation (Strand et al., 2024). The lower bleaching score indicates a higher severity. The data is recorded collectively on a weekly basis. Each red data point represents a singular coral specimen. The black point is the average of all the specimens' bleaching score modeled by a logistic function (green) to demonstrate the overall bleaching trend (Strand et al., 2024).

Even though all the corals are from the same population, they present a big variance in their response to stress. Especially for corals in the box; they have demonstrated a much higher tolerance to stress than others. This is potentially due to molecular factors like genetic diversity. Genetic diversity is a driving factor of sustainability of the species (Elder et al., 2022; Humanes et al., 2022) where even though all the individuals are the same species, they have different mutations in their genome that enable some to be more tolerant to certain stressors than others. In addition to genetic diversity, another factor that may affect their stress response may vary due to the symbiont population that resides in the coral (Palacio-Castro et al., 2023). This may be caused by the diversity of the types of symbionts or the physiology of the symbionts; the temperature may not be accelerating their metabolism with ROS production, thus they are less

likely to harm the coral (Strand et al., 2024). Furthermore, this can also be affected by the location where the corals live; some may live closer to the surface exposed to more sunlight (higher temperature) and some may live deeper. The corals that live in shallower areas are more prone to get bleached than ones that live relatively lower. Deep sea corals do not get bleached because they lack the symbiotic organisms. However, they are still presented to acidification issues. Individual species are likely to respond differently to stress differently (Strand et al., 2024).

Acclimatization vs. evolution

When a change occurs in an environment, it is likely that it will disrupt the balance between the organisms that live in the area and the environment itself. Some will be able to adapt and tolerate the change due to the diversity of traits in their genome. Adaptation and tolerance are the very first phase for evolution to happen within the species. However, this may not occur for every individual. An individual capable of tolerating the heat stress does not mean that its offspring will be able to do so either; it is not certain that its offspring will inherit this trait. This is the difference between acclimatization and evolution. Acclimatization is a physiological adaptation that occurs only within an individual's lifetime. It refers to specific traits or functions that are changing. However, simply improving an individual does not help the population in responding to the change. Researchers are investigating methods to guarantee the inheritance of traits; increase evolvability to protect the species (Logan et al., 2021; Jury et al., 2019).

Natural adaptation vs assisted evolution: What determines the evolvability of a species

Evolution is triggered by genetic variation and a changing environment. Organisms are constantly evolving, and their rate of evolution is varied based on species, physiology, and environmental factors. Nonetheless, the key determinant of evolution is genetic diversity; ensuring a diverse pool of traits that can trigger evolution. For a population of high genetic diversity, some are able to tolerate the change. In an ecosystem, there is an optimal match between the environment conditions and species' traits. As the environment changes, the species also undergoes changes (evolution or acclimatization) to maintain the fit in the environment for its existence. This change may be an improvement to the current present traits or bringing out the hidden traits in the individual. In an organism's genome, some traits are more obviously in use, and some are not and are concealed in its genetic pool. It is possible that a hidden trait will be lost after generations (McGrath 2024). In contrast, it may also come out in response to a change. This whole evolution process is selective; not every individual in the population is capable of evolving, or surviving the change. The individuals that survived will reproduce and their offspring is likely to inherit the trait and improve the tolerance of the whole population. This is natural evolution. The cause of many extinctions is because the species were unable to keep up with the changes happening in the environment. As changes are increasing in frequency and intensity (e.g., temperature), some populations may be able to adapt and evolve with the change (Madeleine et al., 2015). However, climate change is accelerating so the big question is whether corals will have enough time to evolve. For corals, it typically takes 10-15

years to reach sexual maturity, thus it will take approx 100 years to have many generations for evolution (Rapuno et al., 2023). The sea surface temperature (SST) is already increasing with a predicted temperature of 1.5° C above pre-industrial level by 2030 and 2052 (Masson-Delmotte et al. 2018). The ocean is currently warming too quickly for the corals to adapt, thus requiring human intervention of assisted-evolution to trigger evolution.

The potential of assisted evolution in modern conservation practices: an overview

Currently, it is almost an unrealistic approach to alleviate climate change to a degree where it no longer has a significant effect on corals. A solution is assisted evolution: promoting adaptation and evolution triggered by artificial intervention in its genome and/or its environment (Madeleine et al., 2015). This has long existed primarily for commercial purposes through selections for superior phenotypes that bring new allele combinations that are nonexistent in nature (Madeleine et al., 2015). Natural evolution is a slow process with subtle changes passed onto the next generation. However, environmental changes are happening more rapidly where natural evolution cannot keep up, and would require human intervention to trigger evolution. As climate change impacts become more visible in corals, scientists conduct research in applying assisted evolution in coral reef restoration. This is especially noteworthy because genetic manipulation is rarely considered for non-commercial purposes, such as conservation and restoration. The most dire harm that corals are facing is rising temperature which methods of assisted evolution is attempting to increase temperature tolerance in corals. Research conducted by Cleves et al. (2020) has demonstrated that assisted evolution is effective in alleviating the effects of temperature in corals. The objective of assisted evolution is to ensure the change in its physiology and response is effective to sustain the coral, and can be passed down to its offspring. Because the uncertainty is that while scientists are able to make a species acclimatize to the change, they cannot ensure the inheritability of the improved trait. Though genetic modification has been used for a commercial purpose, it has great potential in sustainability especially addressing coral bleaching.

Genetic Rescue: An approach to improve adaptability and resilience in response to environmental change

Genetic rescue is a strategy to prevent extinction by reducing biodiversity loss of a population by means of genetic manipulation of an individual and/or a population (Pavlova et al., 2023). High genetic diversity ensures the overall resilience of the population when presented with an environmental change. Lack of high genetic diversity leads to inbreeding and is often the reason that causes species extinction (Spielman et al., 2004). Genetic rescue has two main components, outbreeding, and de-extinction. Outbreeding is introducing an individual from another population as a way to introduce new genetic material to the inbred population. By doing so, it can reduce genetic disorders from inbreeding of harmful recessive alleles and improve reproductive success (Davinack 2024). Outbreeding is an approach to improve the genetic diversity of a population. In 1995, Florida panthers were a highly endangered population with less than 30 in the wild due to inbreeding. Revive and Restore is a wildlife conservation

organization and in an effort to increase its genetic diversity, they outbred the Florida panther with Texas cougars because they have highly similar phenotypes. The organization was able to improve the population with between 120 and 230 panthers. This is a prime example of successful genetic rescue efforts. Now with a relatively more diverse genetic population, the population is more likely to adapt and evolve in response to an environmental change. However, in regards to corals, this needs to be considered with high caution. There are over 6,000 known coral species that are spread throughout every ocean basin. Each ocean has different environmental conditions due to its geological setting, thus the coral species may have developed an optimal fit with the environment in this specific basin, and may not be accustomed to a completely new environment with different water conditions and surrounding organisms. Furthermore, introducing a new species to a new environment contains many uncertainties; the coral might have a superior trait in this specific environment and disrupts the normal balance of an ecosystem. Despite these considerations, this selective breeding process can potentially improve a threatened coral population; however, the possible consequences must be investigated prior.

The other component is de-extinction, a process to revive already extinct species with the objective to restore lost genetic traits to the ecosystem and enhance biodiversity. This concept was mostly introduced through superlative fiction; however it has gained popularity in recent years. There are three primary methods to achieve de-extinction: back-breeding, cloning, and genetic engineering (Davinack 2024). Back-breeding and genetic engineering both involve trait and gene manipulation of a closely related species. Back-breeding is selective breeding of closely related species to recreate the phenotype of the extinct. And genetic engineering such as using CRISPR-Cas9 gene-editing technology to modify the DNA of an organism to recreate the extinct species (Lin et al., 2022) by editing another individual to have highly similar traits with the extinct species. Scientists will compare the genomes of extinct and living species to identify the area that can be altered to recreate the species. Cloning is taking the preserved cells of the extinct species and creating a genetically identical copy of it. Scientists will take the nucleus of the extinct and insert it into an egg cell of a closely related species. However, this is challenging due to the limited availability and preservation quality of the gene (Pavlova et al., 2023).

There are many forms of assisted evolution and its potential is increasing with the CRISPR-Cas9 genome editing technology. CRISPR-Cas9 is a technology that enables genetic editing by altering sections of the genome. There are many research conducted in attempting to use CRISPR to improve coral resilience.(Jiang et al., 2017). During a CRISPR operation, the Cas9 enzyme will lead an artificially constructed single guide RNA (sgRNA) to the targeted DNA sequence where the sgRNA will make the cut (Jiang et al., 2017). After the targeted DNA sequence is removed, it will be replaced by the desired gene sequence. This has demonstrated potential to improve the tolerance capabilities of corals to address heat-induced bleaching (Cleves et al., 2020). Furthermore, it can provide more information and understanding of the coral's developmental biology to promote further research in applying other genetic approaches. However, there is a temptation of introducing improved corals to the wild that should be

considered with extreme caution especially with our understanding of its mechanisms still remains understudied.

The target of assisted evolution: coral host or symbiotic microorganisms?

Coral bleaching is a stress response that triggers the expulsion of the coral's vital symbionts. Much research regarding approaches to mitigate this response has been targeted towards the coral host itself; however, another direction to consider is to modify its symbiotic microorganisms to mitigate coral bleaching (Maire et al., 2022). This approach can take place through genetic engineering. However, it has clear limitations such as the understanding of the genome and ensuring that the bacteria is genetically modifiable. While experimental evolution has already demonstrated success in improving thermal tolerance in corals. Experimental evolution monitors organisms' evolutionary response under controlled conditions. This strategy overlaps with approaches to preconditioning; after being exposed to mild stress, the individual will be more likely to tolerate stress/improve its tolerance. The experimentation can be done to both the microbiome and the symbionts themselves. The "Coral Probiotic Hypothesis" (Reshef et al, 2006) claims that the microbiome can be manipulated to improve coral health and resilience. This may enable the coral to adapt much quicker to changing conditions than to *only* rely on selection of the coral itself (Maire et al., 2022). *Oculina patagonica*, a coral in the Mediterranean, was able to quickly adapt to changing environment conditions (and developed resistance towards the bleaching-inducing pathogen (*Vibrio shiloi*) by altering the coral's microbiome (Reshef et al., 2006). This requires selection of advantageous microbial composition for a specific environment. For example, an experiential evolution study that targeted oxidative stress mechanisms showed that a constant exposure of *E. coli* to hydrogen peroxide boosts bacterial survival and evolvability against oxidative stress (Rodriguez et. al., 2020). Although this study successfully increased survival, implementing this in coral systems could contain trade-offs like decreased growth rate. Proposed beneficial characteristics of beneficial microorganisms in corals all center around enhancing resilience and maintaining control of its homeostasis. This manipulation can be used in human-assisted evolution associated with other approaches depending on the specific characteristics.

Conclusion

Corals play a vital role in ensuring the health of the ocean and the globe. They lay the foundation for the marine ecosystem; supporting a large population of marine species that all play an intricate and crucial role in a healthy ecosystem. However, climate change induced marine heatwaves are becoming more frequent and intense, resulting in widespread coral bleaching throughout the world. This is a large-scale environmental change that may be lethal to corals over time. Environmental changes occur regularly, leading species to evolve and adapt to the new conditions. Unfortunately, climate change is triggering more severe changes that outpace evolutionary rates that put increased pressure on coral populations. Not to mention, corals are vulnerable and cannot adapt quickly to environmental changes. In order to protect these essential creatures, humans could interfere with their evolution process and perform

assisted evolution to accelerate the process. However, ethical questions may arise from these methodologies. In several methods of assisted evolution, humans are *artificially* changing a *natural* organisms' genome. This topic holds many controversies; however, it is necessary for the purpose of protecting corals. The utility of assisted evolution likely requires more research before implementing for conservation purposes. Important questions to consider are selecting the species to preserve by assessing its environmental value. Resources are limited and it is impossible to save every single organism. Furthermore, after successfully improving a species' tolerance, assessments need to be made whether this species is safe to introduce into the wild. With an improved trait, this organism might be too strong to become invasive. It is important to avoid causing further harm to the already damaged ecosystem. Changes are happening quickly, humans need to take on the responsibility to help the corals; they might not be able to persist by themselves.



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