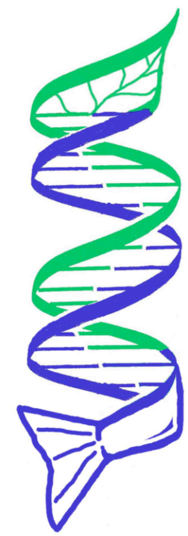
# Experimental measurement and stochastic modeling of multi- generational gene flow from transgenic to wild fish under varying environments

Kelly M. Pennington, Ph.D.
Knauss Sea Grant Fellow
U.S. Senate Committee on Commerce, Science, and Transportation



July 28, 2010

# Acknowledgements


- University of Minnesota (UMN) Doctoral Dissertation Fellowship
- National Science Foundation Graduate Research Fellowship
- UMN Graduate School Fellowship
- USDA Biotechnology Risk Assessment Research Grant
- UMN Conservation Biology Graduate Program

# Outline

- Transgenic fish and conservation concerns
- Methods and models
- What happened compared to what models predicted
- Concluding remarks

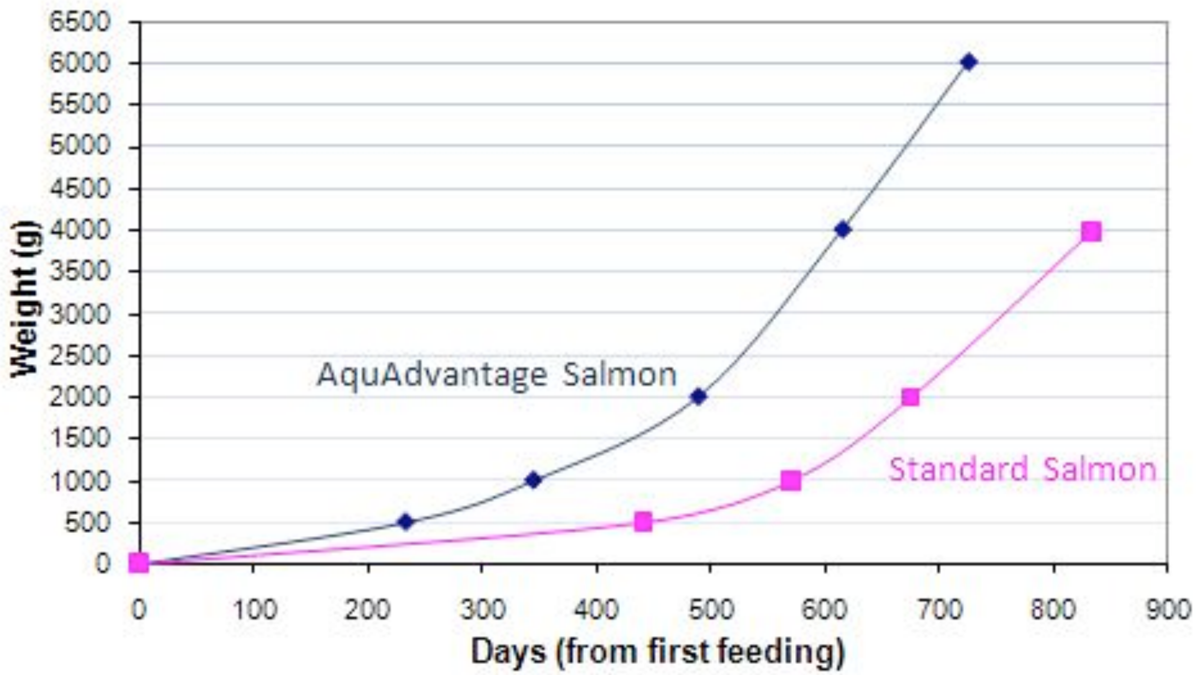
# Transgenic fish

- Recombinant DNA methods applied to structural and regulatory genes to achieve a changed phenotype: i.e. faster growth, disease resistance
- Transgenic, genetically engineered, genetically modified

“auto-transgenic” mud loach, Nam et al. 2001

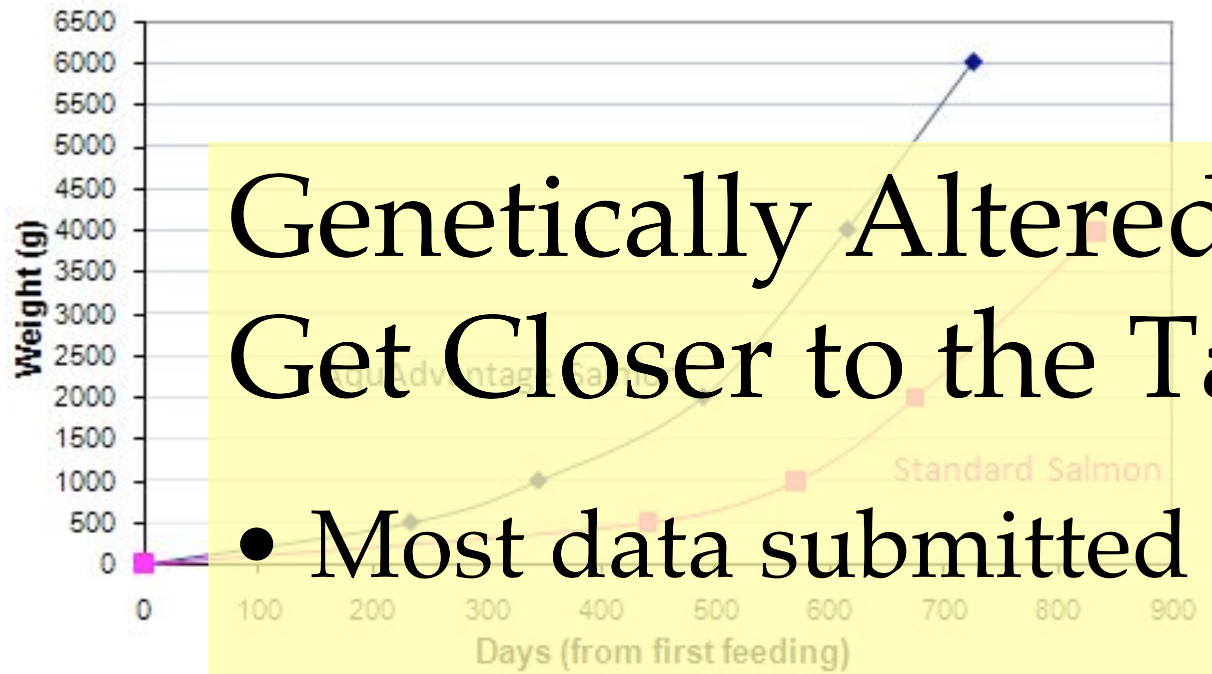

First transgenic animal
marketed in the US

# Transgenic salmon under review




www.aquabounty.com
www.ostc.thaiembdc.org/news_us/Feb52_8.html

# Transgenic salmon under review




## Genetically Altered Salmon Get Closer to the Table

- Most data submitted to FDA
- Hearings possible this fall

*June 26, 2010 New York Times page A1*

www.aquabounty.com
www.ostc.thaiembdc.org/news_us/Feb52_8.html

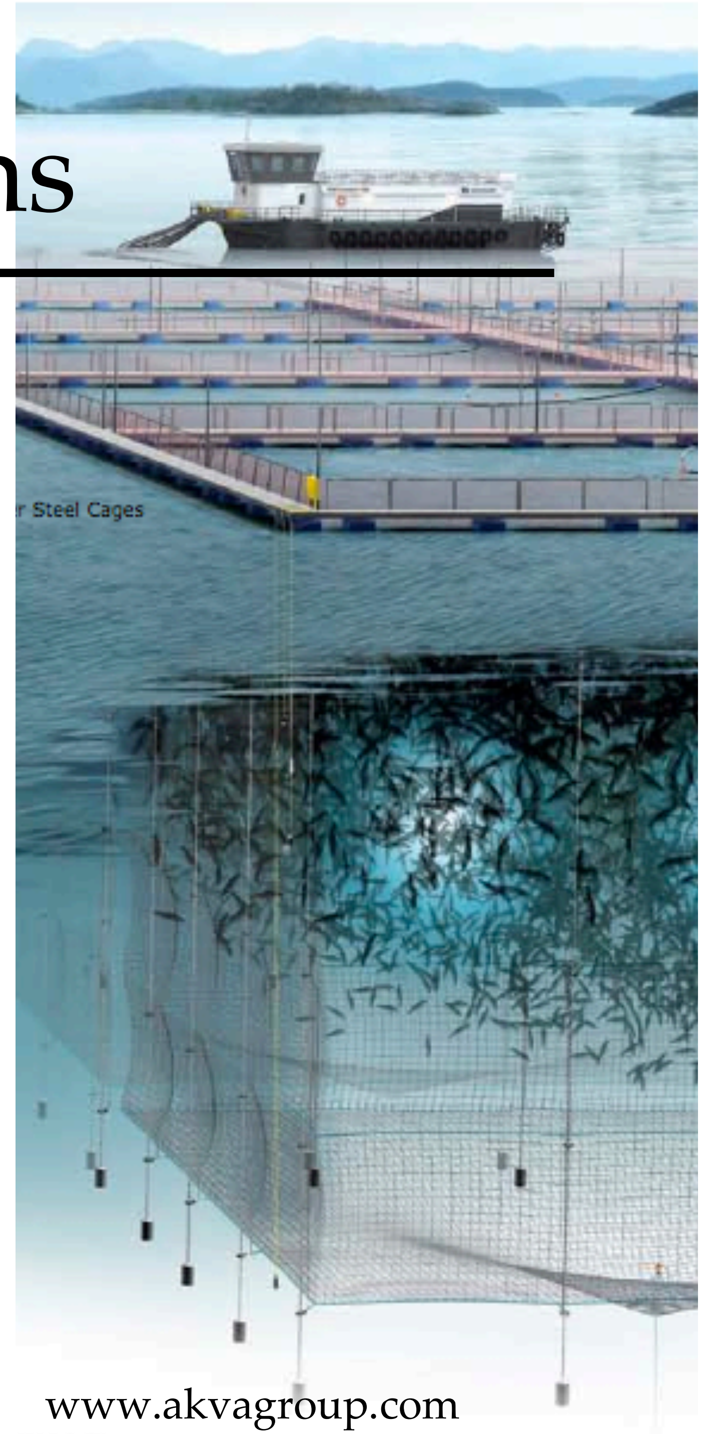
# Conservation concerns

- Farmed transgenic fish could escape into wild environments and…

  …disrupt ecological function

  …act like an invasive species

  …interbreed with wild conspecifics




www.akvagroup.com


# Farmed fish escape

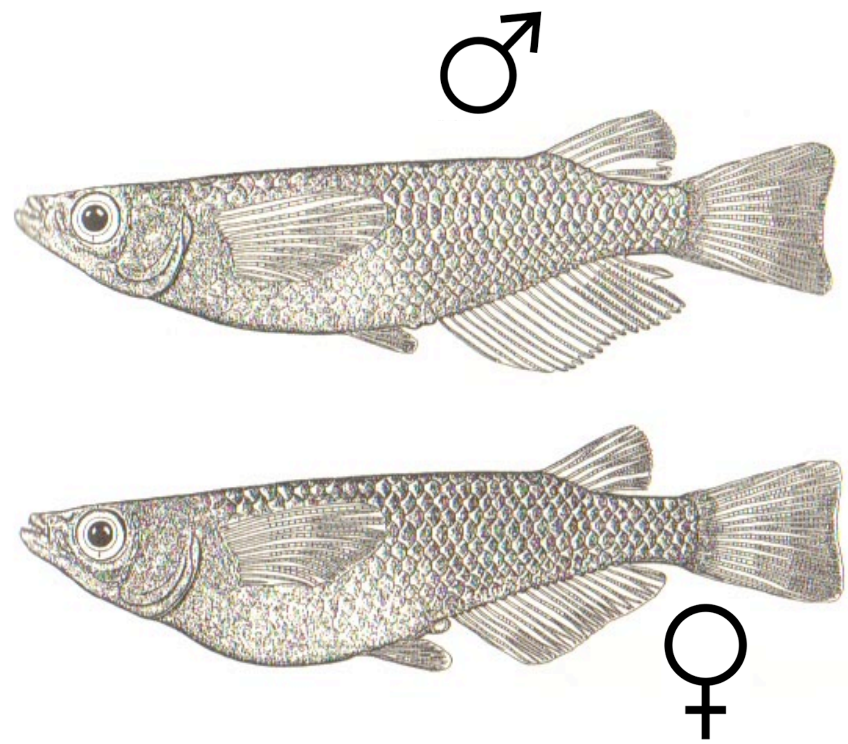
- Mate with wild relatives
- Gene flow from farmed to wild salmon has been shown to depress fitness (Hindar et al. 1991, McGinnity et al. 2003, Naylor et al. 2005)
- Gene flow can reduce ability of wild populations to adapt to environmental change (Hindar et al. 1991, Fleming et al. 2000)

# Will gene flow from transgenic fish will pose a risk to wild populations?

- Use confined population experiments to predict what might happen "in nature"

# Model organism - medaka

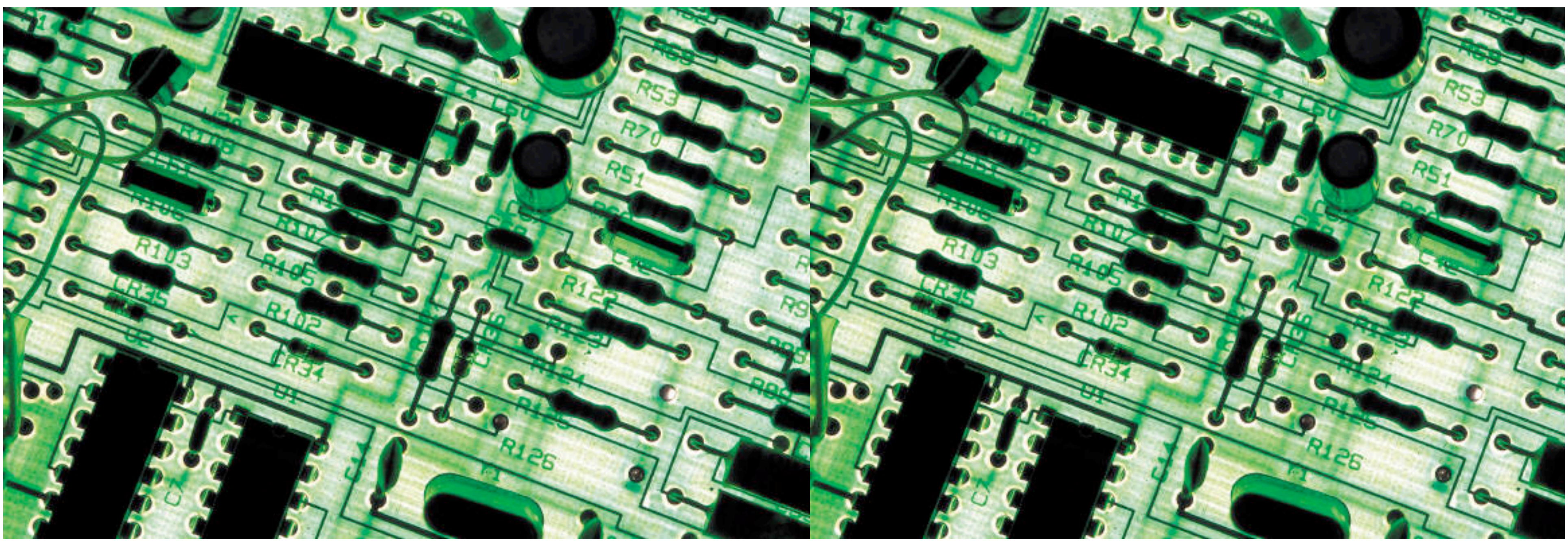
- Japanese medaka (*Oryzias latipes*)
- small (~ 3 cm), ~2 month generation time, sexually dimorphic
- wild-type (W)
- Transgenic (T): growth-enhanced, sockeye salmon growth hormone gene (Devlin 1993)



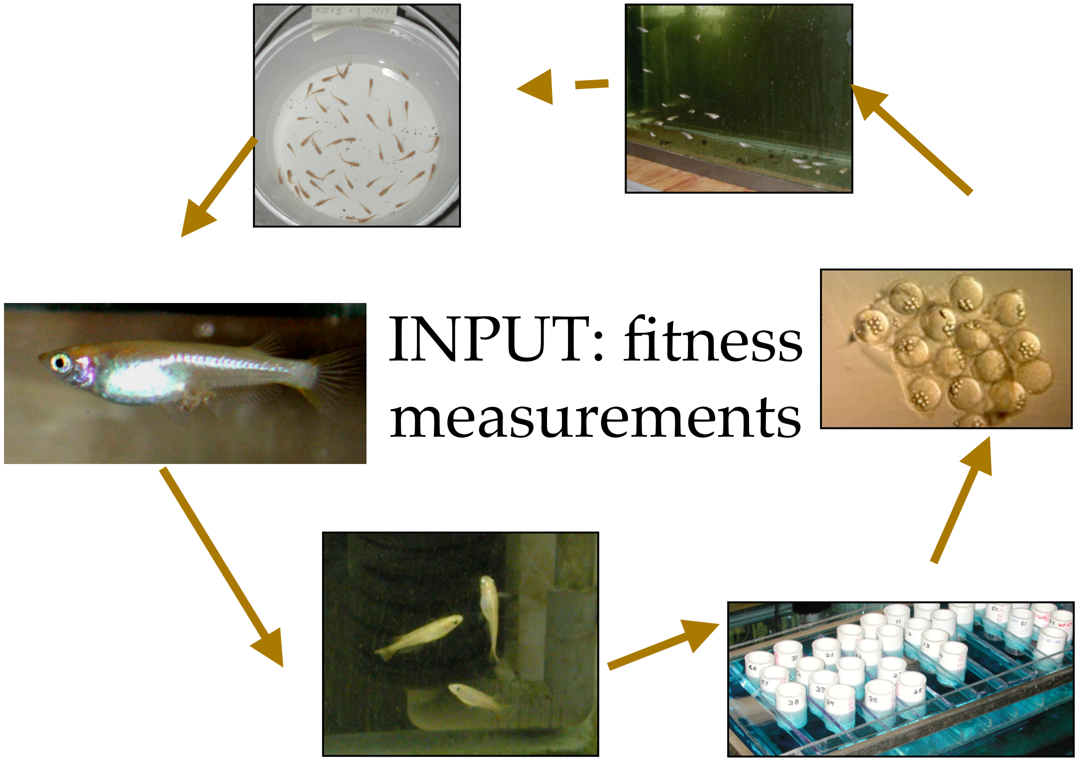
Iwamatsu 2002
(not to scale)

# Population simulation model

- Net fitness model uses fitness component traits to predict gene flow
  (Muir & Howard 1999, Muir & Howard 2001)



# Net fitness model




+ user specifies: initial population size & transgenic fish frequency, number of generations to run

→output:

→Population size of introgressed population

→Frequency of transgene in introgressed pop.

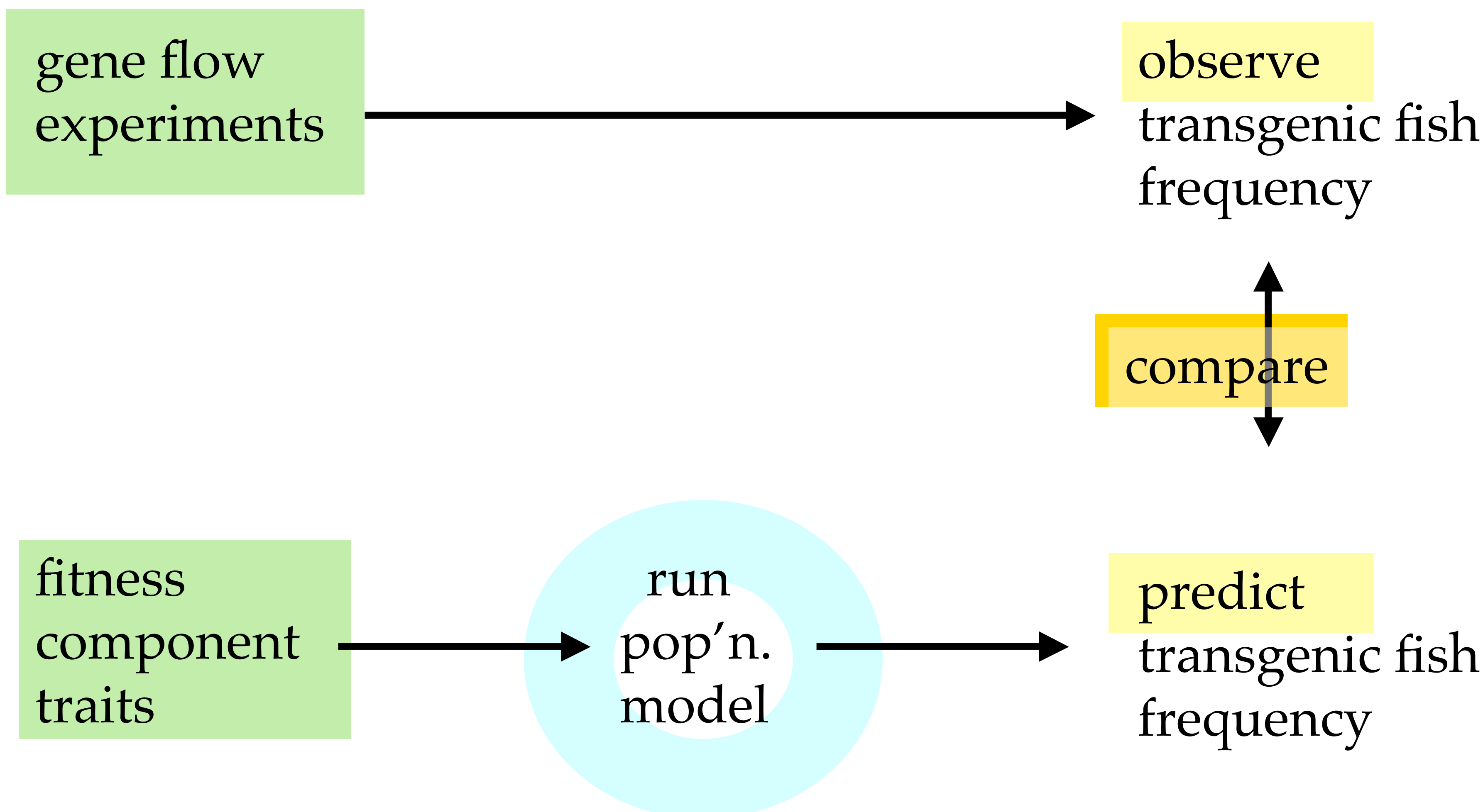
# Objectives

Test the effect of different environments on gene flow from T to W fish

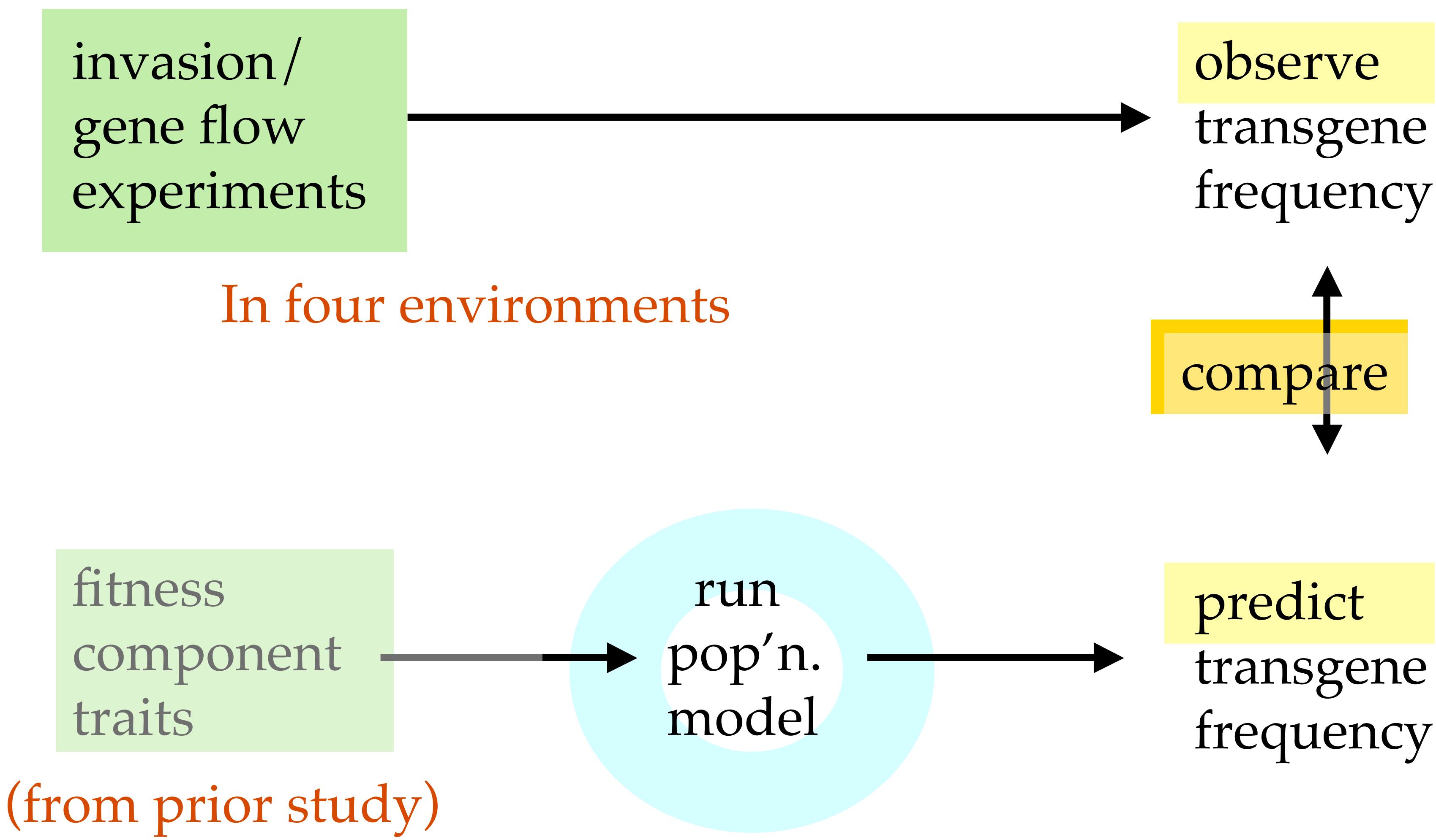
Create a demographic simulation model - parameterized with fitness data collected on medaka under the same environmental treatments

Compare model predictions of frequency of transgenic fish to results observed in populations under corresponding environments

# Conceptual model



# Conceptual model



# Addition of relevant ecological variables

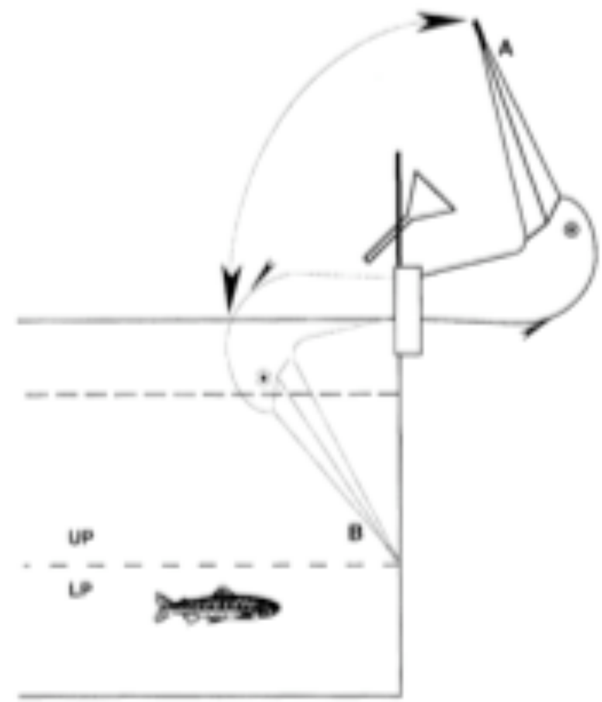
- Most important to growth-enhanced transgenic fish:
  - Food availability
    (Devlin et al. 1999, Devlin et al. 2004, Sundström et al. 2004, others)
  - Vulnerability to predation
    (Dunham et al. 1999, Jönsson et al. 1996, Woodley & Peterson 2003, Peckarsky et al. 2002)

# Relevant ecological variables

- **Food availability**
- Growth-enhanced T fish express higher feeding motivation (Devlin et al. 1999)
- Growth hormone T fish more voracious feeders than W siblings (Sundström et al. 2004)
- Populations with W and T coho salmon crashed under low food availability (Devlin et al. 2004)
- Predation

# Relevant ecological variables

- Food availability
- **Predation**
- Growth-enhanced T channel catfish less able to avoid predators (Dunham et al. 1999)
- Growth-hormone treated rainbow trout displayed higher feeding motivation under simulated predation (Jönsson et al. 1996)

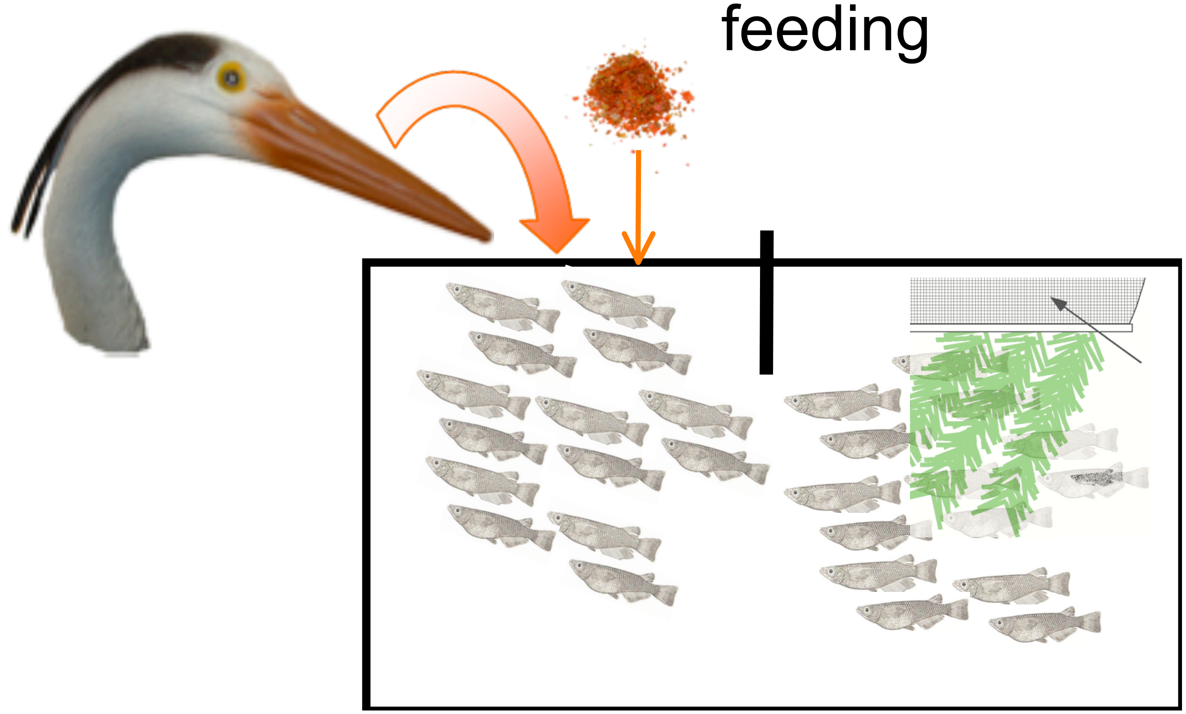


# Addition of relevant ecological variables: Food

| | | |
|---|---|---|
| Food satiating |  | fish fed 10% of body weight in flake food; 1 drop brine shrimp per fish |
| Food limiting |  | fish fed 3% of body weight in flake food; 1 drop brine shrimp per every 2 fish |

# Addition of relevant ecological variables: Predation

| Predation risk absent | Predation risk present |
| --- | --- |



Heron strikes into tank for 5 minutes after feeding

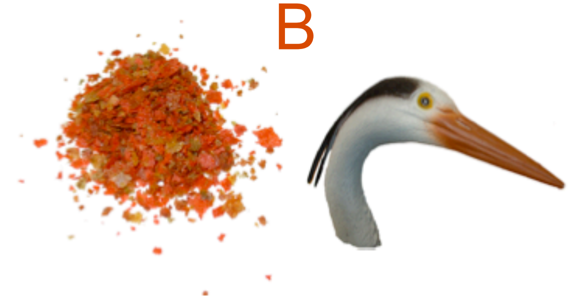
# Environmental treatments

| | Predation risk absent | Predation risk present |
|---|---|---|
| Food satiating |  |  |
| Food limiting |  |  |

# Environmental treatments

| | Predation risk absent | Predation risk present |
|---|---|---|
| Food satiating | A: “control”  |  |
| Food limiting |  |  |

# Environmental treatments

| | Predation risk absent | Predation risk present |
|---|---|---|
| Food satiating |  | B  |
| Food limiting |  |  |

# Environmental treatments

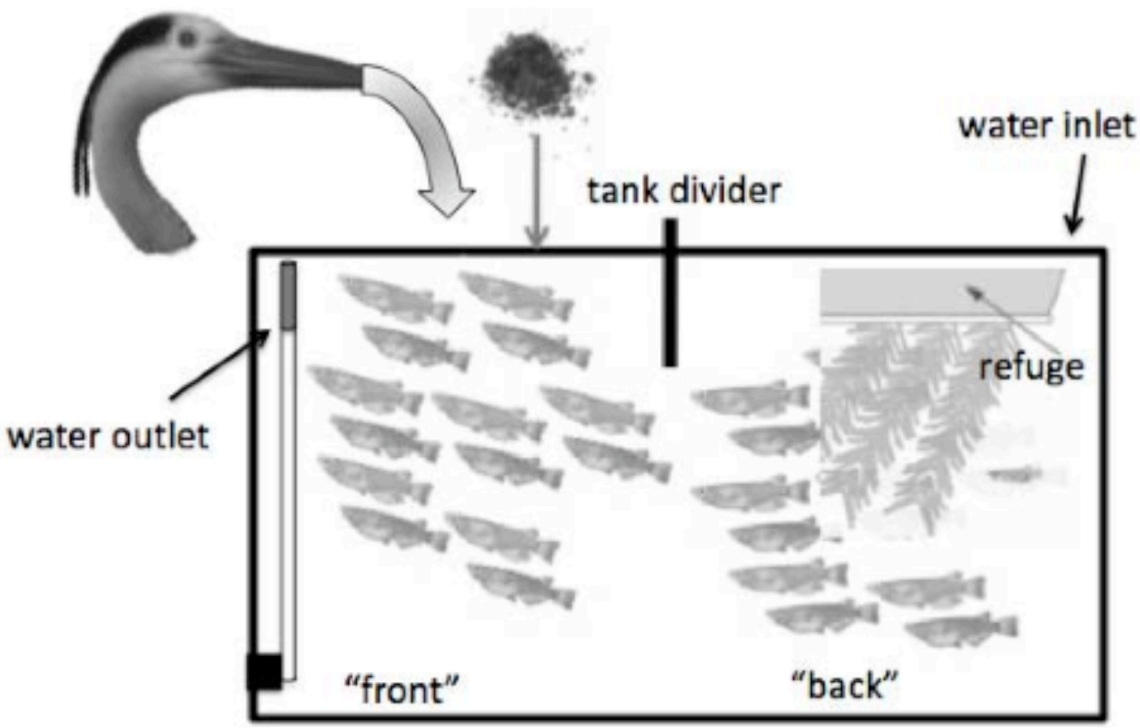
| | Predation risk absent | Predation risk present |
| --- | --- | --- |
| Food satiating |  |  |
| Food limiting | C  |  |

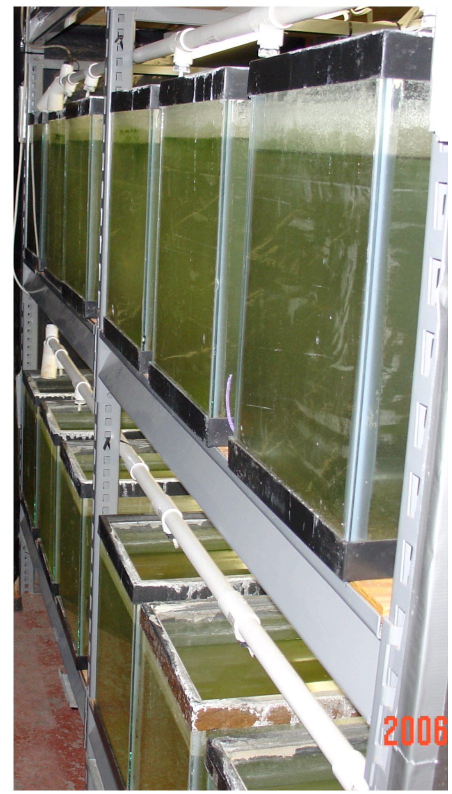
# Environmental treatments

| | Predation risk absent | Predation risk present |
|---|---|---|
| Food satiating |  |  |
| Food limiting |  | D  |

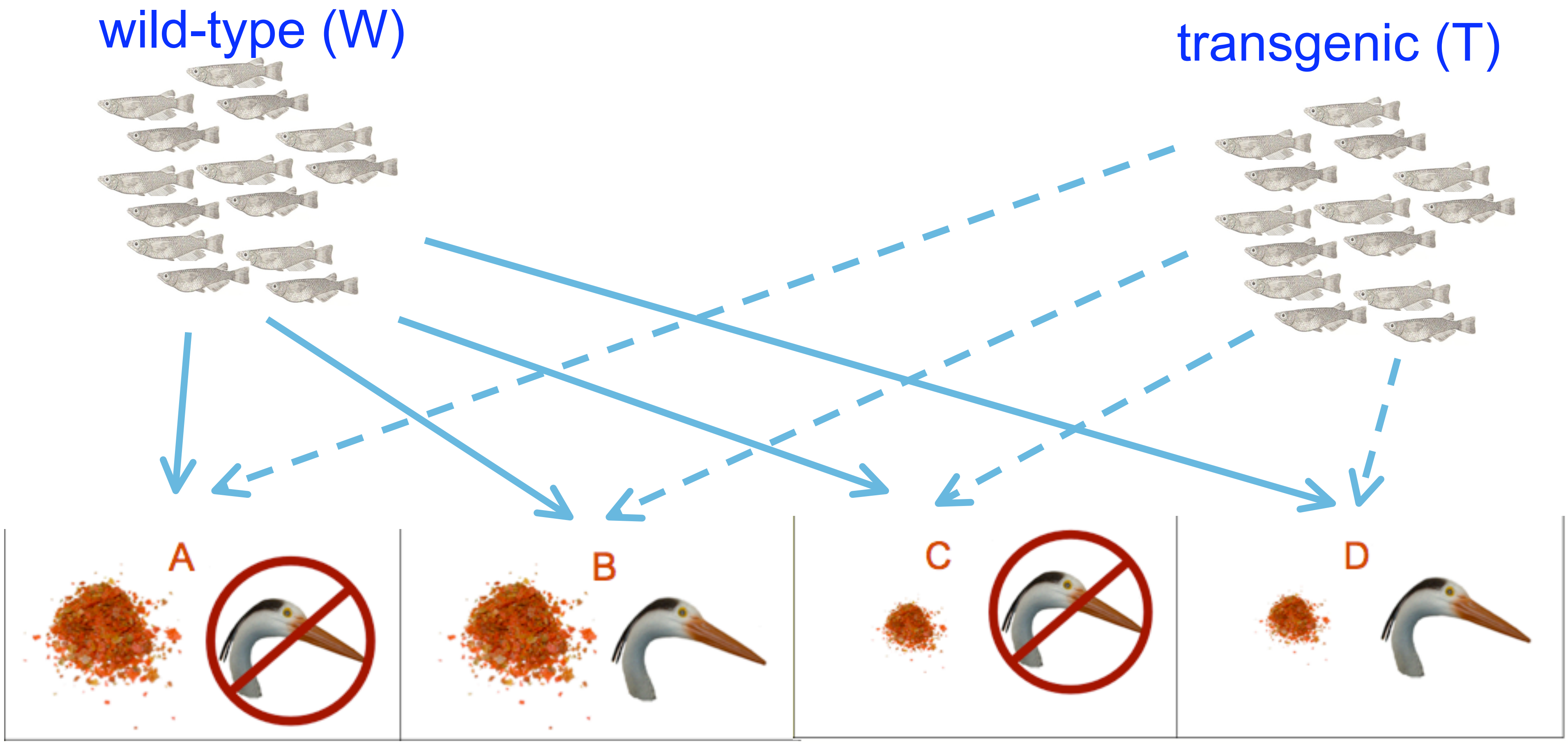
# Experimental units

- 24 30-gallon glass aquaria with shared water



# Gene flow experiment




24 aquaria, 6 aquaria/Env., 16 founders/ aquarium

Experiment lasted 210 days, periodic census, recalc. food

# Demographic model

- 4 Matrix population models, 1 for each Environment
- Modeled females, T and W and offspring of each parental cross in separate matrices
- Started with 24 females of each genotype *j*, 301-306 days old (*d*), max age = 700 days, timestep = 1 day (ran for 1000 days)

# Demographic model

- Sexually mature females produce eggs:

  $N_{d=0} = \Sigma( N_{j,\, d>s} * c_j /2 * r_k * m_k * o_{ijk} )$

- 7 days later, eggs hatch:

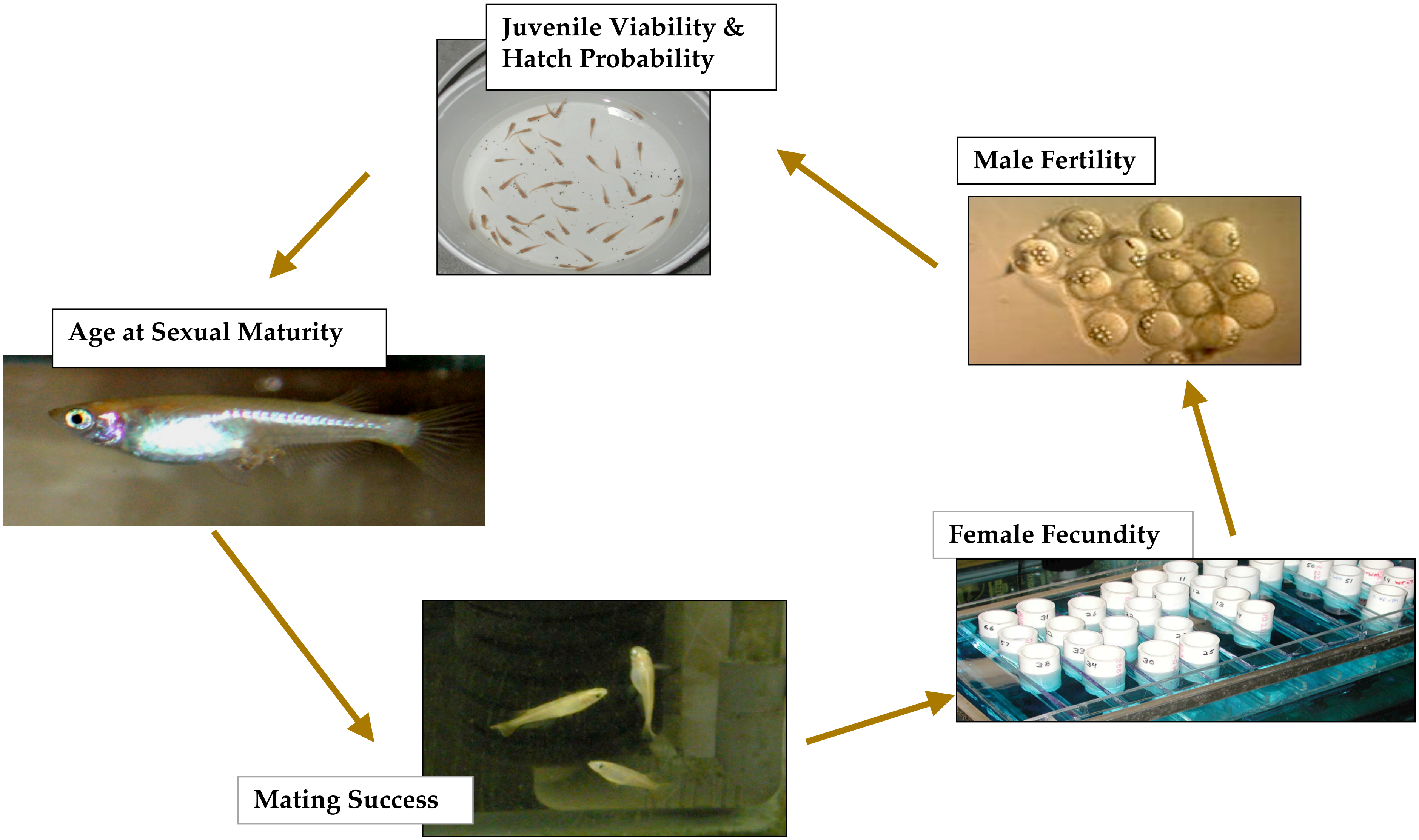
  $N_{d=1} = N_{d=0} * h_{jk}$

# Demographic model

- Reduced juvenile and adult populations with juvenile and adult daily viabilities

- Reduced fecundity from sexual maturity to 300 days with general logistic relationship (Schnute & Richards 1990)

# Demographic model inputs

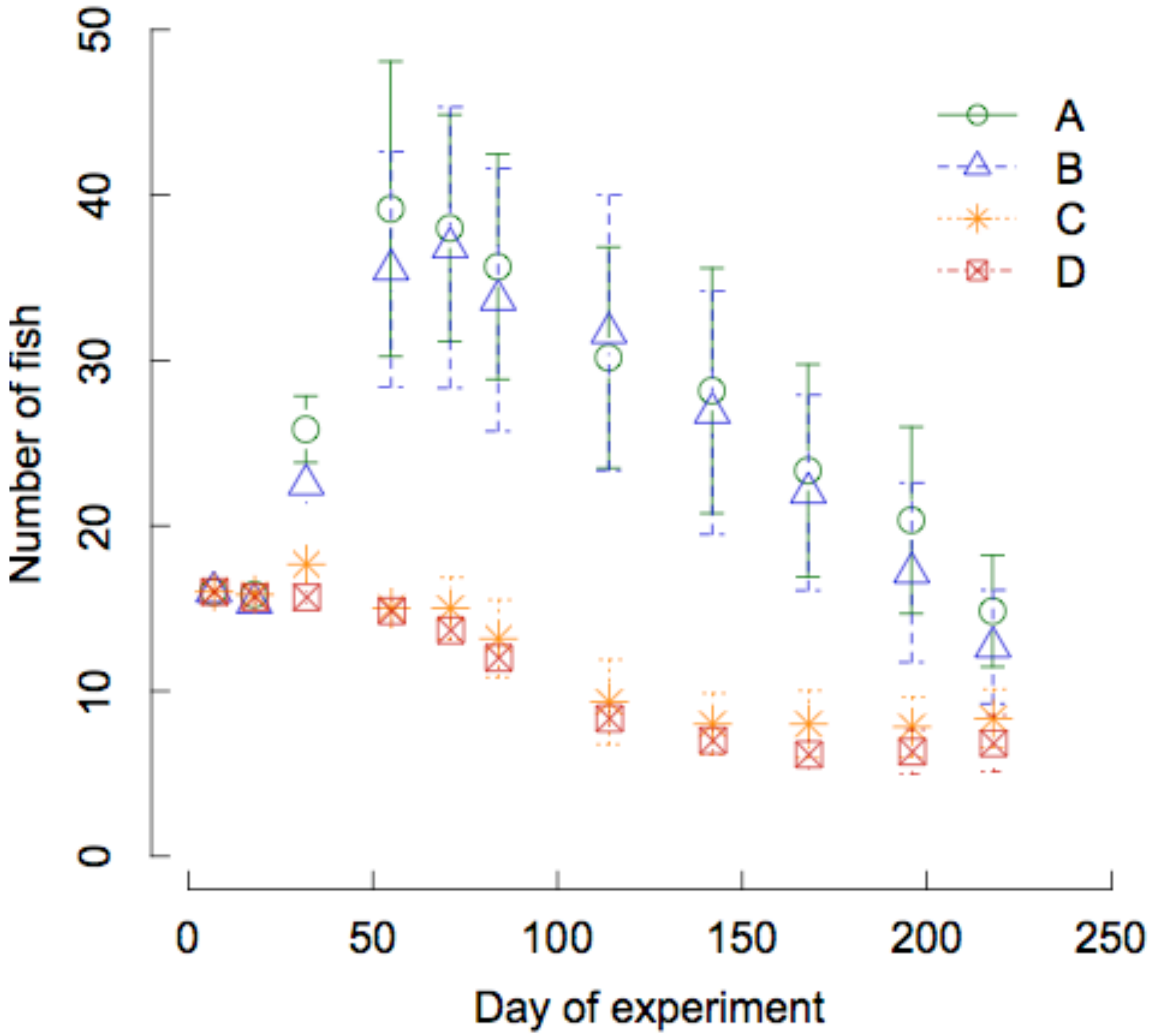


# Demographic model

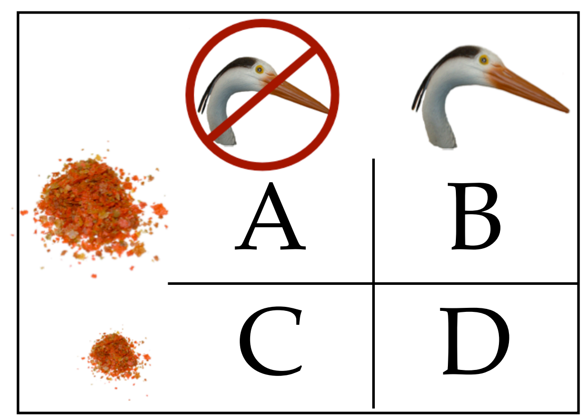
- Monte Carlo simulation to address parameter uncertainty (Regan et al. 2003, Burgman 2005, Hayes et al. 2007)
  - Varied adult and subadult fecundity, male fertility, mating probability, juvenile viability, and hatch proportion over beta distributions using observed variation
  - 1000 iterations per environment

- Model outputs: deterministic and Monte Carlo, transgenic fish frequency & pop. size at $t = 210$ (~experiment's end) and $t = 1000$ days

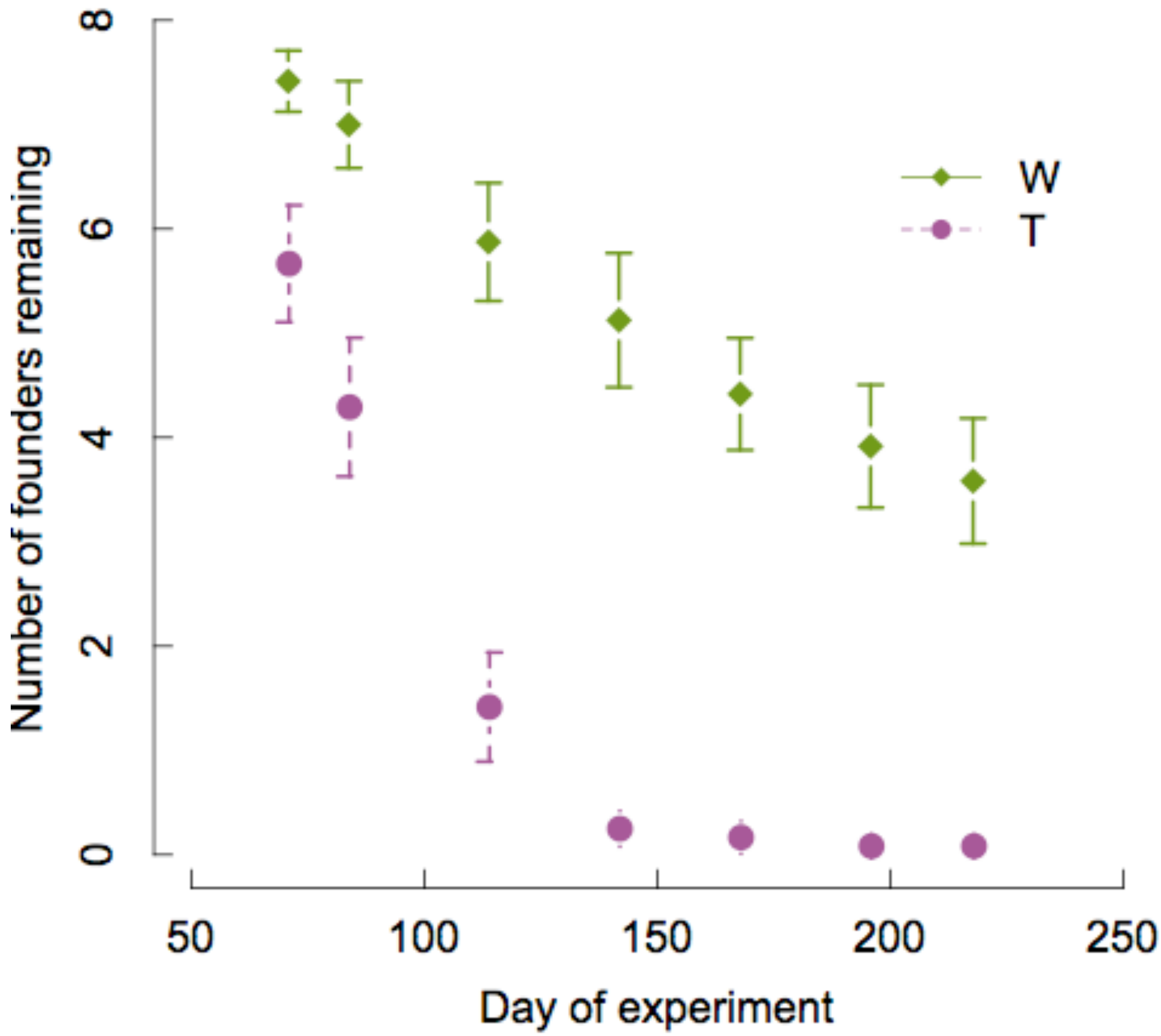
# Gene flow experiment




Pop. size increased in A, B; relatively stable at end

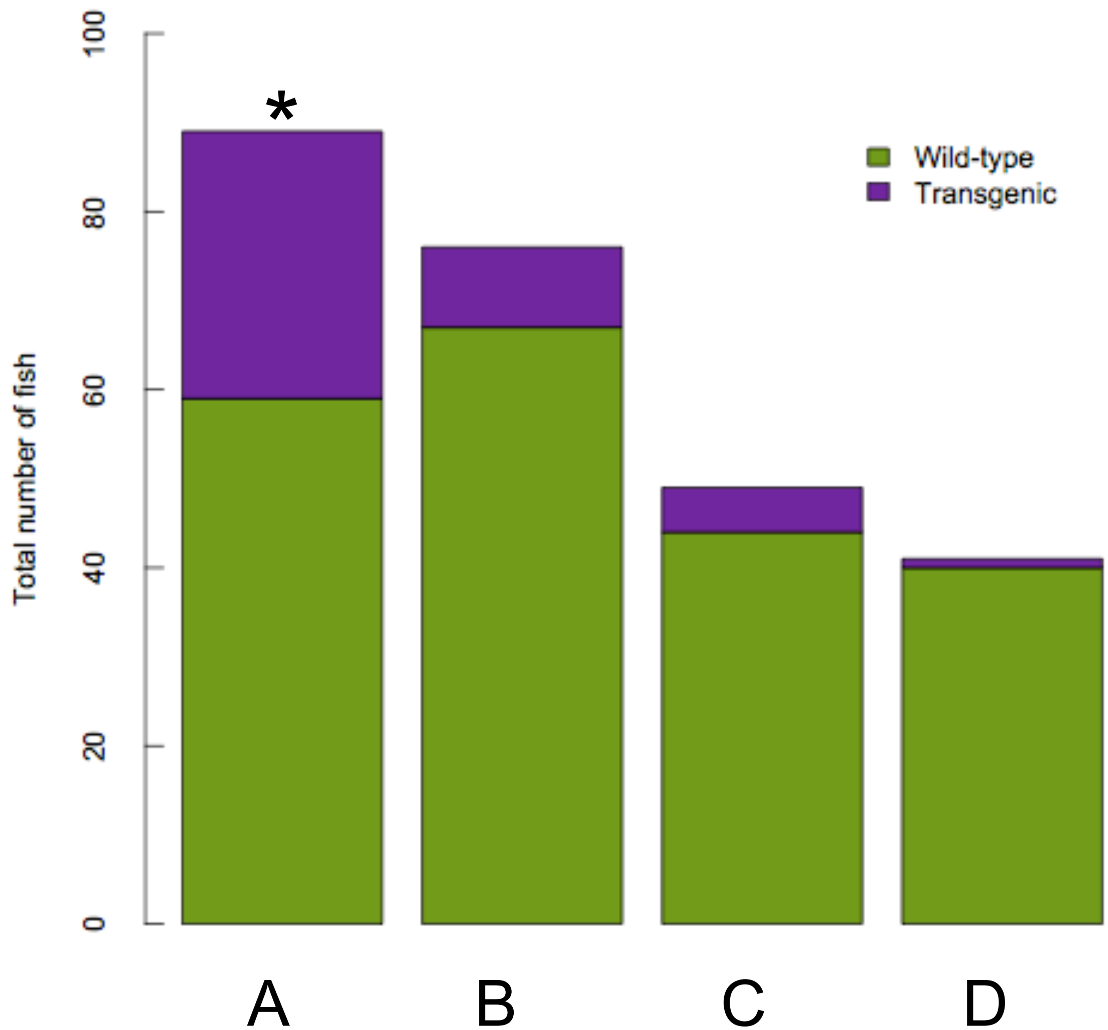


# Gene flow experiment

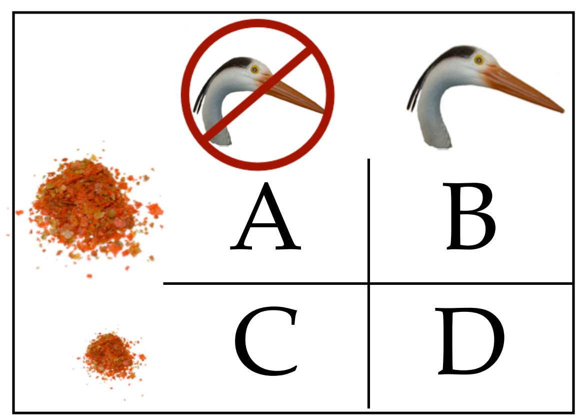



T founder fish died off more quickly than W

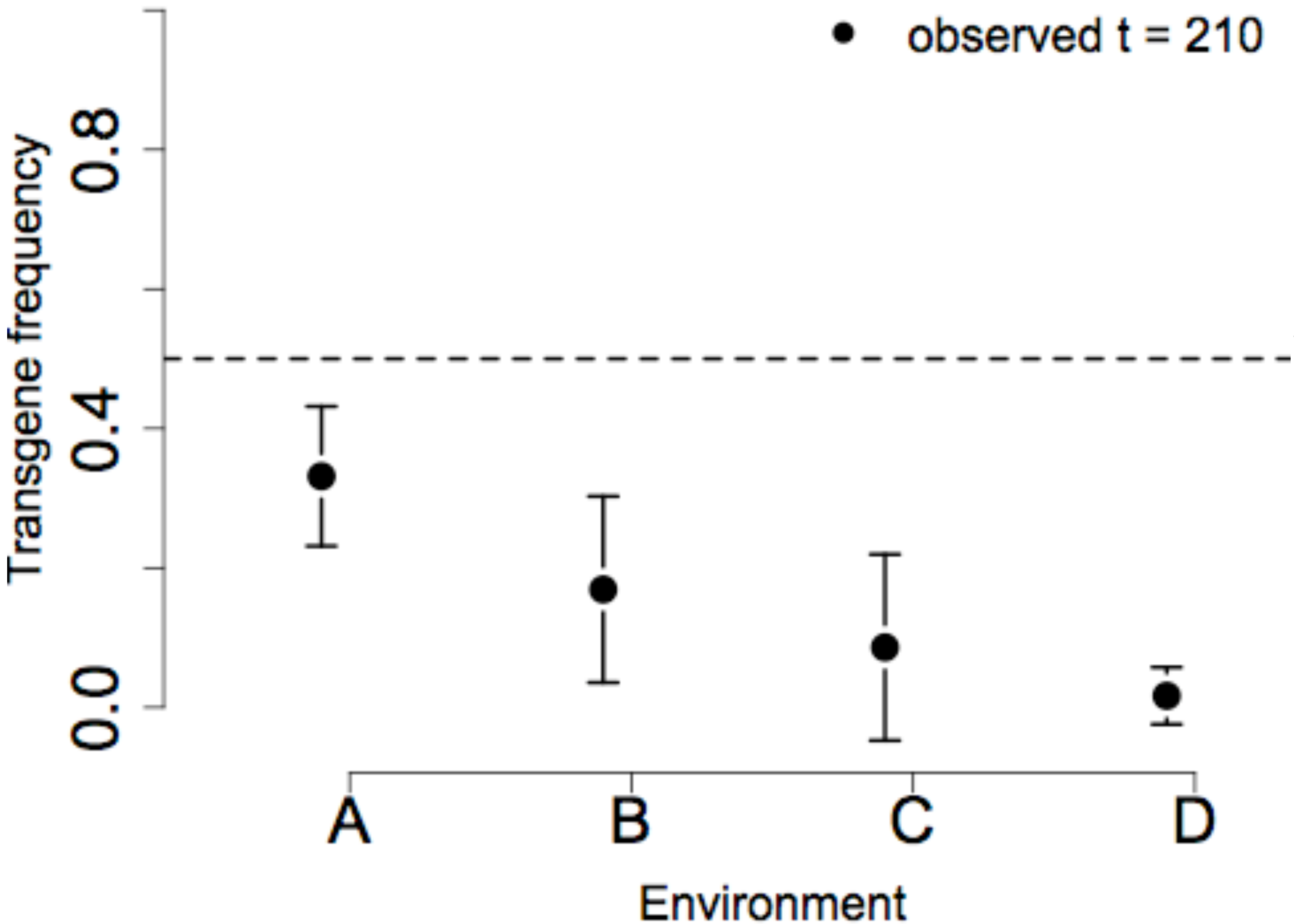
# Gene flow exp. - pop @ 210 days




* number of T fish in Env. A > B, C, or D (all $p < 0.023$)



# Gene flow exp. - T freq @ 210 days

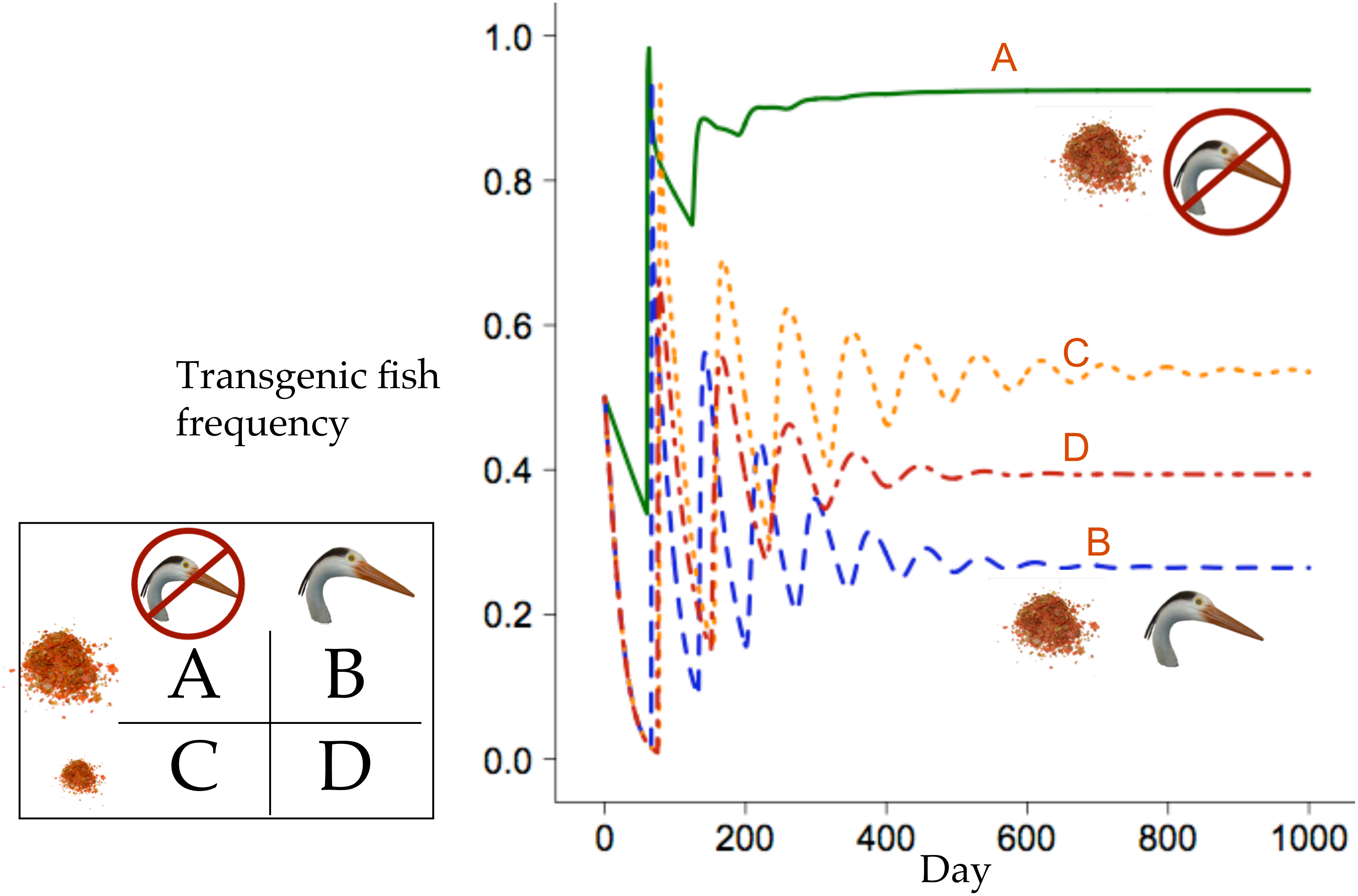



Means ± SD

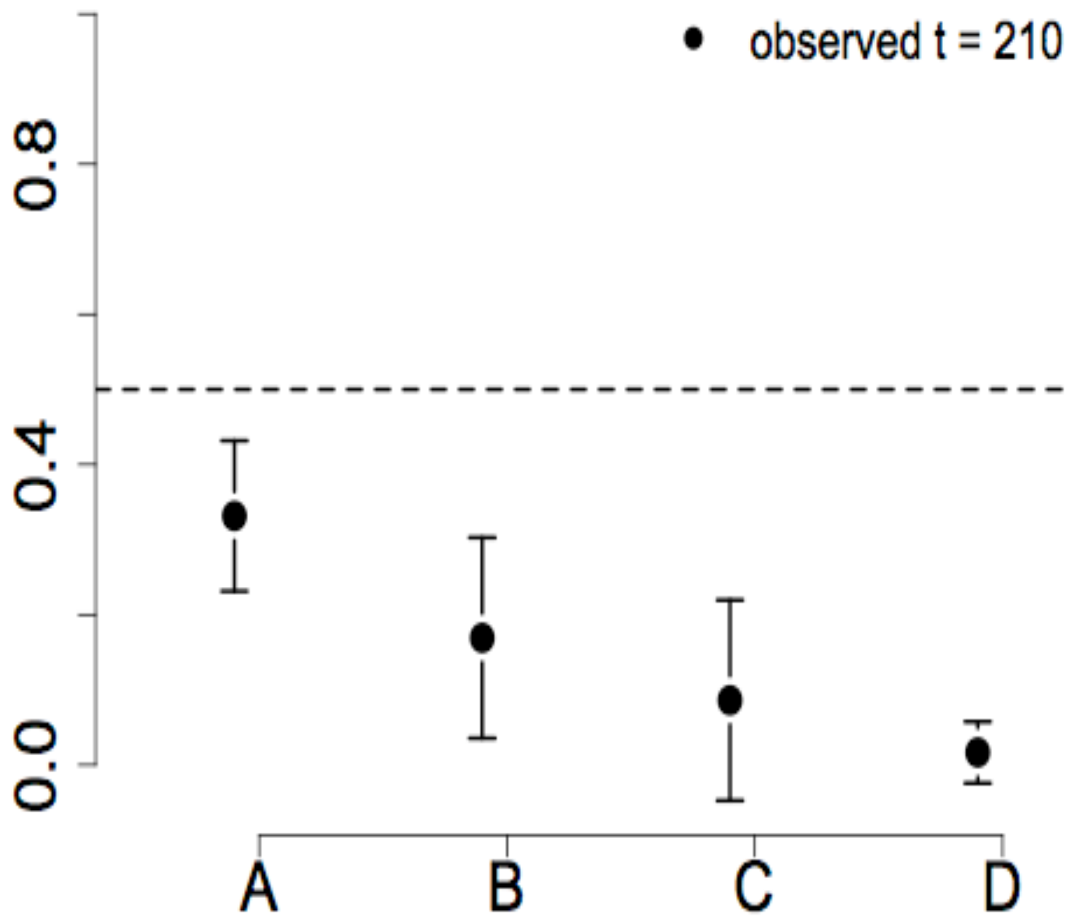
Dashed line = starting T freq 0.5

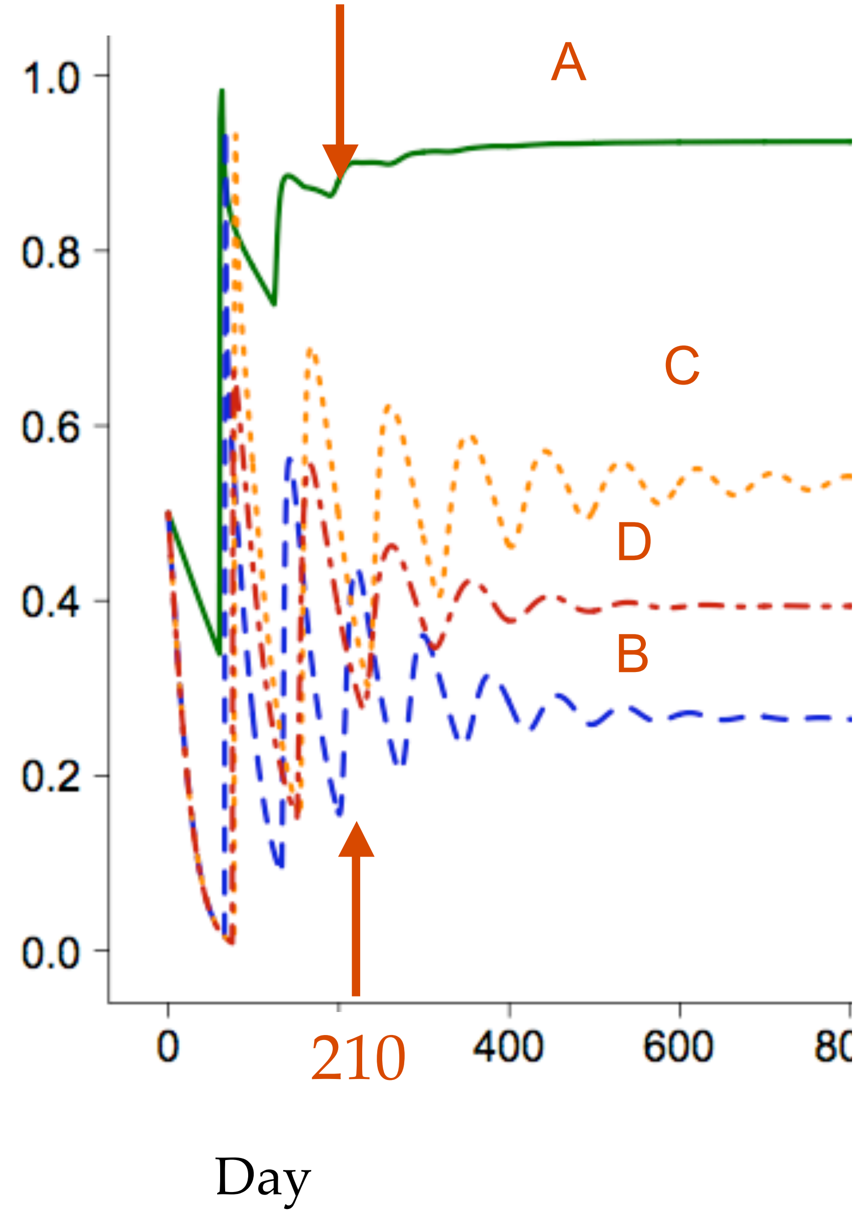
A > C or D (both $p < 0.01$)

# Deterministic model - T frequency



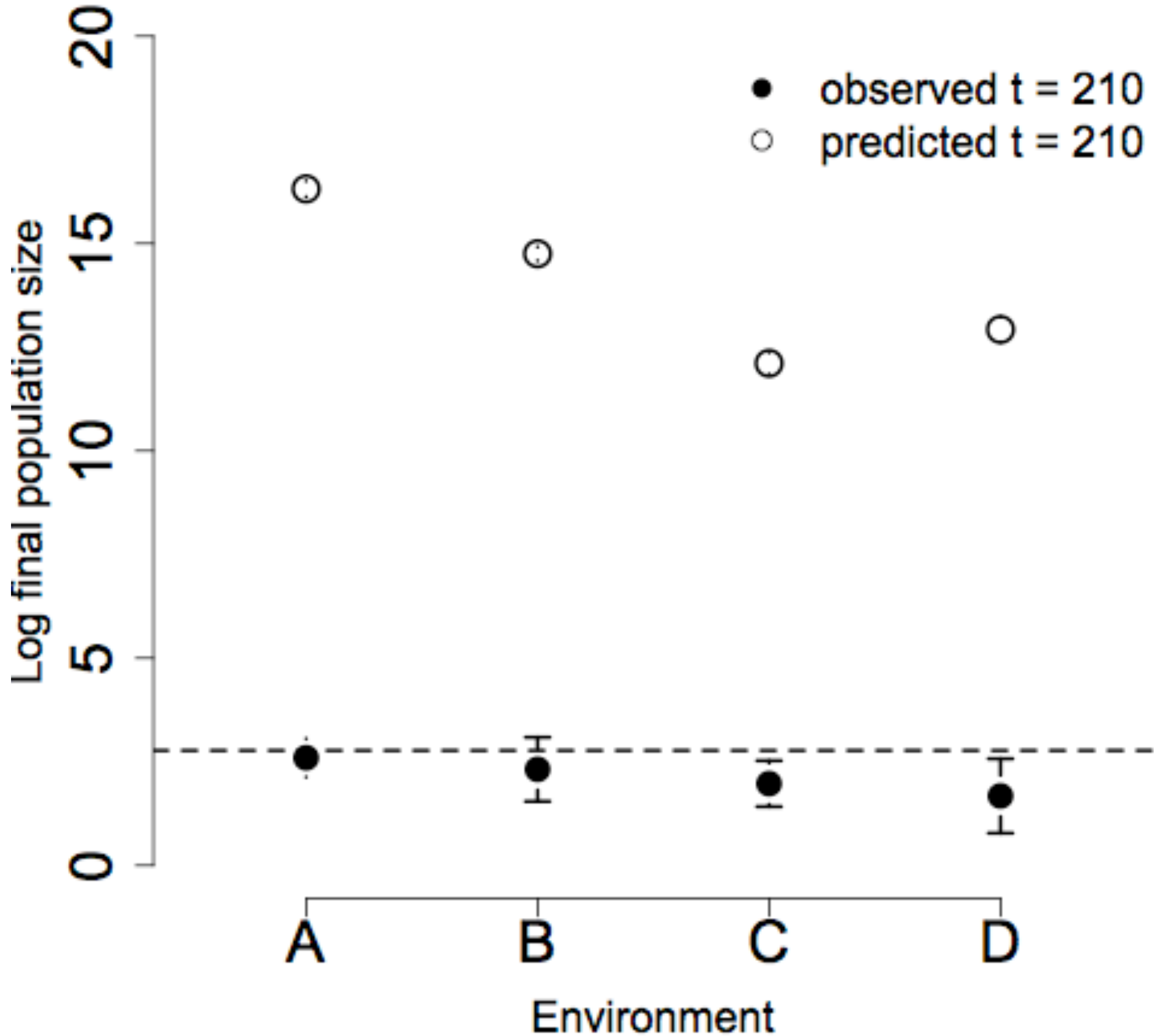
# Deterministic model - T frequency

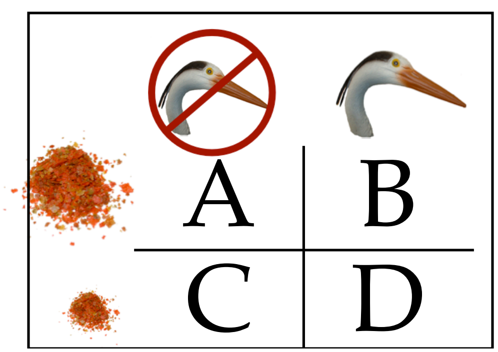


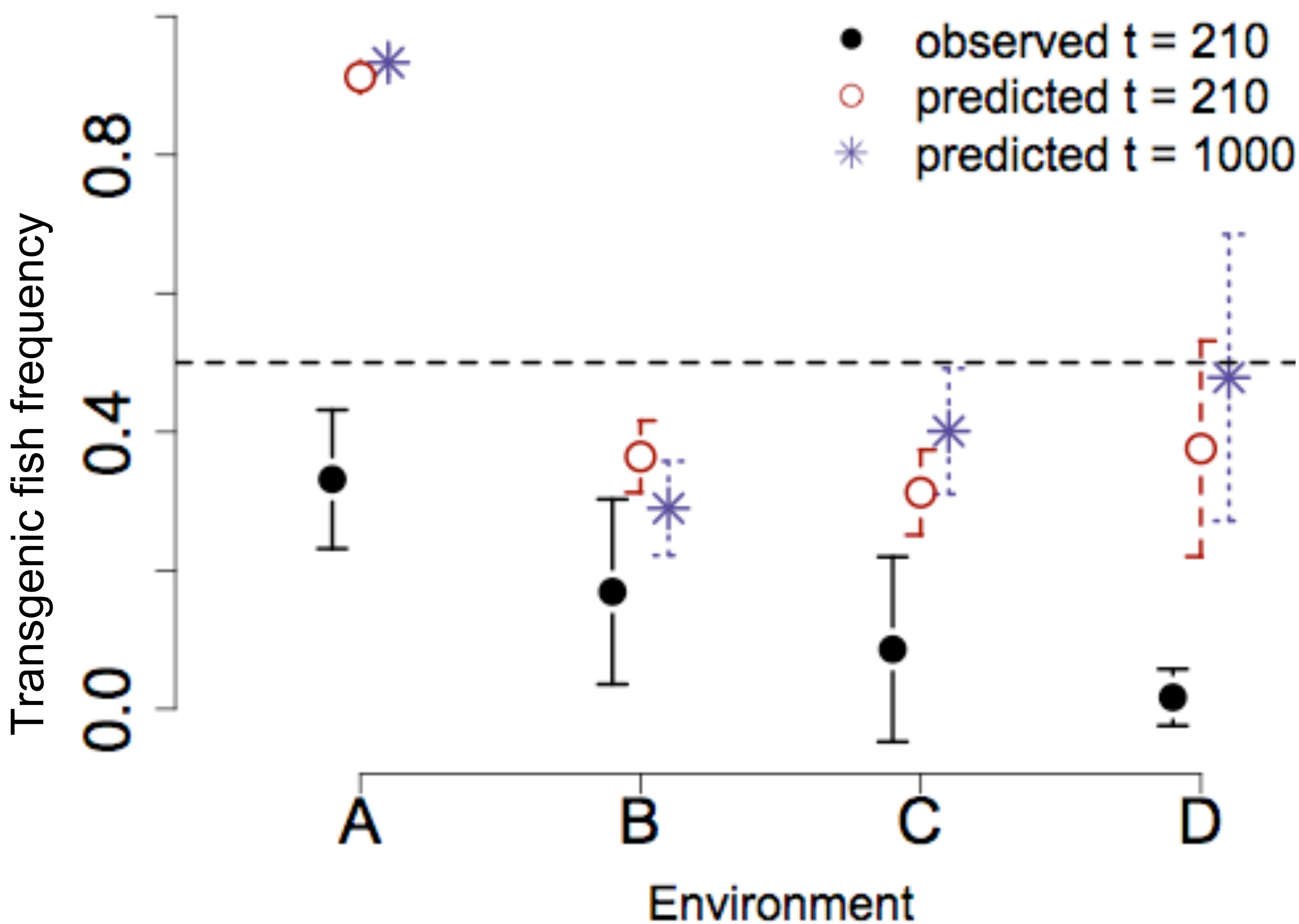
# Stochastic model - pop size

Log (Population size)



# Stochastic model - T frequency



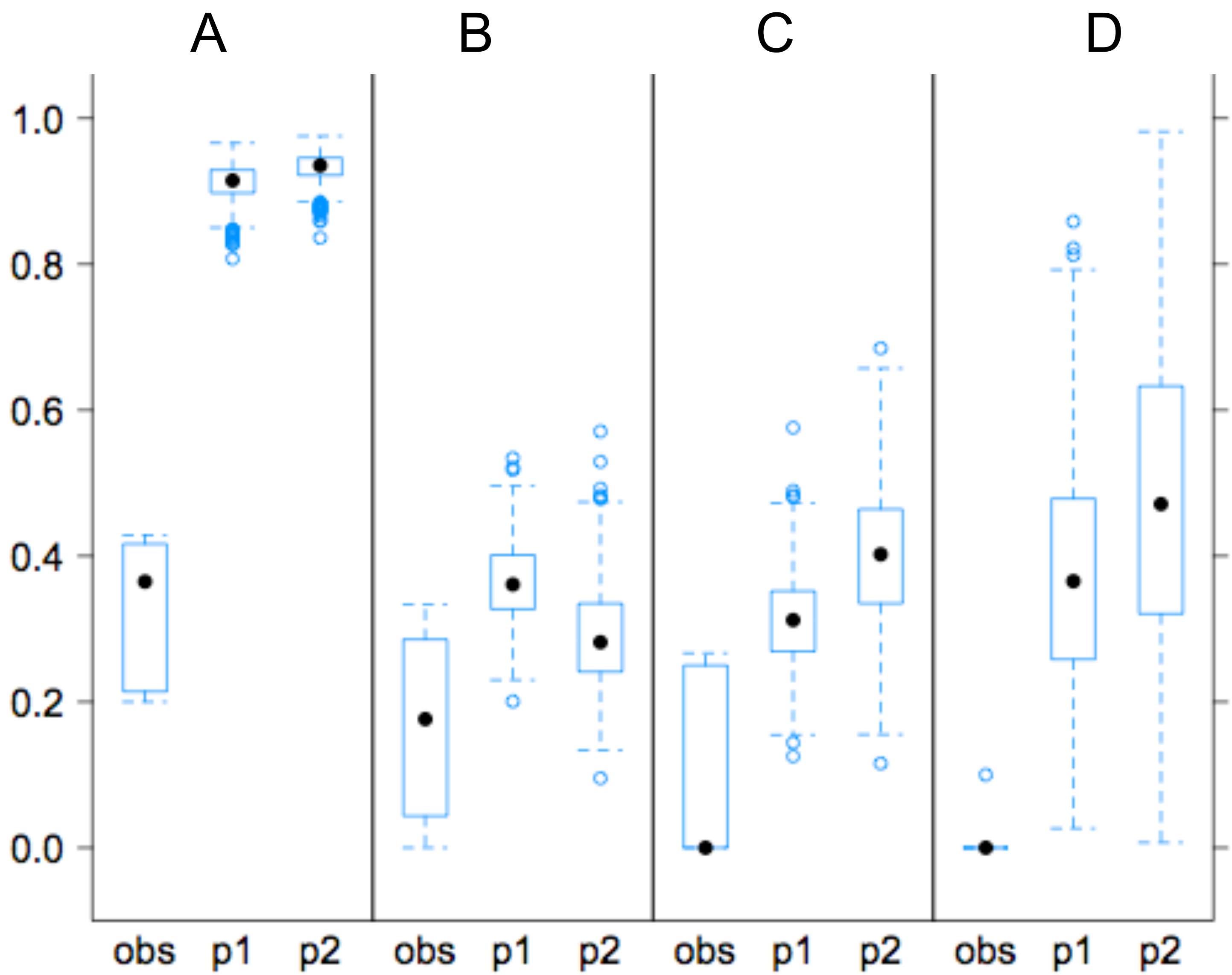

Means ± SD

Dashed line = starting T freq 0.5

# T freq. - observed & predicted

Transgene frequency

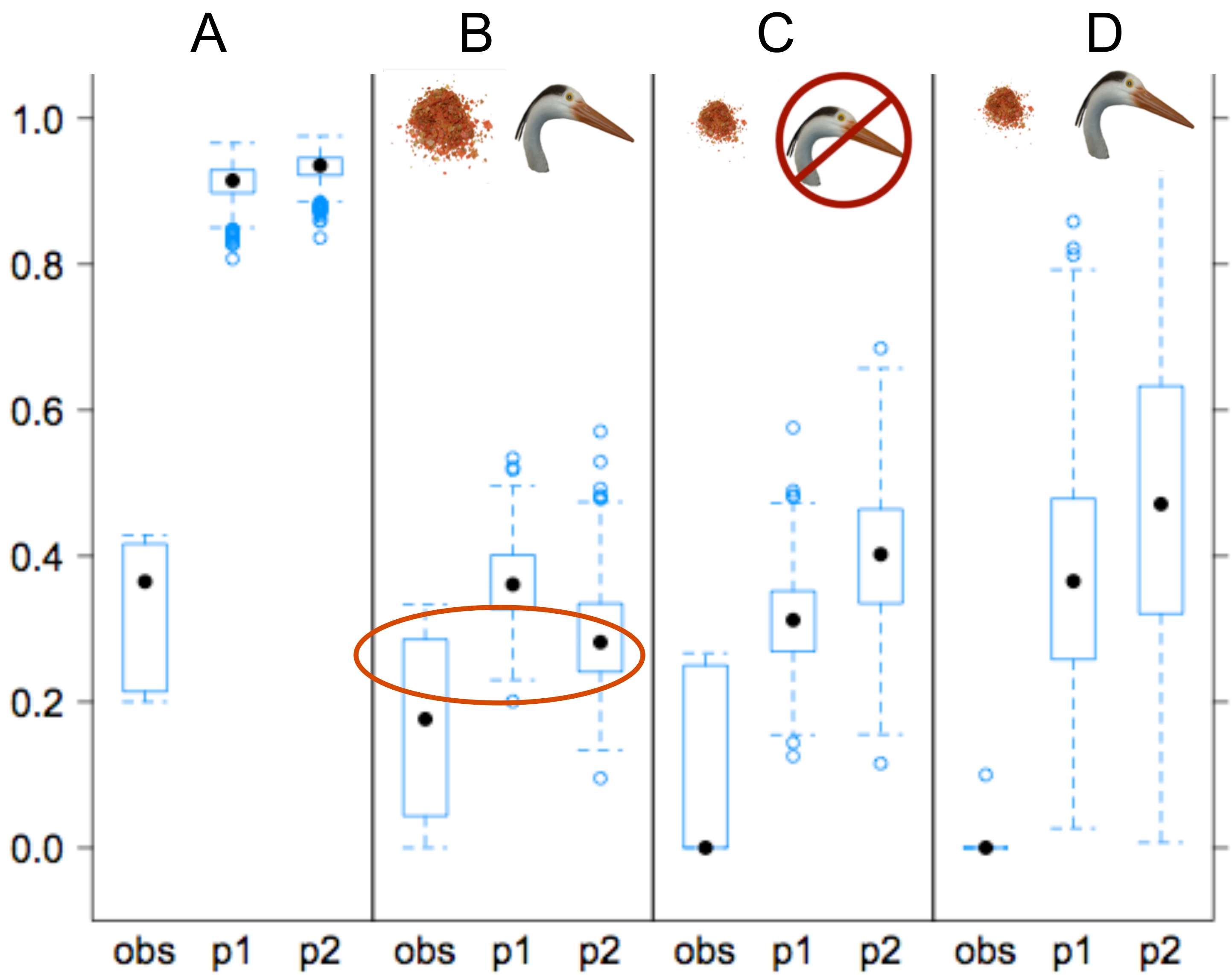
obs @ $t = 210$,
pred @ $t = 210$,
pred @ $t = 1000$



# T freq. - observed & predicted

Transgenic fish frequency

obs @ $t = 210$,
pred @ $t = 210$,
pred @ $t = 1000$



# Recap

- Gene flow experiments w/  & 
  - Reduced survival of T founders
  - Highest T population in Environment A

# Recap

- Gene flow experiments w/  & 
  - Reduced survival of T founders
  - Highest T population in Environment A
- Model of gene flow w/  & 
  - Model predictions better confirmed by data in moderate environments
  - Stochastic model to address parameter uncertainty

# Concluding remarks

- Using fitness component data to predict gene flow may be a promising approach
- *But* model predictions based on fitness trait data from a limited range of environmental conditions may be misleading

# Conclusions in context

- Models can never be correct, but they can be useful
- Models are likely to be used in risk assessment of transgenic fish
- So models should at least incorporate uncertainty about fitness parameters and information from different environments

Thank you!


FIN

Let no one tell you the medaka is uninteresting and unbeautiful. And so far as good qualities for the aquarium go, the medaka can challenge even such a redoubtable fish as the guppy! Champion of the egglayers, I call the medaka!

(Myers 1952: p. 194)

www.kumagaya.or.jp/~tack/medaka/naze.htm

# Acknowledgements

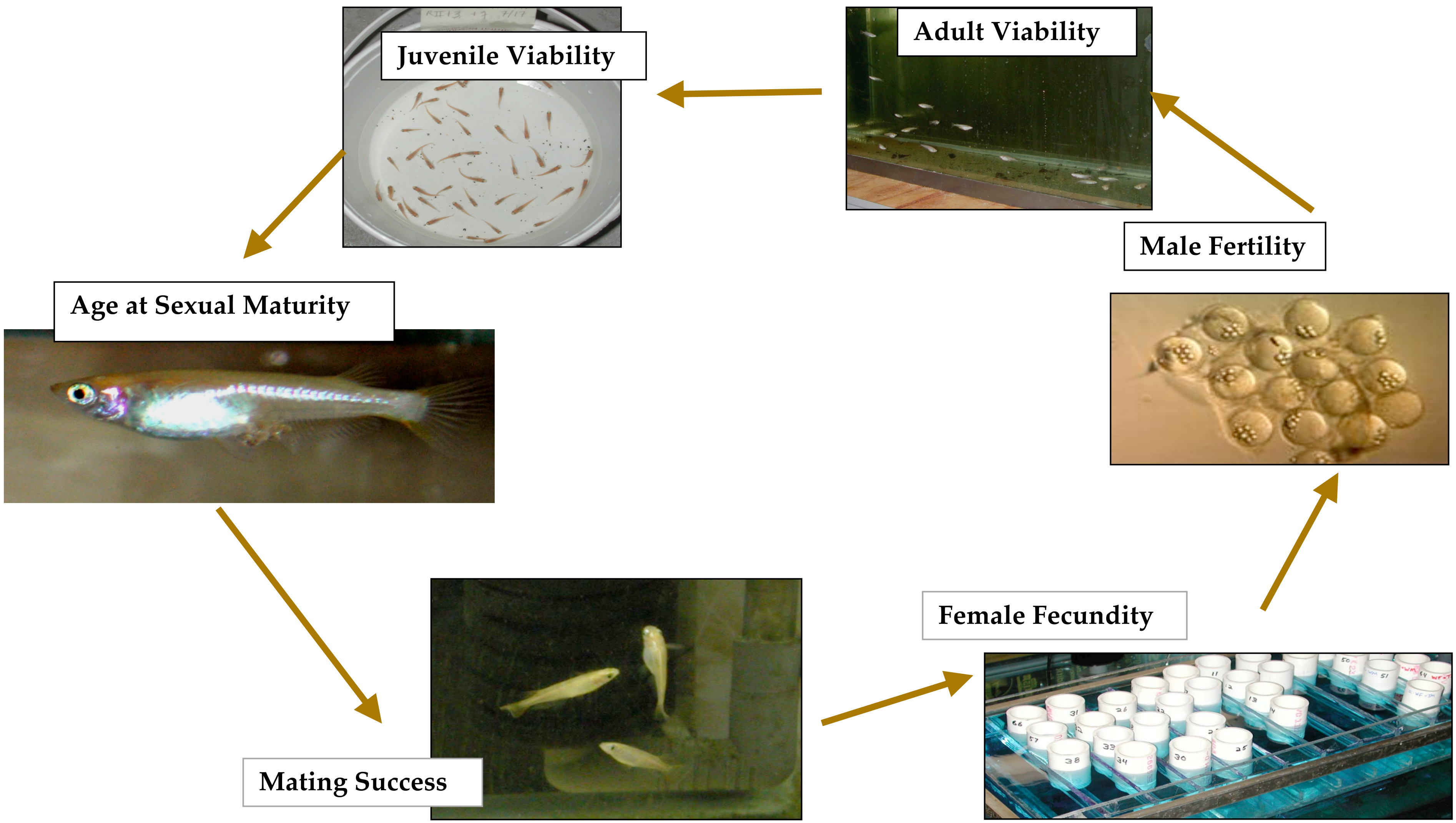

- Anne Kapuscinski, Adviser
- Committee: Jim Curtsinger, Nick Jordan, and Karen Oberhauser
- Aquaculture lab: Jay Maher
- Aqua-Gen lab: Loren Miller
- Mike Morton, Anne Cooper, Bryan Doyle, Ozge Goktepe, Kristen Maccaroni, Aaron McFarlane, Mike Michaelis, Seth Miller, Tyler Pavlowich, Andy Tri.
- Lab: Deborah Brister, Dave Caroffino, Anne Cooper, Genya Dana, Lorissa Fujishin, Mark Hove, Leah Sharpe.
- Tony Starfield, Sandy Weisberg, Aaron Rendahl
- Robert Devlin (University of BC); William Muir and Richard Howard (Purdue)
- Beth Harris and Suelee Robbe-Austerman (USDA)

# Predation and food limitation influence fitness traits of growth-enhanced transgenic and wild-type fish

Objective:

To test whether fitness trait values of W and T fish are affected by different environments

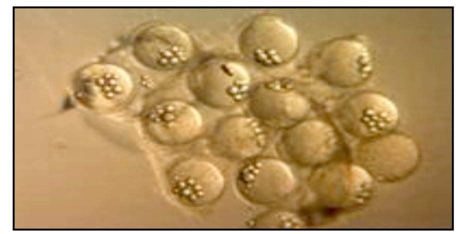
# Net fitness model inputs



# Fitness component measurements

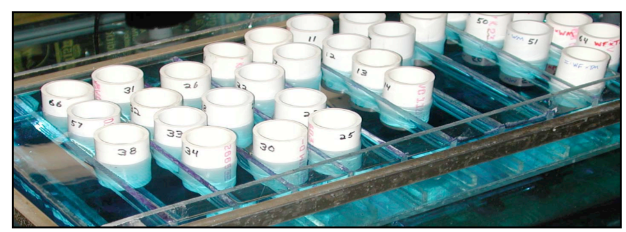
- Fecundity - eggs produced over 10-day trial
- Fertility - proportion of eggs developing 24 hours after copulation
- Mating advantage - T v. W male, successful copulation with W female

**Male Fertility**



**Mating Success**

**Female Fecundity**



# Fitness component measurements

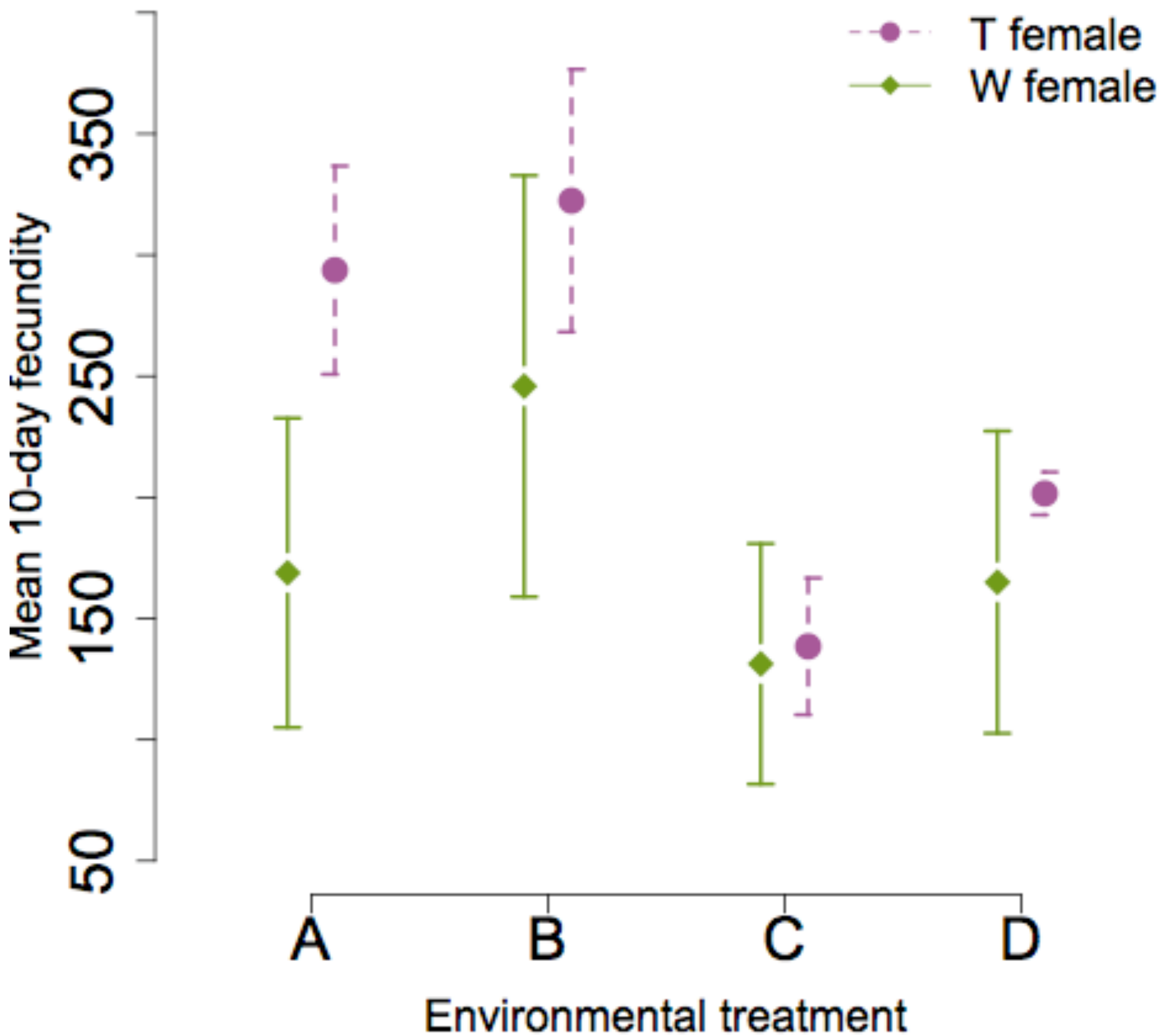
Hatch proportion x


= survival to maturity



- Age at sexual maturity - 1st to <u>9th</u> females to bear fertile eggs
- Hatch proportion x juvenile viability = survival to maturity

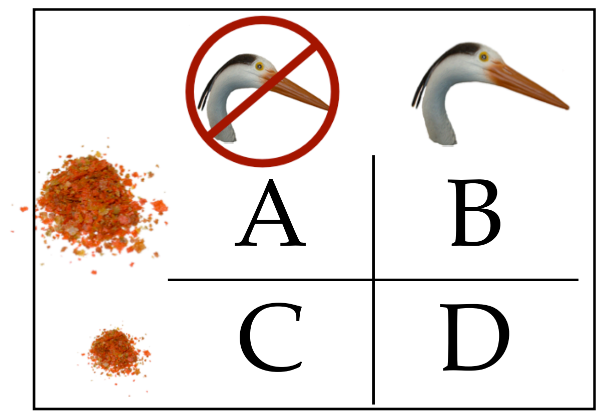
# Fecundity




T > W ($p = 0.023$)

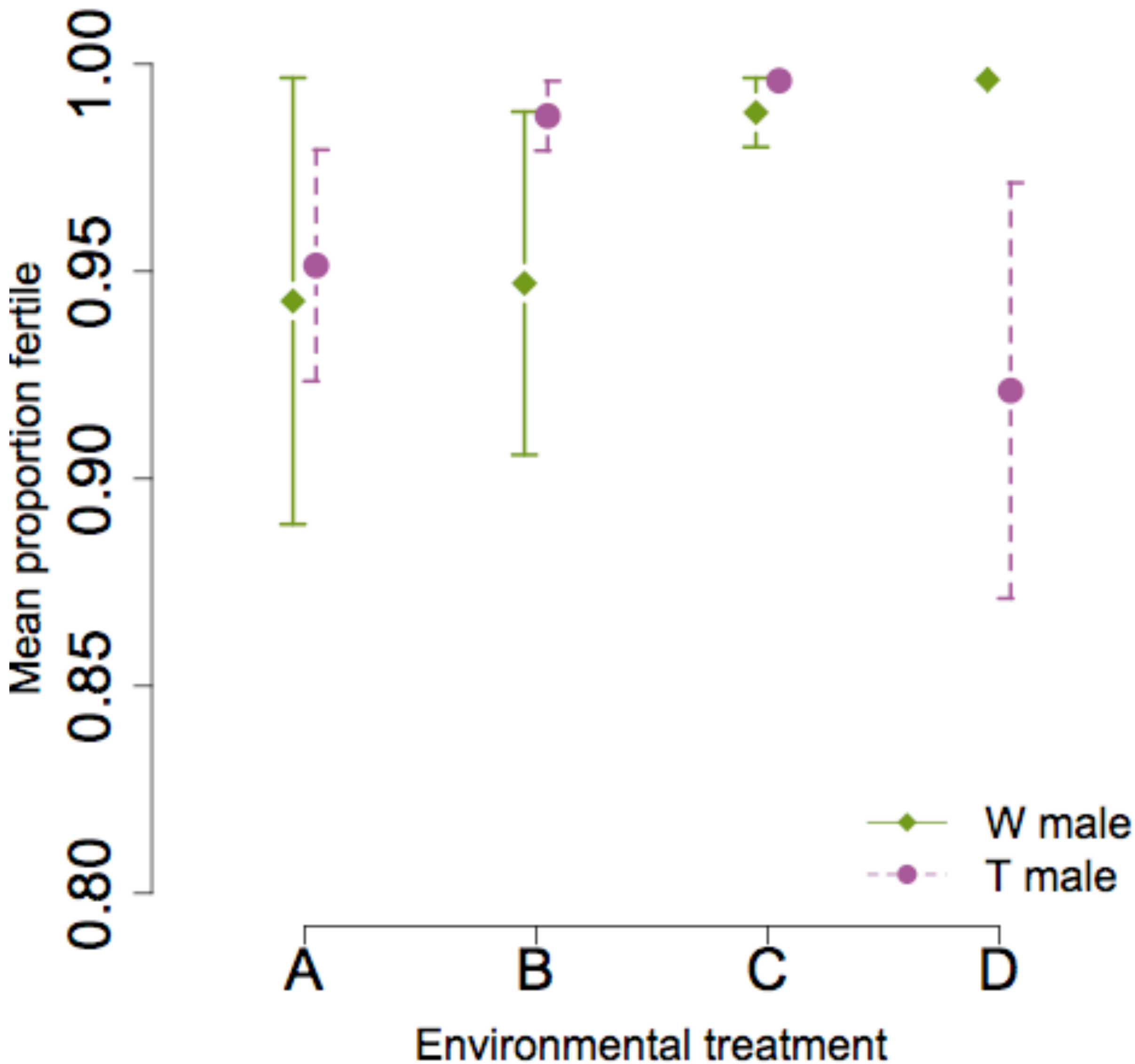
B > C & D ($p < 0.026$)

No statistical G x E

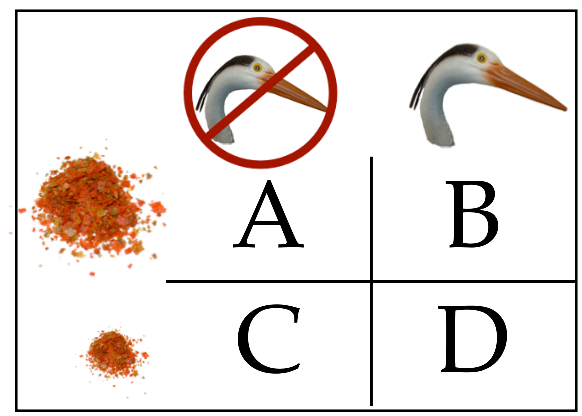



Means ± SE

# Fertility

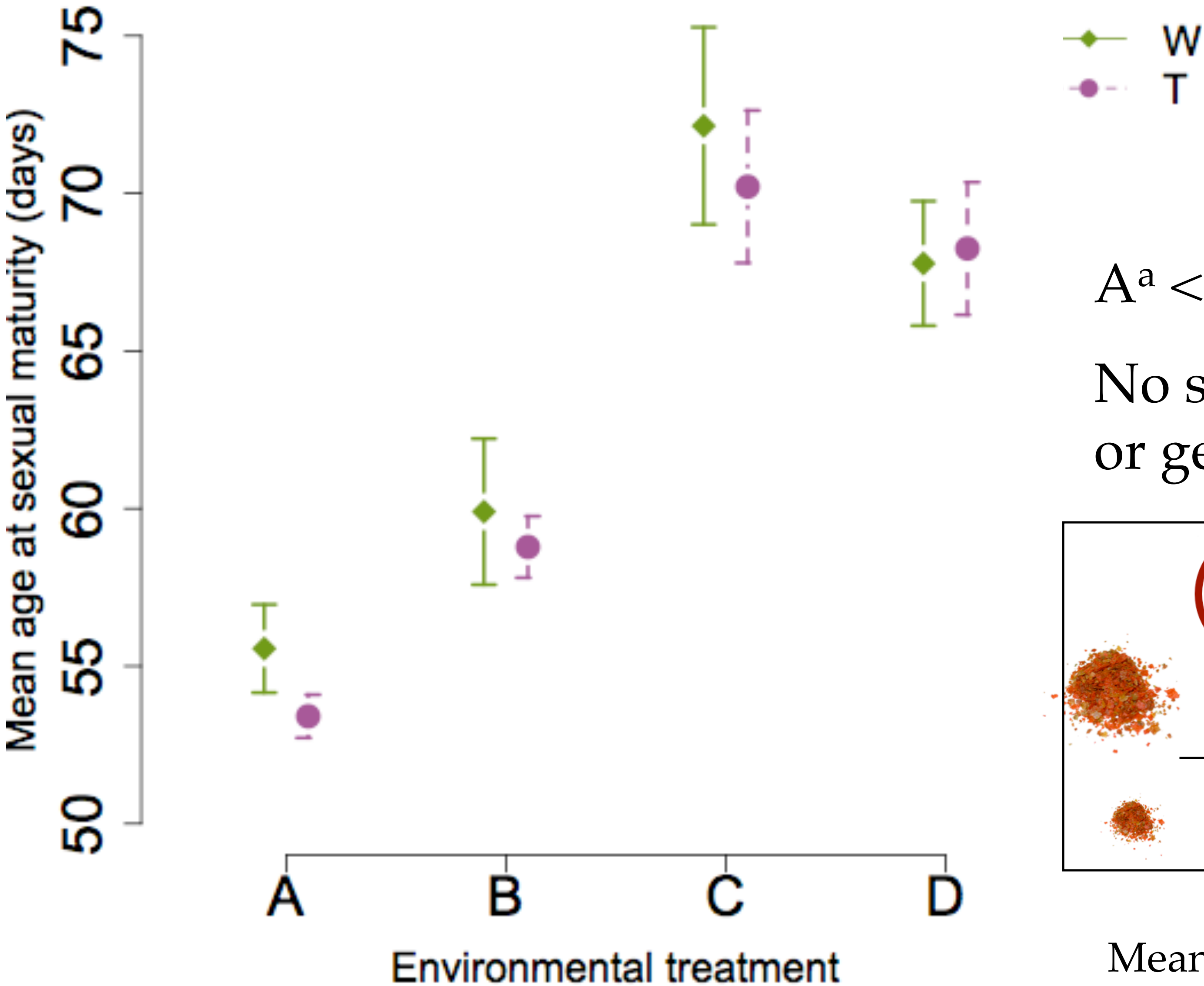



Statistical G x E in model



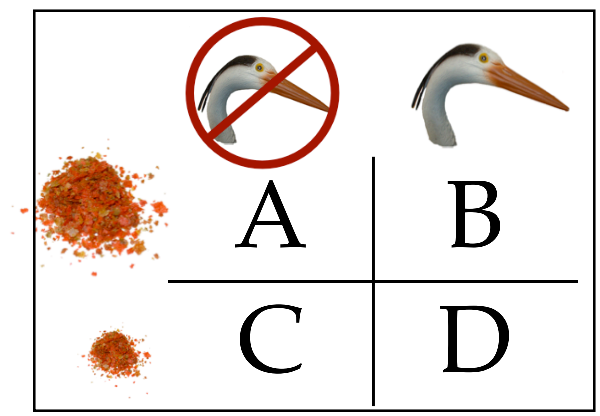

Means ± SE

# Age at sexual maturity



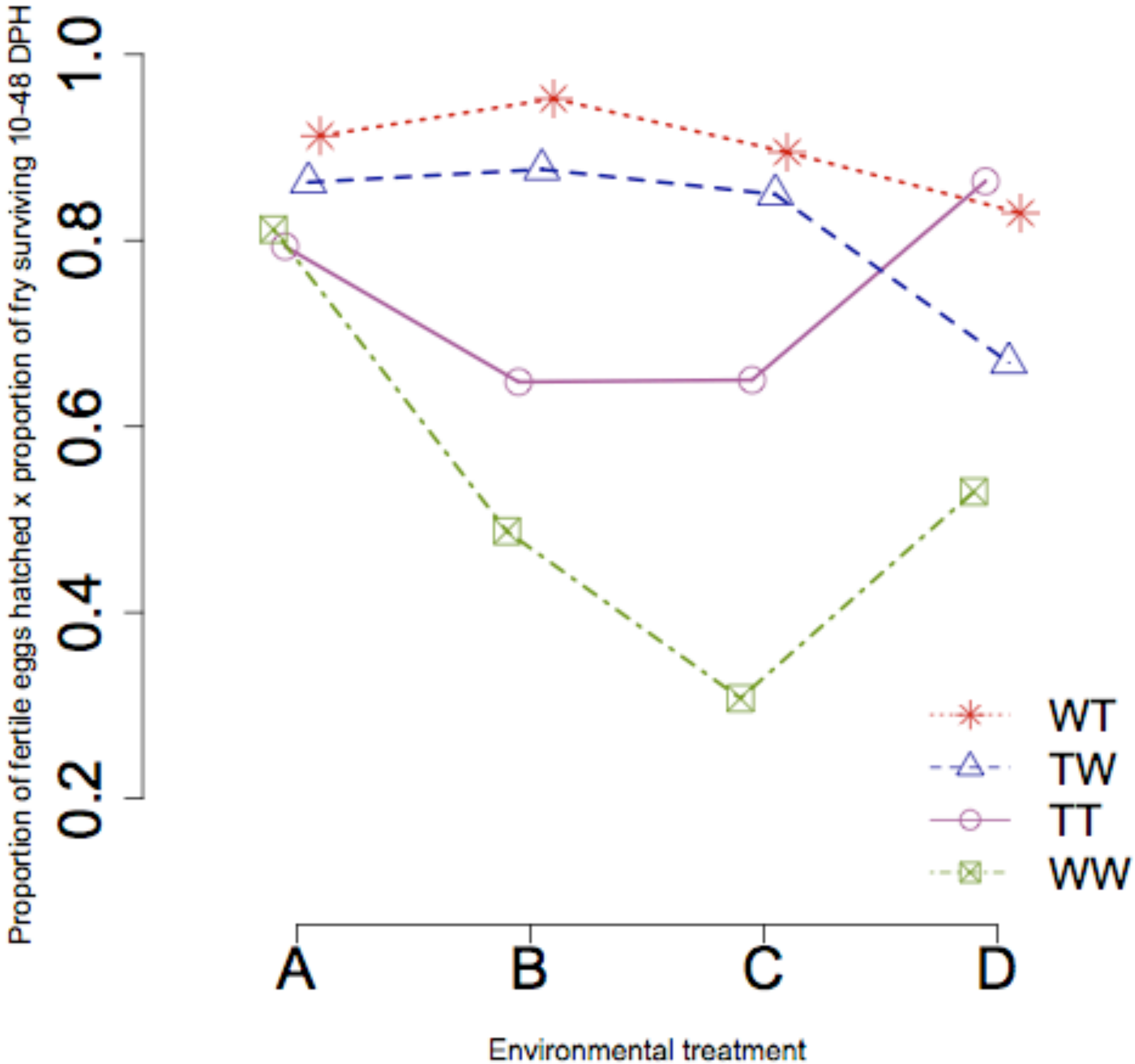

$A^a < B^b < C^c < D^c$

No statistical G x E or genotype effect



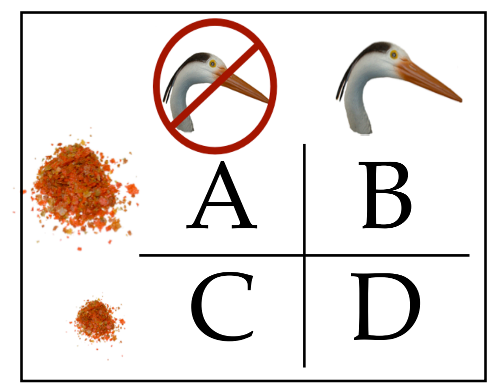

Means ± SE

# Survival to maturity



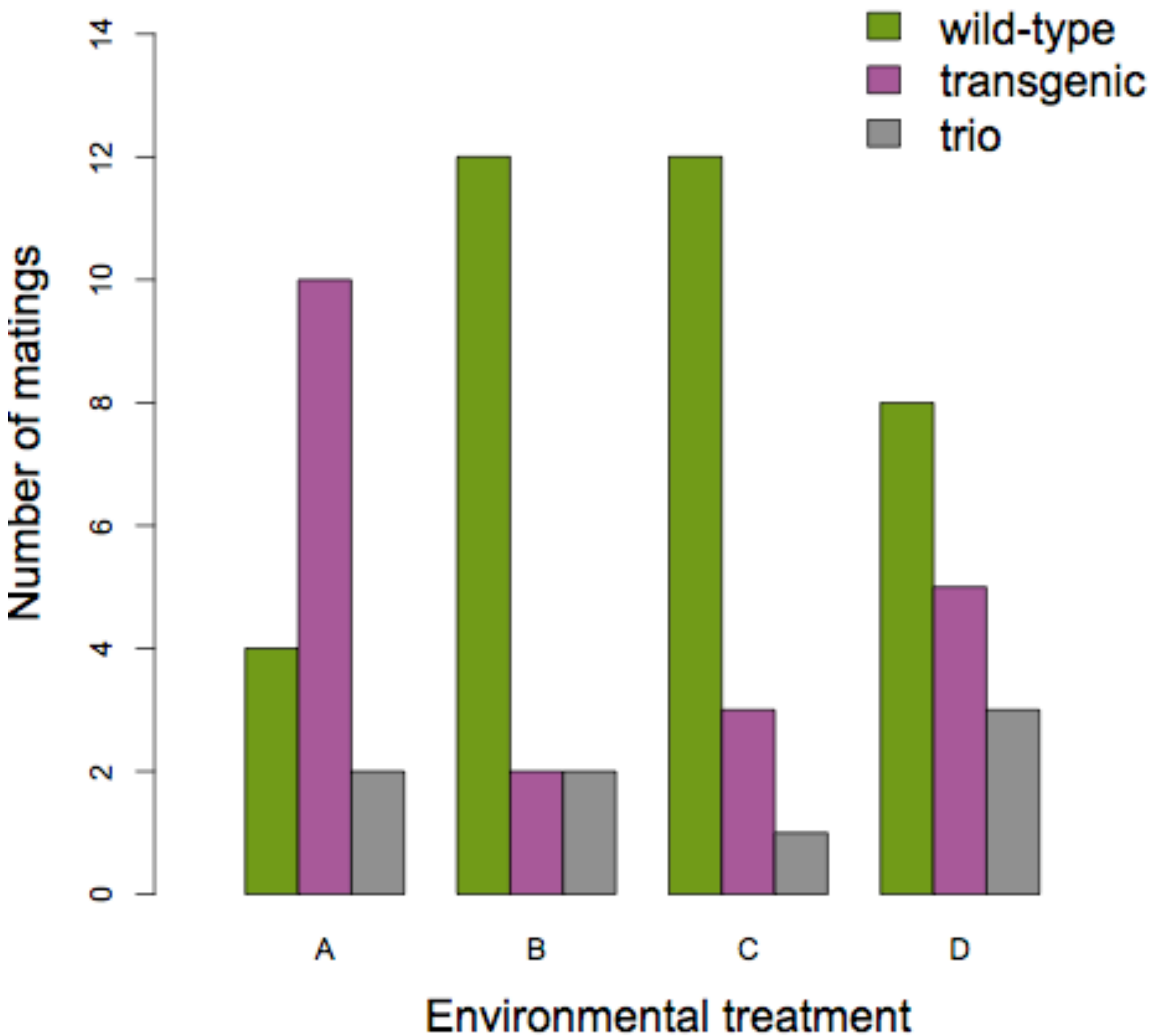

TW & WT > WW
($p < 0.020$)

Env and G x E *n.s.*




Means, parental genotype connected by line

# Mating advantage

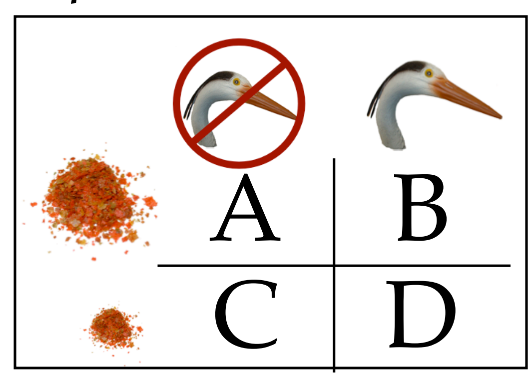



Overall, W > T mating ($p = 0.035$)

W > T in Env. B & C

T & W advantages in Env. A & D *n.s.*

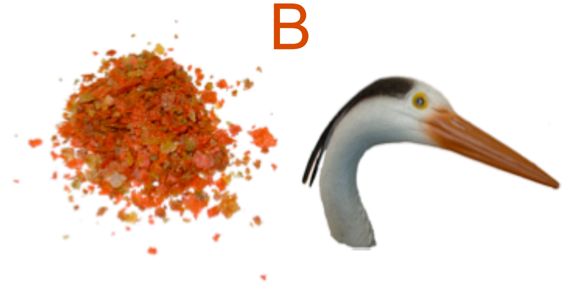
T weighed more than W ($p < 0.001$)



# Fitness trait summary

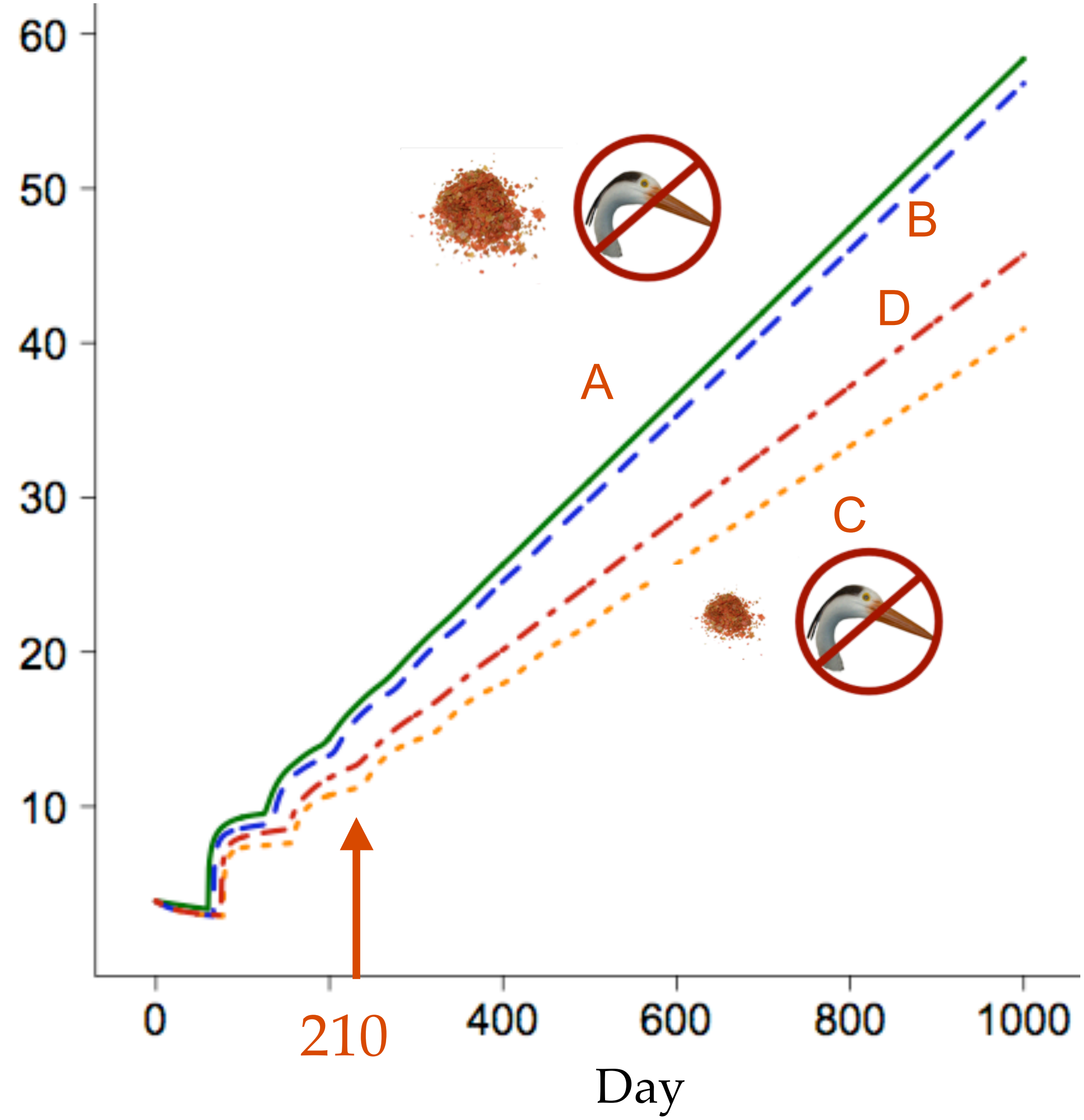
- One trait with a G x E interaction
  - Fertility
- Genotype effects:
  - Fecundity T > W
  - Survival to maturity $WT^{a} > TW^{a,b} > TT^{b,c} > WW^{c}$
  - Mating advantage W > T in Env. B and C
    - Unexpected: smaller W outcompeted larger T males
- Environment effects:
  - Fecundity $B^{a} > A^{a,b} > D^{b} > C^{b}$
  - Age at sexual maturity $A^{a} < B^{b} < C^{c} < D^{c}$

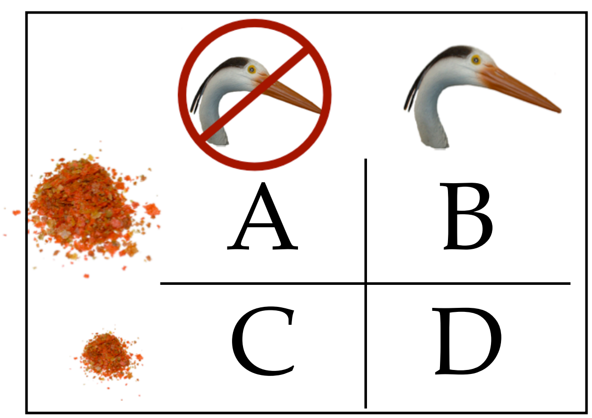
# Environmental treatments

| | Predation risk absent | Predation risk present |
| --- | --- | --- |
| Food satiating | A  | B  |
| Food limiting | C  | D  |

# Deterministic model - pop size

Log (Population size)



# Multi-generational gene flow trials consistent with fitness differences in transgenic and wild-type Japanese medaka fish (*Oryzias latipes*)

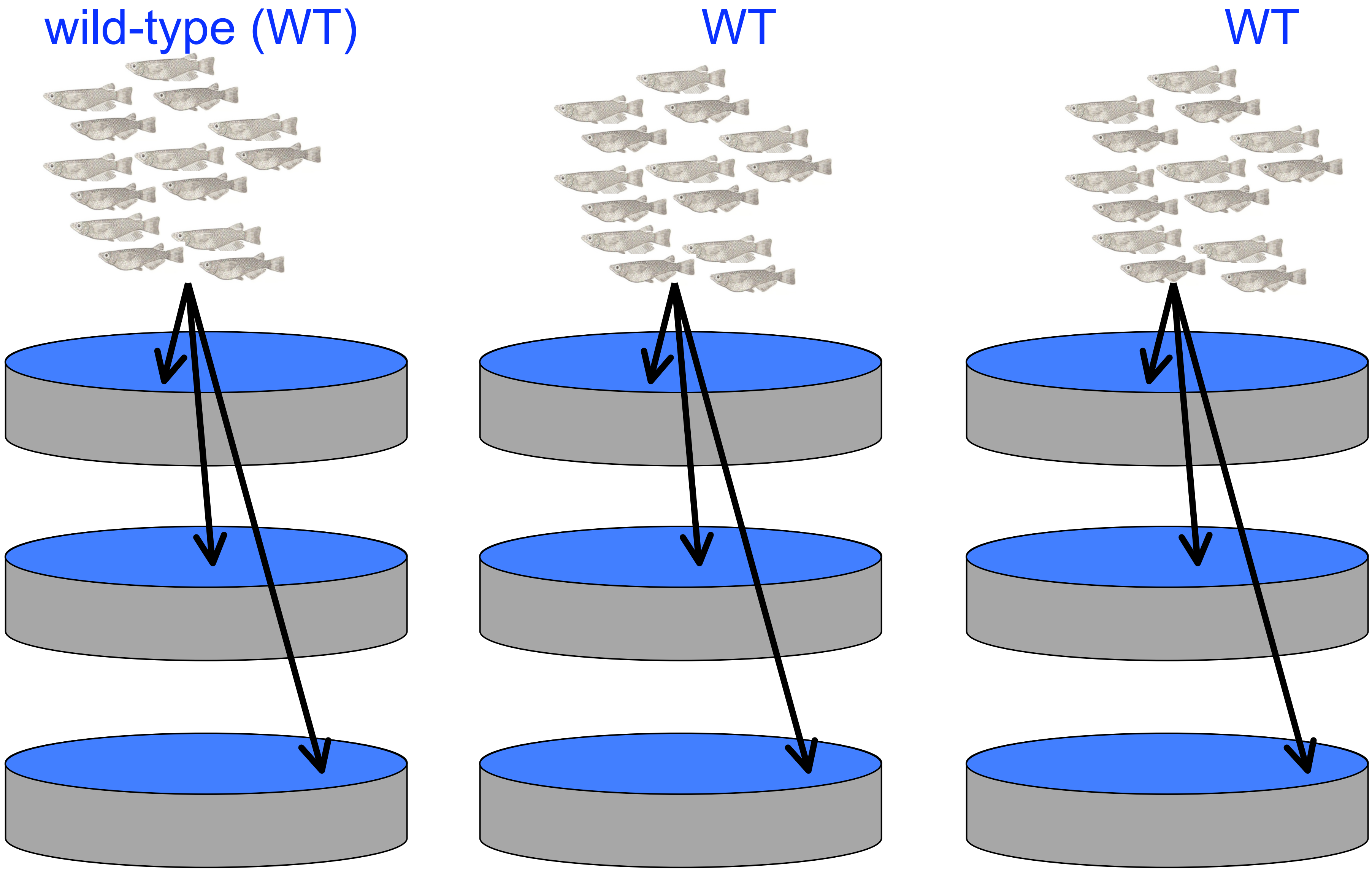
**Objective:** To test whether the relative fitness of transgenic fish could provide useful information about the outcome of an invasion of transgenic fish into a wild-type population.

# First experiments

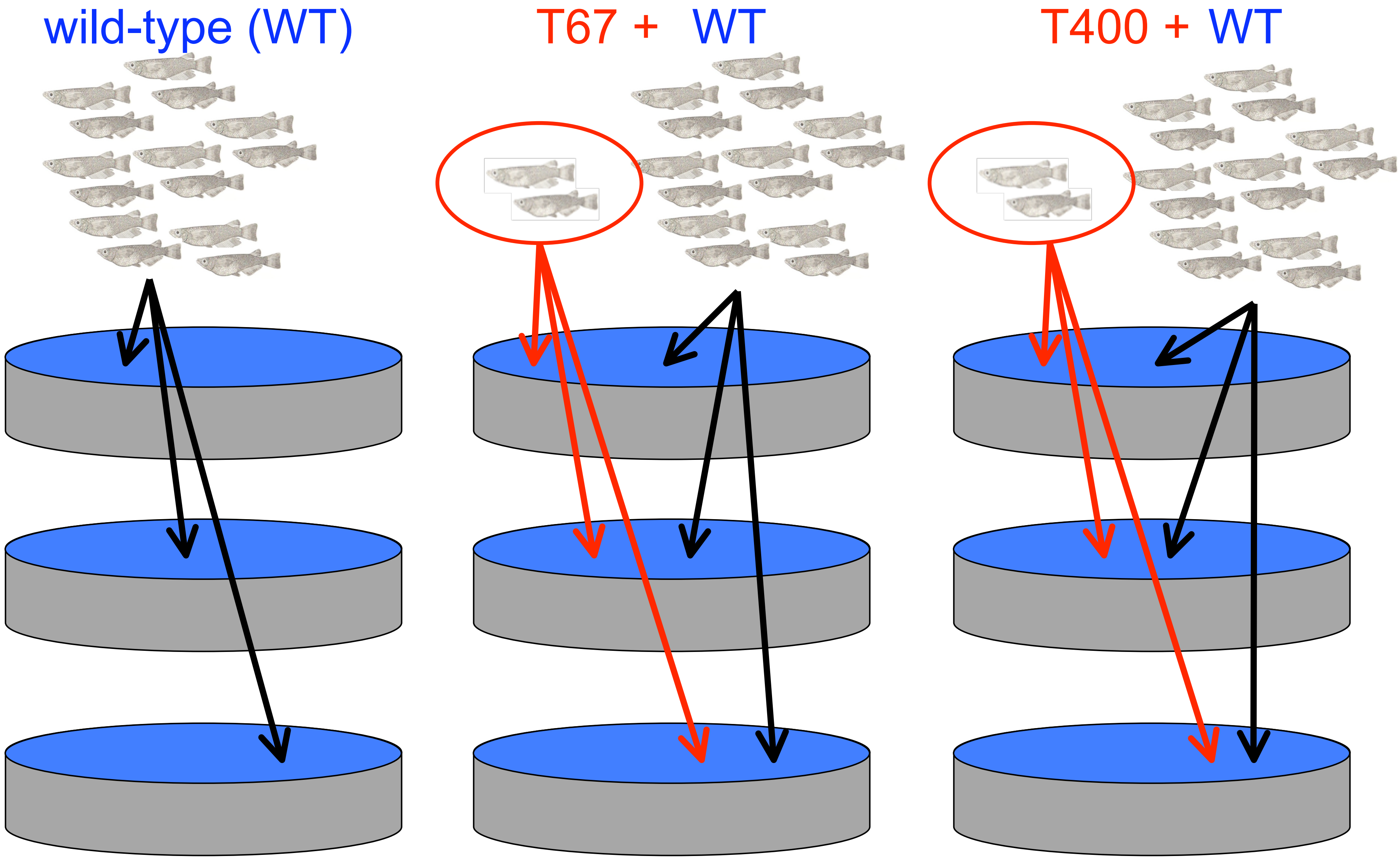
invasion experiments → observe transgene frequency

fitness component traits → observe relative fitness of W and T fish

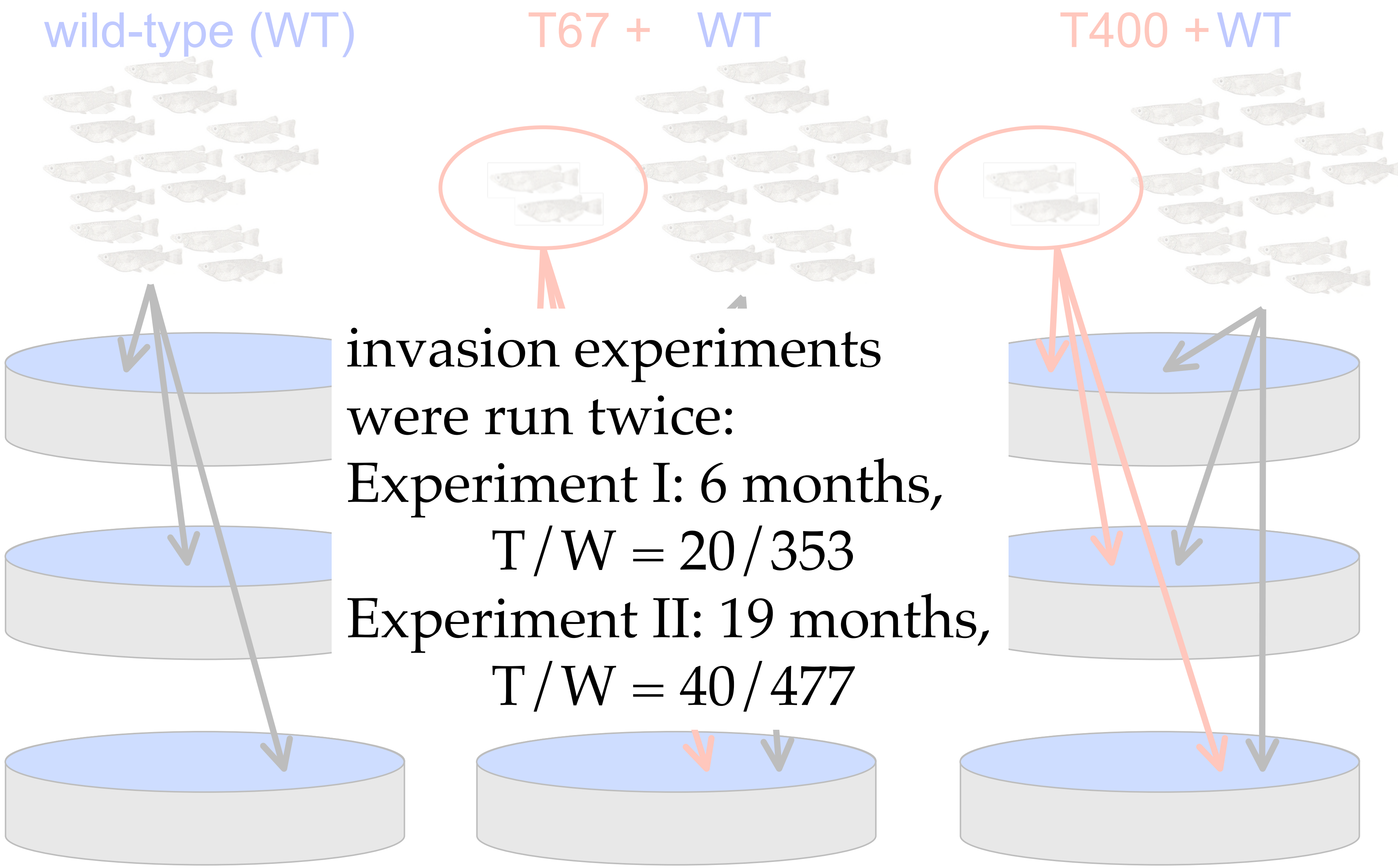
# Invasion experiment design



# Invasion experiment design



# Invasion experiment design




invasion experiments were run twice:

Experiment I: 6 months,

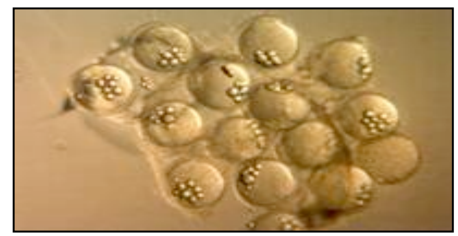
T/W = 20/353

Experiment II: 19 months,

T/W = 40/477

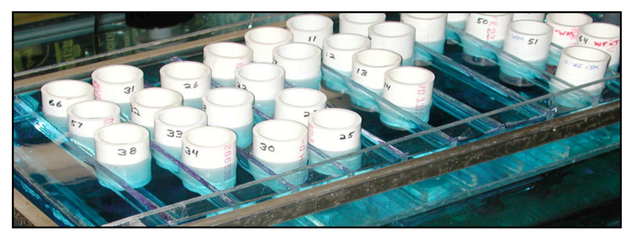
# Fitness component measurements

- Fecundity - eggs produced over 10-day trial
- Fertility - proportion of eggs developing 24 hours after copulation
- Mating advantage - T v. W male, successful copulation with W female



Male Fertility

Mating Success



Female Fecundity

# Fitness component measurements

Juvenile Viability

Adult Viability

Age at Sexual Maturity

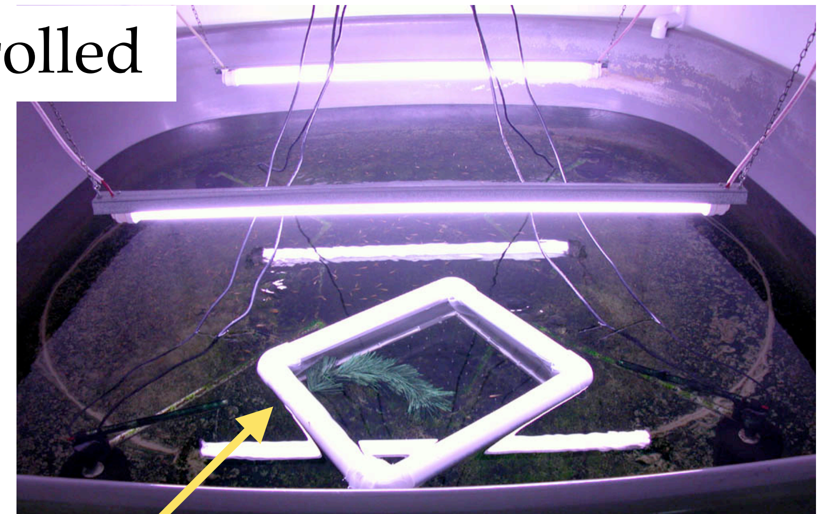
- Juvenile viability - proportion of fish survived to maturity
- Age at sexual maturity - 1st to 5th females to bear fertile eggs
- Longevity - days survived

# Mesocosm design

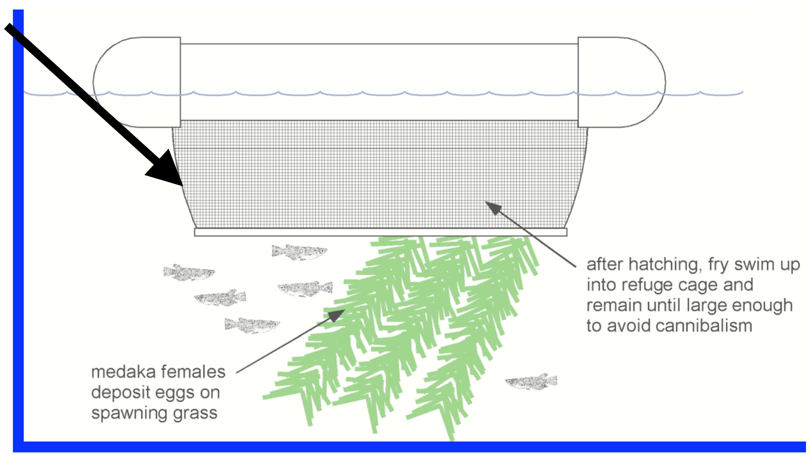
photoperiod and temperature controlled


1900 L fiberglass tanks

photos: Mike Morton



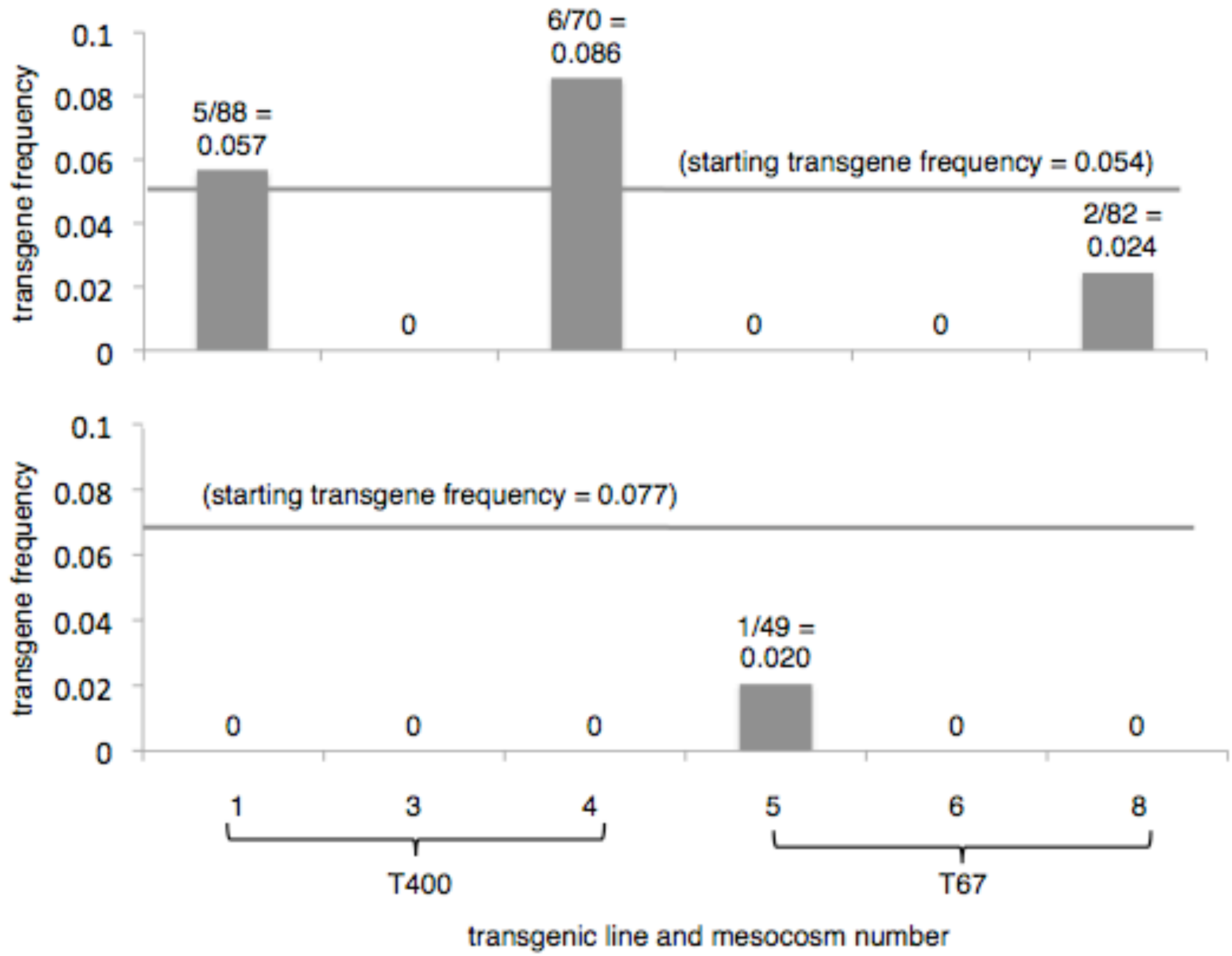
refugia for fry & juveniles



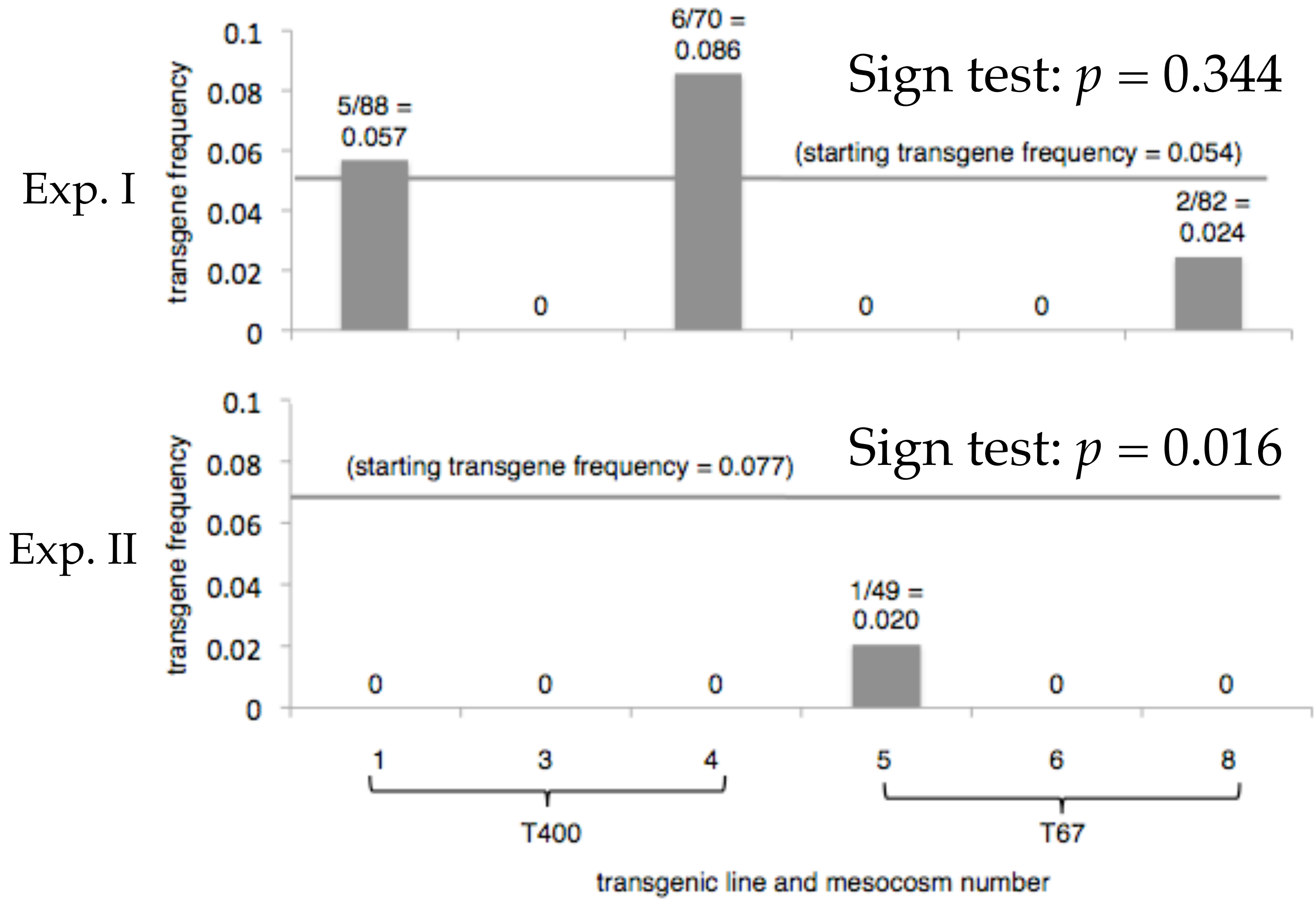
# Final transgene frequencies

Exp. I

Exp. II

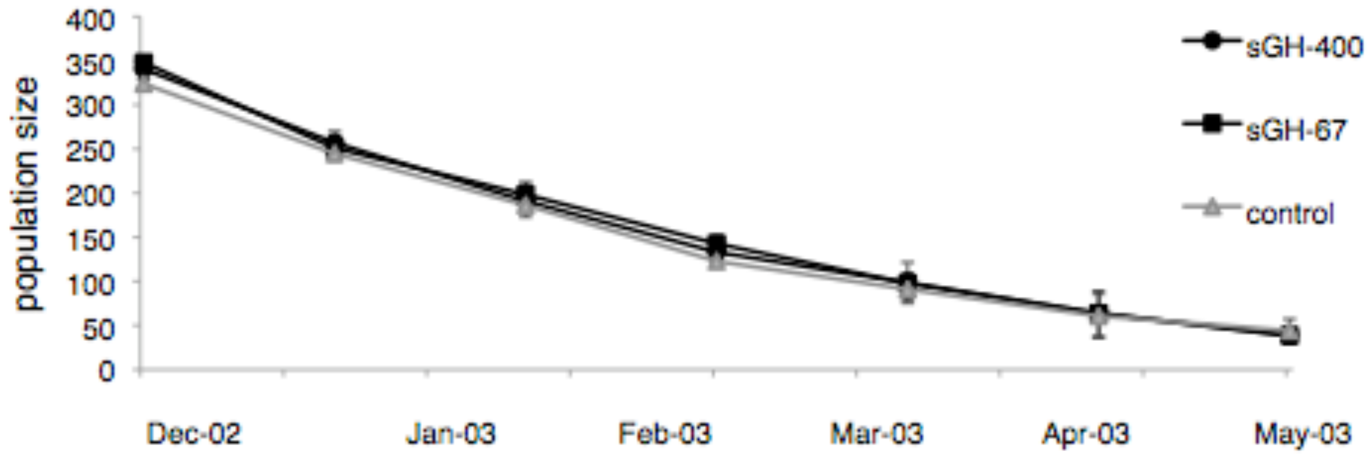


# Final transgene frequencies

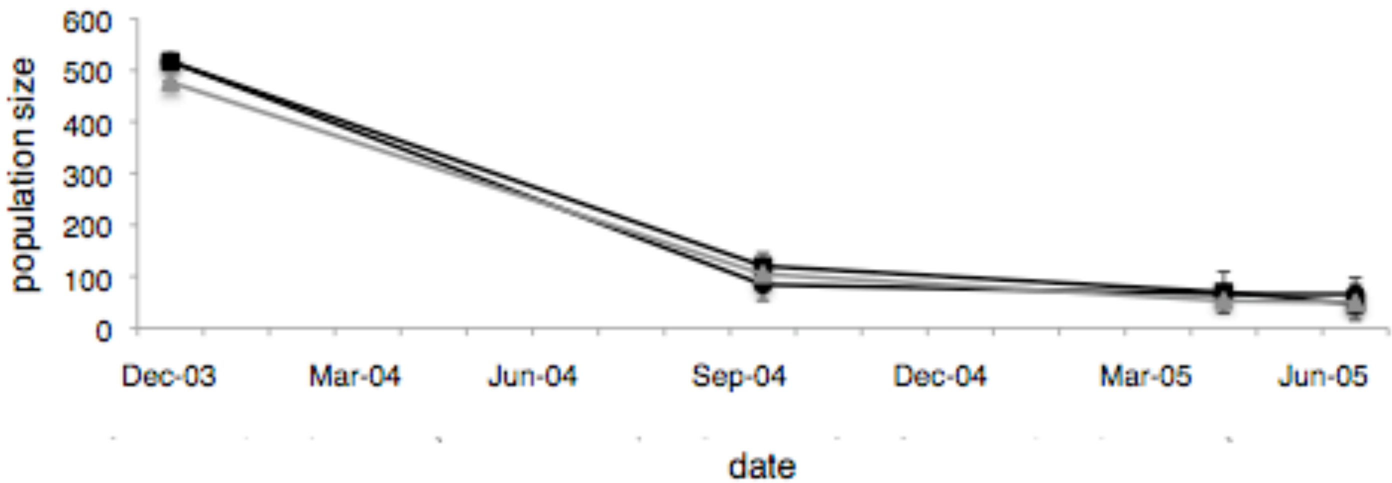


# Population decline in invasion trials

Exp. I

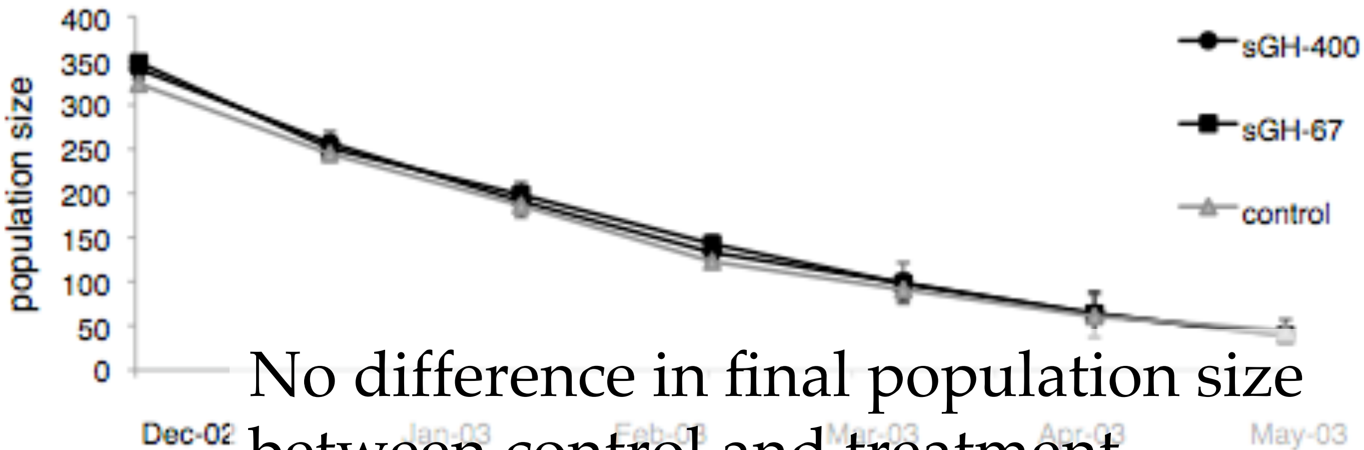



Exp. II



# Population decline in invasion trials

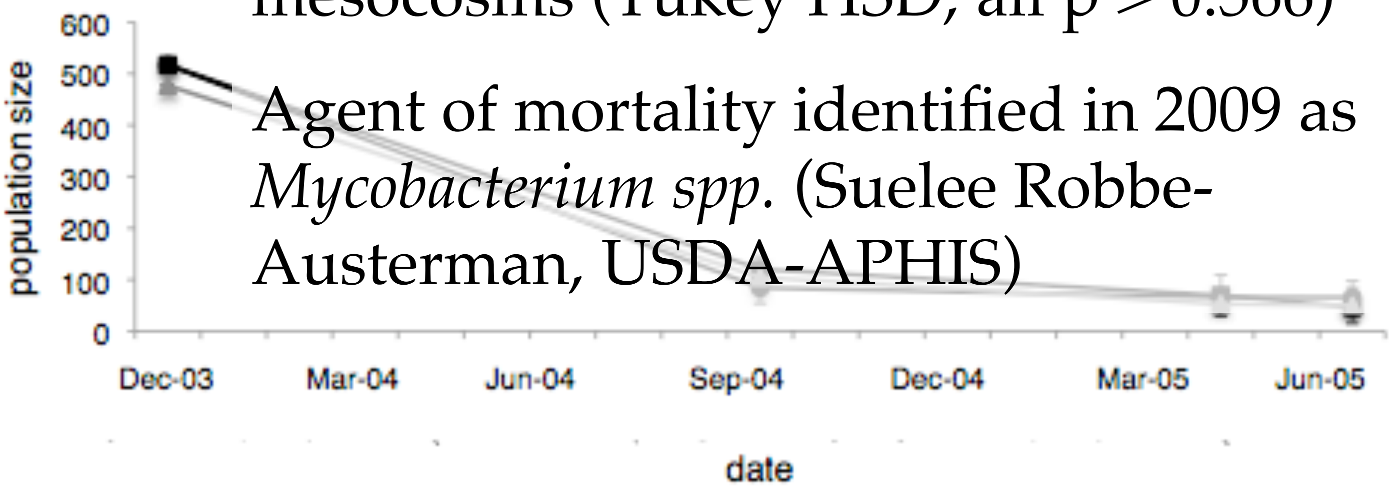
Exp. I




No difference in final population size between control and treatment mesocosms (Tukey HSD, all $p > 0.566$)

Agent of mortality identified in 2009 as *Mycobacterium spp.* (Suelee Robbe-Austerman, USDA-APHIS)

Exp. II



# Fitness component measurements

| Fitness trait | Genotype rank |
|---|---|
| Fecundity | $T67^{a} > W^{a} > T400^{a}$ |
| Fertility | $T400^{a} > T67^{a,b} > W^{b}$ |
| Mating advantage | $W^{a} > T400^{a}$<br>$W^{b} > T67^{c}$ |
| Age at sexual maturity | $T67^{a} < T400^{a} < W^{a}$ |
| Juvenile viability | $W^{a} > T67^{a}$ |
| Longevity | $W^{a} > T67^{a,b} > T400^{b}$ |

# Summary

- W > T67 mating advantage and W > T400 longevity advantage, but T400 > W fertility advantage…
- Invasion experiment II suggests no T advantage
- Lessons:
  - Low starting T frequency
  - Uncontrolled environmental variation

# Recap

- Mesocosm invasion experiments
  - Transgenic disappearance in Exp. II
  - No great T fitness advantage

# Recap

Fitness trait measurements w/  & 

- G x E in fertility, all other traits affected by genotype and/or environment
- W mating advantage surprise (again)

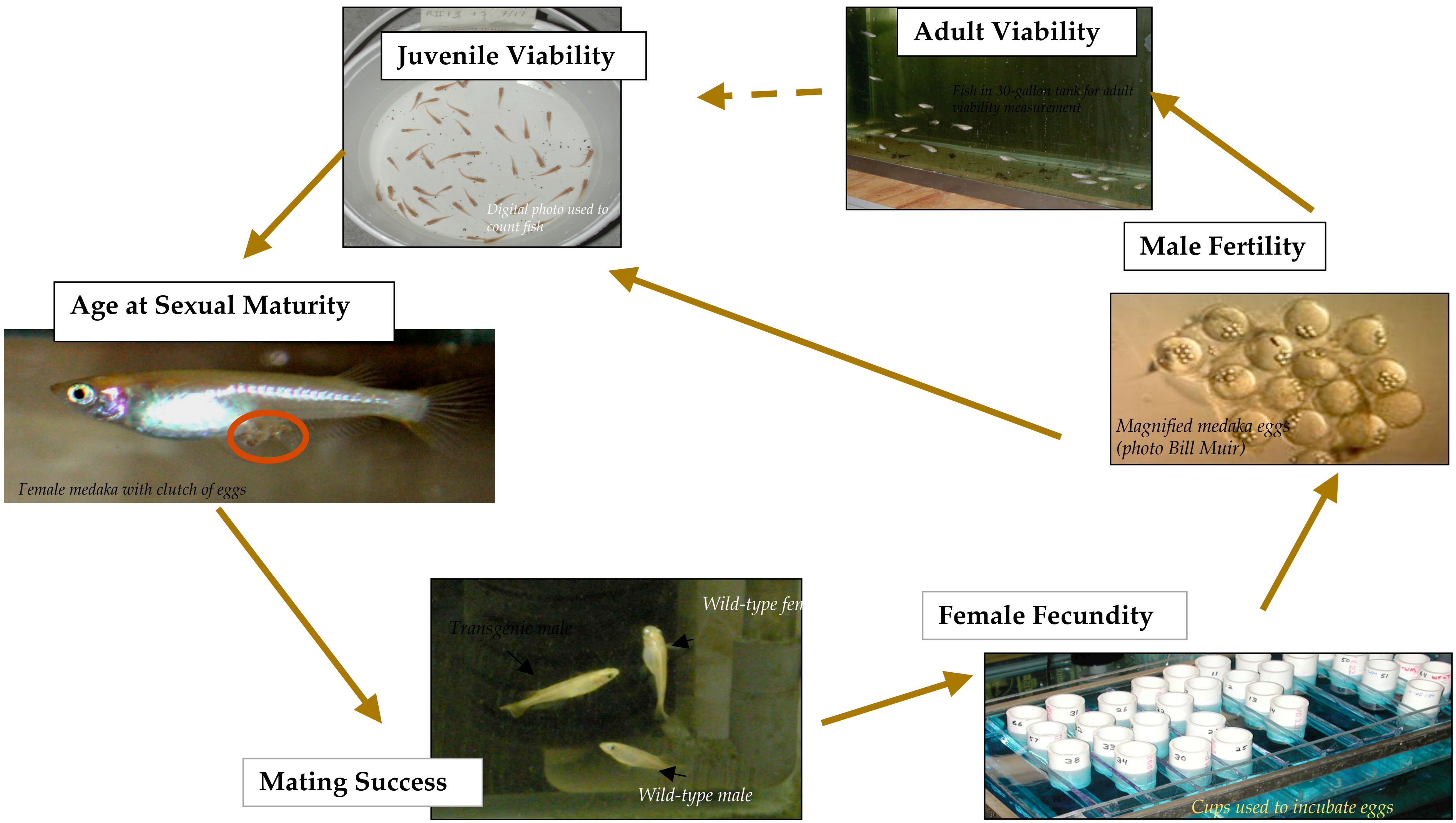
# Recap

- Mesocosm invasion experiments
- Fitness trait measurements w/  & 
  - G x E in fertility, all other traits affected by genotype and/or environment
  - W mating advantage surprise (again)
- Gene flow experiments w/  & 
  - Reduced survival of T founders
  - Highest T population in Environment A

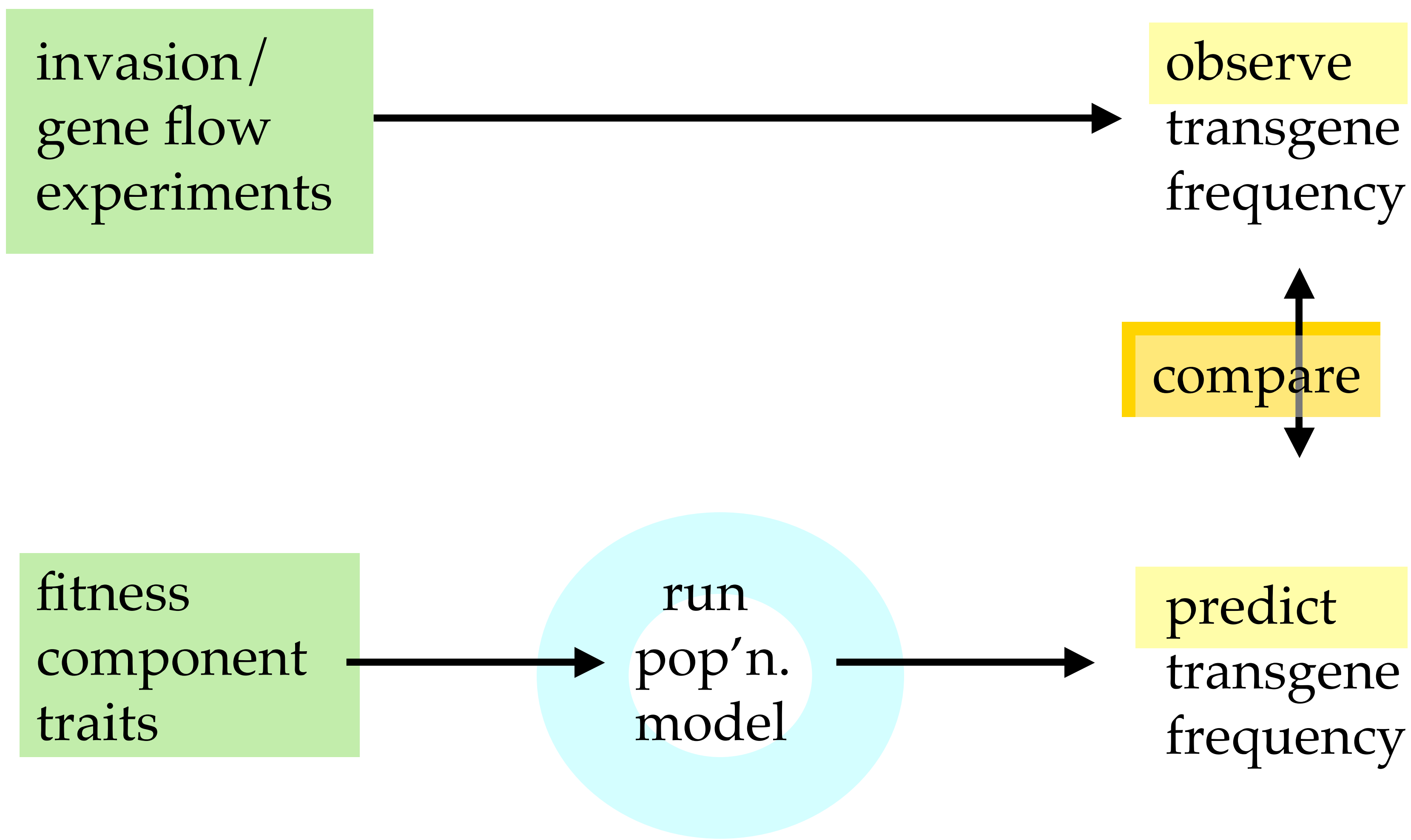
# Recap

- Mesocosm invasion experiments
- Fitness trait measurements w/  & 
- Gene flow experiments w/  & 
  - Reduced survival of T founders
  - Highest T population in Environment A
- Model of gene flow w/  & 
  - Model predictions better confirmed by data in moderate environments
  - Stochastic model to address parameter uncertainty

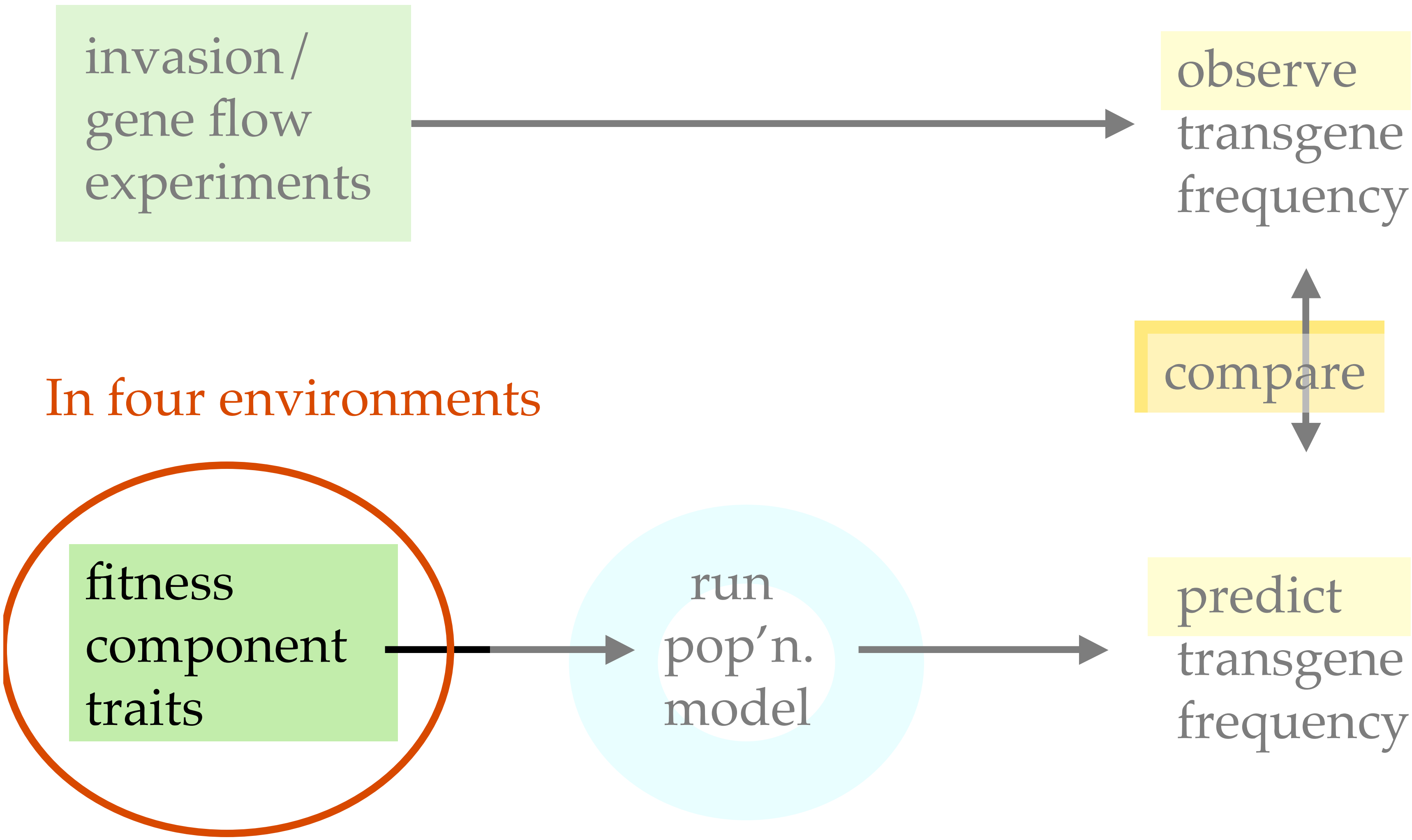
# Net fitness model inputs



# Conceptual model



# Conceptual model



# Acknowledgements


- D&D: Mary Williams and Barbara Martinez
- Olivia LeDee, Laura Phillips-Mao, Julia Frost Nerbonne, Emily Pullins, Mike Rentz, Erika Rivers, Courtney Tchida, Adam Zeilinger
- Aldemaro Romero, Mary Macleod
- Minnesota Department of Natural Resources
- Ordway Center for the Performing Arts
- Twin Cities Velo Bellas
- Saint Paul Vocal Forum
- Derric Pennington
- Alan and Cyndy Paulson