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Trends in Biotechnology

Opinion

Harnessing synthetic biology to enhance ocean health

Peter Q. Nguyen,[1,11] Xiaoning Huang,[2,11] Daniel S. Collins,[1,3,11] James J. Collins,[1,4,5,6,*] and Ting Lu[2,7,8,9,10,*]

Ocean health is faltering, its capability for regeneration and renewal being eroded by a steady pulse of anthropomorphic impacts. Plastic waste has infiltrated all ocean biomes, climate change threatens coral reefs with extinction, and eutrophication has unleashed vast algal blooms. In the face of these challenges, synthetic biology approaches may hold untapped solutions to mitigate adverse effects, repair ecosystems, and put us on a path towards sustainable stewardship of our planet. Leveraging synthetic biology tools would enable innovative engineering approaches to augment the natural adaptive capacity of ocean biological systems to cope with the swiftness of human-induced change. Here, we present a framework for developing synthetic biology solutions for the challenges of plastic pollution, coral bleaching, and harmful algal blooms.

Highlights

Synthetic biology tools can be used to develop novel sustainability solutions for global ocean health challenges.

Tailored microbial consortia can be designed to target plastic waste for biodegradation and upcycling.

Engineering and altering coral holobiont dynamics may enhance coral reef resilience in the face of climate-induced extinction.

CRISPR-based nucleic acid sensors can enable the rapid and inexpensive identification of toxic HAB events.

Pressing ocean challenges

The ocean covers over 70% of the Earth's surface and provides a range of ecosystem services. It regulates biogeochemical dynamics for global carbon and nitrogen cycling, oxygen production, and heat distribution that underpin the climate and environment of the entire planet [1–5]. The ocean also provides diverse living and nonliving resources including food, natural products, and renewable energy, offers defenses against flooding and erosion, and supports regional and global economies via coastal tourism and seaborne trade [6–8]. However, the health of the ocean is under severe threat due to myriad industrialization activities (Figure 1), including the discharge of **plastics** (see Glossary), chemicals, metals, nutrients, and pesticides that directly pollute the ocean and the emission of greenhouse gases that indirectly impair it [5]. The consequent harm is manifestly evident by impacts on marine ecosystem functioning and diversity, as well as human health. Researchers are increasingly alarmed at the rate these adverse effects are transpiring. Current conservation strategies such as waste regulations, fishing restrictions, and emerging sustainability practices are essential for restoring ocean health. However, these approaches alone are not sufficient to address the monumental threats that are rapidly unfolding; this dire situation calls for novel solutions to mitigate and monitor ongoing ocean deterioration and establish actionable strategies for global environmental stewardship. In this opinion article, we submit that synthetic biology holds tremendous potential to ameliorate the catastrophic consequences facing the ocean and could offer powerful strategies that complement long-term marine conservation policies.

Synthetic biology applies engineering principles to design and build synthetic gene circuits, biomolecular components, and programmable cells with well-defined dynamics and functionality [9]. By incorporating design and modeling approaches to biological systems, artificial gene networks with complex regulatory behaviors such as toggle switches [10] or oscillators [11] can

[1]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, USA
[2]Department of Bioengineering, University of Illinois Urbana-Champaign, Urbana, IL, USA
[3]Department of Biology and Nicholas School of the Environment, Duke University, Durham, NC, USA
[4]Institute for Medical Engineering & Science, Massachusetts Institute of Technology, Cambridge, MA, USA
[5]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA
[6]Broad Institute of MIT and Harvard, Cambridge, MA, USA
[7]Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana, IL, USA
[8]Department of Physics, University of Illinois Urbana-Champaign, Urbana, IL, USA
[9]Center for Biophysics and Quantitative Biology, University of Illinois Urbana-Champaign, Urbana, IL, USA

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be assembled. This approach has blossomed into the engineering of advanced biological systems that enable population coordination, living materials synthesis, cellular spatial patterning, biological computation, and ecosystem functioning, amongst others [12–15]. This powerful capability for programming living and cell-free biological systems has led to the application of synthetic biology to various sectors of our society, ranging from medical therapeutics to biosensing, material fabrication, energy production, and environmental remediation [16–18]. Here, we propose enhancing ocean health by leveraging the principles and tools of synthetic biology to develop solutions for mitigating pollutants, augmenting ecosystem functions, and monitoring dynamic biosystems (Figure 2). Specifically, we examine the potential utilization of synthetic biology for ocean repair and maintenance through the lens of three urgent ocean health issues. First, we explore the pervasive problem of plastics pollution in the ocean and discuss how biodegradation pathways can be engineered to convert these plastics into biocompatible green carbon sources. Next, we tackle the crisis of coral reef collapse, examining synthetic biology avenues for buttressing the failing symbiotic dynamics that underpin **coral bleaching**. Finally, we dive into **harmful algal blooms (HABs)** and describe how rapid, programmable biosensors could be developed and deployed for predicting and monitoring their progression.

[10]National Center for Supercomputing Applications, University of Illinois Urbana-Champaign, Urbana, IL, USA
[11]These authors contributed equally to this work.

*Correspondence:
jimjc@mit.edu (J.J. Collins) and
luting@illinois.edu (T. Lu).

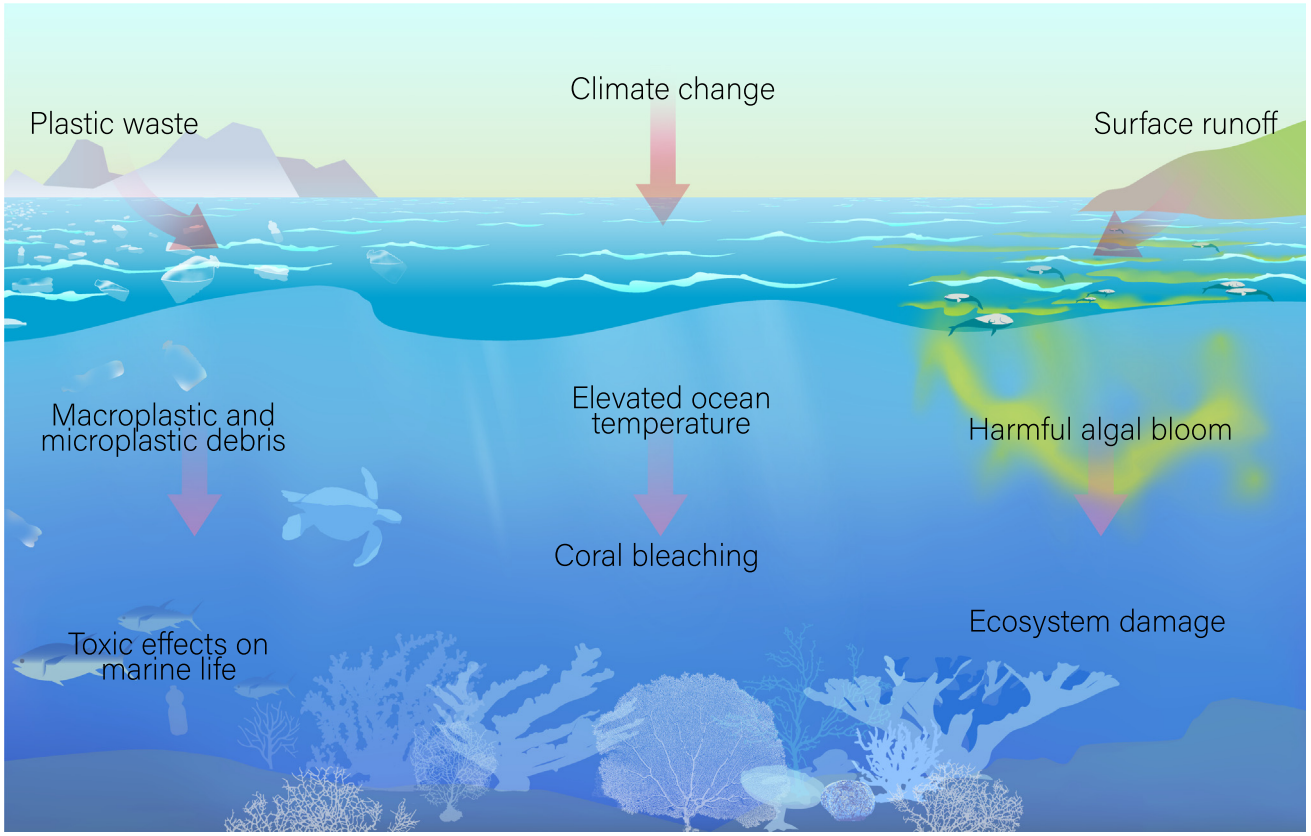


Figure 1. Three key ocean health challenges. Plastic pollution from landfills and rivers turns into macro- and microplastics in the ocean, which wreaks havoc on marine life through entanglement of sea life and absorption into organs and tissues, causing toxic effects. Climate change has increased ocean temperatures, causing increased stress on coral reefs, eventually leading to the bleaching and death of corals. Nutrient-rich runoff such as sewage and agricultural discharge fuels the explosive growth of harmful algae; the resulting bloom causes dead zones in the oceans and the accumulation of toxins in the ocean food chain.

Engineering biological solutions to ocean plastic pollution

Plastic materials are omnipresent in modern life due to their durability, versatility, and low cost. However, their improper disposal has caused environmental contamination worldwide, especially in the ocean [19,20]. Plastic waste constitutes ~80% of all human-made ocean debris, with an estimated 200 million metric tons of plastics percolating throughout oceanic gyres, polar waters, and even deep seas [21]. These plastics are fragmented into debris of varying dimensions, of which the most concerning are tiny fragments called **microplastics** (Box 1). While larger plastics have been known to impact the ocean through ingestion by and entanglement of marine life, microplastics have recently been shown to extensively permeate not only ocean habitats, but also into the very tissues of living organisms, threatening ocean biomes, food safety, and human health.

As most plastic ocean waste originates from land, current approaches for addressing this problem largely focus on reducing plastic use and increasing the efficiency and economics of plastic recycling. Thus, thermal, mechanical, and chemical methods have been extensively explored for plastics recycling [22,23]. However, these processes typically generate toxic by-products and require energy-intensive temperature and pressure conditions as well as complex infrastructure. Recently, the search for more efficient solutions has expanded to **biotransformation** routes using living systems. For example, Yoshida *et al.* screened soil samples from a recycling facility and identified a bacterium, *Ideonella sakaiensis,* that has evolved to natively assimilate polyethylene terephthalate (**PET**) plastics as its sole carbon source using a novel hydrolase named **PETase** [24]. Examination of other species from the genera *Rhodococcus*, *Pseudomonas*, *Acinetobacter*, and *Bacillus* were also found to possess innate abilities for degrading synthetic **polymers** such as polyethylene and polystyrene [25,26]. While the associated microbial enzymatic pathways have exciting potential, they have very low catalytic performance in their native form. In principle, engineered versions of these enzymatic pathways may offer a novel route for efficient polymer biodegradation. Supporting this notion, recent **directed evolution** of microbial hydrolases has yielded variants with improved catalytic activity and thermostability, and strains carrying these mutated enzymes exhibit an enhanced efficiency in substrate transformation [27,28].

We envision developing, optimizing, and utilizing synthetic polymer biodegrading pathways to address ocean plastic pollution in two modes: (i) within industrial bioprocessing facilities, and (ii) out in the open ocean environment. In the former case, plastic waste collected from oceans and landfills would be transported to a facility where it would be bioprocessed with engineered microbes harboring designer metabolic pathways. These biotransformation reactions would degrade the plastic waste and convert it into value-added products such as chemicals and materials. This bio-upcycling approach to end-of-life plastics could serve to generate inexpensive and carbon-rich feedstocks to produce valuable products, establishing a closed-loop plastics bioeconomy. For instance, a consortium composed of *I. sakaiensis* and *Pseudomonas putida* could be developed to degrade PET and upcycle it into **biodegradable plastics** by building synthetic circuits that encode engineered PETases and MHETases and anabolic pathways that convert PET-derived building block molecules into polyhydroxyalkanoate. In our latter scenario, these rationally designed microbes could be deployed directly in oceans to biotransform plastic debris *in situ*. From this process, carbon derived from plastics would be converted into nutrients that would seamlessly flow into marine microbial metabolic pathways to drive the ocean food web. Here, different microbes could be engineered to degrade different synthetic polymers, thereby enabling them to collectively and cooperatively degrade mixed plastics. Members for such communities can be selected from bacterial isolates such as *Exiguobacterium* sp., *Halomonas* sp., *Ochrobactrum* sp., and fungal strains, such as *Zalerion maritimum*, which have shown the ability to degrade different plastics. Cooperativity among these members can be designed through

Glossary

Amnesic shellfish poisoning (ASP): an illness in humans caused by domoic acid exposure through ingestion of contaminated seafood that causes excitotoxic brain damage and organ failure in mammals, including humans.
Biodegradable plastics: plastics that can be broken down by the action of living organisms, usually microbes, producing biomass, water, and carbon dioxide as products.
Biotransformation: the biological conversion of an organic chemical or contaminant into a metabolite that can be utilized by natural metabolic processes.
Chaperones: a functional category of proteins or protein complexes that assist in a cell's protein folding functions.
Coral bleaching: a process in which the coral–algal symbiosis collapses, resulting in expulsion of the algae from the coral host. The corals become energy starved and are at risk of death. The bleaching effect is due to the loss of the algae, which provides the color to many coral species.
CRISPR-Cas nucleic acid sensors: biosensors that use CRISPR enzymes to recognize nucleic acid sequences and produce an output signal. The enzymes currently in use include Cas12a for dsDNA detection and Cas13a for RNA detection.
Directed evolution: an optimization method in which variations of a biomolecule of interest are generated and subjected to a selection scheme. Desired 'improved' variants are selected and then used to seed subsequent cycles of variant generation and selection. In this way, a path can be traced along the fitness landscape to obtain optimized versions of the parent molecule.
Domoic acid (DA): a small-molecule neurotoxin produced by some species of algae, most notably *Pseudo-nitzschia australis* and *Chondria armata*. It enters the food chain through contaminated shellfish and small fish that feed off the algae and bioaccumulates in higher trophic levels.
Ecoengineering: the bioengineering of ecosystems, with the goal of maintaining or restoring the health of a niche or general biome.
Eutrophication: an influx of nutrients and/or minerals into a body of water which affects the natural ecosystem. Usually caused by sewage discharge,

division of labor or metabolic cross-feeding to achieve both plastic degradation and upcycling [13]. Microbial communities can be further enhanced with synthetic genetic circuits that encode substances to protect and stabilize individuals to facilitate their degradation function [29]. Such '**ecoengineering**' approaches could potentially enable *in situ* ocean plastic degradation and establish a paradigm for the long-term self-regulating management of plastic pollution by living systems. This strategy could also address the difficult challenge of microplastics, which cannot be easily extracted from ocean waters and for which no remediation solutions currently exist.

We envision several key synthetic biology focus areas that would be necessary for advancing plastic biotransformation platforms. The first undertaking would be more comprehensive gene annotation for natural microbial communities reported to degrade plastics, with a focus on enzyme and pathway prediction. Through the elucidation of functional contributors in native plastic-degrading communities, researchers could derive datasets for use in establishing genetic toolkits, guiding engineering strategies, and searching for other plastics-degrading populations. A second focus is the systematic optimization of biotransformation enzymes for performance improvement. Several classes of enzymes have recently been reported to depolymerize plastics, including laccases, manganese peroxidases, lignin peroxidases, cutinases, suberinases, and carboxyl esterases [27,30]. Protein engineering could be deployed to enhance the catalytic rate, improve thermal stability, increase robustness, and alter the substrate specificity of these enzymes. Specific engineering strategies here include random or targeted mutagenesis and recombination approaches for directed evolution, rational design based on bioinformatics-guided molecular simulations, and *de novo* engineering through machine learning.

A third focus area we would like to highlight is the design of metabolically integrated, synthetic microbial consortia that support both the biodegradation of plastics and the biosynthesis of value-added products. A leading obstacle in plastics recycling is the wide diversity of polymer structures and high variability of physical and chemical properties. Thus, an efficient bio-upcycling scheme would require the integration of select microbes with complementary innate phenotypes for either hosting designer biotransformation pathways or providing auxiliary functions that benefit the net process. Through a division-of-labor approach, these designed microbial consortia could leverage metabolic specialties and reduce burdens of individual strains, enhance overall biodegradation efficiency, and broaden the spectrum of plastic substrates that can be degraded. These tailored microbial consortia would be superior for bio-upcycling tasks owing to their versatility and adaptability compared with engineered monocultures. An additional further step in this area would be the creation of engineered consortia that are robust and self-sustaining in marine environments, a key step for implementing designer microbial units in the open ocean for *in situ* operation. This could be accomplished by conferring to engineered microbes a selective advantage through designer metabolic pathways, that allow efficient utilization of plastic debris as an additional carbon source inaccessible to native organisms, or perhaps synthetic gene circuits that enhance microbial robustness to environmental changes such as rising temperatures. Alternatively, the goal can be achieved by introducing social interactions that facilitate the integration of engineered strains into native ecologies or creating synthetic communication networks such as quorum-sensing circuits that reinforce the consortia integrity [31]. We envision these *in situ* ecoengineering microbial communities could then be developed as synthetic ecosystem nodes for the conversion of plastics into harmless biological metabolites and nutrients that would flow into and benefit ocean trophic networks.

Synthetic biology for coral reef resilience

Other key pollutants derived from our use of hydrocarbons include greenhouse gases from burning fossil fuels. The resulting global warming has an outsized effect on the ocean, which has absorbed

industrial waste dumping, or fertilizer runoff from farms.

Guide RNA (gRNA): a synthetically derived RNA that binds to a CRISPR enzyme to program its sequence specificity.

Harmful algal bloom (HAB): an explosive overgrowth of unicellular algae populations in a localized area, caused by favorable nutrient conditions, warm waters, and certain weather conditions. The resulting bloom and decomposition of dying algae cause hypoxic zones, which can kill fish and other organisms in the area.

Heat shock proteins (HSP): a highly conserved family of chaperone proteins that have evolved to play key roles in maintaining proper protein folding.

Holobiont: an ecological unit composed of the entire aggregate network of an organism, including the host and any mutualistic partners, colonizing protists, microbes, fungi, or viruses.

Microbiota: the contingent of microorganisms that colonize a host organism and may consist of bacteria, archaebacteria, fungi, and viruses.

Microplastics: fragments of plastic waste less than 5 mm in size. Microplastics can readily contaminate food chains and permeate throughout the ecosystem.

PET: polyethylene terephthalate, colloquially known as polyester. PET is one of the most widely used plastics in the world and is highly recyclable.

PETase: an esterase class of enzymes that efficiently catalyze the hydrolysis of polyethylene terephthalate (PET) plastics to monomeric mono-2-hydroxyethyl terephthalate (MHET).

Plastics: synthetic polymers typically made from petroleum-based compounds.

Polymers: natural or synthetic substances consisting of very large molecules, composed of many repeating subunits.

SHERLOCK: specific high-sensitivity enzymatic reporter un-locking, a CRISPR-Cas diagnostic platform that uses a gRNA-programmable CRISPR nuclease and a nucleic-acid probe reporter to detect RNA or DNA sequences with high sensitivity and specificity.

Thermotolerance: the ability of an organism to survive changes in temperature.

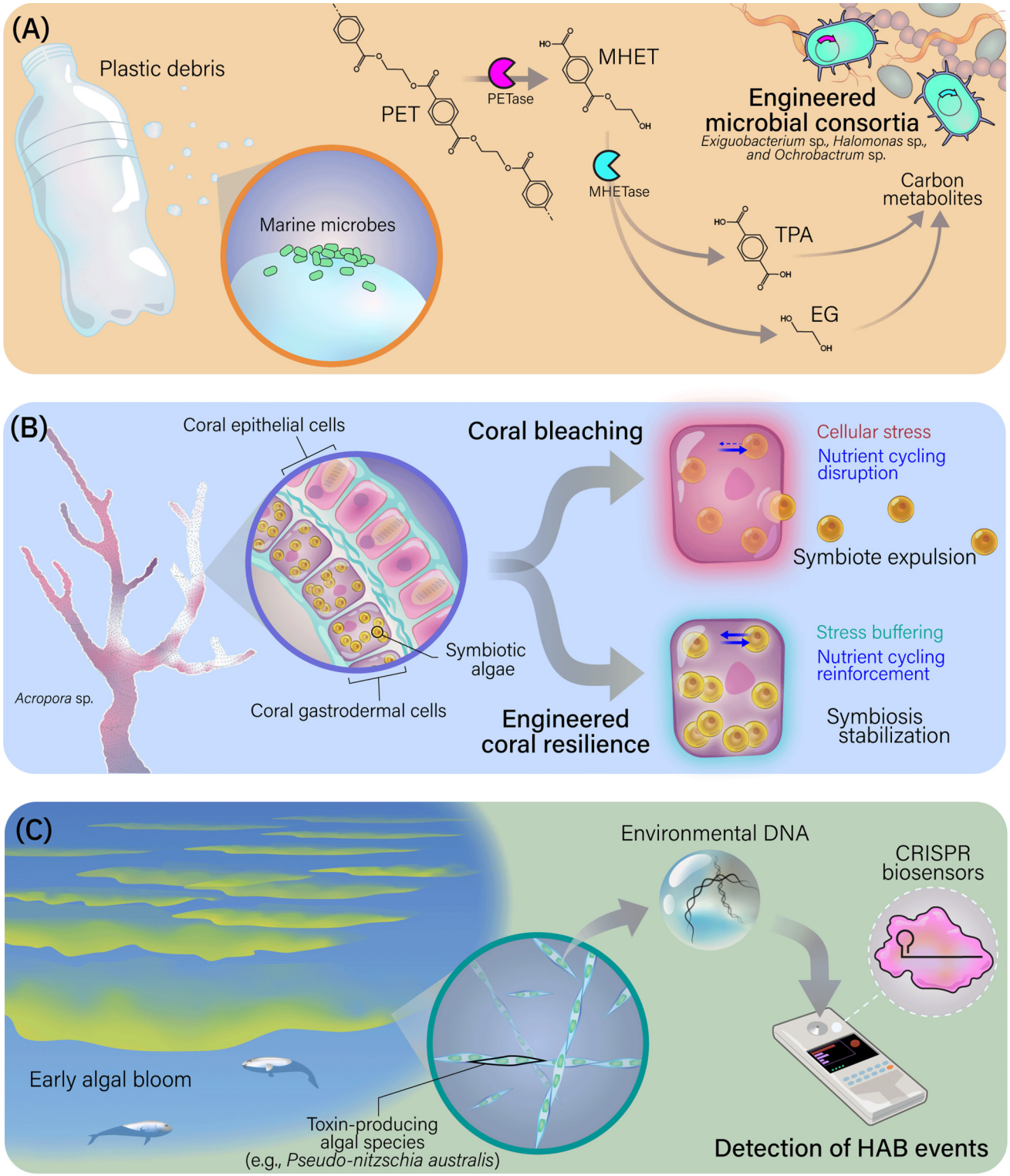
Upwelling: an oceanic phenomena whereby nutrient-rich deeper and colder

much of the excess heat. Such anomalous warming of the ocean waters has impacted various marine organisms, including corals, which are keystone organisms in the ocean. Corals are sessile marine invertebrate animals that self-organize into massive structures composed of individual polyps ensconced in a biomineralized calcium carbonate exoskeleton. They are characterized by their unique symbiotic relationships with photosynthetic **zooxanthellae algae**, which convert sunlight and coral wastes into metabolites such as sugars, amino acids, lipids, and oxygen. Approximately 90% of these nutrients are exported to the corals; in return, the corals provide protection and nutrients for the algae, thus enabling both organisms to thrive in nutrient-poor ocean regions [32]. This complex integration of metabolic processes is delicately balanced and susceptible to breakdowns due to various environmental stressors, including UV radiation, pollution, microplastics, insufficient light, algal blooms, and infections [33]. The ensuing dysfunction is known as coral bleaching, in which the algal symbionts are expelled by coral tissues, resulting in the significant loss of a coral's source of metabolic energy [34]. Eventually, bleached corals weaken and die, although there is a period during which corals may recover if the symbiosis is re-established [35].

water rises to the ocean surface to replace warmer surface waters.
Zooxanthellae algae: unicellular photosynthetic dinoflagellates known to serve as endosymbionts to a wide range of marine invertebrate organisms.

Climate change is thought to be the dominant causative factor, triggering coral bleaching when prolonged sea surface temperatures exceed just 1°C of the baseline [36,37]. There have been ever-worsening global coral bleaching events, with the frequency of major bleaching events having increased fivefold over the past 40 years [38]. Shockingly, it is estimated that half of the planet's corals have already perished since the 1950s [39]. As full coral recovery from bleaching damage takes 10–15 years, the scale and frequency of current bleaching events is reaching unsustainable levels, exceeding the natural recovery capacity of corals. At the current rate of global warming, it is anticipated that 90% of coral reefs will be lost by 2050 [40]. This is alarming, especially given the key role of coral reefs in maintaining healthy ocean ecosystems by providing shelter and habitats for a quarter of all marine organisms. Notably, corals possess the highest concentration per unit area of biodiversity on the planet, earning them the title 'the rainforests of the sea' [41]. While their role as a natural engine of global marine biology is invaluable, coral reefs also have tangible economic contributions to a variety of industries, including fisheries, tourism, coastal erosion protection, and drug discovery. At this critical juncture, we must consider more direct assistance to fortify the resilience of coral organisms, providing a buffering window for their natural adaptative responses and mitigating the extent of impending coral reef collapse.

This challenge is well suited for synthetic biology with its established capacity for engineering metabolic functions, artificial symbiosis, and microbial communities. Recently, studies have identified key molecular mechanisms behind the bleaching process, which paves the way for exploring synthetic biology solutions. Here, we outline multiple synthetic biology approaches for improving coral resilience. Our first strategy is centered on engineering organismal **thermotolerance**. Transcriptomic analyses have revealed that **heat shock proteins (HSPs)** and protein folding **chaperones** are upregulated in both host and symbiont during coral stress, indicating that poor protein stability is an important molecular mechanism in bleaching events [42,43]. Rationally designed HSP and related genetic modules from thermophilic organisms have been successfully developed to improve temperature adaptation in various organisms, underscoring the plausibility of screening heat resistance genes and integrating the best candidates into corals and algae to bestow protective effects [44,45]. In addition, recent evidence suggests that thermotolerant strains of symbiotic zooxanthellae can imbue corals with increased heat tolerance [46]. Currently, there are large-scale efforts to naturally breed hybrid heat-tolerant corals and evolve thermoadaptive zooxanthellae [47,48]. These efforts could be accelerated with synthetic biology approaches that provide corals and zooxanthellae with a genetic diversity difficult to achieve in nature, endowing them with a buffering capacity against heat stress via engineered antioxidant or protein chaperone pathways.



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Box 1. Microplastics in the ocean

Plastic debris from 1 μm to 5 mm in diameter are classified as microplastics and are a pervasive constituent of man-made ocean pollution [85]. The presence of small plastic particles in the oceans was reported for the first time in the 1970s, while the term 'microplastic' was coined around 2004 [86]. There are two distinct subclasses (primary and secondary) of microplastics. Primary microplastics include small items that are originally manufactured with these miniscule dimensions and make their way into the environment; these include microbeads, synthetic particles, and microspheres, which are commonly used as abrasives in industrial and domestic applications. Secondary microplastics, which make up the majority of microplastics, are particles fragmented from conventional plastic debris that enter the marine environment from terrestrial sites and are vulnerable to fragmentation by UV light, mechanical abrasion, and possible *in situ* biodegradation. Due to their small size, microplastics have become widely distributed across ocean waters, seabed sediments, coastal sediments, beaches, and are even found embedded in Arctic and Antarctic sea ice. Of all plastic-based waste accumulating at the ocean surface, microplastics account for more than 90% by weight [87].

Marine microplastics cause a wide range of ecological damage in the ocean. These microplastics are inhaled and ingested by fish, turtles, squid, shrimp, seabirds, and nearly any other marine animal examined [88]. Once consumed, microplastics reside in the tissues and then are transmitted throughout the food chain. As the microplastics accumulate in their bodies, chemicals and contaminants may leach out, leading to growth inhibition, oxidative damage, and developmental abnormalities of marine life. The long-term ecosystem consequences of this pervasive population poisoning are a matter of intense study. For instance, microplastics have been shown to inhibit the reproductive capacity of the oyster *Crassostrea gigas* [89], negatively affect gamete fertilization of coral reefs [90], and disrupt microalgal growth at high concentrations [91], among many other effects. The increasing ubiquity of microplastics in the environment raises growing concerns about their implications for human health as well. As these microplastics cascade up the food chain, they reach beyond the oceans and penetrate the human body through the consumption of contaminated seafood. A recent study found microplastics in the blood of 80% of sampled people [92]. Uptake of relevant concentrations of microplastics can damage human health through a variety of mechanisms, including DNA and cellular damage, increased inflammation, carcinogenesis, and neurotoxic effects [93]. Over the past decade, microplastic pollution has been recognized as a significant global threat and the topic continues to receive growing interest. To date, research on microplastics mainly focuses on its distribution, analysis methods, and harmful effects. Unfortunately, there are currently no technologies or efforts that can safely remove or degrade microplastics from the open environment. Advanced oxidation processes are being investigated for the decomposition of microplastics [94], but this is an energy-intensive process and it is not clear how this could be implemented on a planetary scale. The use of *in situ* biotransformation, as we outline in this opinion article, can leverage the self-replicating and self-organizing properties of living organisms to confer ocean ecosystems the ability to convert microplastics into biocompatible metabolites.

A second, targeted synthetic biology approach could be taken through the engineering of specific metabolic pathways, as investigations have shown that dysfunctions in the symbiotic metabolite dynamics are critical to the bleaching process [49]. When there is a disruption of the symbiosis involving the transfer of metabolic nutrients (Box 2), a pathological feedback cycle takes hold, leading to a shift from a symbiotic state to a parasitic state, which ultimately leads to expulsion of the algal symbiont [50]. Here, synthetic biology modules such as RNA logic circuits could be integrated into metabolic pathways of the host and the symbiont to correct this disruption. The coral glutamate metabolism and zooxanthellae nitrogen assimilation pathways in particular are key targets for engineering, as they play decisive roles in symbiosis maintenance [51]. Along these lines, reinforcing the nitrogen-limiting state of the zooxanthellae algae has been proposed as a tactic for maintaining the supply of algal sugars to corals [49].

We acknowledge that there are manifold challenges in augmenting coral health, including our incomplete understanding of bleaching mechanisms, limited knowledge of underlying symbiotic

Figure 2. Synthetic biology strategies for ocean health challenges. (A) Engineered microbial consortia with artificial enzyme pathways can be used to biotransform ocean microplastics into biocompatible metabolites. Shown is a proposed degradation pathway for polyethylene terephthalate (PET) polymers into mono-2-hydroxyethyl terephthalate (MHET) monomers, which are further transformed into terephthalic acid (TPA) and ethylene glycol (EG), which can be used as microbial carbon sources. (B) Coral bleaching is caused by cellular stress and nutrient cycling disruption between the coral host and zooxanthellae algal symbiotes, leading to coral death. Synthetic biology circuits can be used to engineer stress adaptation and metabolic pathway reinforcement into the coral holobiont to combat the bleaching process. (C) Environmental DNA extracted from ocean waters can be rapidly analyzed by CRISPR-based sensors to provide on-demand monitoring of toxic algal species. This could enable the detection of harmful algal bloom (HAB) events at early stages, allowing for swift restriction of fishing activities.

Box 2. Symbiotic metabolism dysfunction and coral bleaching

Heat-induced oxidative stress has been implicated as a major driver of coral bleaching events [43]. However, recent investigations have found that a dysfunction of the symbiotic metabolic exchange systems involving the coral host and the algal symbiont also plays a critical role in the bleaching process [49]. Core to the symbiotic relationship is the nutrient cycling of nitrogen and carbon metabolites. When this dynamic is properly functioning, waste nitrogen from coral metabolism is transferred to the algae, which utilize it as their dominant inorganic nitrogen source. In return, algal photosynthesized sugars are transferred to the coral for use as its major energy source. It has been discovered that heat stress causes systemic changes in both host and symbiont metabolic genes, leading to an eventual breakdown of this nutrient cycling. In the early stages, heat stress causes increased metabolic requirements for the coral host, leading to its energy starvation. This may reflect an increased need for repair mechanisms to offset heat-mediated oxidative damage or protein destabilization [95]. To compensate for this increased energy expenditure, coral catabolic pathways are upregulated to utilize amino acids as a supplemental energy source [49]. This metabolic shift generates increased nitrogen waste, which is consumed by the algae, leading to its elevated growth. As the algal symbionts proliferate, they retain and consume their photosynthetically generated carbon, thus starving the coral host of energy. These events lead to a positive feedback cycle, which increasingly pushes the coral holobiont to a carbon-limited state, triggering a transition from a symbiotic state to a parasitic state and leading to the eventual expulsion of the algal symbiont [50]. These findings support an earlier hypothesis that the host coral may use nitrogen limitation as a means to regulate zooxanthellae growth [96]. Engineered alterations of these metabolic pathways using synthetic biology approaches can potentially be designed to arrest and prevent this dysfunctional progression. Further discoveries of how heat alters metabolic pathways and the consequent breakdown in coral symbiosis could highlight additional engineering targets for coral therapies.

relationships, and the lack of established methods for coral genomic editing. This highlights a third additional area of focus: developing key technologies for genetic manipulation and synthetic genetic parts for the coral hosts and symbionts, which will be essential for the proposed synthetic biology strategies [52]. Only recently has CRISPR-mediated genome editing been demonstrated in coral hosts [53]. Considering this limitation leads us to an alternative fourth strategic pathway for engineering coral resilience, namely, through alteration of the coral **holobiont microbiota**. This is the totality of bacteria, archaea, fungi, and viruses that colonize and coexist with corals through overlapping mutualistic, commensal, and parasitic relationships. Current synthetic biology tools are well established and suited for engineering these microbial systems, making them more tractable targets. Encouragingly, recent evidence has demonstrated that altering the microbiome confers an increased degree of thermotolerance to the coral holobiont, raising the hope that microbiome engineering may be a viable means to restore coral reefs [54].

Molecular surveillance of HABs

While pollutants often have negative impacts on ocean organisms, in some cases particular organisms will thrive and their overpopulation results in an ecosystem imbalance. Here, we focus on algae, which comprise the base trophic energy level in the ocean and play foundational roles in global aquatic ecosystems. However, not all species of algae are benign; some can produce harmful toxins that have devastating effects on wildlife and humans. The rapid proliferation of toxin-releasing algae is referred as HABs, which can occur naturally under specific ocean conditions involving a combination of ocean **upwelling** and warm water. Recent studies have indicated that, globally, HABs are becoming more common and increasingly potent [55]. The non-natural cause of this increase is thought to be anthropogenic **eutrophication**, or nutrient pollution, from agricultural and storm runoff that terminates in the ocean and triggers algal growth [56,57]. Climate change is also thought to progressively intensify HABs through increased ocean temperatures and enhanced precipitation leading to more runoff [58,59].

HABs have profound effects on their surrounding environments by rapidly depleting the water of oxygen, resulting in extensive hypoxic dead zones and hence drastically reducing local biodiversity [60]. HABs also reduce ecosystem diversity by blocking light from reaching underwater flora and smothering corals. Notably, HAB species produce a wide diversity of toxins, including dermatoxins, hepatotoxins, and neurotoxins. A HAB toxin of particular interest is **domoic acid**

(DA), a potent neurotoxin produced by the microalgae diatom *Pseudo-nitzschia australis* [61,62]. When ingested, this toxin can cause memory loss, seizures, and death in birds, marine mammals, and humans (Box 3). DA infiltrates food sources through a process known as biomagnification, whereby it becomes further concentrated in organisms at each successive trophic level [63].

While the multitude of challenges posed by HABs remains to be tackled, one immediate concern is detecting the formation of HABs in their early stages to prevent the processing of contaminated seafood catches. Typical laboratory testing of ocean water samples for identifying HAB organisms requires specialized instruments and takes hours to days to complete. There are automated detection sentinels known as environmental sample processors, which are buoys equipped with sampling and analysis instrumentation, that provide real-time monitoring at sea [64]. However, these large and complex floating laboratories are expensive, cannot be deployed at large scale for high-resolution monitoring, and are not accessible to typical fisheries and fishers. As a result, although these sensor technologies have been in place for decades, HAB outbreaks and poisoning continue to occur. There is thus an unaddressed need for rapid and inexpensive lab-free solutions that can detect HAB organisms in the field to provide immediate assessment of fishing zones, while minimizing downtime and economic impact.

Recently, CRISPR-Cas systems in the context of synthetic biology have been exploited to create highly robust and efficient nucleic acid diagnostics. Such systems harness a unique property of certain CRISPR-Cas enzymes: the activation of nonspecific 'collateral' nuclease activity upon the programmed recognition of a target nucleic acid via a **guide RNA (gRNA)** component [65,66]. When paired with nucleic acid cleavable probes, this feature enables ultrasensitive signal amplification from highly specific target detection. A palette of different output modes has been developed, including simple visual lateral-flow outputs, sensitive fluorescent detection, and electrochemical signaling [67–69]. This CRISPR-Cas molecular biosensor platform, known as **SHERLOCK**, has been successfully utilized to detect Ebola and Zika viruses as well as bacterial

Box 3. Domoic acid (DA) and amnesic shellfish poisoning

DA is a metabolite produced by the single-celled photosynthetic microalgae diatom *Pseudo-nitzschia australis* with no clear ecological function, but is a highly potent neurotoxin to mammals [62]. DA levels in the water, although greatly increased during a HAB, remain low in terms of toxicity as there are no documented cases of fatal poisonings solely from contaminated water exposure. The greatest danger occurs when DA enters the food chain by absorption at the lower trophic levels by organisms that consume algae, such as filter-feeding bivalves, crustaceans, and small fish. The toxin begins to accumulate in their tissues and becomes biomagnified in larger predatory fish. This concentration of toxin can then reach levels orders of magnitude higher than would ever occur naturally in the water during a large algal bloom. Molluscs, shellfish, and fish can tolerate the effects of concentrated amounts of DA in their tissues without any observable detrimental effect; thus, highly contaminated organisms may appear healthy when in fact they are actually a ticking neurotoxin time bomb to other animals. When ocean mammals or humans ingest these DA-infused marine organisms, it can lead to the development of a devastating illness known as **amnesic shellfish poisoning (ASP)**.

Structurally, DA exhibits glutamate and pyrrolidine moieties and is classified as a kainoid, a class of molecules known for their neurotoxic effects [97]. In the mammalian central nervous system, DA acts as an agonist to the kainic acid glutamate receptor, leading to excitotoxic effects on neurons, which can result in widespread cell damage [98]. This particularly impacts regions of the brain that are rich in synaptic glutamate signaling, leading to neural degeneration and localized necrosis of the hippocampus and amygdala. The clinical manifestations of ASP are short-term memory loss, anterograde amnesia, seizures, coma, and even death. In 1987 on Prince Edward Island, there were 150 reported human cases of ASP, leading to 19 hospitalizations and four deaths; these cases were linked to the consumption of cooked wild mussels. Interestingly, DA cannot be removed by cooking and, if ingested, there is no neutralizing treatment, which further stresses the importance of preliminary testing for seafood toxins early in the fishing/fisheries supply chain. In addition, DA contamination from HAB events extends beyond the human sphere, with significant impacts on marine wildlife, such as mass die-off events of sea lions, seals, dolphins, and whales. In 2015, a die-off of 343 endangered baleen whales near the coast of Patagonia was attributed to DA exposure [99]. Marine and coastal bird populations can also be affected, making DA a widespread ecological threat.

samples [70]. Most recently, this technology has been adapted to create low-cost and rapid severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostics [71,72]. A distinct advantage of this synthetic biology platform is its modularity and programmability, allowing for the rapid development of biosensors for novel targets just by replacing the gRNA.

We propose the development of a suite of field-deployable SHERLOCK biosensors for detecting the nucleic acid signatures of toxic HAB algae species, which can be assembled into a high-frequency biomonitoring network. The first undertaking would be biosensors development through the design and screening of gRNAs for sensing genes or transcripts unique to HAB algae. These sensors would be refined to accurately discriminate between toxic and nontoxic strains of algae that have high genetic similarity (e.g., such as *P. australis* versus other *Pseudo-nitzschia* sp.). As we envision a point-of-sampling detection system, the second effort would be the engineering of an inexpensive device that would house the sensor and also perform the required ocean water sampling, filtration, and processing functions. In contrast to existing testing practices, the detection system should require no specialized infrastructure or additional equipment. Hence, the design parameters for the device should focus on facile use, low cost, and robustness to the marine environment. The third effort would be incorporating an efficient multiplexing scheme to allow the simultaneous detection of many different genes or algal species from a single sample. This would enable the detection of interspecies correlations and their contributions to the formation of HAB events, which is an emerging area of research [73]. Such data would also be useful for the final fourth focus area, establishing infrastructure for network data generated from our biosensors that are taken from individual fisheries and boats, to obtain a high-resolution spatial and temporal record of algal population changes. These rich data could be combined with DA environmental sampling to develop machine learning algorithms towards predicting the progression and extent of a HAB from early patterns of algal growth.

These rapid and low-cost biosensors would enable the fishing and aquaculture industry ensure that their catch is HAB toxin-free, provide novel tools for oceanographers to assess HABs, and establish a surveillance-and-response approach for ocean biome stewardship. These SHERLOCK rapid diagnostic technologies could also be applied beyond the surveillance of HABs, such as for the detection of harmful marine pathogens, quality monitoring of aquaculture enterprises, and the routine monitoring of marine ecology. CRISPR-based sensors could even be integrated into *in vivo* synthetic biology circuits as regulatory modules for developing future *in situ* ecoengineering endeavors to inhibiting HAB formation in its early stages.

Concluding remarks and future directions

Our oceans are currently under siege from multiple threats of anthropomorphic origin. We have proposed here a concerted effort to apply synthetic biology strategies towards ocean health, with a specific focus on addressing the challenges of plastic waste degradation, coral reef resilience enhancement, and HAB surveillance. Such tailored bio-based approaches are sustainable, environmentally friendly, and can be uniquely deployed as ocean ecoengineering schemes that operate in a self-perpetuating fashion, enabling long-term and cost-effective *in situ* living solutions. The current hurdles in realizing such an ecoengineering vision can be divided into three categories, fundamental biology, engineering tractability, and ethics/regulatory concerns, which we briefly discuss here (see Outstanding questions).

While these platforms will be challenging to develop, we believe our current understanding of the underlaying biology and assortment of synthetic biology tools are sufficient to make significant headway towards these goals. As we have presented, the present knowledge of these ocean challenges highlights multiple avenues that can be targeted immediately for synthetic biology

Outstanding questions

Can synthetic biology strategies be deployed on a large scale in the open ocean?

Is it possible to integrate artificial biological systems to create a green plastic biotransformation process?

What synthetic biology strategy would be most effective for improving coral reef resilience?

How broadly can **CRISPR-Cas nucleic acid sensors** be applied to detect multiple biological contaminants and ocean biome health?

What should be done to improve the genetic stability and containment of engineered microbes in the natural environment?

What are the guiding principles for the division of labor in microbial communities toward desired functions?

How many microbial members can be involved in an engineered consortium while maintaining its functional robustness and organizational coherence?

What are the appropriate strategies for engineering cooperative behaviors and stable organismal interactions between engineered microbes and naturally occurring populations of a target niche for the development of modular 'drop-in' synthetic biology systems?

engineering. Ongoing elucidation of the biological mechanisms will allow further refinement of our proposed strategies. In terms of bioengineering tractability, while there already exists a sufficient synthetic biology toolkit for starting these engineering efforts, there are several areas that require further development. Specifically, establishing these approaches will require the expansion and refactoring of key synthetic biology technological areas to enable robust operation in marine environments. Notably, genetic circuits for biomaintenance should be explored to ensure the long-term functional performance of engineered microbes [74]. These schemes would counteract mutational pressures and reinforce gene stability to prevent the loss of desired, engineered traits. Directed evolution and machine learning approaches could also be applied in such cases to fine tune microbial functional robustness [75,76]. Alternatively, a long-term functional stability could be conferred by establishing fitness advantages for engineered organisms, such as through the utilization of plastic waste as novel carbon sources or the increased resilience of cellular physiology against ocean temperature fluctuations. Additionally, the ecological fitness demands on engineered microbes adapting to the open ocean should be considered. Attempting to extrapolate successful laboratory tests for practical *in situ* implementation in the field will likely lead to failure if the modified organisms are not adaptive to their desired operational context. For example, plastic waste streams are commonly accompanied by additives, such as antioxidants, metals, dyes, and plasticizers, which can inhibit microbial growth and enzyme activity. Furthermore, interactions and competition with innumerable native organisms in the ocean will surely impact the establishment and maintenance of designer microbes. This is exemplified in coral communities, where vast dynamic interactions exist between animals, algae, protists, microbes, fungi, and viruses, which constitute the component denizens of the coral holobiont. Thus, identifying strategies that enduringly integrate engineered biosystems with existing ocean ecosystems will be a critical area of research. One approach is to broaden the niche range for engineered and partner indigenous microbes through targeted metabolic engineering to increase ecological compatibility, offering the potential to improve the efficiency and stability of the desired functions.

In our estimation, the most significant challenge for realizing these goals is the ethical and regulatory considerations which must be established, debated, and agreed upon. Thus, it is of the utmost importance that the practical and ethical implications of releasing engineered biological systems into the ocean be considered and discussed straightaway (Box 4). For instance, releasing highly efficient synthetic polymer-degrading microbes into natural environments without proper consideration may lead to undesired destruction of susceptible maritime structures. To allay this concern, engineered strains should be restricted to degrade polymers not found in composites used in marine structures or colonize in habitats whereby susceptible structures are absent. Another potential risk that must be considered is unintended alterations to the native ecosystem, a central concern for work involving genetically engineered organisms. Disruption of the ecosystem can be minimized by using native microbial species as the starting chassis and limiting the alterations to a very narrow desired phenotype. Furthermore, during development it would be critical to examine the performance of engineered microbes and their impacts on microbial community dynamics in contained model ecosystems. Ecosystem diversity, composition, and gene transfer should be key metrics here. It is quite possible that the challenge these endeavors will face may actually be insufficient persistence of engineered strains in the competitive native ocean biome. Upon satisfactory impact assessments, these engineered microbes could be deployed in limited test pilot experiments in the open ocean. At these developmental stages, the prototype microbes should possess biocontainment systems to prevent them from spreading in the environment. Examples in this area include recently developed transcriptional 'Deadman' or 'Passcode' circuits, CRISPR-based kill switches, or recoded genomes for the control of microbial populations and gene transfer [77–80]. A full deployment of such engineered microbes require them to integrate into natural ecosystems and robustly thrive in sufficient

Box 4. Ethics of ecological engineering

For the proposed synthetic biology solutions that operate in the natural environment, there are important considerations regarding the ethical implications of this 'ecoengineering' undertaking. The unintentional release of genetically modified organisms beyond the confines of a laboratory has been a major concern of the biotechnology field, with scientists pushing to establish regulatory frameworks soon after the advent of recombinant DNA technology. The Asilomar Conference in 1975 brought together scientists, lawyers, and physicians to consider the hazards that recombinant DNA technology pose [100]. From this event, several regulatory principles and experimental best practices were established for the containment of recombinant DNA. More recently, similar assessments have been undertaken for synthetic biology in the 2010 Presidential Commission for the Study of Bioethical Issues [101]. These examples of self-governance by proactive researchers have formed the basis for similar regulatory laws governing recombinant DNA technology at the local, federal, and international levels. While these considerations focus on unintended biomedical hazards that may directly impact human health, significant consideration is also given to ecological risks.

However, in an era of impeding human-induced ecological collapse, dialogue and debate should be invoked anew to consider the limited use of synthetic biology technologies in the open environment for repairing ecological damage. This intentional bioengineering of ecosystems may be a crucial strategy when faced with the destruction of core organs of the biosphere that we ultimately depend on to survive. Modeling efforts from key leaders in the fields of biology and ecology have raised alarming predictions about the ecological damage that is already well underway. Coral reefs have so far declined by 50% and are estimated to become extinct when climate change levels reach 2°C of warming, a threshold we are expected to hit even in a limited scenario which assumes that all countries can implement and keep their global climate emissions pledges [40]. Numerous studies have also predicted that even under this nominal case, approximately 30% of all plants and animal species will become extinct [102]. The cumulative effects on the ecosystems of the earth and concomitant biodiversity collapse will exacerbate the challenges we face and directly threaten humanity's food and water supply chains. We are already witnessing the beginnings of such knock-on effects from climate change. Many researchers have warned that we are blind to the worst-case scenarios for climate change, as such risk assessments in academic studies are avoided so as to not seem alarmist [103].

One option is to do nothing and hope that the natural ecosystems are able to adapt and self-repair. While idling is the easiest option, it places us in a perilous position of self-imposed powerlessness. Furthermore, studies have demonstrated that natural ecosystems are not endlessly robust, with numerous examples of species becoming extinct from acute environmental changes [104,105]. A second option is to limit the damage using non-bioengineering solutions. While this approach may be more conservative and such schemes certainly need to be pursued, relying on them alone severely limits our abilities to ameliorate the extent of ecological collapse. The third option is to consider planetary stewardship as a responsibility that requires the consideration of all technological options in the face of existential challenges. This last choice is not only the most prudent, but also supported by moral principles that form the ethical frameworks we use to develop research guidelines [106].

For example, the principle of beneficence is central in medicine and research, stating actions must maximize the benefit to a patient and minimize the harm. Thus, providing potentially toxic or unproven treatments to a patient is considered unethical. However, the moral calculus is altered when the status of the patient changes to a potentially fatal condition, for example, in the case of cancer chemotherapy or 'compassionate use' cases of experimental therapies. If we consider the ecological threats facing humanity as approaching a state that will cause long-term effects leading to significant human mortality (as indicated by our current predictive power), then is it not morally incumbent upon us to minimize this unfolding disaster using technologies as expansive as synthetic biology? Similar arguments can be made from the principle of self-preservation (we are morally justified to ensure our own survival) and the principle of rectification (we are morally obligated to correct injustices; in this case, humans are unequivocally the cause of these environmental challenges and we have the responsibility to act to restore these imbalances). Perhaps the most appropriate doctrine to apply here would be the principle of environmental stewardship, a concept which is not fully formalized in the field of ethics but proposes that as beneficiaries and as an integrated part of the planet, humans have a responsibility to prevent its collapse using any means necessary [107]. These considerations should be integrated with weighing the risks of impacting the natural environment, taking into consideration that most of the technologies we fundamentally rely on already impact into the planet's chemistry and biosphere with negative consequences. The perspective of an applied synthetic biology approach to environmental stewardship attempts to rebalance human-induced impact by creating a counterpoint of technologies designed to be explicitly beneficial. As we move towards an era of increasingly extreme climate dynamics and dwindling biodiversity, it is critical to ensure our discourse is revised on the benefits, hazards, and implications of responsibly using synthetic biology for ecoengineering.

numbers in order to exert positive ecological benefits. There will thus always be some level of risk, as potential impacts, which may arise at that level of ecosystem integration in the vastly complex mélange of the ocean environment, may not be fully predicted. As synthetic biology technologies are currently being explored for disease vector population control and other ecoengineering

applications [81,82], ethical discussions are needed to ensure that bioengineers, scientists, policymakers, and the public are in consensus on responsible applications of these advanced technologies in ocean settings and the concomitant risks [83,84]. One of the biggest challenges here is defining who it is that should be tasked with organizing and driving these international discussions. Which countries should be involved and how will that be determined? What of differing cultural views on altering natural systems? Which organization, if any, has jurisdiction over the implementation of ecoengineering efforts that could have planetary effects? These difficulties notwithstanding, it is important to advance cautiously and not succumb to inaction, while we still have a window for ecosystem repair and recovery. Key to this cost–benefit analysis of using engineered microbes on a global scale is considering that humans have already artificially disrupted ecosystems by our negligent activities. Thus, as a species we have assented to the widespread use of technologies detrimental to the planet but have yet to apply beneficial technologies that limit and repair on an Earth-wide scale. At some threshold, catastrophic damage such as the complete extinction of ocean species will not be reversible. Even with these engineered solutions in hand, it will likely require a decades-long effort to fully implement them, from pilot studies to scaling efforts to monitoring their persistence and efficacy in the natural environment. While we have focused here on three ocean challenges that are in pressing need of solutions, we believe that synthetic biology will find broad utility in addressing other issues of ocean health, including oil spill remediation, degradation of fertilizer and pesticide pollution from agricultural runoff, heavy metal contamination of ocean life, mangrove forest protection, ocean acidification, offshore aquaculture enhancement, amelioration of climate-change spread pathogens, nucleic-acid monitoring of ecosystem dynamics, seafood traceability chains for sustainable fish harvesting, and ocean-based carbon sequestration, just to name a few. With the power to retool life itself, synthetic biology's potential is tremendous, necessitating greater dialogue on its expeditious application for reversing the ecological damage we continue to exert on the ocean.

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Declaration of interests

J.J.C. is a cofounder and director of Sherlock Biosciences. The other authors declare no competing interests.

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