



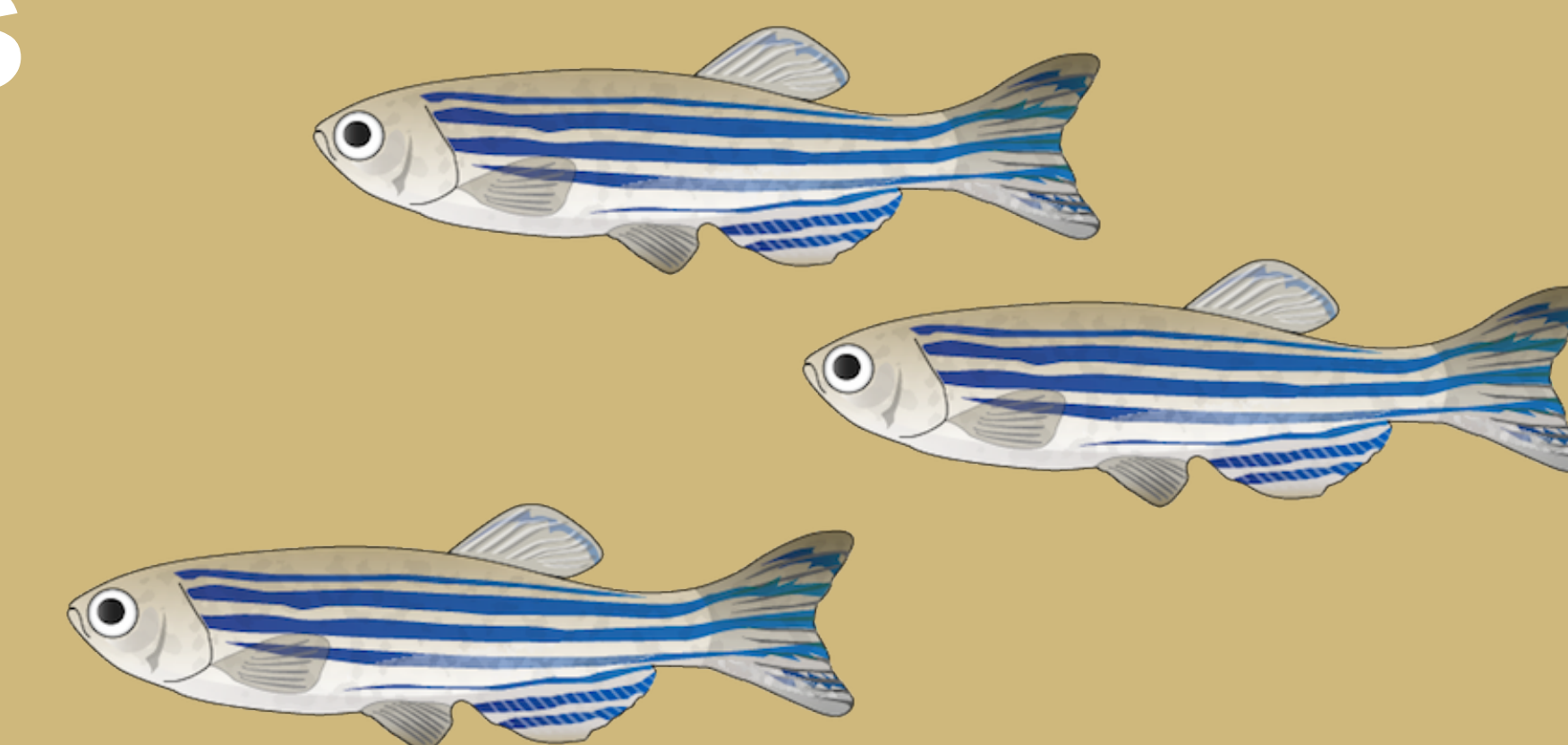
Office of Laboratory Animal Resources

UNIVERSITY OF COLORADO
ANSCHUTZ MEDICAL CAMPUS

Assessment of a gel-based diet for zebrafish: water quality, growth rate, and reproductive success

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Background

- Standard meal-style feeding of laboratory zebrafish:
 - Time consuming for care personnel
 - Unlike natural zebrafish behavior
- Ad libitum feeding is not feasible in common husbandry systems
- Development of a commercial gel-based diet with standard nutritional content and good palatability could overcome these challenges

Hypothesis

Adult zebrafish fed a new gel diet will have similar growth rates and reproductive success compared to those fed a commercial pelleted diet

Methods

- Impact of diet on water quality (Figure 1)
 - Diets added to tanks with no fish
 - Water quality (total ammonia nitrogen, dissolved oxygen) assessed at 0 and 24 hr
- Feeding trial
 - 200, 23-wk old AB (ZIRC, F1) zebrafish randomly assigned to 10 different 2.8 L tanks and 2 diet groups
 - Control diet:** GemmaMicro 300 (Gemma) fed twice daily, 3% body weight/day
 - Test diet:** AquaFeed[®]Z_β (AZ) 1cm³ cube
 - Wk 0-2: 1 cube/10 fish
 - Wk 2-15: 1 cube/tank (20 fish) to reduce spoilage
- Growth assessment q2 wk (Figure 2-4)
 - Group mass by tank, averaged per fish in tank
 - Nose-to-caudal peduncle length
- Fecundity (Figure 5)
 - Bred w/in diet group q2-4 wk, starting wk 5
 - Live embryos counted on 0 and 1 days post fertilization (dpf)
- Body composition (Figure 6)
 - EchoMRI to estimate % Lean and % Fat
 - Assessed post-euthanasia in groups of 10 fish
- 1 M + 1 F from each tank were fixed, sectioned, and stained with H&E for histopathology (Figure 7)
- Statistics: Prism v9.3.1. Parametric or non-parametric tests selected based on data distribution, sample size

Results

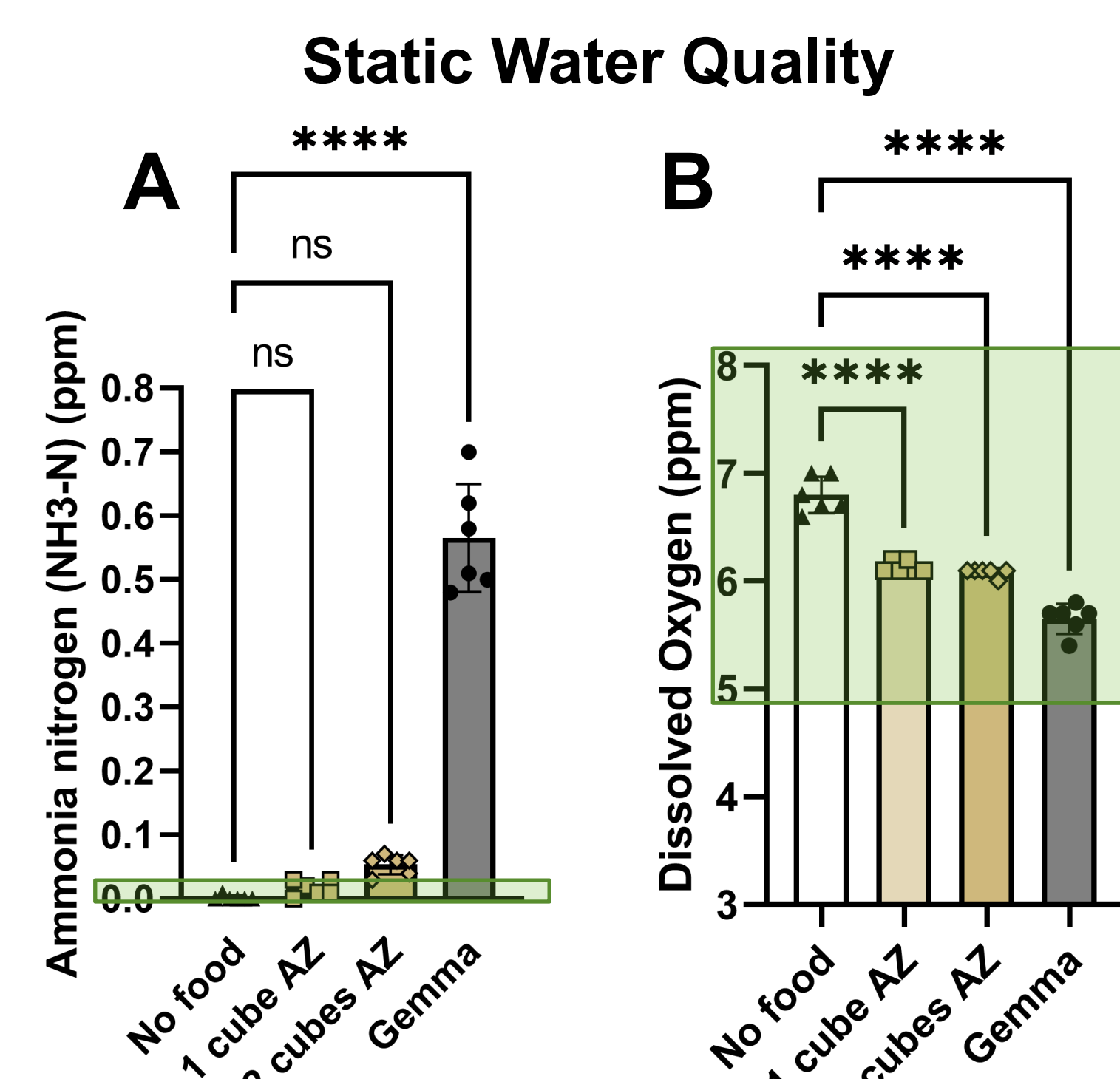


Figure 1: Water quality after 24 hr in static tanks without fish. (A) Total ammonia nitrogen, (B) Dissolved oxygen. Green boxes = target values for zebrafish. (Mean +/- SD) ****p<0.00005



Figure 3: Example of fish length measurement in ImageJ. Measurement of nose to caudal peduncle (yellow line) is calibrated to ruler in tank.

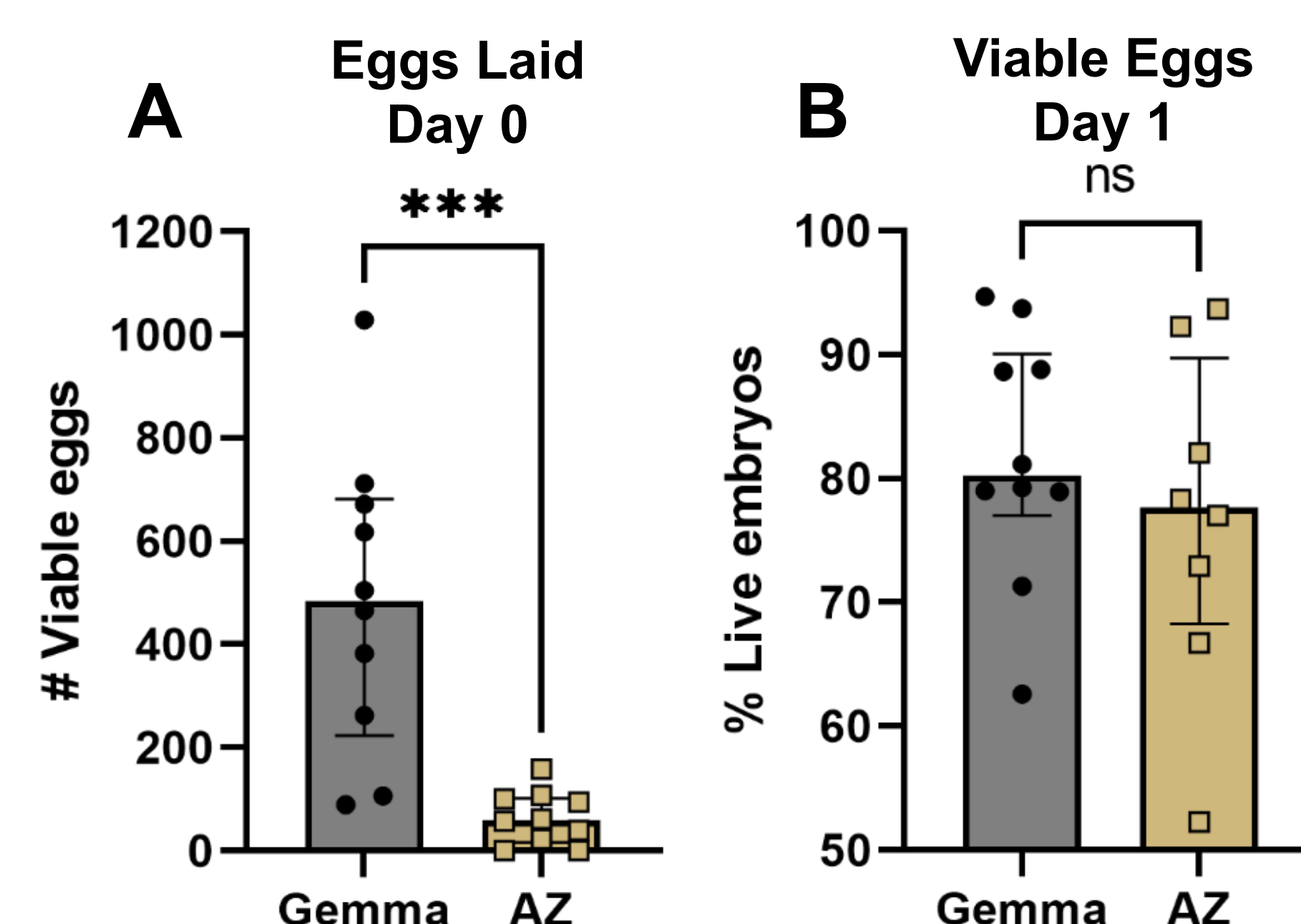


Figure 5: Fish fecundity as number of (A) viable eggs per mating and (B) percentage of live embryos 1 dpf. Data points are the mean of two replicates. (Median and IQR) ***p<0.0005

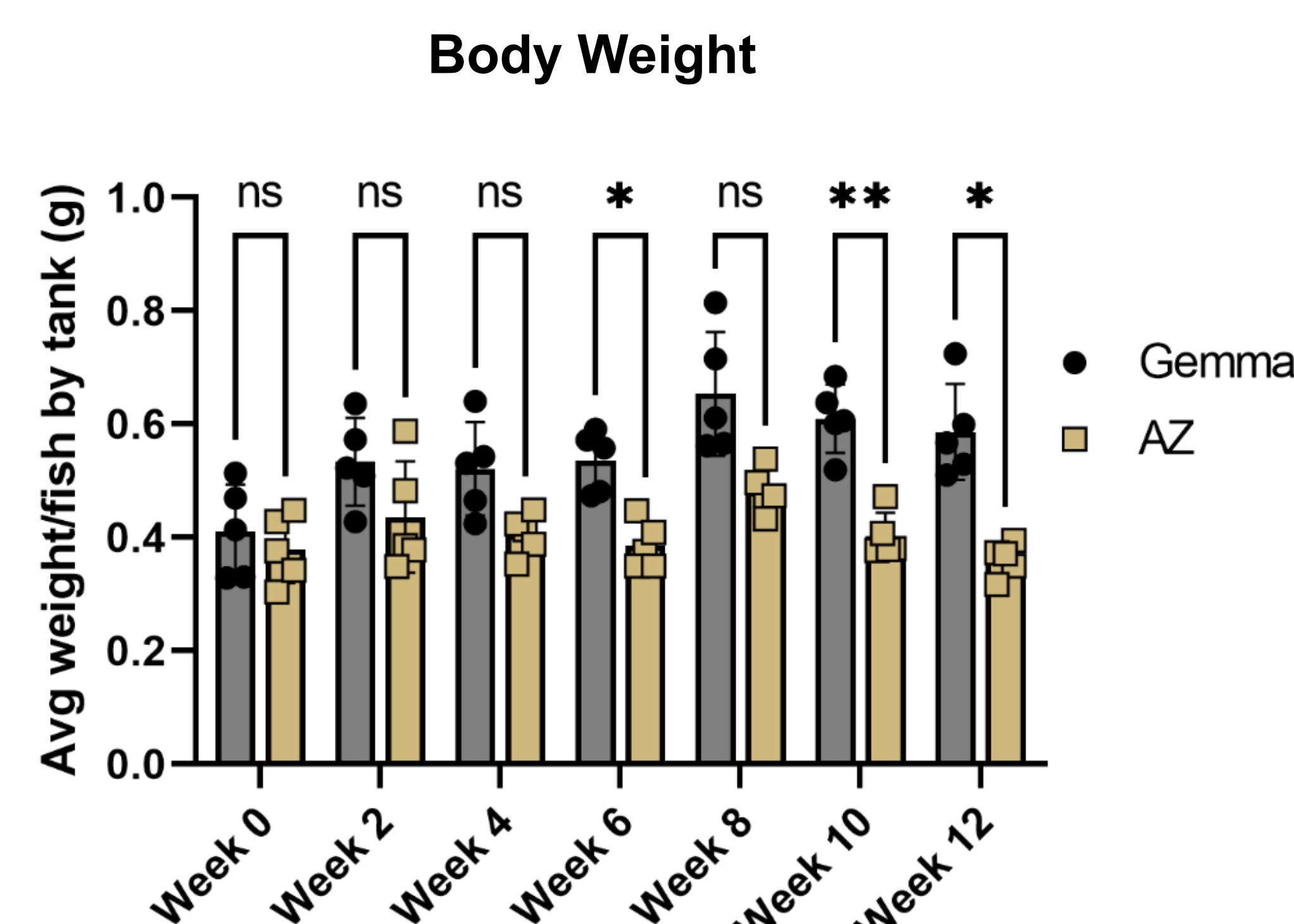


Figure 2: Average weight of individual fish by tank. Gemma and AZ groups were compared at each timepoint. (Mean +/- SD) *p<0.05, **p<0.005

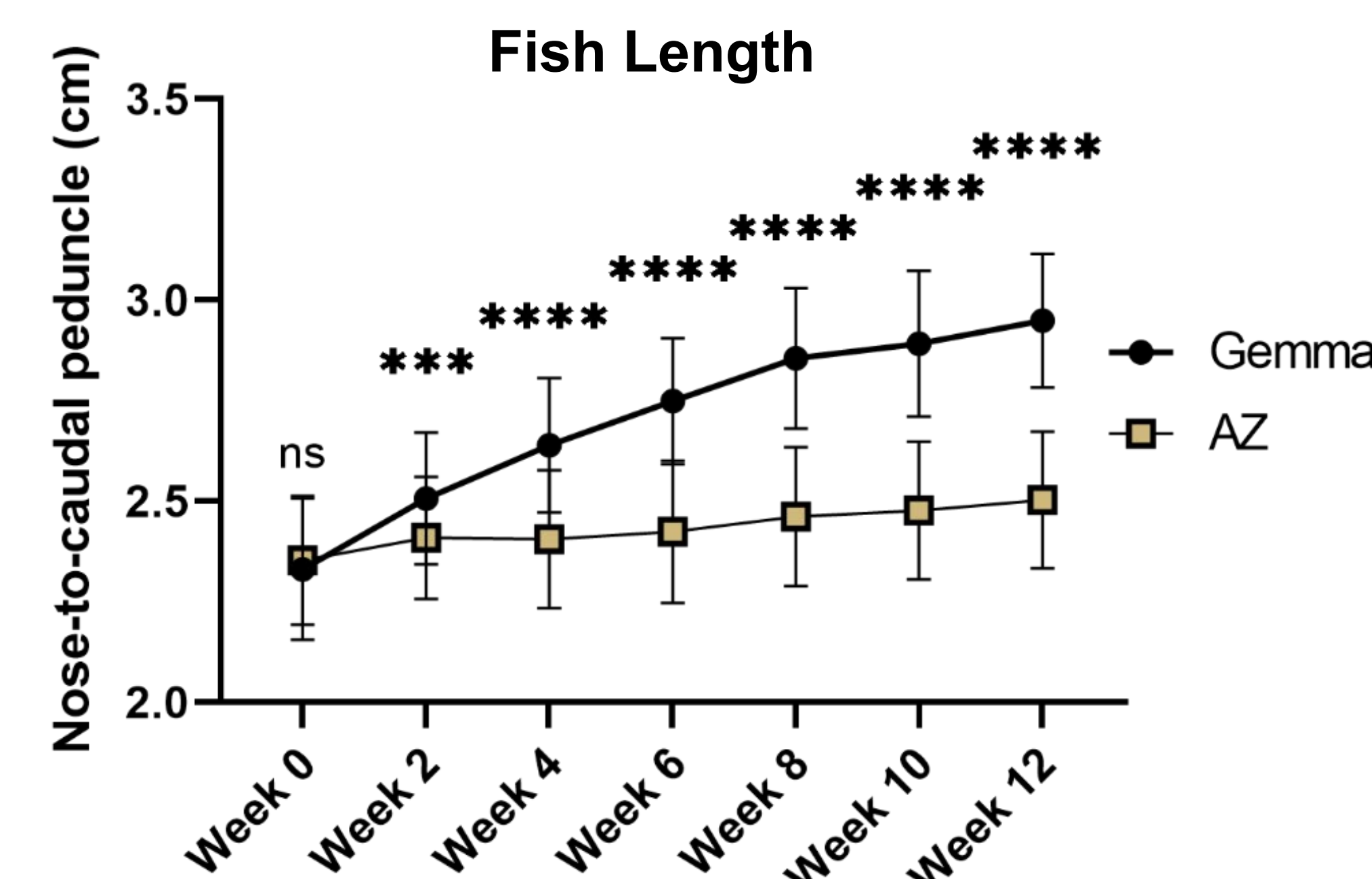


Figure 4: Fish lengths during feeding study. Gemma and AZ groups were compared at each timepoint. (Mean +/- SD) ***p<0.0005, ****p<0.00005

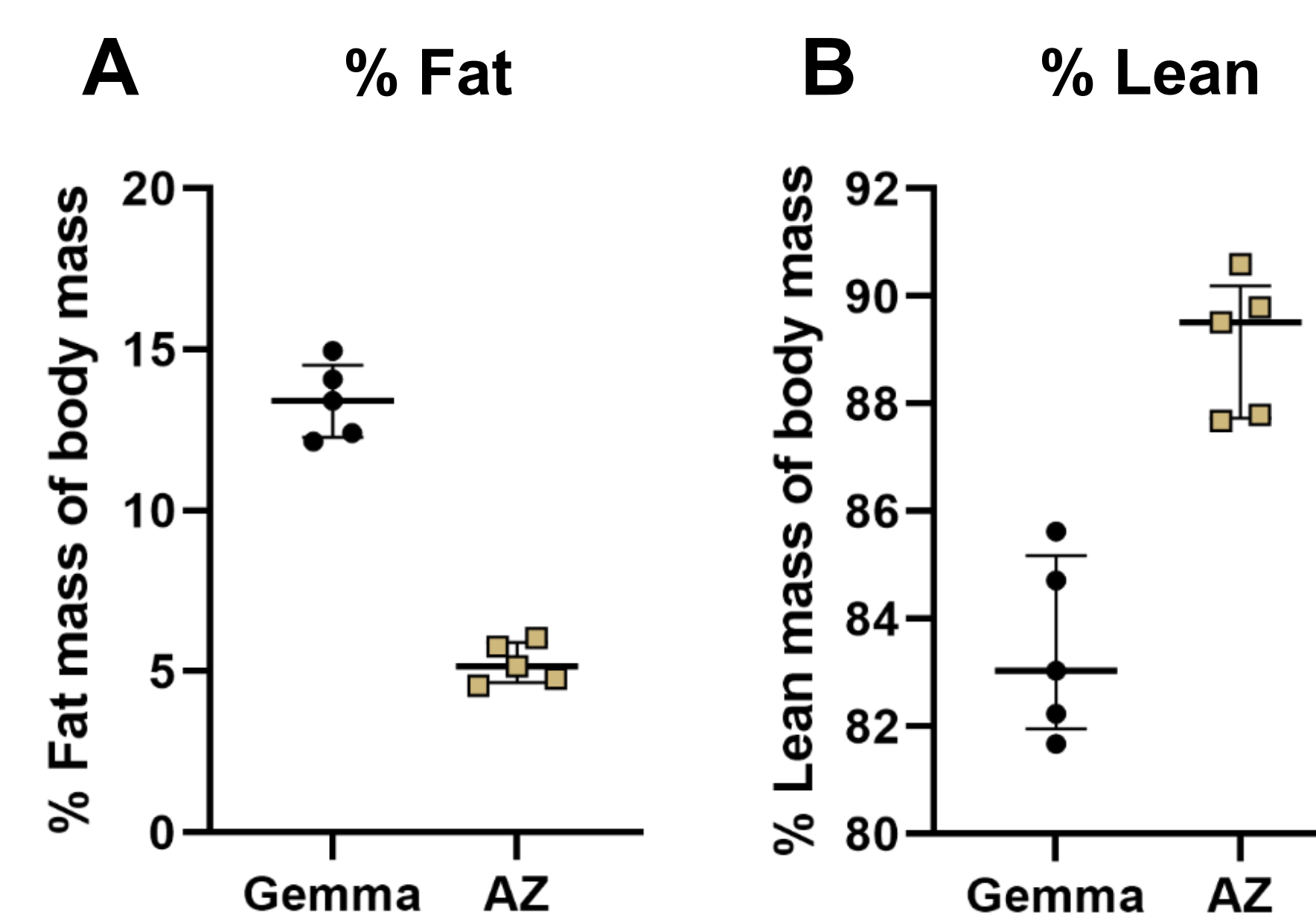


Figure 6: EchoMRI body composition of fish fed Gemma or AZ diet as (A) % Fat and (B) % Lean. (Median and IQR)

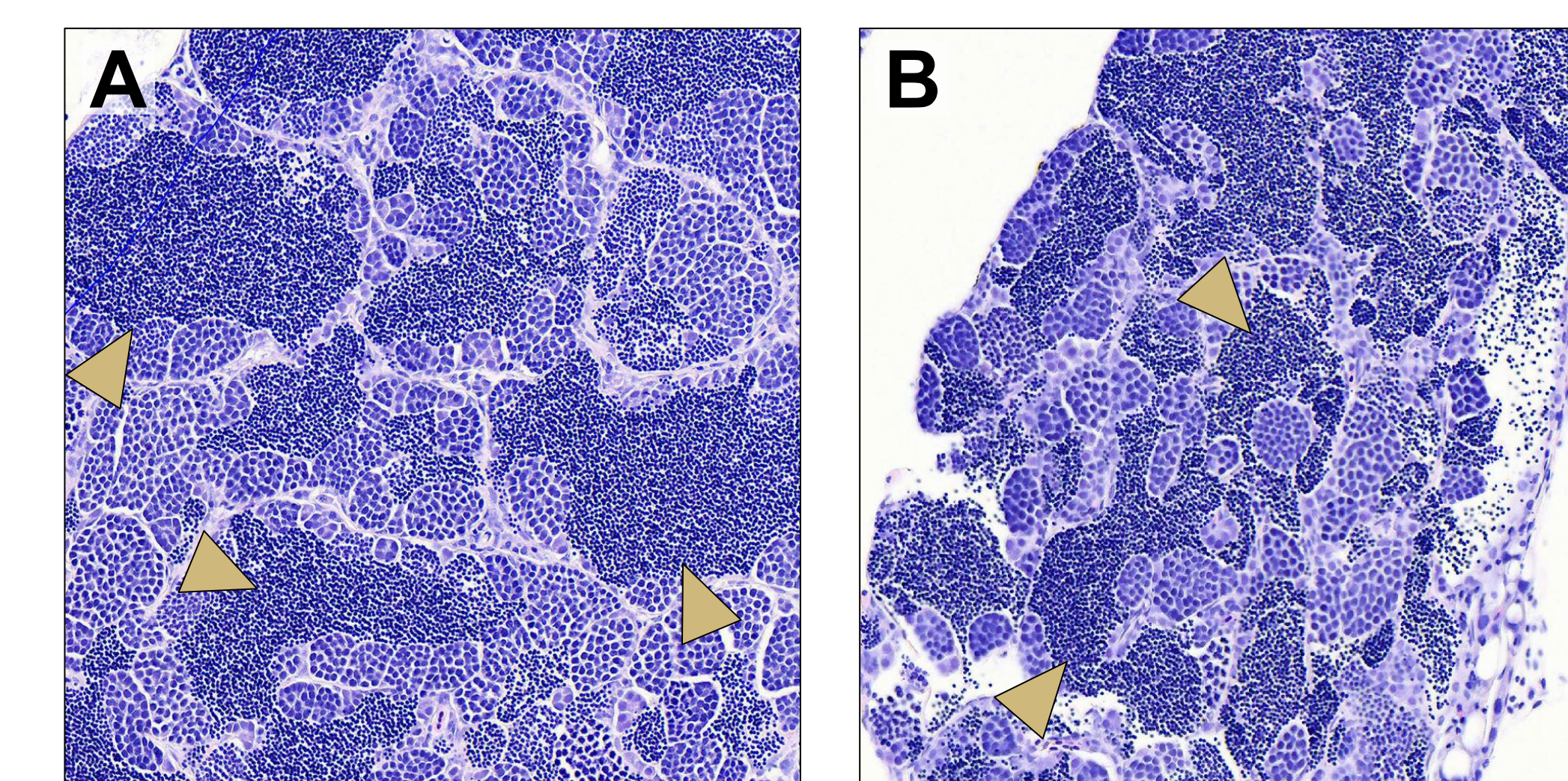


Figure 7: Histopathologic comparison of testes from fish fed (A) Gemma or (B) AZ. (20x) Note differences in size and sperm abundance (arrowheads).

Results Summary

- Water quality was impacted more quickly with Gemma in a static tank than AquaFeed[®]Z
 - Calculated un-ionized ammonia for all groups was non-toxic at 24 hr for all groups
- Zebrafish ate less AZ than expected
- 12 weeks of feeding adult zebrafish AZ resulted in smaller, leaner fish that laid fewer eggs and were less sexually mature than those fed Gemma
- The percentage of viable eggs was similar whether fish were fed Gemma or AZ
- No significant lesions were found in either group by histopathology

Conclusions

- AquaFeed[®]Z diet is safe for adult zebrafish fed for at least 12 weeks
- Early formulations of AquaFeed[®]Z may not be palatable to laboratory zebrafish raised on a pelleted GemmaMicro diet
 - Since this study, AquaFeed[®]Z has been reformulated to increase palatability and nutrient availability
- Because of slower changes in static water ammonia levels, AquaFeed[®]Z could be considered for feeding zebrafish in extended emergencies without water flow

Acknowledgements

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- Thank you to Dr. Jennifer Brazell of IDEXX BioAnalytics for histopathology support
- Zebrafish illustration: © 2016 DBCLS TogoTV / CC-BY-4.0