RAINBOW TROUT EYED EGGS AND YOLK-SAC LARVAE as a platform for early *in vivo* preclinical screening: a pilot feasibility study

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Repeated immersion from eyed egg to yolk-sac larvae was operationally feasible.
No detectable final mortality differences were observed between *Bacillus subtilis* isolates and PBS vehicle.

276 individuals | **27** flasks | **5 x 24 h** baths | **Endpoint: 409** degree-days

WHY THIS STUDY

Early *in vivo* preclinical screening needs exposure protocols that can be implemented reproducibly. This pilot assessed the feasibility of using rainbow trout (*Oncorhynchus mykiss*) eyed eggs and yolk-sac larvae as a platform for developing immersion assays.

CONDITIONS

- Isolate 72
- Isolate 339
- PBS vehicle

(Spores suspensions)

EXPERIMENTAL DESIGN

9 flasks per condition | 92 initial eggs per condition (8 x 10; 1 x 12) | Temperature: ~6.5 - 6.8 °C | Nominal bath concentration 10^7 CFU/mL | Water renewal 90% every 2 days


Eyed eggs (*Oncorhynchus mykiss*)


Flask setup (immersion baths)


Yolk-sac larvae

Eyed egg stage → **Yolk-sac larval stage**

Allocation: 234 DD (start)	Bath 1: 266 DD	Bath 2: 286 DD	80% hatched 325 DD	100% hatched 339 DD	Bath 3: 359 DD	Bath 4: 277 DD	Bath 5: 297 DD	Endpoint: 409 DD
								22 days after the First bath

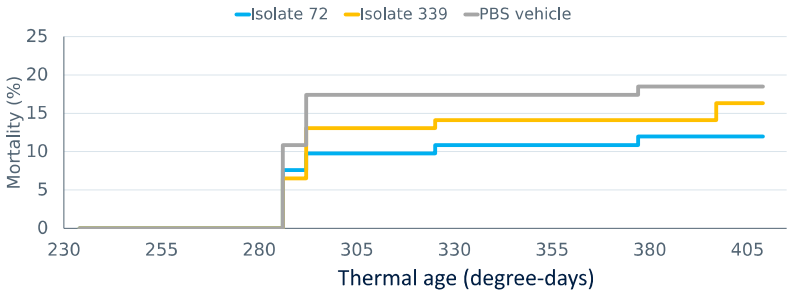
MORTALITY OUTCOMES

Isolate 72	Isolate 339	PBS vehicle
11.96%	**16.30%**	**18.48%**
11 dead / 92 initial	15 dead / 92 initial	17 dead / 92 initial

Flask = experimental unit
Kruskal-Wallis H(2) = 1.465, $p = 0.481$
Stratified permutation p = 0.513

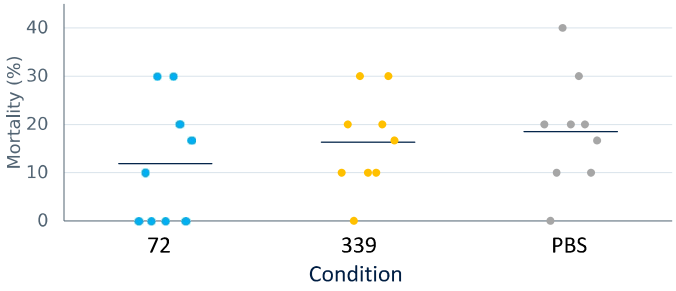
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A Cumulative mortality



Steps locate losses at census times, not exact death times.

B Final mortality per flask



Nine points per condition; bars show unweighted flask means.

CONCLUSIONS

- This pilot supports the operational feasibility of mantaining and monitoring a cohort from the eyed-egg to the yolk-sac larval stage under repeated immersion.
- These results provide an experimental basis for developing an early *in vivo* toxicity-screening platform using this vertebrate model.

NEXT STEPS

- Concentration series
- Independent batches
- Positive toxicity control
- Exposure verification in water

Predictive value for fish toxicity requires specific validation

SCOPE & LIMITATIONS

- Single egg batch and single nominal concentration.
- No water-only or positive toxicity control.
- Exposure concentration unverified in water.
- Assay sensitivity and reproducibility require validation.