Potential Genetic Strategies to Eradicate Quagga Mussel in the Colorado River Basin: Formation of a Risk Assessment Working Group

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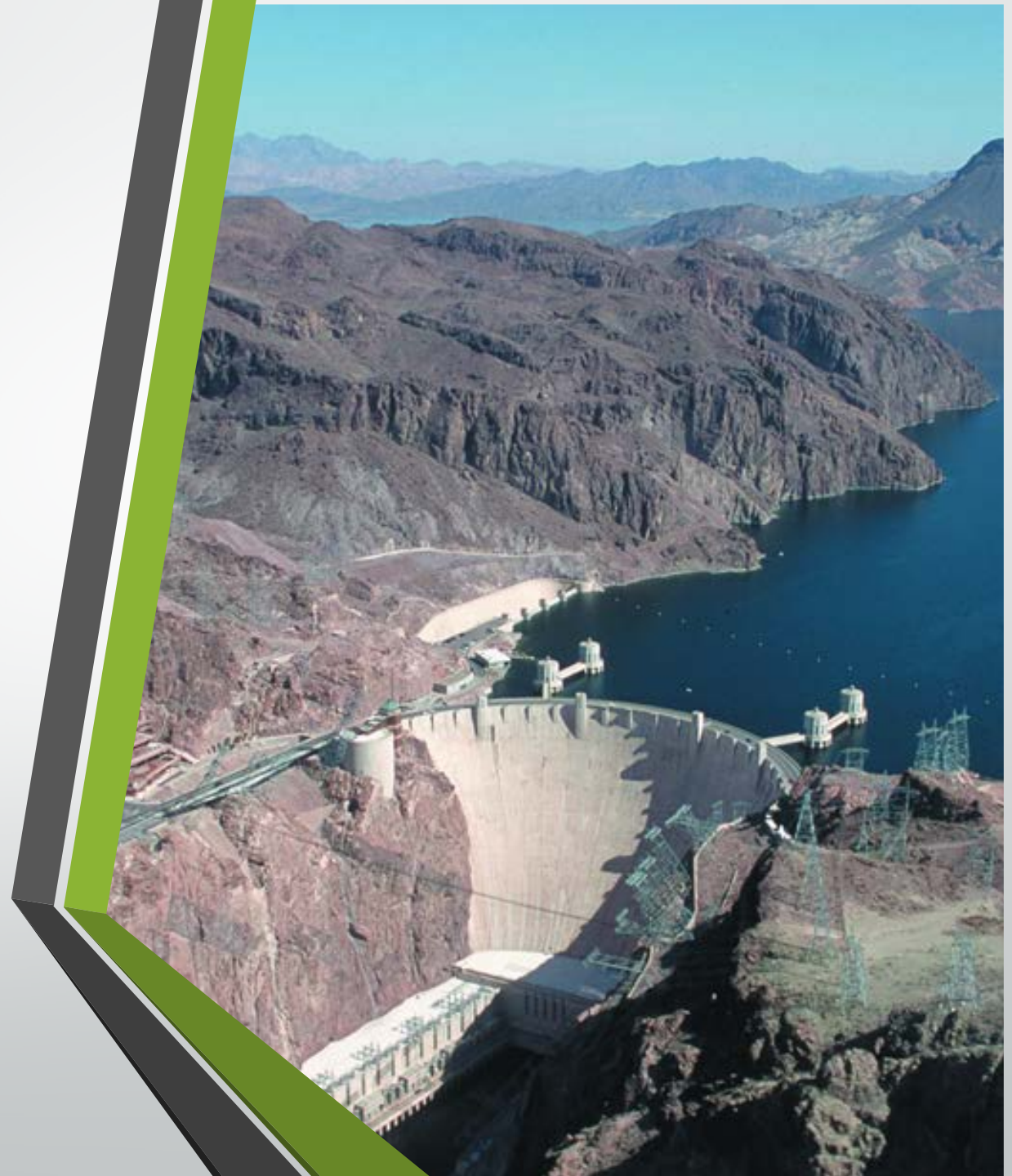
Vulnerability of the Colorado River to AIS

- Perhaps the youngest large-scale engineered ecosystem in the U.S.
- Assemblages and species are still jockeying for position.
  - Impossible to accurately predict what assemblages may one day look like.
  - Especially true under changing climatic conditions.



Founding effects and bottlenecks do not stop AIS that have evolved under strong selection pressures

Genetic variability of quagga mussel in the Basin

- Studies (Jennet 2013, Lindsey et al, 2017) have shown heterozygosity to be relatively low.
  - Compared to infestations in the Great Lakes Region and to their native range.
- Heterozygosity will likely slowly increase over time.
- Containment is extraordinarily important(!)
  - However, statistics are not kind to even the best of containment efforts.
  - Constantly having to work against the ecological grain of natural selection processes.

Spatial Variability in Gene Drives and Risk Assessment

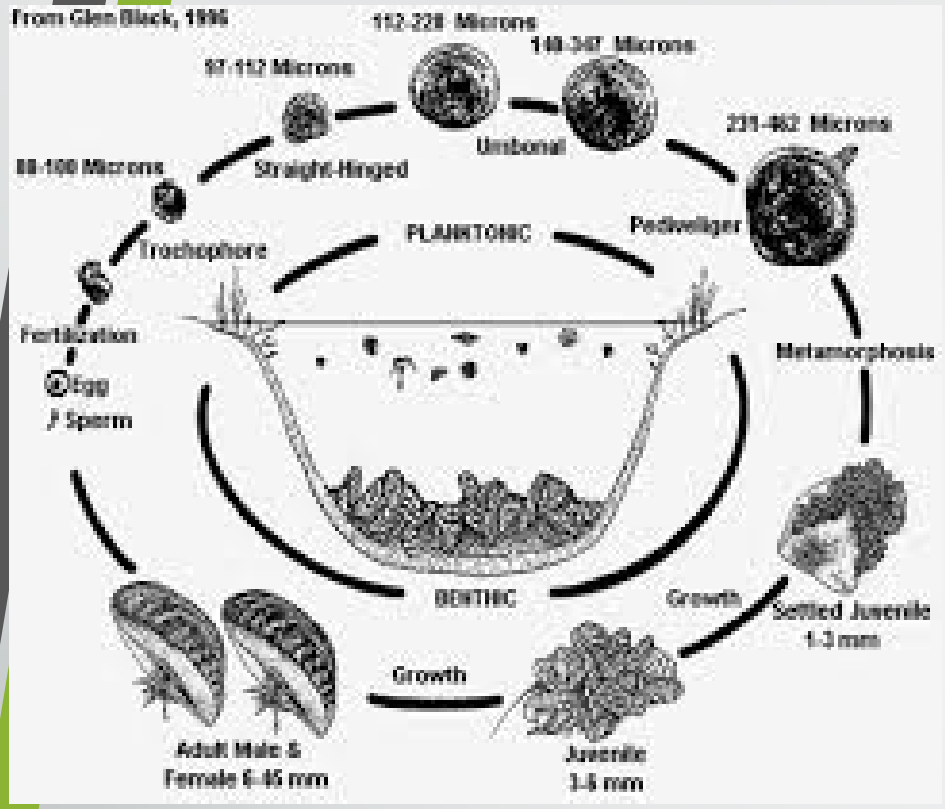
- Gene drives and risk assessments idealized for one area, may not work in others.
- Ecosystems all contain unique sets of variables (inter- and intra-specific variability).
  - Gene drives and risk assessments should be done on a watershed or smaller scale.
- In order to be as fully protective as possible, and to minimize as much risk as possible, gene drives and risk assessments should be ecosystem-specific.

Non-lethal Gene drives

- "Non-lethal" in this context doesn't mean such a drive wouldn't be capable of eradicating a species.
  - It means it doesn't target reproduction (or the lack thereof).
- Life History aspects such as development of byssal threads, calcium uptake, etc. could be targeted.
- Pros: Relatively Low risk. Low potential of spread to other areas or species. Less likely to be stopped due to genetic variability.
- Cons: Labor intensive and logistically difficult to introduce.
  - Re-introduction would be required.
  - Requires genetic "swamping".

Potential Non-Lethal Genes to Target

- Quagga mussel have an absolute requirement for dissolved calcium in water to complete their early life stages as veligers and then adults.
  - Invasion risk factors for both quagga and zebra mussels in certain areas have been performed using calcium concentrations from waterbodies.
- This calcium requirement increases post-trocophore stage.



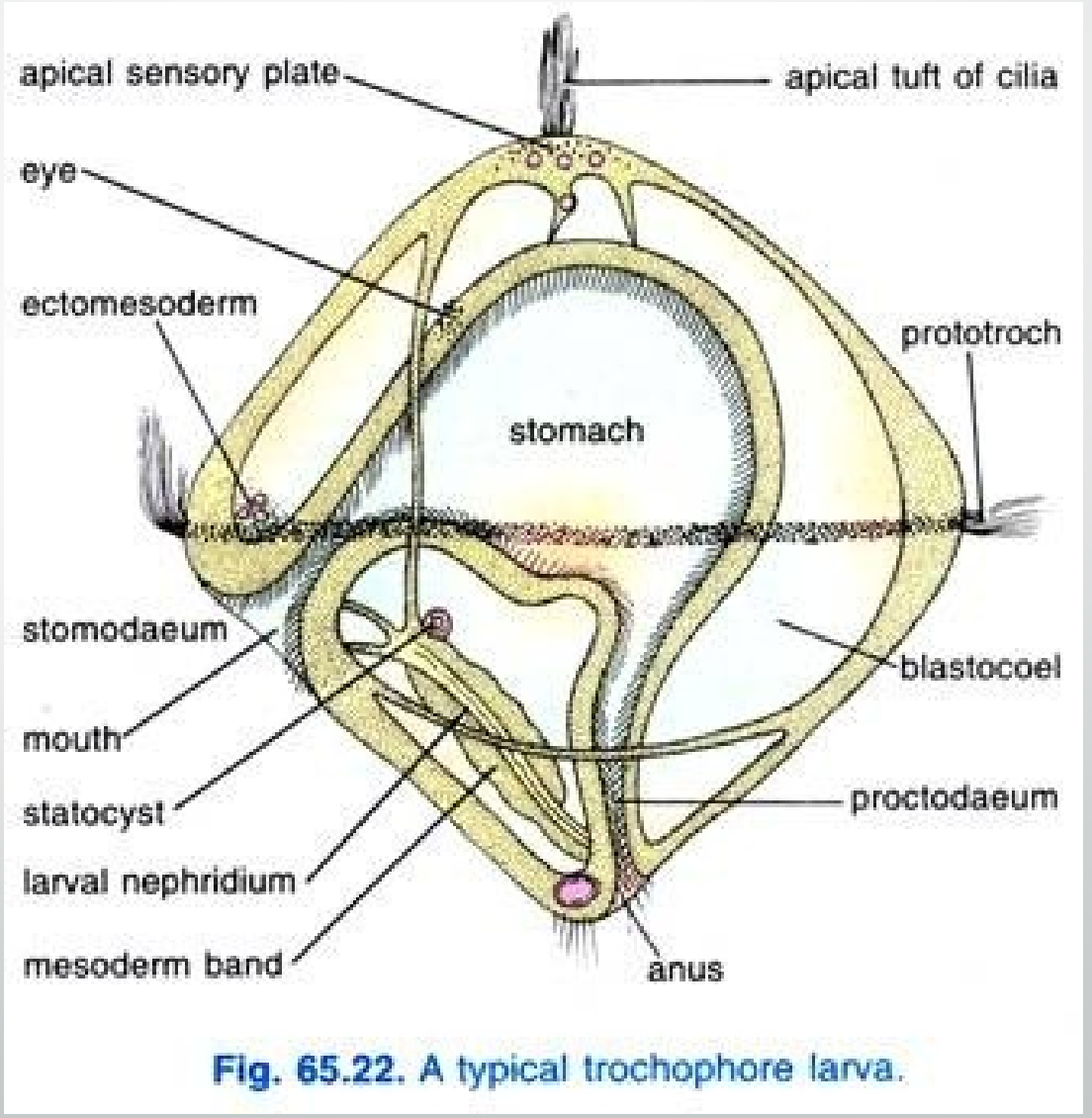


Fig. 65.22. A typical trochophore larva.

Calmodulin ("Calcium-Modulated protein" or "CaM")

- Vital in most living organisms for biomineralization of calcium.
  - Especially important in bivalves and mollusks for shell development.
- Also dependent upon "calmodulin-like proteins" or CaLP).
- Both CaM and CaLP are vital to shell regeneration and formation in all mollusks.
- calcium-bound calmodulin forms a critical subunit for the regulatory enzyme phosphorylase kinase, which in turn is a regulator for glycogen breakdown.
- Calmodulin also binds and activates other kinases and phosphatases that play significant roles in cell signaling, ion transport and cell death

Lethal (Sex-Based) Gene Drives

- Would directly affect reproductive potential in subsequent generations.
- Still requires a large number of F^0 organisms to be introduced into the environment.
- Pros: Directly reduces population numbers through infertility.
- Cons: Reproduction is one of the most highly-conserved traits of organisms.
  - Highly susceptible to genetic variation within a population.
  - Still requires genetic swamping.

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Gene drives thwarted by emergence of resistant organisms

Until this obstacle is overcome, the technology is unlikely to succeed in the wild.

Ewen Callaway

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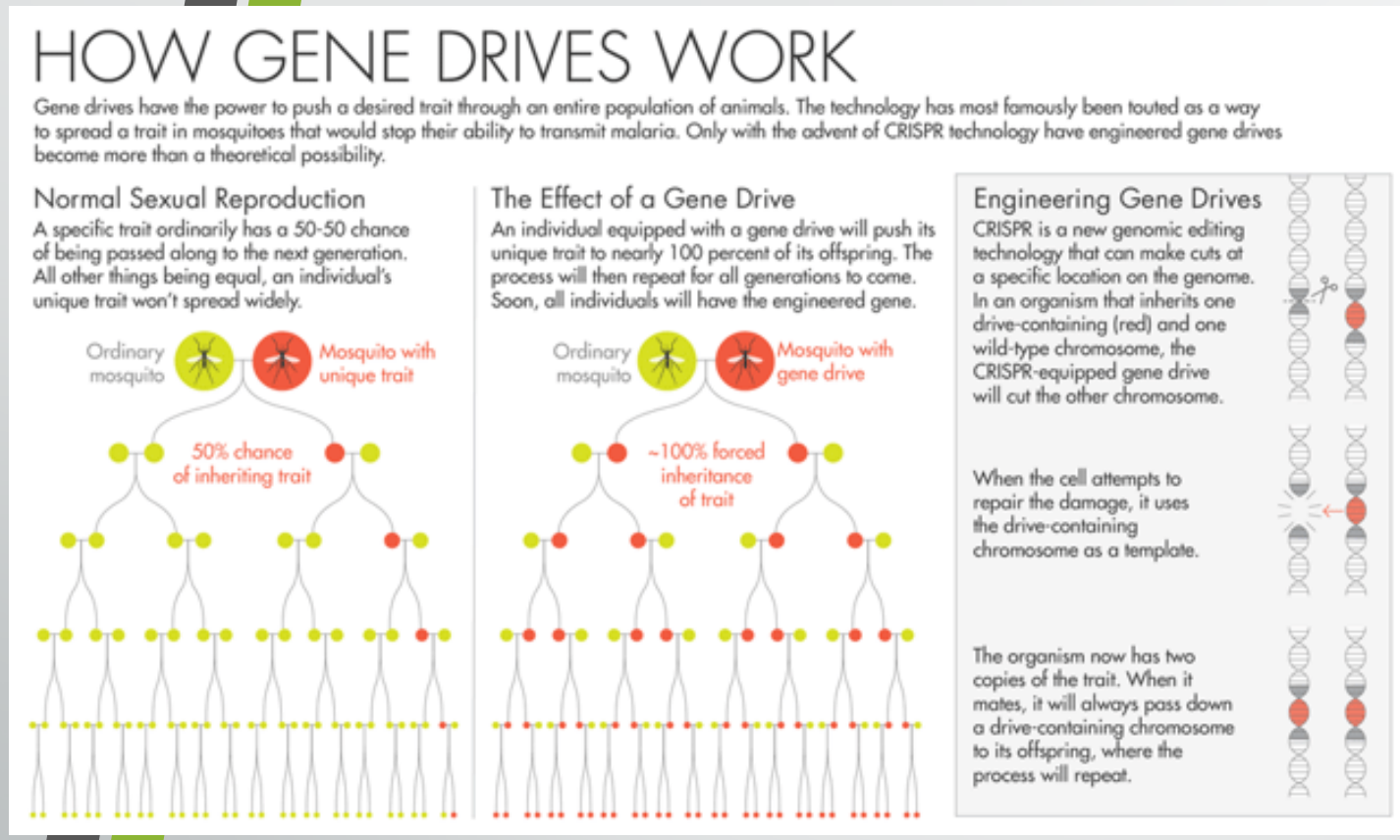
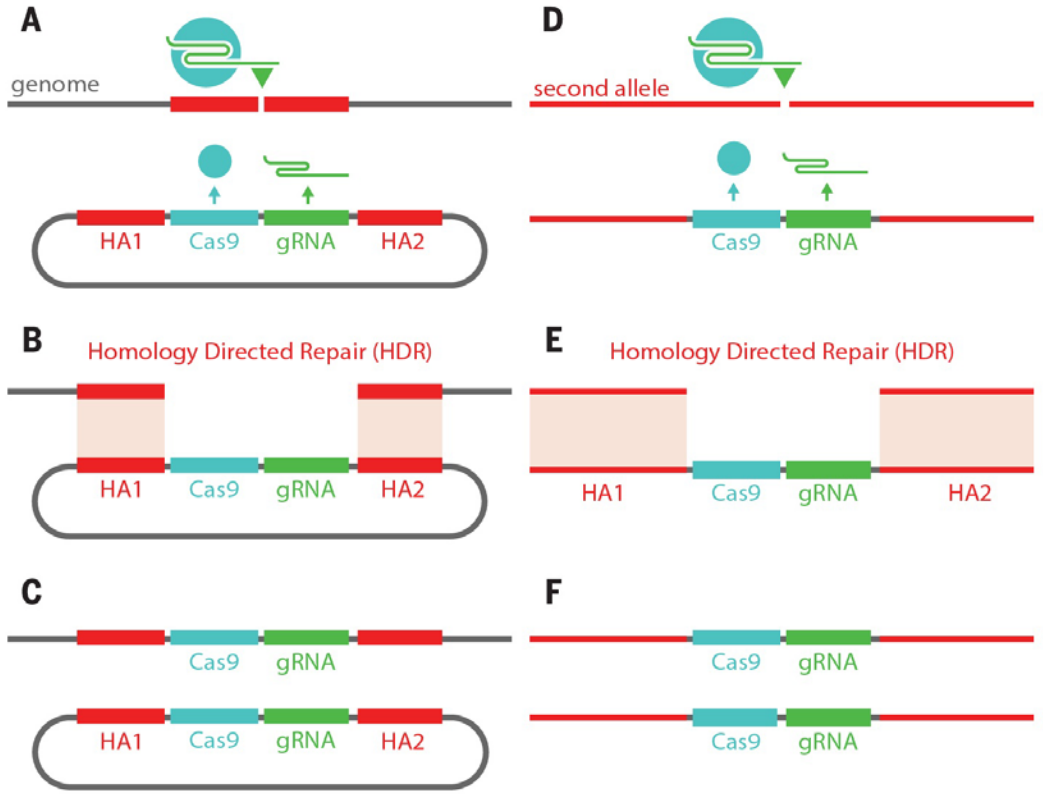
How are Mutant Alleles or Transgenes Carried on via a Gene Drive?

- How do we ensure the spread of the engineered genetic material through a population?
  - How do we drive a trait from heterozygosity to homozygosity?
- Mutagenic Chain Reaction (MCR) using homology directed repair (Gantz & Bier, 2015).
  - Often used synonymously with the term "gene drive".
- Loss-of-function mutations may only produce a mutant phenotype when both copies of the gene are mutated.
- MCR uses the initial mutated allele to cause a mutation in the allele on the opposing chromosome and thus the homozygosity of the trait.

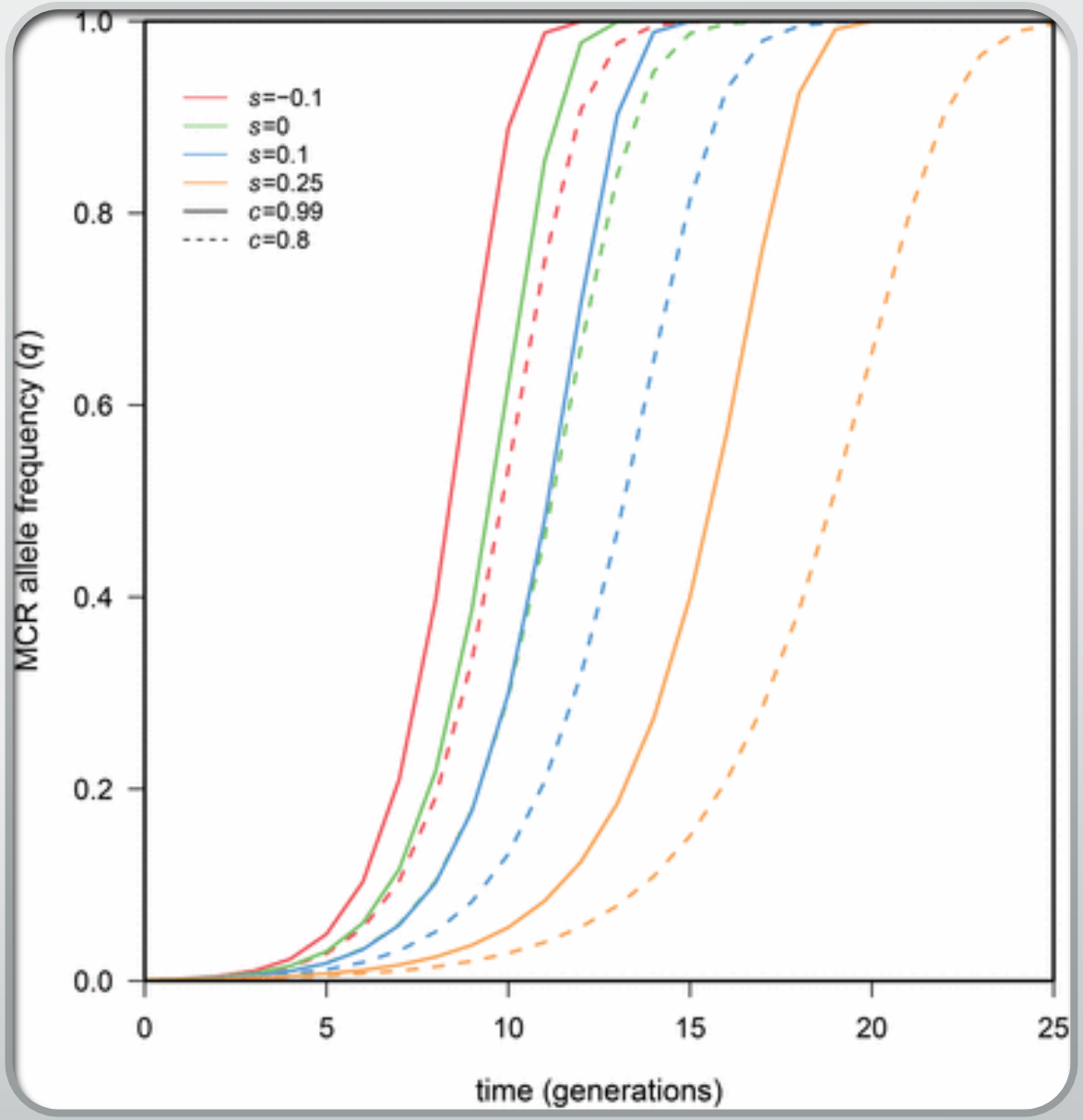




Mutagenic Chain Reaction. (A) DNA containing CRISPR components is injected into a fruit fly. The components are expressed, leading to cleavage of the DNA. (B) Through *homology directed repair*, the injected DNA is incorporated into the genome. (C) Now, there is a set of CRISPR components permanently incorporated into the genome. (D) The CRISPR components are then expressed from the new genomic insert, and DNA on the second chromosome is cleaved. (E) Homology directed repair occurs a second time, leading to (F) a homozygous mutant genome in one generation. *Modified from Gantz and Bier, 2015.*

Unckless et al., 2015

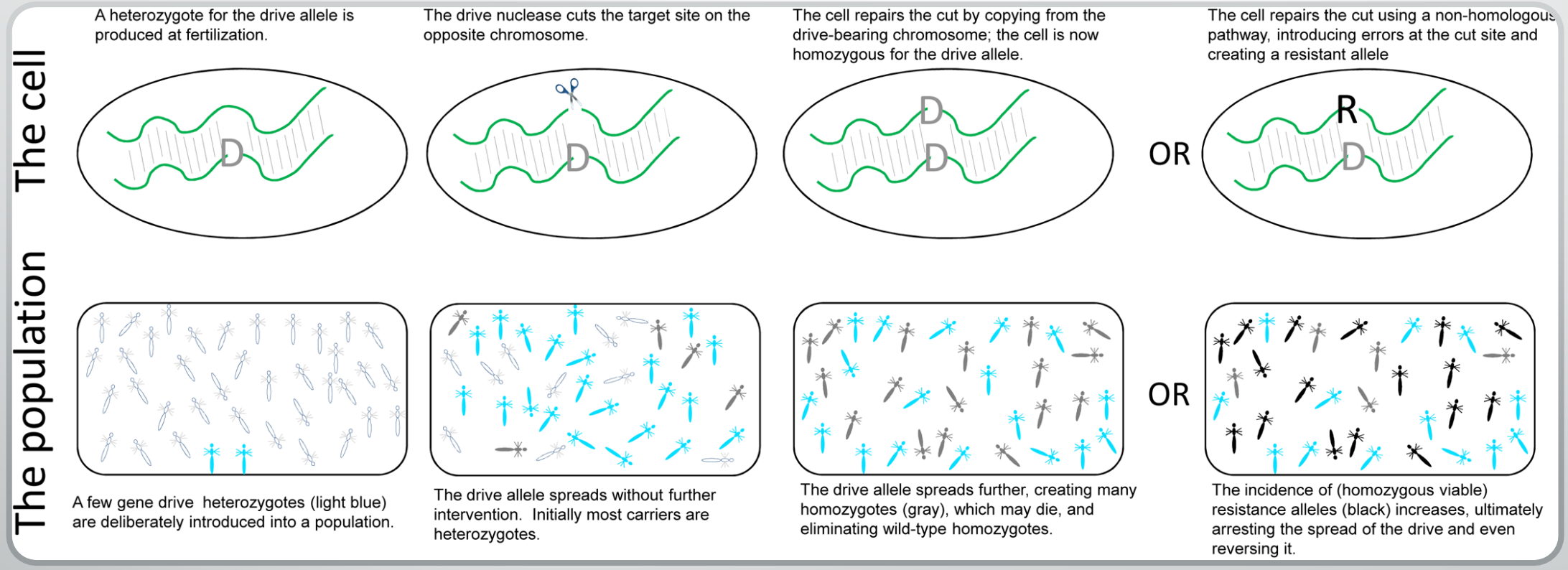
- Speed depends on its effect on individuals fitness, on the rate of allele conversion, and on the population structure.
- Only 30% reproduction rate is required for a gene drive to progress.



Gene Drive Containment

- Current boots-on-the-ground containment efforts in the Basin and between and amongst states is vital.
- An added layer of protection would be to have *reversibility* as a vital component to any quagga-based gene drive.
  - Engineered reversibility should be an incorporated counter-measure in any planned gene drive.

Bull & Malik, 2017



Using Fecundity Against Quagga Mussel

- Fecundity and fertility in any population capable of sexual reproduction is a highly-conserved trait.
  - Up to a million eggs/year produced by 1 female quagga.
  - Due to, generally, warmer water temperatures in the Basin than the Great Lakes or the Dneiper drainage, fecundity may actually be greater here than elsewhere.
- This fact may mean that a gene drive based on fertility/fecundity alone could be stopped by even small amounts of heterozygosity in a wild population.
- However, initiating a gene drive in a highly fecund species, such as quagga mussel, may mean that traits other than fertility/fecundity could be carried deep into gene drive.

Using Aridity as a Gene Drive Safeguard in the Basin

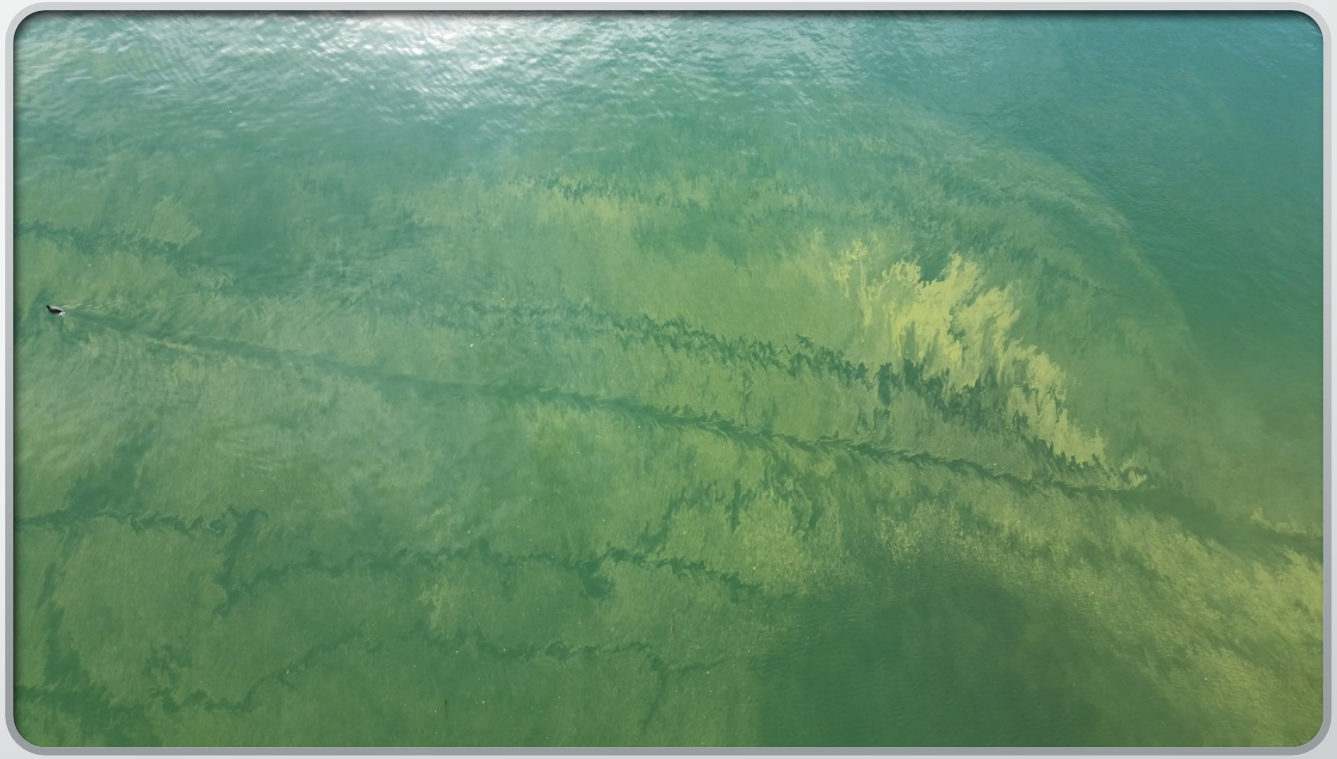
- Colorado River Drainage Area = 242,897 mi^2
- Quagga mussel currently only occupy a fraction of this.
- As difficult as containment currently is, it would likely be far more difficult in a more mesic environment.

Potential for Interbreeding with Other Species

- The most closely-related species to quagga mussel in the world are zebra mussel.
- Zebra and quagga mussel do not produce viable offspring.
- The most-closely related native species to quagga in the Basin would be the California Floater (*Anodonta californiensis*).
  - Compared to zebra mussel, California floater is not closely related to quagga mussel.
- Any quagga-based gene drive should still safeguard against any chance of inter-breeding with any native species.

Ecosystem/Trophic Level Effects

- Difficult to predict.
- Although the exact cause of *Microcystis* outbreaks in the Colorado River cannot attributed directly to quagga mussel, they certainly can't be helping.



- There are many reasons why a gene drive in the Colorado River Basin should result in a large-scale decrease of fitness in the quagga mussel population.
  - Eradication should always be an ultimate goal.
- Any gene drive and risk assessment should be designed for the ecosystem in question.
  - Ecosystems, and their subsequent management, differ greatly.
  - There should never be a one-size-fits-all approach regarding gene drives.

- Coordination between those well-versed in the science of risk assessment and those researching effects, positive and negative, of gene drive research, is essential.
- This coordination and cooperation should be a vital first step toward increasing efficacy, while simultaneously reducing risk, as much as possible.
- Such a group should likely be coordinated specifically for the Colorado River Basin.

Discussion