Assessing Nutrition in Zebrafish

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Facility & System Conditions

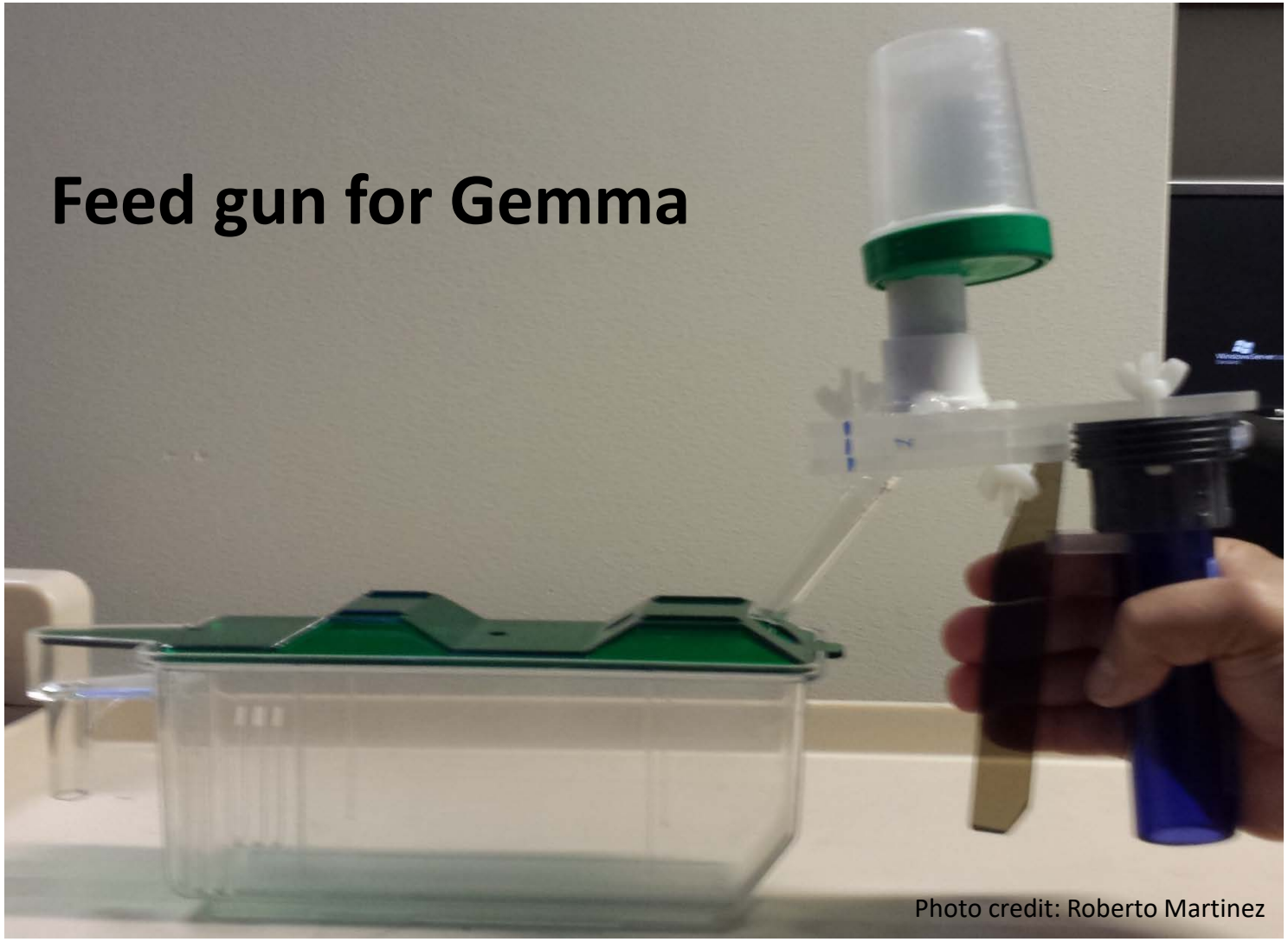
- 100,000+ liters broken into four, separate zebrafish systems
- 12% daily water changes
- RO water with added salt

- Mechanical- bead filter
- Biological- sand bed filter
- 20 micron polishing filters
- UV bulbs- 3900 total Watts

Parameter	Typical values
pH	7.0
Conductance (µSiemens/cm)	1000
Dissolved Oxygen (mg/L)	7.3
Alkalinity (mg/L $CaCO_3$)	16
Total Hardness (mg/L)	60
Ammonia (mg/L)	<0.02
Nitrite (mg/L)	0.040 - 0.120
Nitrate (mg/L)	30.0 - 37.0

Should we switch the diet of the juvenile fish?

Squirt bottles or Pressure pumps for Flake



- In an effort to improve upon husbandry practices and protocols for the zebrafish, *Danio rerio*, we looked at the effects of a new feeding regiment and diet upon juvenile zebrafish.
- Our previous feeding protocol, FKA (Flake commercial diet + Krill + Artemia), was compared to a proposed feeding protocol, PA (Processed commercial diet + Artemia), to determine any difference in fish development and reproductive success.

*Data and Charts from NCAB AALAS poster presented in 2016 by Charles River Lab Staff
Stephen Frederickson, Mark Steinmiller, Tiffany Blaylock, Mike Wisnieski, Daniel Castranova

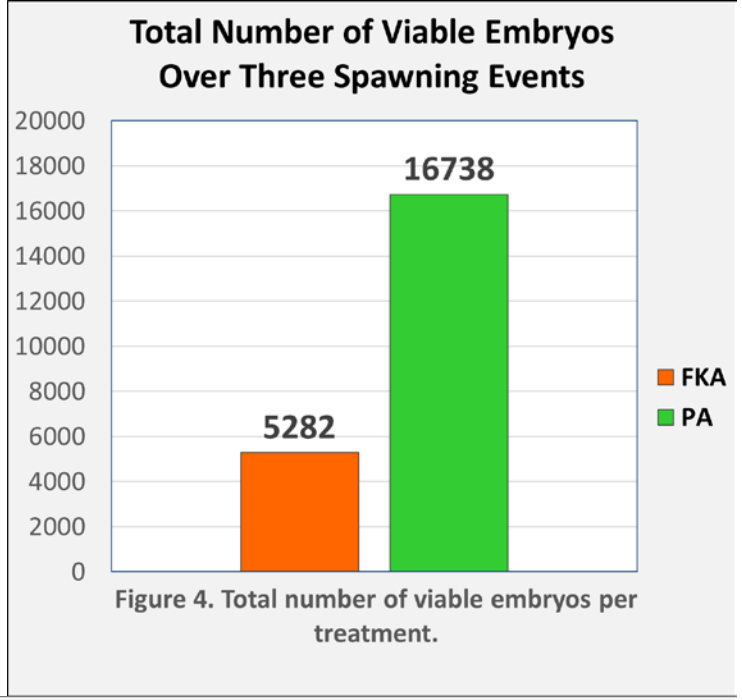


Figure 4. Total number of viable embryos per treatment.

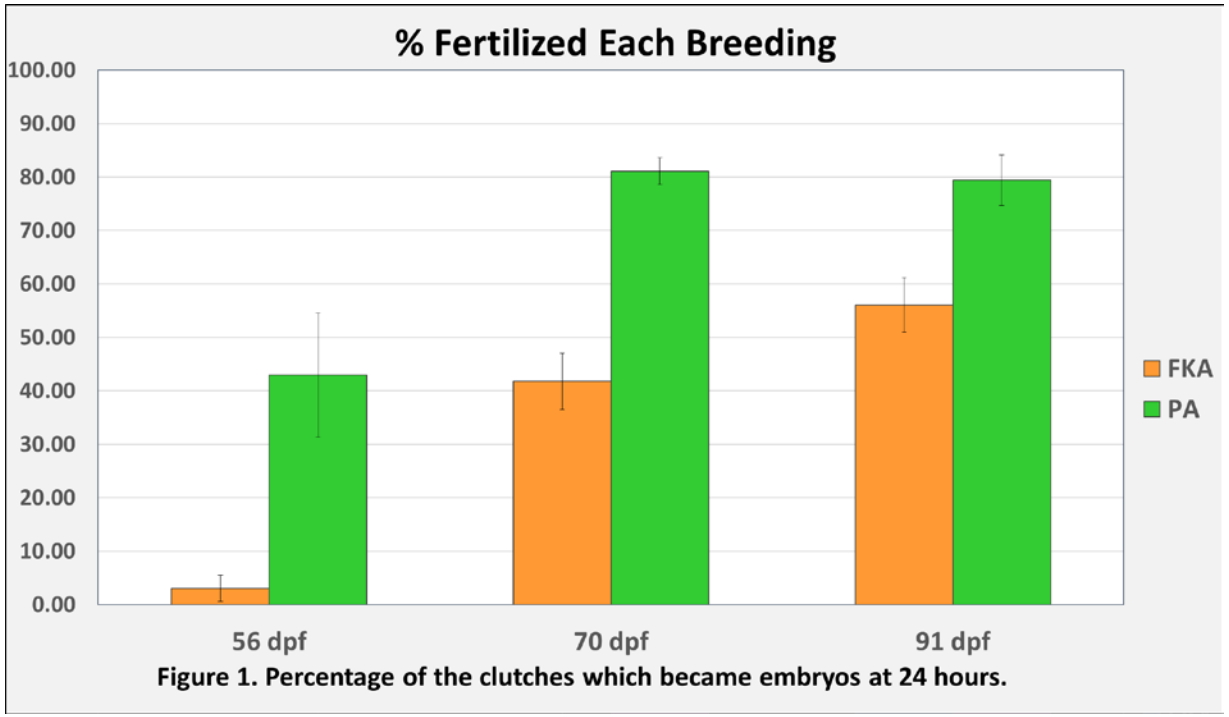


Figure 1. Percentage of the clutches which became embryos at 24 hours.

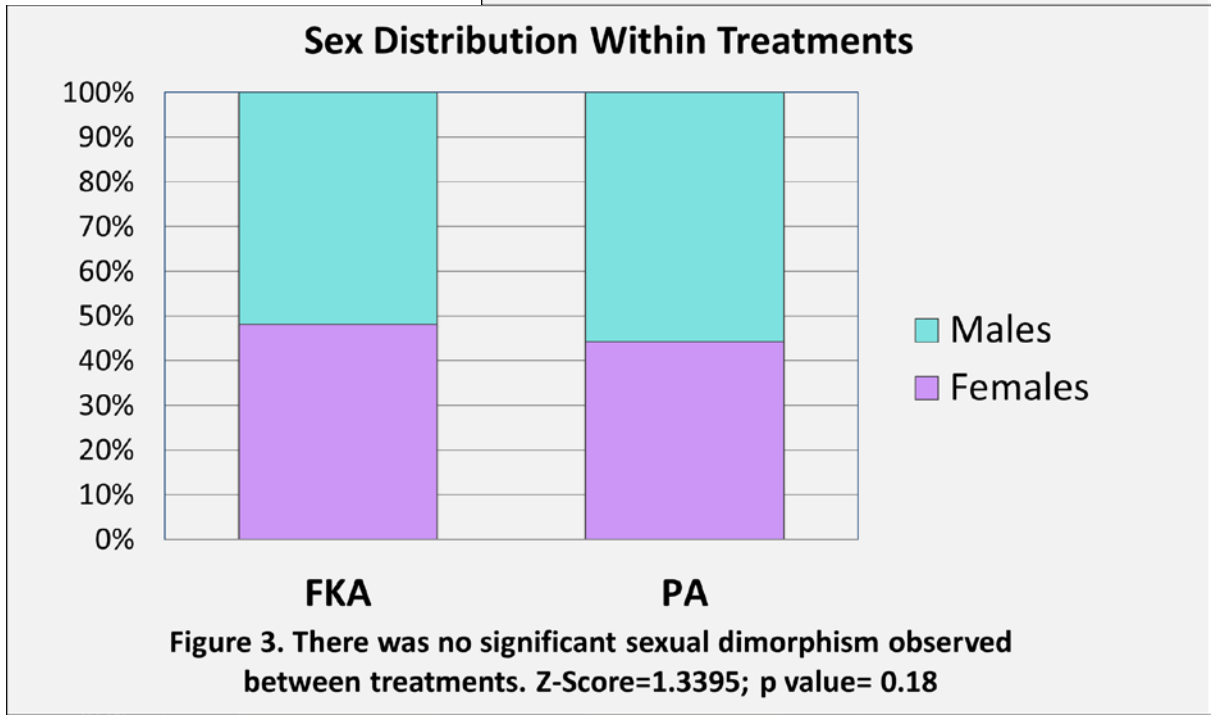


Figure 3. There was no significant sexual dimorphism observed between treatments. Z-Score=1.3395; p value= 0.18

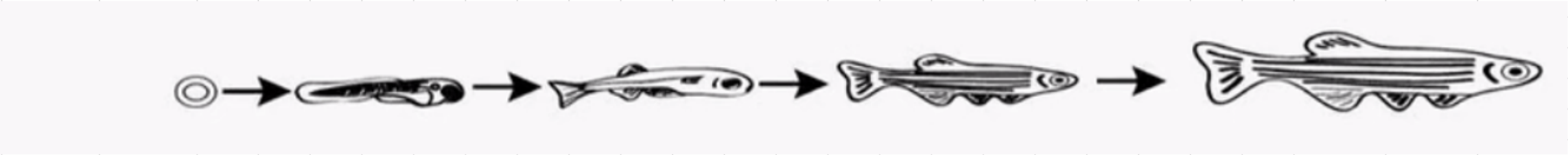
Artemia is not available- How do we move forward?

- Option 1
  - Deal with the current artemia market – cost and availability not stable
    - Company A $66/can has metal shavings – currently halted production
      - If it becomes available again mitigation is possible but at labor cost
    - Company B Premium- expensive unvetted product $260/can
    - Change from SFB to GSL- too big for fry/juv
      - Low in HUSFA , unknown profile
- Option 2
  - Replace artemia regime with rotifer regime
  - Known and controllable supply and nutrition profile
  - Cost controlled
- Option 3
  - Replace live feed for juveniles and adults with Gemma completely

Artemia Drawbacks

- No dietary nutrition control
  - grown in the wild
  - Nauplii cannot be enriched because they do not consume feed
  - GSL has a lower fatty acid profile than SFB strain
- Increased demand community-wide
- Increased and variable cost of product
- Multiple consecutive years of poor harvest currently
  - Limited supply and availability shortages
  - Varying quality

Current Diet at NIH Central Zebrafish Facility



Days post- fertilization	0	5	9	15	20	25	30	35	40	45	50	55	60	65	70	75	80
		Early Larval Stage		**Fry Grow Out Stage**		**Juvenile Stage**							**Adult Stage**				
Feeding Regiment #1		Rotifers in Green H20 Culture		Rotifers 2x a day diluted in System Water		GEMMA ~3% of Adult BM (AM)											
50% of 6L and 1.8L tanks				Zeigler AP110 in AM		Flake (PM)											
				Argent Hatchfry in PM													

*Diet Chart Provided by Charles River Lab Staff
*Fish Development Image borrowed from Christian Lawrence

Thank you!

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- NIH/NICHD RAMB Dpt.
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- Dr. Tannia Clark DVM
- Charles River Laboratories
  - Mike Wisnieski
  - Kristin Merrill
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