Zebrafish as a Model System for Developing a Gene Drive to Eradicate Invasive Fish

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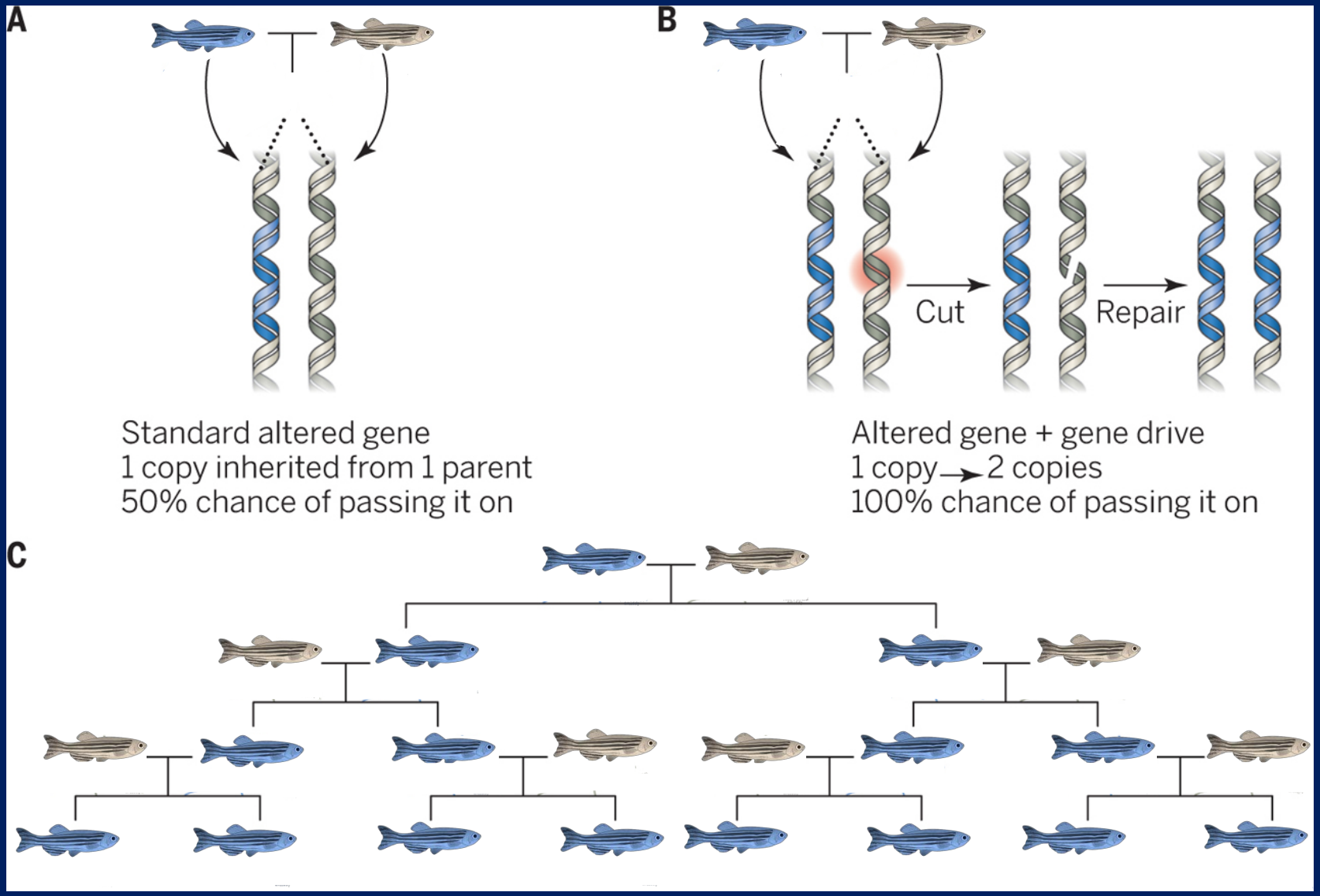
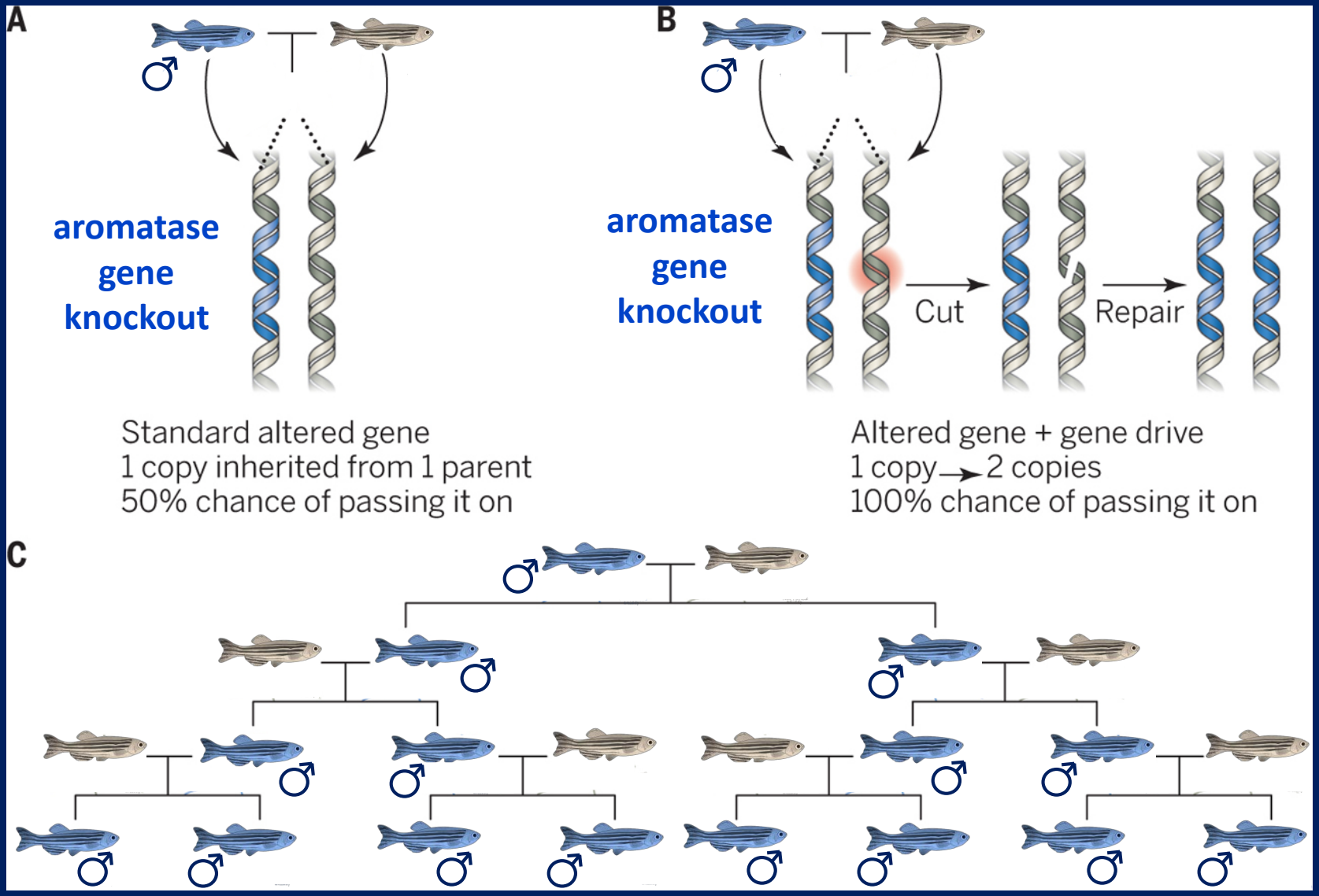
A Gene Drive for Fish



A “Daughterless” Gene Drive for Fish



Gene Drive Risks and Benefits

Pros:

- Species specific
- Self perpetuating
- Feasible for most organisms that reproduce sexually
- Can be used to produce a “Daughterless” eradication strategy
- Eradication possible in large systems

Cons:

- Off-target cutting (cas9)
- Once started, it can’t be stopped
- Cannot be limited geographically
- Could endanger invasive species in their native range

Questions that Need to be Answered

1. Can a gene drive be set up in a fish to study the dynamics of the system?

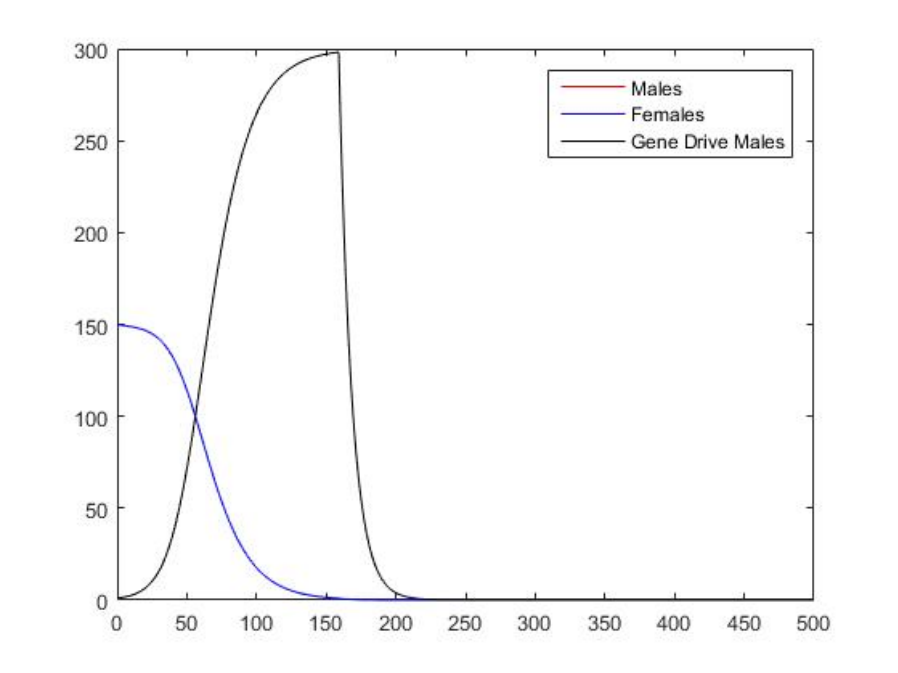
2. Can the gene drive be constructed as an inducible system to limit the environmental risk?

3. Can a prototype for an inducible gene drive be produced based upon the Daughterless carp eradication Strategy?

Modeling a Gene Drive

Initial conditions:
150 males
150 females
1 Gene Drive male
All fish equal viability

Birth rate = 0.1
Death rate = 0.1
Carrying capacity= 300



The Gene Drive fish is produced at twice the rate of normal males and females.

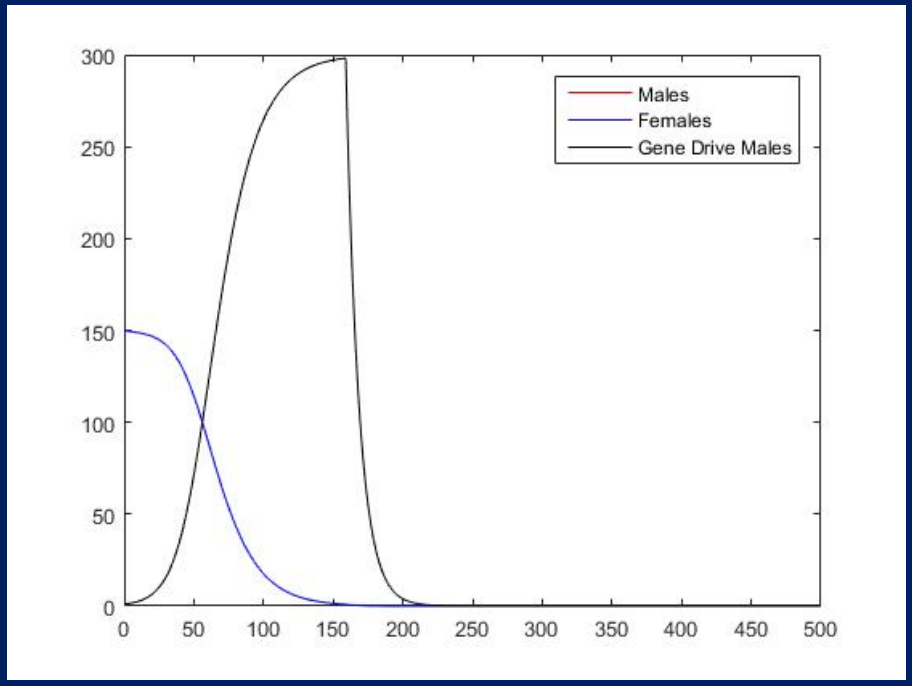
Eventually the Gene Drive fish fills the carrying capacity and females go to zero.

A single Gene Drive fish can cause the target population to go to extinction.

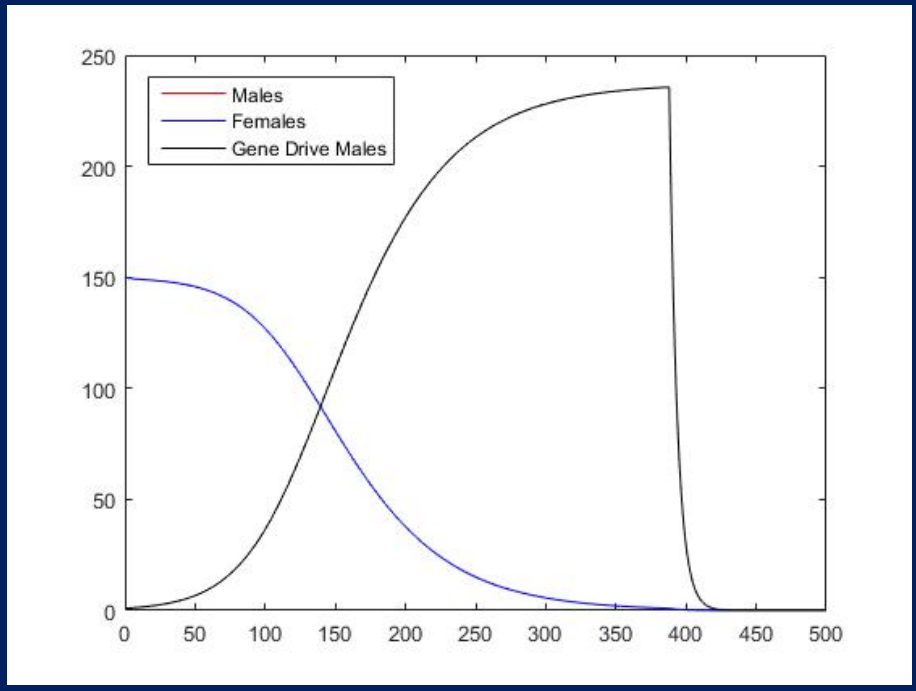
Can the parameters be changed in some way to prevent extinction?

Modeling a Gene Drive

Initial conditions:
150 males
150 females
1 Gene Drive male
All fish equal viability



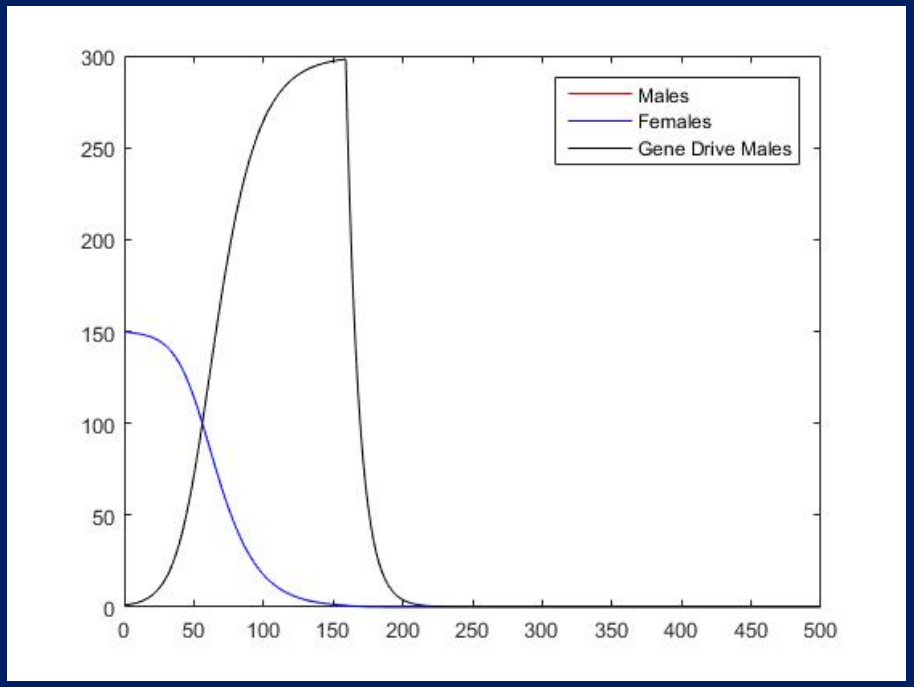
Initial conditions:
150 males
150 females
1 Gene Drive male
Gene Drive fish reduced viability
(death rate 1.6X wild type)



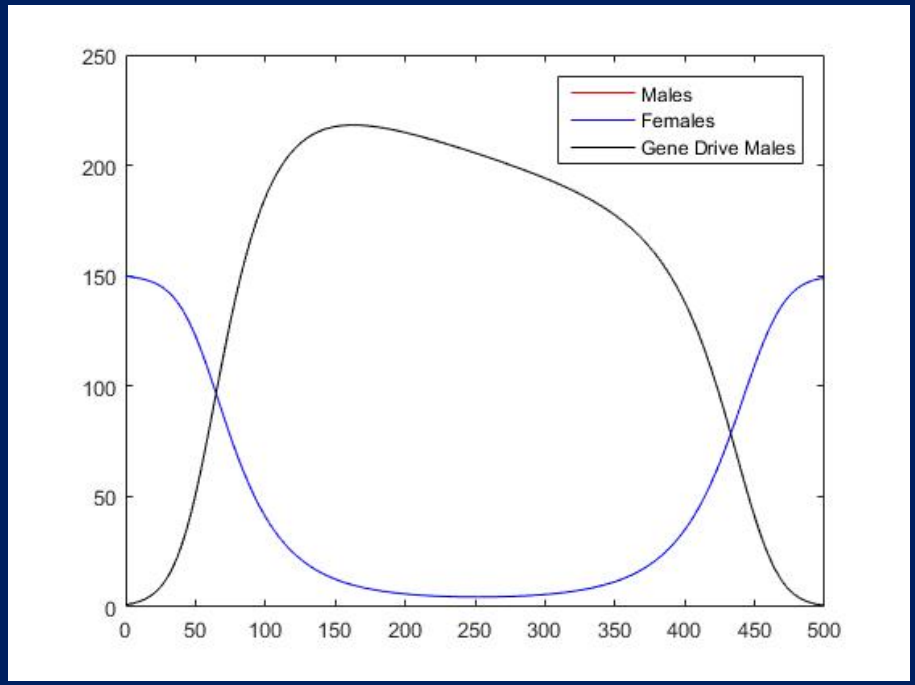
Reducing the viability of the Gene Drive fish (increasing the death rate by a constant over each generation) increases the time required for extinction, but does not prevent extinction.

Modeling a Gene Drive

Initial conditions:
150 males
150 females
1 Gene Drive male
All fish equal viability



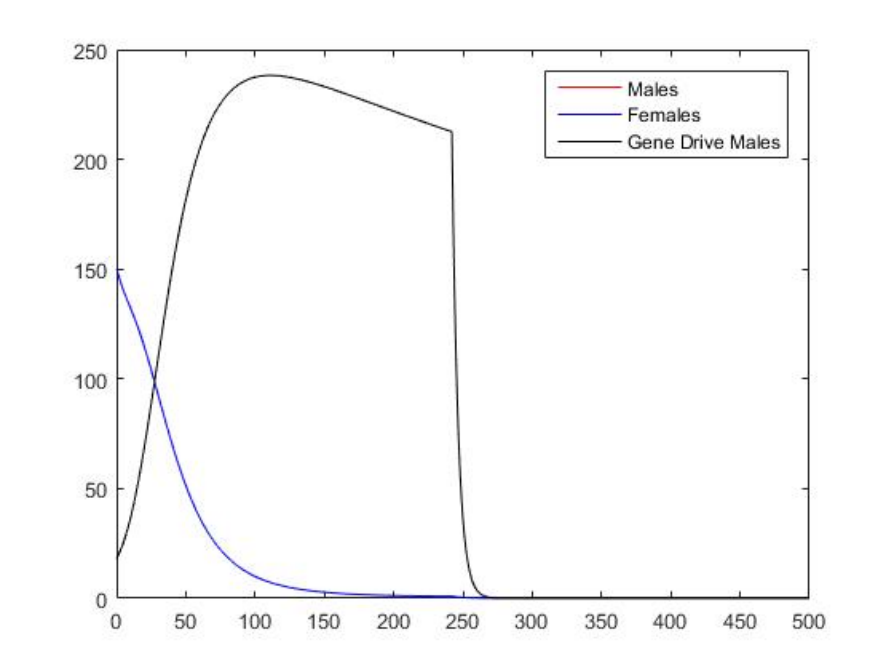
Initial conditions:
150 males
150 females
1 Gene Drive male
Gene Drive fish reduced viability (death rate increasing 1.004X wildtype at every generation)



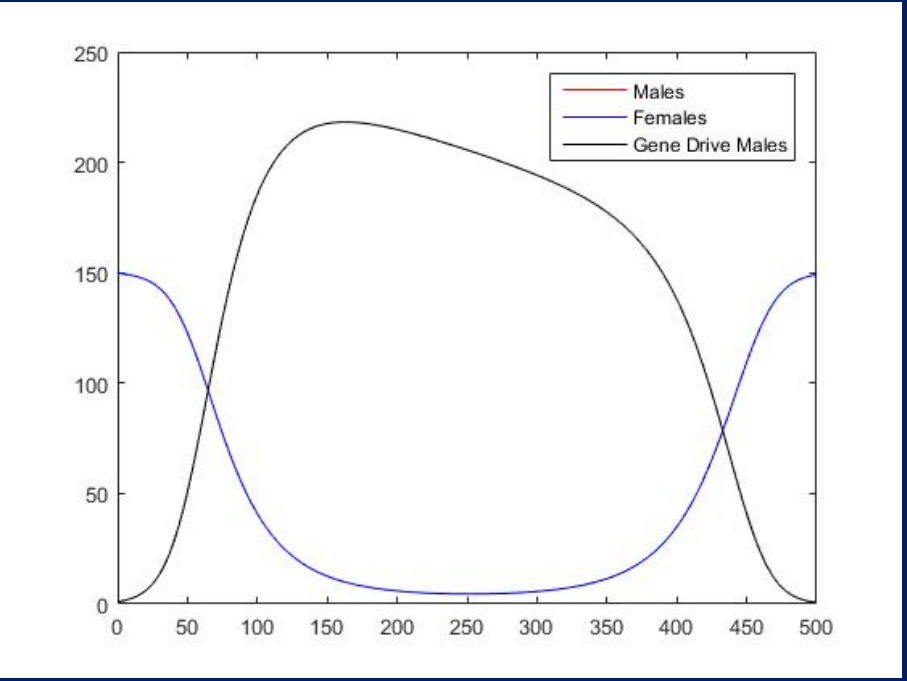
Reducing the viability of the Gene Drive fish (increasing incrementally over each generation) prevents extinction.

Modeling a Gene Drive

Initial conditions:
150 males
150 females
18 Gene Drive males
Gene Drive fish reduced viability (death rate increasing 1.004X wildtype at every generation)



Initial conditions:
150 males
150 females
1 Gene Drive male
Gene Drive fish reduced viability (death rate increasing 1.004X wildtype at every generation)



Reducing the viability of the Gene Drive fish (increasing incrementally over each generation) prevents extinction, unless a sufficiently large number of GD fish are added.

Can the Gene Drive Risks be Mitigated?

Pros:

- Species specific
- Self perpetuating
- Feasible for most organisms that reproduce sexually
- Can be used to produce a “Daughterless” eradication strategy
- Eradication possible in large systems

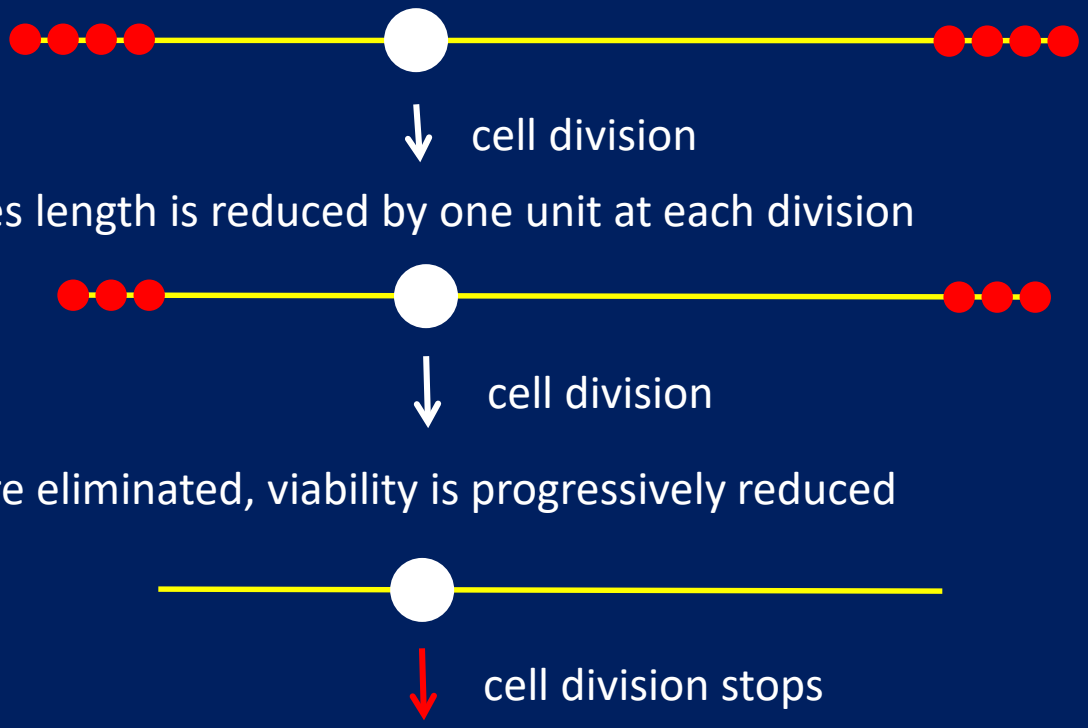
Cons:

- Off-target cutting (cas9)
- Once started, it can’t be stopped
- Cannot be limited geographically
- Could endanger invasive species in their native range

What if it were possible to stop the gene drive after a fixed number of generations?

The Way Cells Count Generations: Telomeres /Telomerase

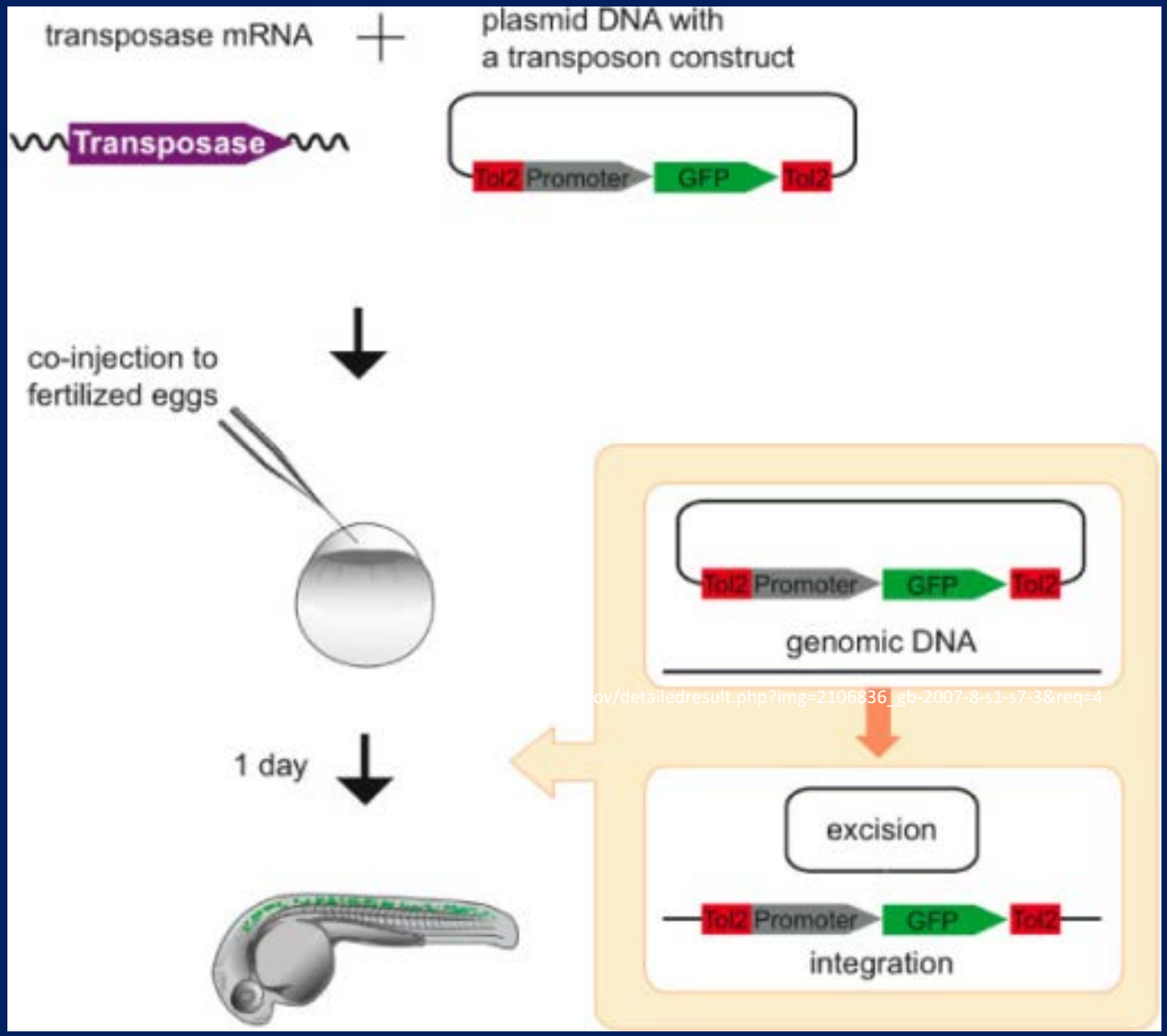
Chromosome ends (telomeres) have a unique structure composed of repeating DNA units

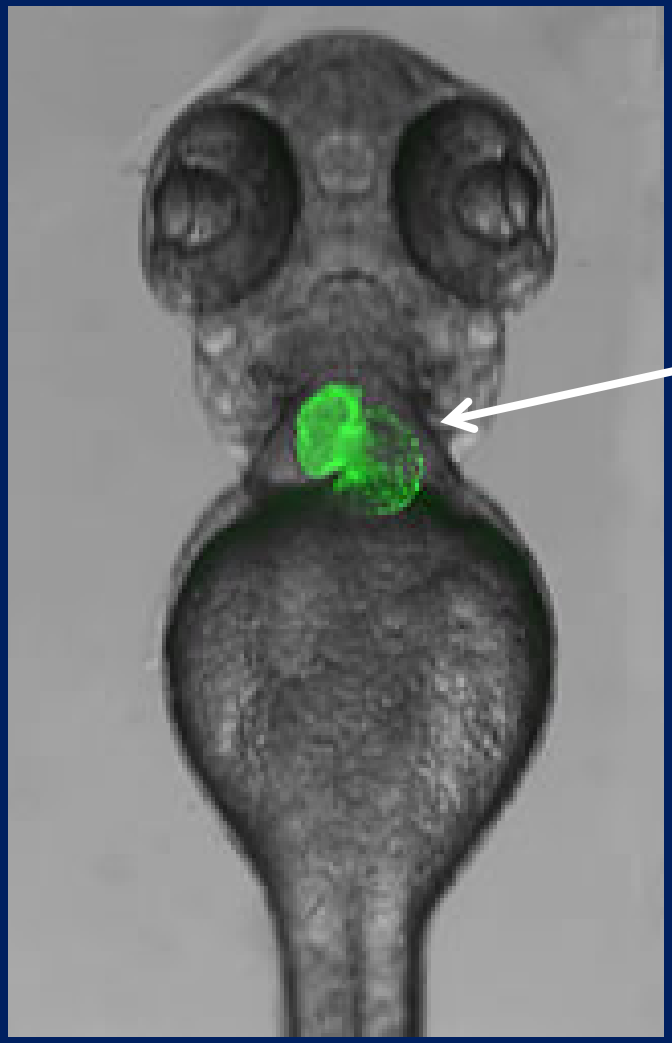


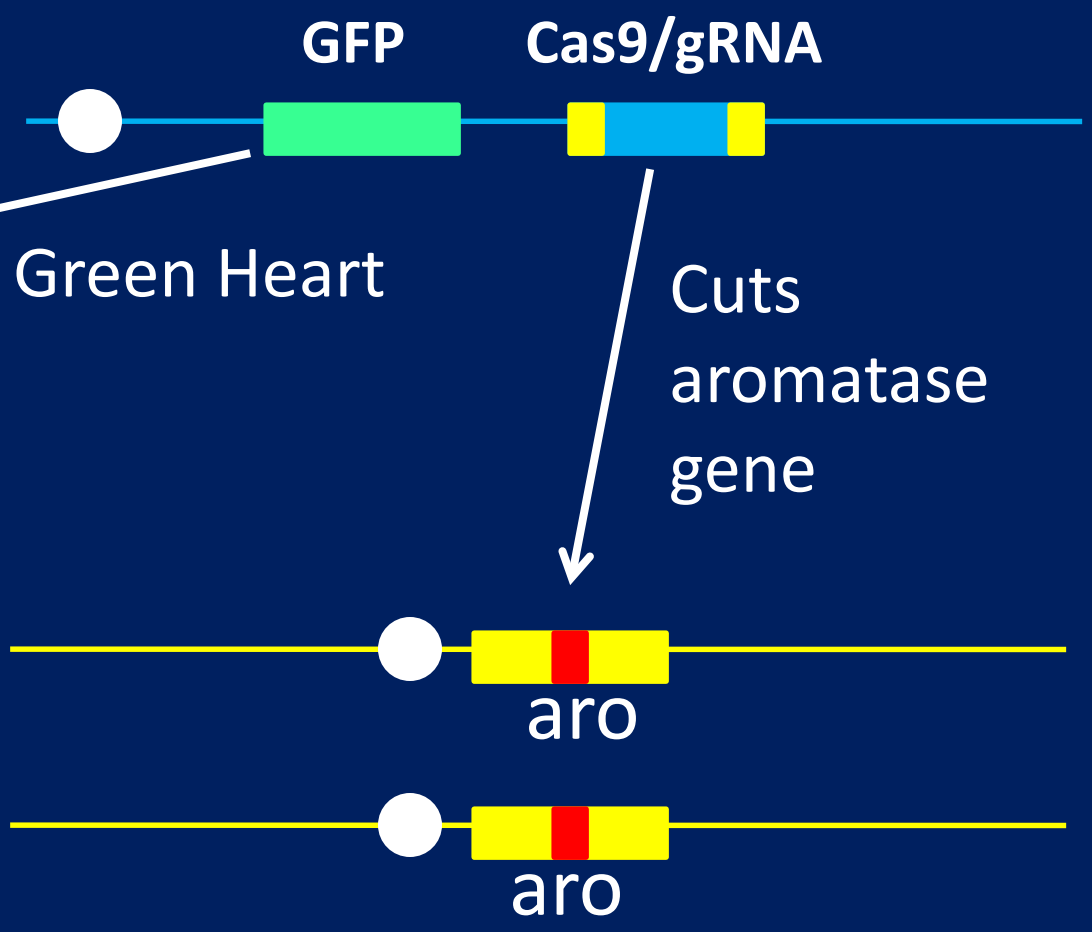
Could shortening telomeres stop the gene drive after a fixed number of generations?

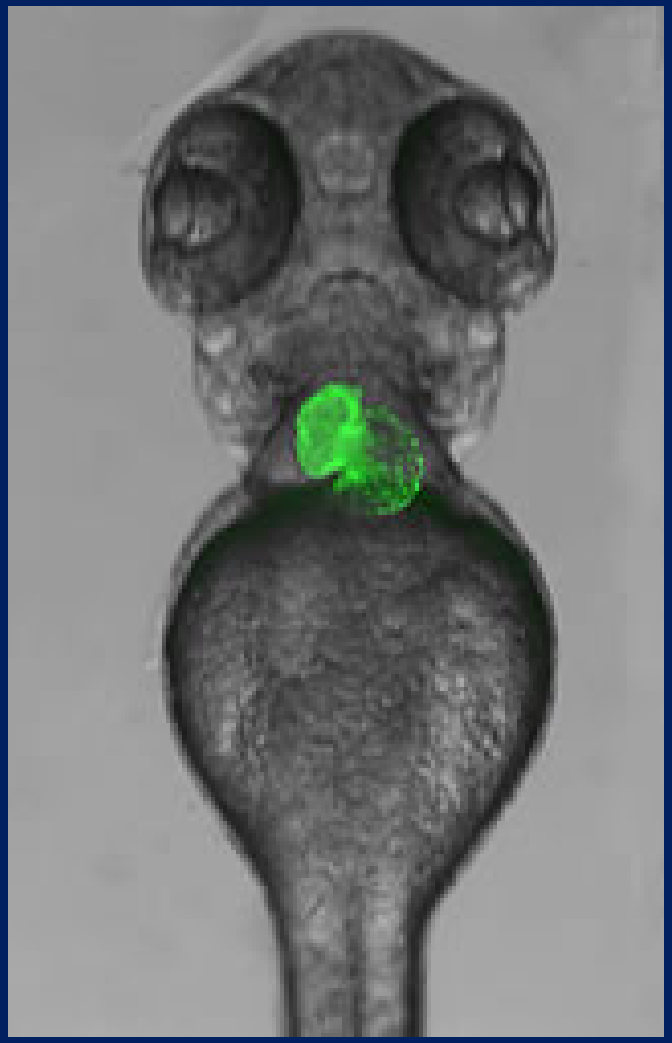
Could this be tested experimentally in a model system?

Making a Prototype Gene Drive In Zebrafish

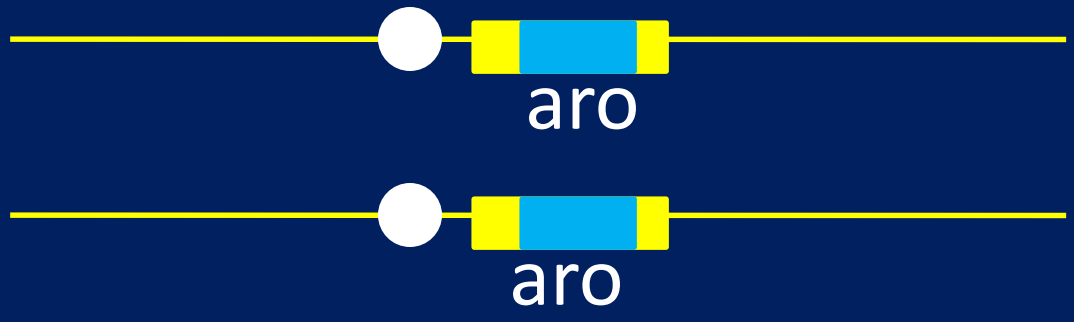





GFP
Cas9/gRNA
Green Heart
Cuts aromatase gene
aro
aro



Both aromatase genes are disrupted by cas9 , the fish becomes male



Next Steps

Cross existing fish with green hearts to find stable transgenic fish.

Induce cas9 and see if a cut is produced at the aromatase gene.

Screen by PCR for aromatase knockouts with cas9 inserted.

See if the proportion of males increases if cas9 is induced at each generation.

Long Term

Make telomerase conditionally expressed in zebrafish.

Determine the number of generations that fish can attain in the absence of telomerase.

Determine whether telomerase expression can be manipulated to control telomere length in a Gene Drive fish, allowing regulation of a gene drive.