

OHRC Triploid Experiments 2007-present

OHRC Advisory Committee
Update
12/10/12

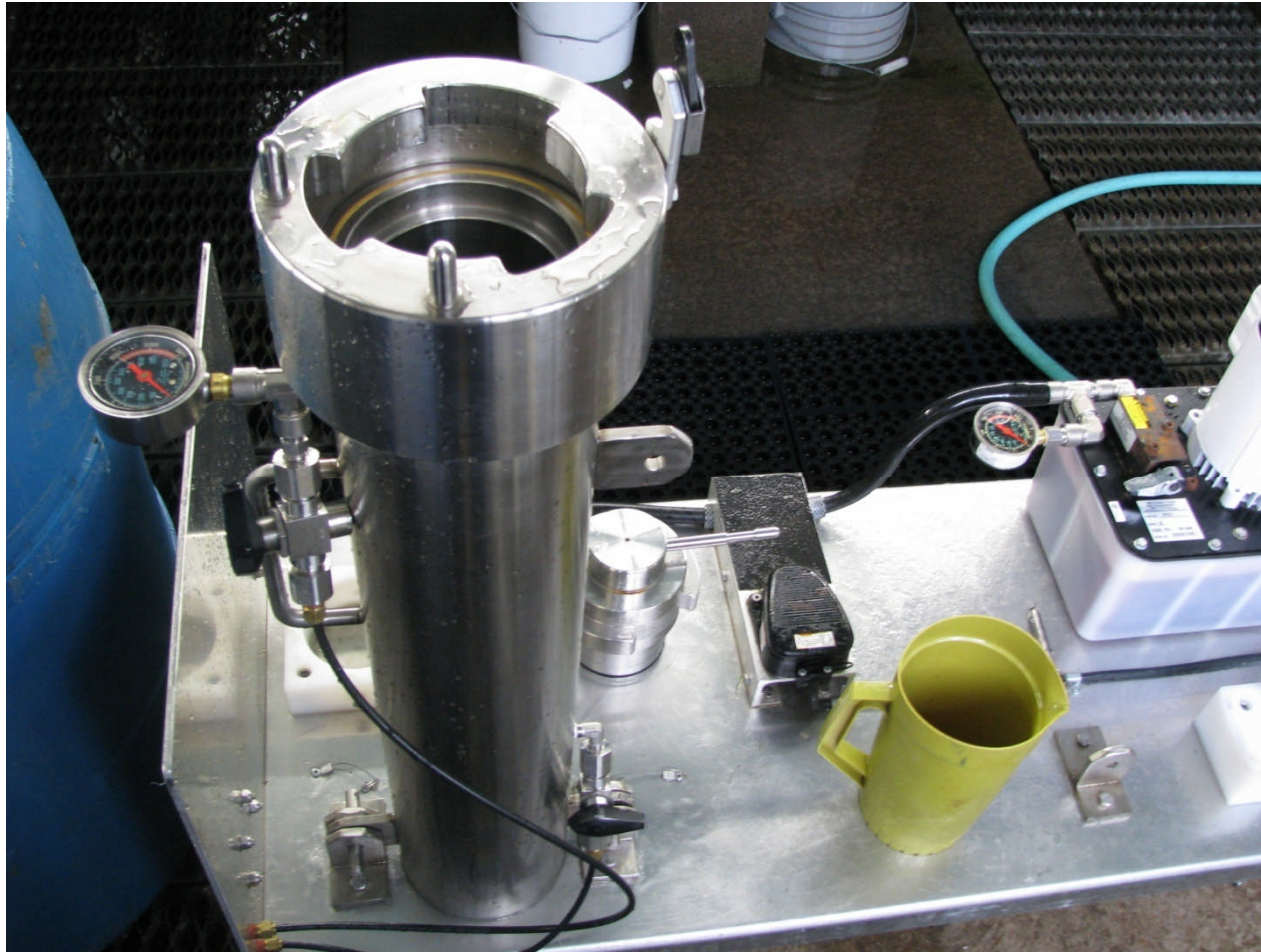
What are Triploids?

- Polyploid organisms are those containing more than two paired sets of chromosomes.
- Polyploidy occurs due to abnormal cell division during mitosis.
- Addition of heat or pressure “shocking” during mitosis disrupts cell division and causes the cell to retain the polar body (typically shed in $2N$ organisms) creating a $3N$ or triploid organism.

Triploid Production

- Started at production scale with HEAT at Roaring River in 2003
 - Inconstant induction rates
 - Inconsistent mortality
 - High cost of heating water
- Purchase of Hydrostatic pressure chamber in 2007

Hydrostatic Pressure Chamber



Chronological List of OHRC Triploid Projects

- 2007 - Pressure Shocking at Roaring River
- 2008 - Pressure Shocking at Roaring River
- 2009 - Pressure Shocking at Oak Springs Hatchery
- 2010 - Triploid “Crane-bows” at Fall River
- 2011 - Triploid Spawning at OHRC
- 2012 - Brook Trout at Wizard Falls Hatchery
- 2013 - Triploid “Crane-bows” at Fall River Hatchery

Effect of pressure on induction and survival

- **2007 - OHRC/Roaring River Preliminary Experiment**

Objective: Establish working range of pressure/time combinations

- **“Shot-Gun” type experiment w/ no replication**
 - **To determine appropriate range of treatments**
 - **Varied Pressure and Time in chamber**

Conclusions:

- **Pressure 9000-10000 PSI = good induction**
 - **Comparable to literature**
- **Egg and Fry Loss generally higher**
 - **Expected additional 10% loss**
 - **Actual additional loss was 25.6%**
- **4-6 min treatment = acceptable induction/mortality**

Effect of pressure on induction and survival

- 2008 - OHRC/Roaring River

Objective: Evaluate effect of pressure on induction and survival of egg/fry

– Varied Pressure and TTUs



Conclusions:

- **Optimal induction (100%) is achieved at:**
 - **9,500 to 10,500 psi**
- **Average additional loss was 13.0%**
 - **Treatment with the lowest increase was 375TTU at 10,000psi (4.3% additional loss)**
- **Pressure or time can be varied**

OHRC Triploid Research

- **2009 OHRC/Oak Springs Hatchery experiment w/ Pressure Shocking**
 - **18 test groups (triplicate treatments)**
 - **Varied Pressure (9,500 and 10,000 psi) and Time Temperature Units (300 and 375 TTU's)**
 - **Variable Loss (due to sperm viability?)**



Conclusions

- **2009 OHRC/Oak Springs Hatchery experiment w/ Pressure Shocking**
 - Results similar to RR
 - Good induction
 - Acceptable survival



OHRC Triploid Research

- **2010 OHRC/Fall River Hatchery experiment w/ Pressure Shocking**
 - **1st Triploid experiment with “Crane-bows”**
 - **Varied TTU’s (300 and 375 TTU’s)**
 - **Egg survival (~38-50%) on treatment groups and 67% on control groups**
 - ~17% additional loss for 375 TTU groups
 - ~31% additional loss for 300 TTU groups
 - **Good induction rates (>98%)**



OHRC Triploid Research

- 2011 Triploid Spawning at OHRC
 - 3 diploid females into each stream
 - 10 diploid males into streams B & D
 - 10 triploid males into streams A & C
 - Individually tagged
 - Triploidy verified by blood sample
- Hourly observations
 - Location of individuals
 - Spawning behavior
 - Redd site locations

Conclusions

- The channels appear to be useful for evaluating spawning behavior in trout
- Diploid females spawned in all channels
- Triploid and diploid males exhibited spawning behavior

OHRC Triploid Research

- **2012 OHRC/Wizard Falls Hatchery**
- **Experiment w/ Pressure Shocking**
 - 1st Salvelinus exp in Oregon
 - Poor survival due to egg quality (high diploid loss)
 - GREAT induction rates (12 of 13 had >99%)



OHRC Triploid Research

- **2013 OHRC/Fall River Hatchery experiment w/
Pressure Shocking**
 - **March 2013**

Questions?

Evaluation of artificial stream channels for evaluating spawning interactions among diploid and triploid rainbow trout

Ryan Couture & Shaun Clements



OREGON HATCHERY
RESEARCH CENTER

Goals

- This study is intended to be a preliminary investigation into:
 - 1) the use of the artificial streams at the Oregon Hatchery Research Center to evaluate interactions between wild and hatchery reared stocks of rainbow trout
 - 2) whether diploid “domesticated” rainbow trout spawn in a semi-natural environment
 - 3) whether male triploids exhibit spawning behavior in a semi-natural environment

Methods

- 3 diploid females into each stream
- 10 diploid males into streams B & D
- 10 triploid males into streams A & C
 - Individually tagged
 - Triploidy verified by blood sample
- Hourly observations
 - Location of individuals
 - Spawning behavior
 - Redd site locations

Analysis

- Compare the proximity of male trout to female trout among treatments.
- Document the % of triploid and diploid males that develop mature gametes.
- Estimate the number of eggs deposited per stream and by type (diploid/triploid)
- Estimate the number of redds built per female
- Estimate the fertilization rate of both diploid x triploid and diploid x diploid matings.

Initial Observations

- Males and females pairing in all streams within 8 h
- Male aggression
 - Chasing other males
 - Territoriality
- “Quivering” by both triploid and diploid males
 - “Quivering” appeared to trigger females to spawn

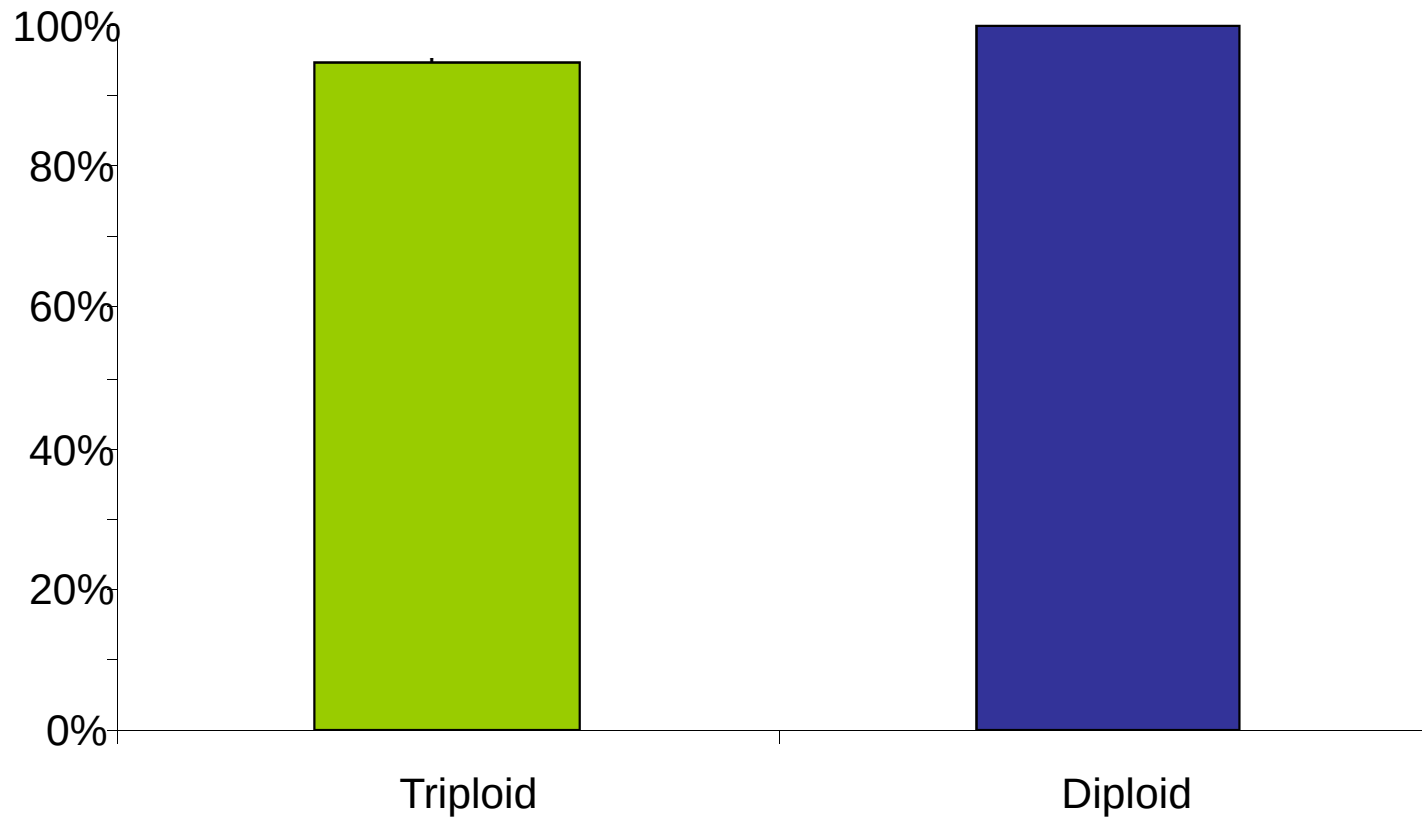
Male Distribution

- We tested for differences in the proximity of males to the nearest female.
 - No effect of stream and date
 - No difference between diploid and triploid males ($p=0.77$)



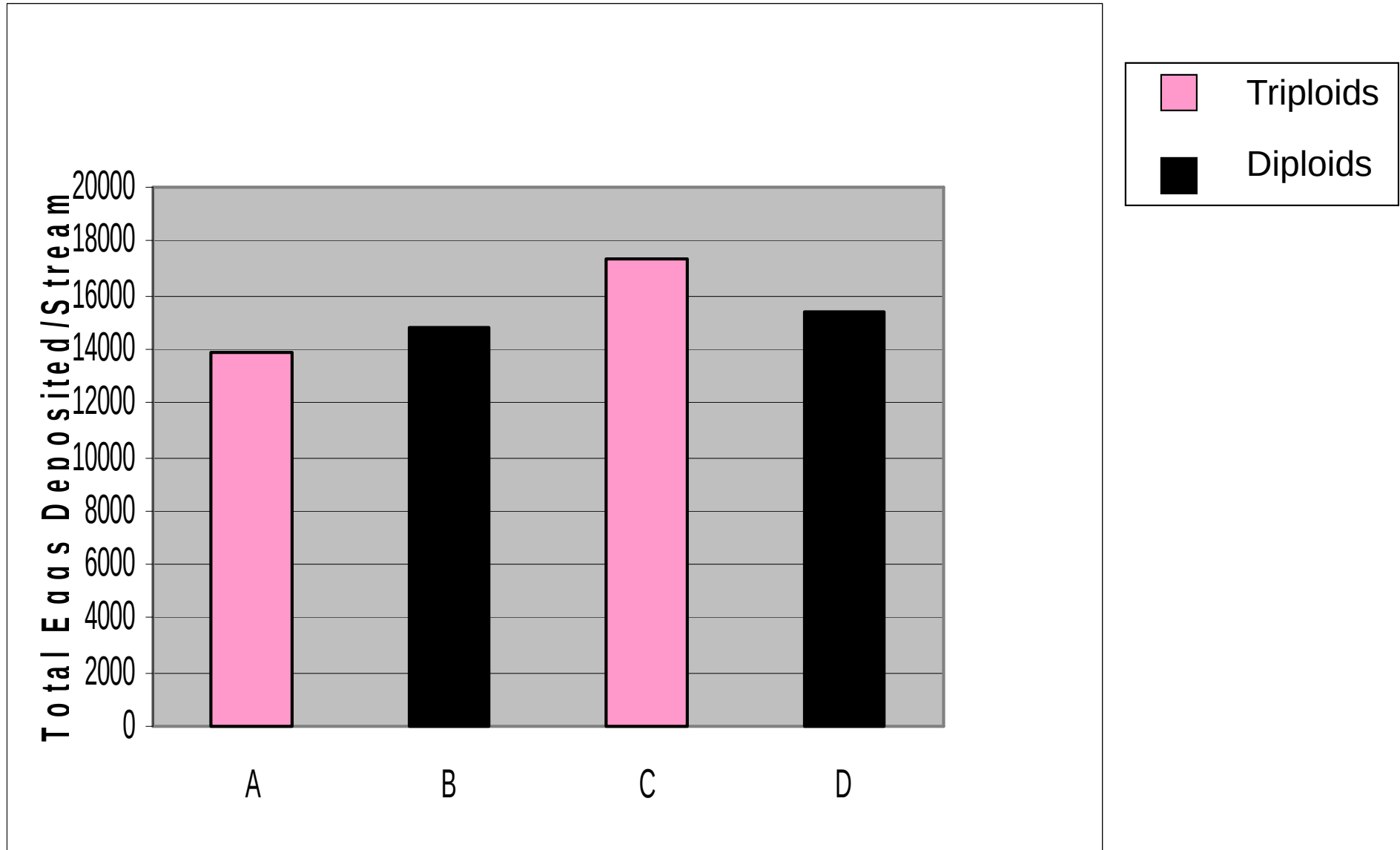
Ploidy had no effect on proximity to females

Gonad Development

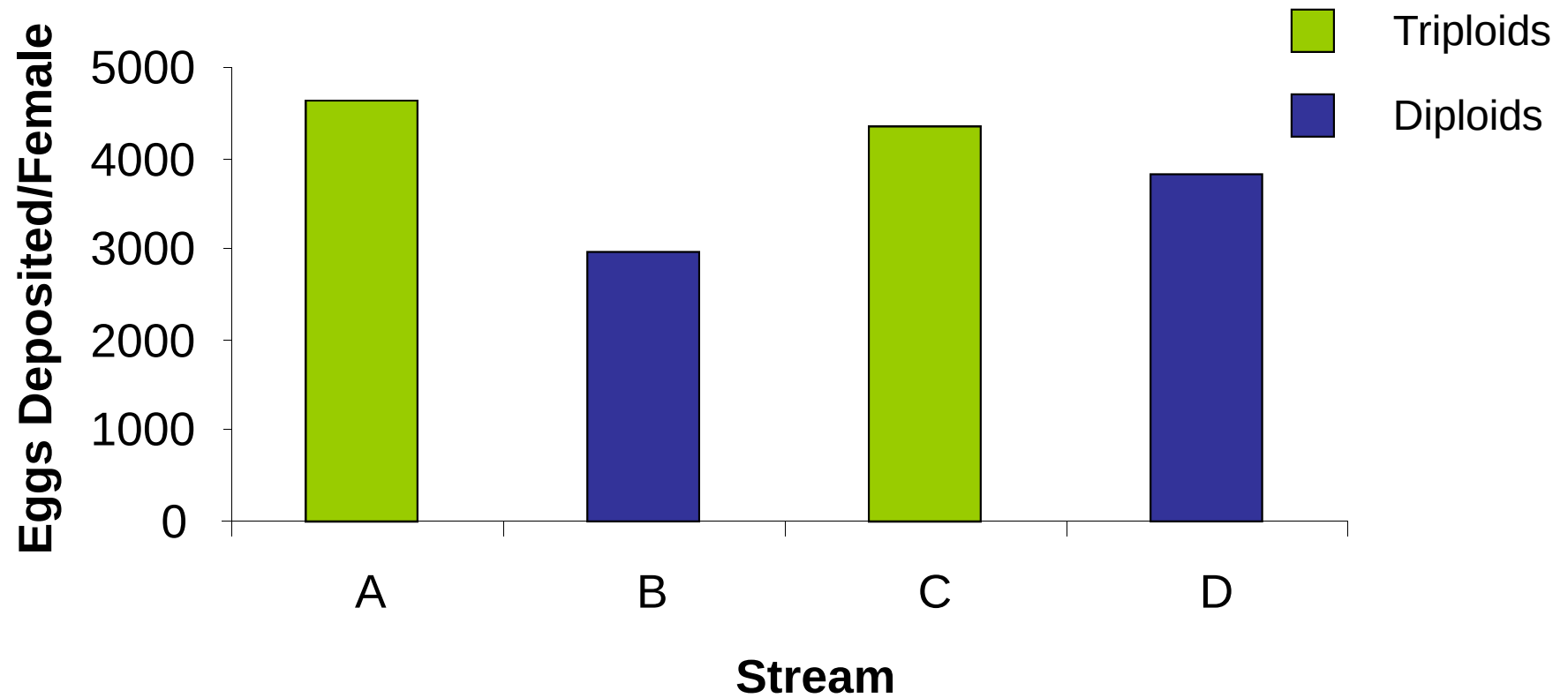


*95% of triploid males had developed gonads

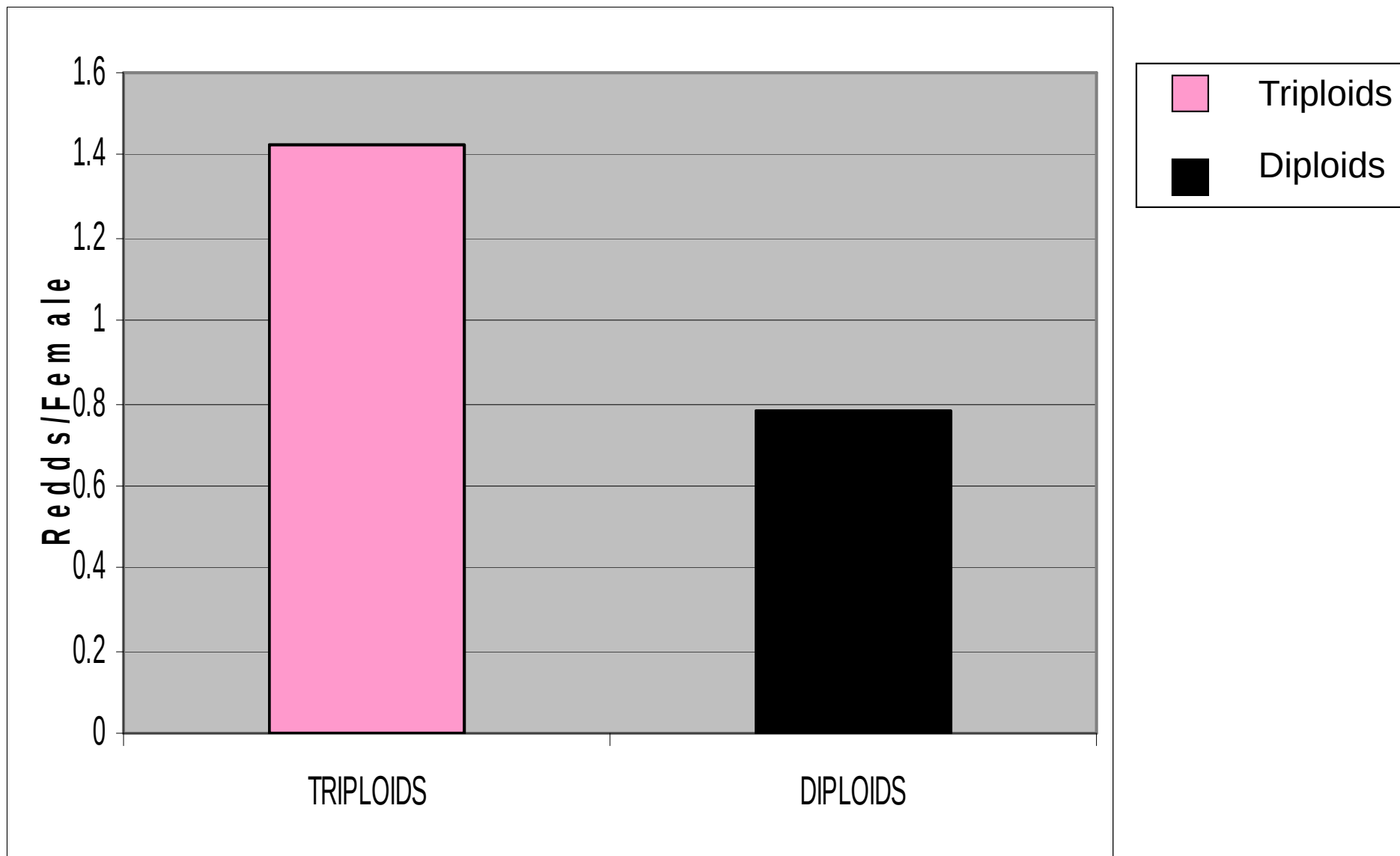
Total Eggs Deposited per Stream



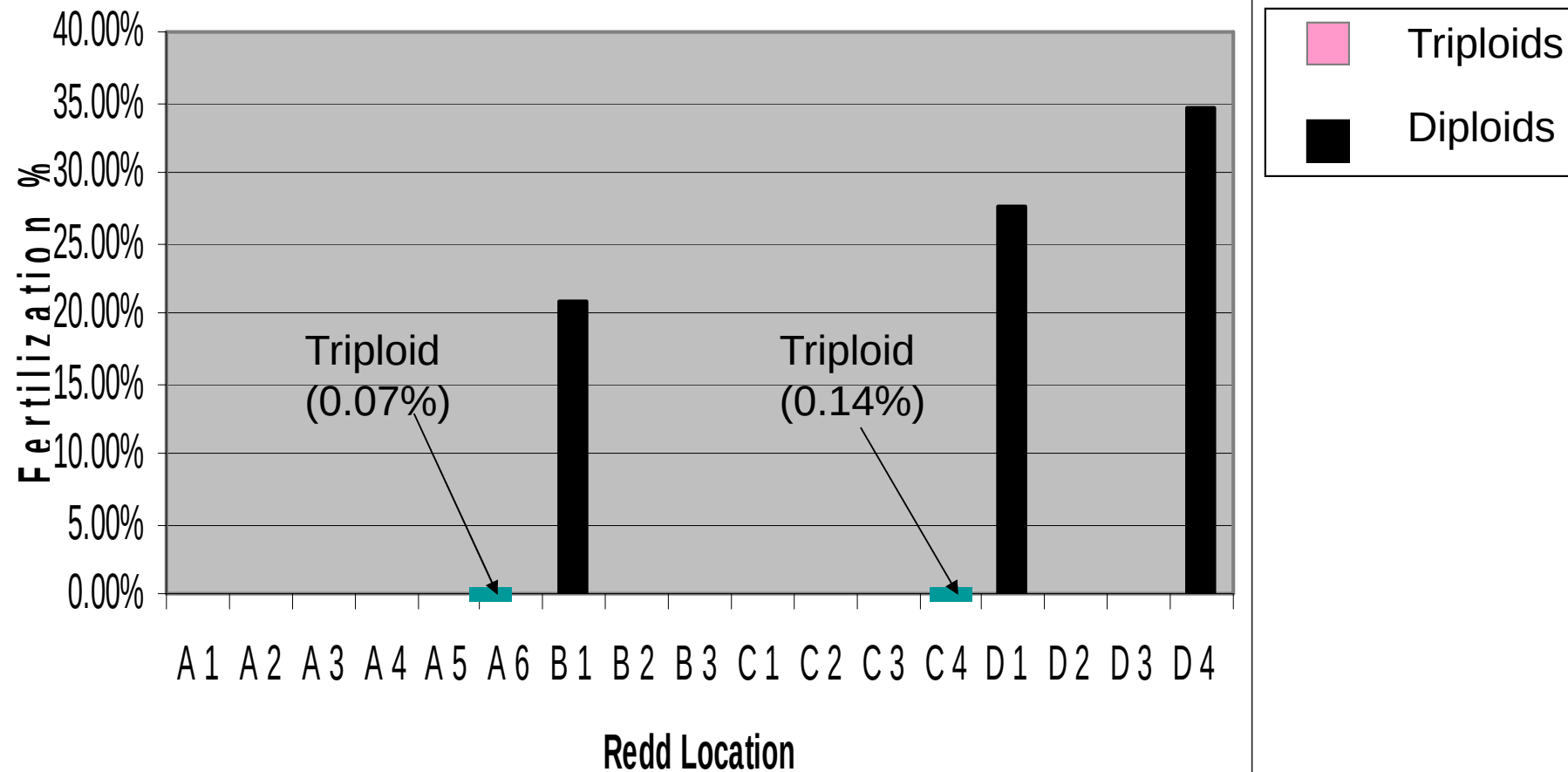
Did male ploidy affect egg deposition?



Redds per Female



Egg Fertilization



Conclusions

- The channels appear to be useful for evaluating spawning behavior in trout
- Diploid females spawned in all channels
- Triploid and diploid males exhibited spawning behavior

Future Research

- Use of wild females, spring and fall spawners
- Develop research to investigate other ecological impacts of triploid trout stocking
- Use of all-female triploids

Thank You!

- Craig Banner for assisting with the blood samples and flow cytometry
- OHRC staff and OSU interns for observations and data collection