STUDY DESIGN

**Hypothesis**: Polyethylene (PE) fiber ingestion at environmentally relevant concentrations will induce changes to Japanese medaka reproductive health.

**Scheme**:

PE fiber exposure was conducted per previous publication: DiBona et al., 2020 (<https://www.frontiersin.org/articles/10.3389/fphys.2021.668645/full>)

Briefly:

* Juvenile medaka (30 days post hatching) exposed to 5 concentrations of PE fibers for 21 days through the feed.
  + 0 PE fibers/fish/day
  + 0.5 PE fibers/fish/day
  + 1.5 PE fibers/fish/day
  + 3 PE fibers/fish/day
  + 6 PE fibers/fish/day
* After exposure, fish were allocated and kept in 2L tanks at a density of 10 fish per tank until reproductive maturity (40 days).
* No PE fibers were administered during this maturation period resulting in a 40-day depuration prior to breeding.
* Fish were then sexed and paired for breeding.
* 2 pairs (4 fish) were bred in a 2L tank.
* 5 replicates of breeding tanks per PE fiber exposure concentration.
  + Ex: 5 tanks of juvenile fish who were exposed to 0 fibers/fish/day (total of 20 fish per exposure concentration).
* Eggs were collected for2 sets of 5 consecutive days (10 replicates) from each tank.
* Fecundity, fertility, and hatching rate were monitored.
* After breeding, fish were sacrificed and 10 (5 males, 5 females) fish were allocated for histological assessment of gonadal tissue and 10 (5 males, 5 females) fish were allocated for gonadal tissue extraction for RT-qPCR assessment.

STRESSOR

**Chemical**: Blue low-density polyethylene multifilament yarn was obtained from Lumat USA.

Polymer was confirmed to be an 86% match to low density polyethylene using FTIR analysis.

400µm increments of fibers were obtained via manual cutting with a scalpel. Fibers were then counted and combined with dry feed for administering daily to fish tanks.

TEST SUBJECT

Japanese Medaka (*Oryzias* *latipes*) Orang-red were used as a model organism.

Embryos/larvae were obtained from the Seemann lab at TAMUCC.

Juveniles were exposed to PE fibers at 30 days post hatching.

EXPOSURE CONDITIONS

PE fiber exposure:

* Medaka were kept in 2L tanks at a density of 50 fish per tank for the duration of the PE fiber exposure (21 days).
* Food during the duration of the exposure was dry artemia 2x daily (one feeding containing PE fibers at designated concentration) and live artemia 1x daily.
* Fish tanks were aerated to maintain water flow and keep any PE fibers in the water column.

Breeding experiment:

* Fish were moved to clean 2L tanks and kept at a density of 10 fish per tank until reproductive maturity (40 days).
* Fish were then sexed, and 2 males and females were kept in a tank at a density of 4 fish per tank. The other 6 fish were maintained and kept in a 2L tank but not included in the breeding study for egg collection.
* Food during the duration of the breeding period was dry artemia 2x daily and live artemia 1x daily.
* Tanks were continued to be aerated to maintain consistency in the environment for the fish.

Water quality for the entire experiment was maintained according to IACUC regulations for the general husbandry of medaka in the Seemann lab at TAMUCC.

ENDPOINTS

* Fecundity (number of eggs per female)
* Fertility (number of fertile eggs/total eggs)
* Hatching rate (number of eggs hatched/total fertile eggs/day)
* Histology
  + Gonadal tissue assessment
    - Males: testis stage, Leydig cell counts
    - Females: oocyte stage, oocytes per area
* RT-qPCR
  + Relative expression of gonadal genes in gonadal tissue
    - Males: StAR, SOX9a, SOX9b, AMH, HSD11B2, ARa
    - Females: OLVAS, FOXL2, CYP19A1, LHR, FSHR, mPRa

DATA ANALYSIS

Unless otherwise stated. R-Studio and R version 4.0.3 were used to analyze all data.

Comparisons were made between the control of 0 PE fiber/fish/day and all other concentrations.

ANOVAs (one-way, and nested) were used for comparisons. When differences found, TukeyHSD was used as posthoc.

Survival analysis was done for hatching rate using survial package and survimer package.