Saving California's kelp forest by reducing purple sea urchin population with the use of CRISPR

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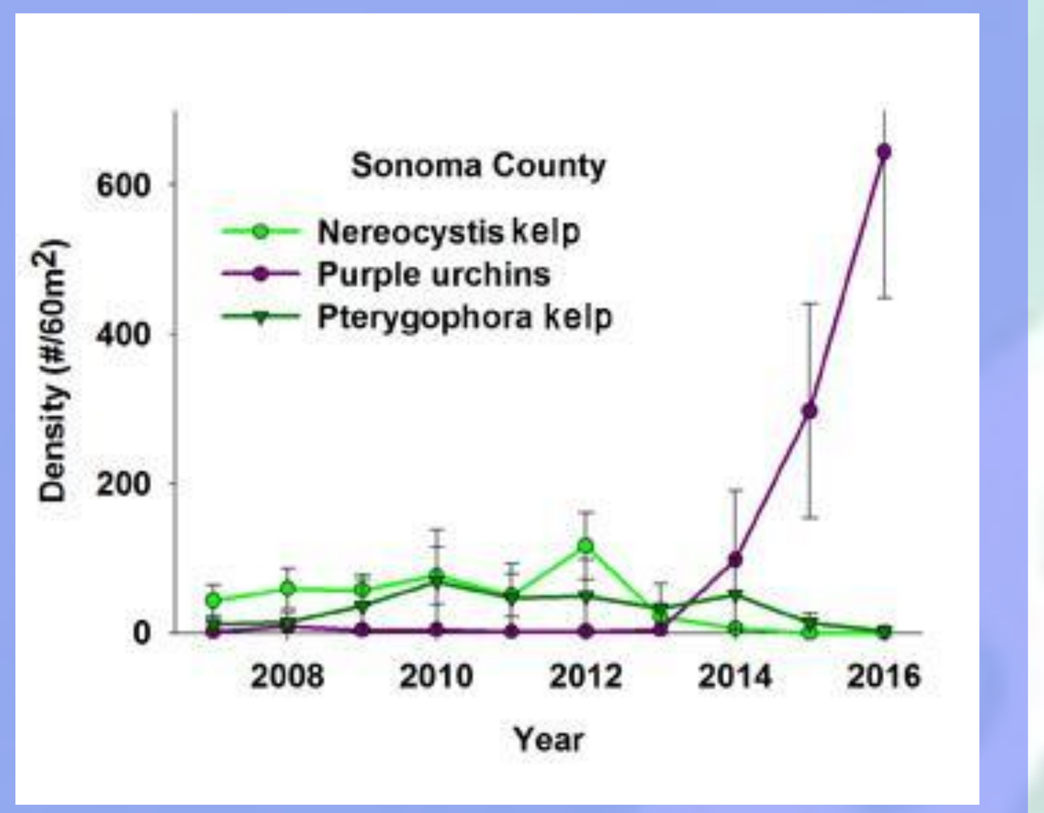
Problem and Solution

The uncontrolled increase in sea urchin (*Strongylocentrotus purpuratus*) population has led to the destruction of kelp forests at a rate of 30 ft per month along the Western coast of the United States every year. The massive loss of kelp forests have devastated the ecosystem. In order to help save the diminishing kelp forests, we propose genetically engineering the gonads of adult female sea urchins so they competitively impede the production of healthy offspring population in the wild by producing embryos that do not fully develop effectively decreasing the entire population over time.



History and Alternatives

Since the past couple of decades, oceans have gotten warmer by three degrees Fahrenheit. Due to human carbon-dioxide emissions in the air, the sea has been getting more warm and acidic, causing plants and animals to die off or become more aggressive to survive. In the Pacific Ocean, human behavior has led to the decline of starfish and sea otters, two of the biggest predators for sea urchins. Due to this, purple sea urchins overpopulate and kill stalks of kelp before the kelp can recover to grow. These issues combined contribute to the destruction of bull kelp in Northern California. Organisms rely on these kelp forests, creating red sea urchin and abalone industries to plummet and even closing the abalone fishery in 2018.

Alternatively, divers have spent hours underwater used hammers to crush sea urchins one by one. However, even if some divers can smash ten thousand in ten hours, it would take those divers five years straight, without breaks, to eradicate the problem. Even if divers could remove the sea urchins physically, they would have done so by now. Conclusively, because of the exponential growth of urchins, the hammers can not simply keep up.

Ethical and Societal Concerns

Ethically, systematically stopping the growth of a whole population of an organism may seem wrong to the general population. However, stopping the embryo from developing is much more ethical than just destroying the sea urchins. A concern with genetically modifying sea urchins with society is its impact on fisheries. Some fisheries that depend on sea urchin will be forced to focus less on quantity of product, but quality, leading to raised prices in the uni market. The full effects of dramatically reducing the purple sea urchin population is unknown, posing a concern that we cannot predict at this moment. So many organisms are intertwined in the ocean habitat, and having humans manipulate nature may not be justified. Determining if it's ethical to change the current direction of the food chain depends on how it affects other species whether that be positive or negative, and weighing out the net gain or loss. An ethical concern arises from another potential fear of infecting more than just sea urchins. Applying a solution of viruses all over a sea urchin may not guarantee that all of the viruses will be used. Some may still be remaining on the surface of the sea urchin, and when it is released back into the ocean, it may spread to other organisms. However, there has been no testing to see if the viruses we are using can survive when released in the open ocean. The cost of the viruses we are using may be a concern. People may not be willing to spend millions of dollars on stopping sea urchins, and instead want to focus it on humans. This all comes down to whether it is worth it to save the ocean environment or spend our resources elsewhere.

Detailed Solution and Sketches

In our bioengineering solution, we propose using the CRISPR/Cas9 gene editing technique and viral based gene delivery to disable a maternal effect gene called Bicaudal-C in female sea urchin gonads. When the mutated female population produces eggs, the eggs will also contain the disabled Bicaudal-C gene. This will inhibit the development of the females' offspring's anterior neurogenic ectoderm, resulting in the embryo not completing gastrulation.

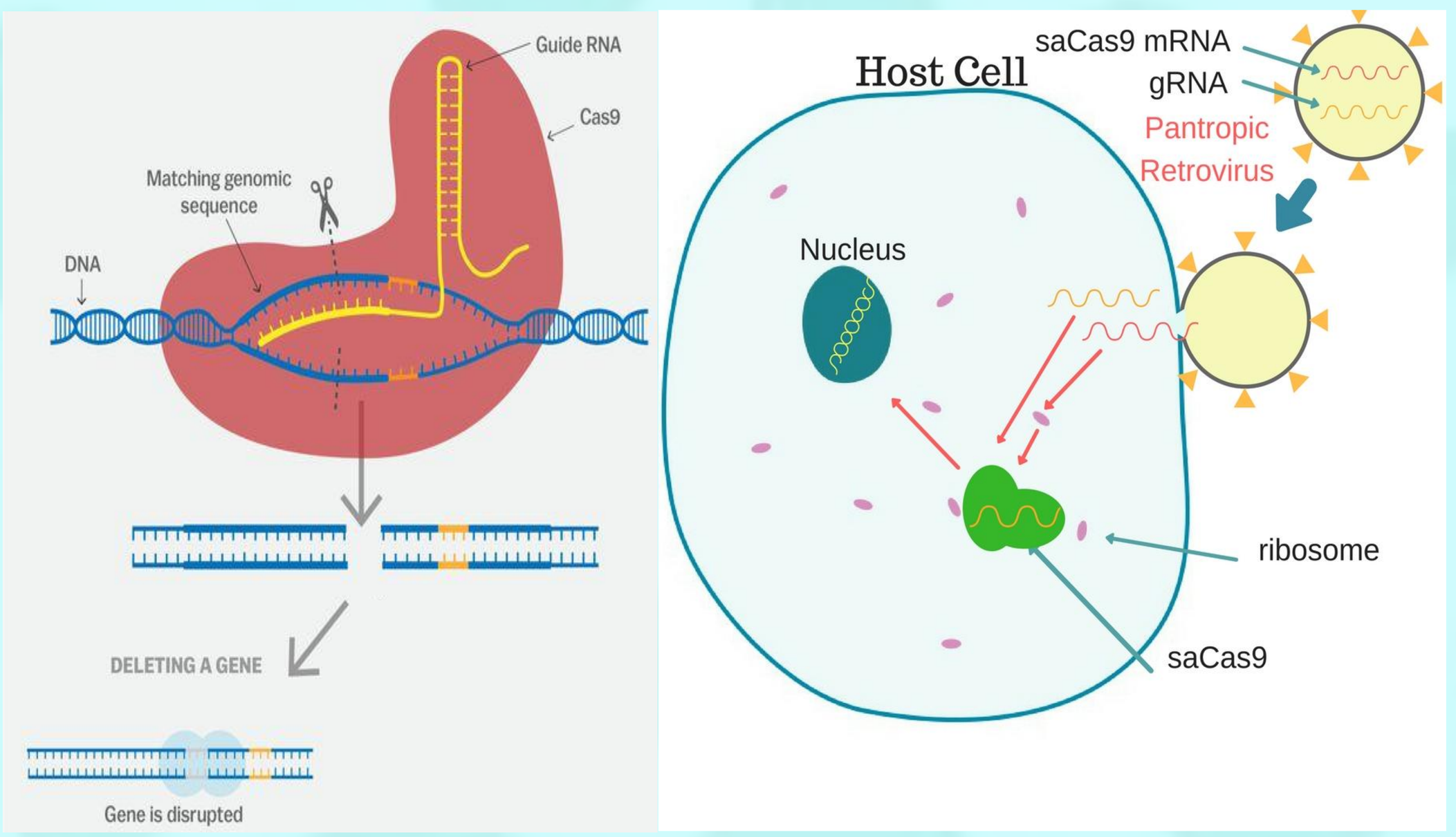
Why is it possible to disable the development of the offspring by just altering the genome of the mother? The answer is maternal effect genes, such as the Bicaudal-C gene. During the maturation of oocytes, specific maternal mRNAs are produced that are necessary for post fertilization. If the mother is mutant for these maternal effect genes, the mutation will result in a particular offspring phenotype, regardless of the offspring's own genotype.

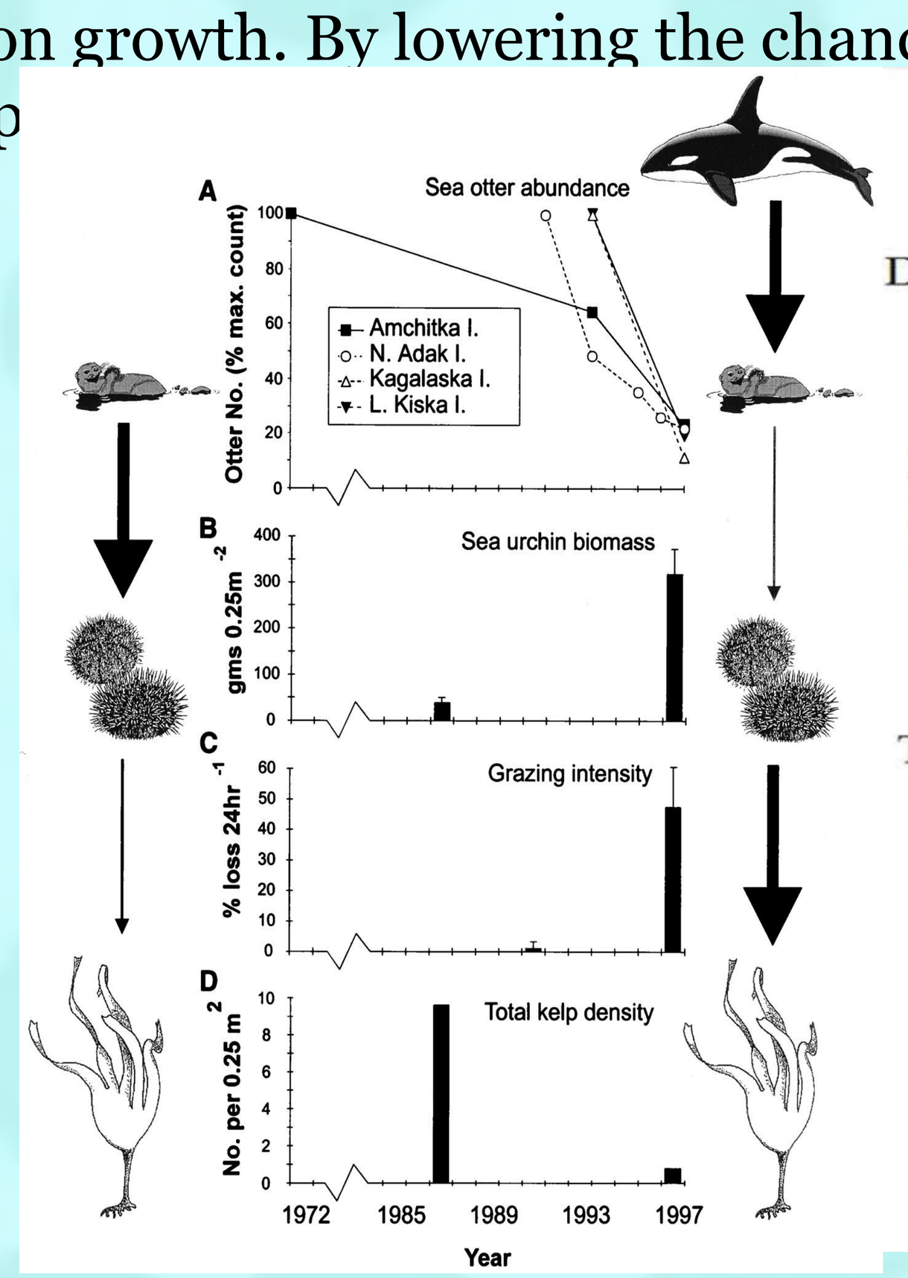
To biologically engineer the Bicaudal-C gene, we will use the CRISPR/Cas9 gene editing system consisting of the saCas9 endonuclease and the guide RNA (gRNA). The saCas9 endonuclease is an enzyme capable of precisely cutting the double strand host DNA. Using a saCas9 enzyme is more effective than other Cas9 enzymes because of its small size and its ability to carry the Bicaudal-C target sequence. The gRNA, a 20 bp-long RNA at the five prime end, binds to the target sequence on the DNA leading the saCas9. This target sequence must be immediately upstream of a five prime NNGRRT protospacer adjacent motif (PAM) sequence. Once the saCas9 enzyme locates the complementary sequence of the gRNA preceding the required PAM sequence, it will make a double strand cut approximately three nucleotides before the PAM sequence in the targeted sequence. As the cut DNA repairs itself automatically by non-homologous end joining, small insertion or deletion mutations can occur and disable the Bicaudal-C gene by creating a frameshift.

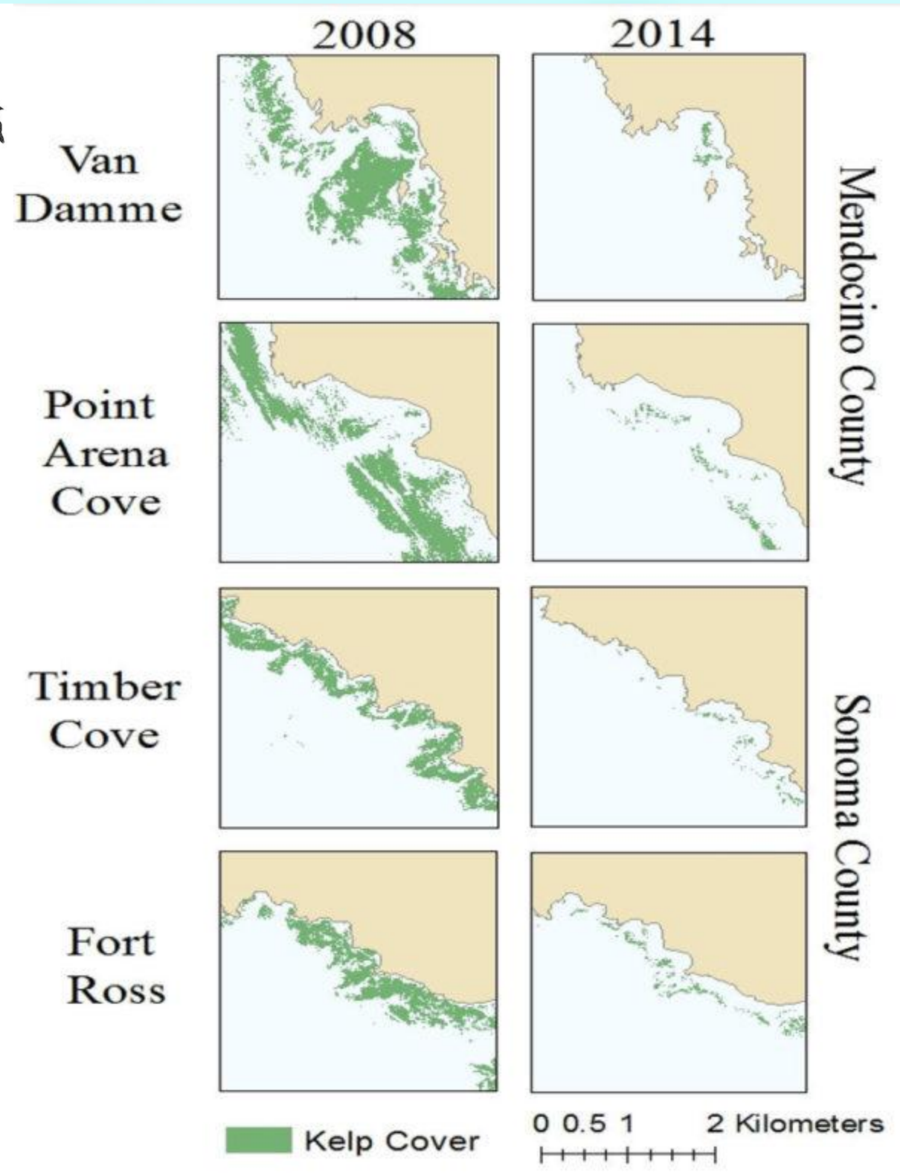
Because of the frame shift in the protein coding gene sequence, the nucleotide codon groupings change. This prevents a correct amino acid sequence in the Bicaudal-C protein or causes an early termination codon to occur, ending the protein synthesis prematurely and causing the gene to be ineffective.

To deliver the CRISPR/Cas9 system, the female sea urchins' gonads are injected with a solution of modified pantropic retroviruses carrying the saCas9 enzyme coding mRNA and the gRNA. Pantropic retrovirus are large enough to carry all the genetic material necessary for the CRISPR/Cas9 editing technique, and has already been successful in infecting sea urchin cells that are dividing. A viral delivery technique works by high jacking the natural process of viral infection where viruses connect to host cell membrane receptors and insert their genetic material into the cell's DNA. In our use of the pantropic retrovirus, we would replace the typically harmful genetic material of the virus with our CRISPR/Cas9 complex genetic material. This allows us to use the virus's method of delivering genetic material to deliver the gene editing material to silence the Bicaudal-C gene in the cells of the female gonads.

By editing adult purple sea urchin mothers, we are able to have wild male sea urchins fertilize both the eggs that develop successfully and the eggs that will not develop. This creates a type of competitive inhibition that keeps unmutated sea urchins in the ecosystem while also limiting their population growth. By lowering the chance of a successful fertilizations, we will allow the regrowth of California's kelp







Testing, benchmarking, and Comparison to Alternatives

Small Scale Testing

Before releasing our genetically modified sea urchins into the ocean, we must first modify and test sea urchins on a smaller scale. The goal is to see if disabling the Bicaudal-C gene via induced mutation will still allow female purple sea urchins to produce healthy eggs. We also want to confirm that stopping the expression of the maternal effect gene Bicaudal-C still allows for fertilization to occur, but is able to inhibits embryo development. Moreover, testing is necessary to see if the pantropic retrovirus will infect all of the cells in the sea urchin's gonad and successfully stop Bicaudal-C. The quantitative data we want to collect from these test are a percentage of how many cells in the gonad are successfully altered to have an ineffective Bicaudal-C gene, and also the amount of embryos that are unable to develop into an adult sea urchin.

Test have been done that prove inhibiting Bicaudal-C stops embryo growth, however, it is still unknown if removing the target sequence of the gene will inhibit the production of Bicaudal-C alone. Since we are relying on the cell to repair its own DNA after using CRISPR, we do not know if this will affect other genes in the sea urchin genome.

Benchmarking

To benchmark our method, we must see how effective stopping future generations is compared to the current method of destroying sea urchins now. Currently, people are limiting mosquito population by creating a generation of males that fertilize females so they lay eggs that would not hatch.

Comparison to Alternative

Current alternative methods, such as destroying sea urchins, have their benefits and disadvantages. Although destroying purple sea urchins with hammers is an immediate way to decrease the sea urchin population, it is also very ineffective and requires many skilled divers. In comparison, we propose to use genetic engineering techniques (see "Detailed Solution" for details) to introduce mutant female sea urchins that produce modified eggs. When the defective eggs are fertilized, the offspring will not survive. In this way, the mutant female urchins compete with wild type females to inhibit the reproduction of healthy zygotes. This method requires less long term manual labor and is more effective in decreasing the sea urchin population systematically to prevent further kelp forest destruction.

Technical Challenges

Our road to a plausible solution contained many technical obstacles. A primary challenge was to find a mutation that interfered with crucial developmental stages in the sea urchin mother's offspring, but did not kill the mother herself. This is important because we did not want the mother to die before reproducing since her mutated offspring are necessary to compete with the wild type sea urchin population. Furthermore, we had to find a way to mutate the Bicaudal-C gene in a large number of the cells in the mother's gonads. Introducing mutations in a mature sea urchin is much more difficult than in sea urchin embryos because of the sheer number of cells that must be mutated and the unknown immune responses that can occur. This will be a point of emphasis in our future tests. Lastly, we want to ensure that the viral vector used to deliver the CRISPR/Cas9 complex does not kill the sea urchin mother before she reproduces for the same reason mentioned above.

References and Acknowledgements

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