Impacts of Chronic PE Fiber Exposure in Larval and Juvenile Fish

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Plastic Pollution: Microplastics

Primary source of microplastics is degradation in beach environment
(Andrady et al., 2011)

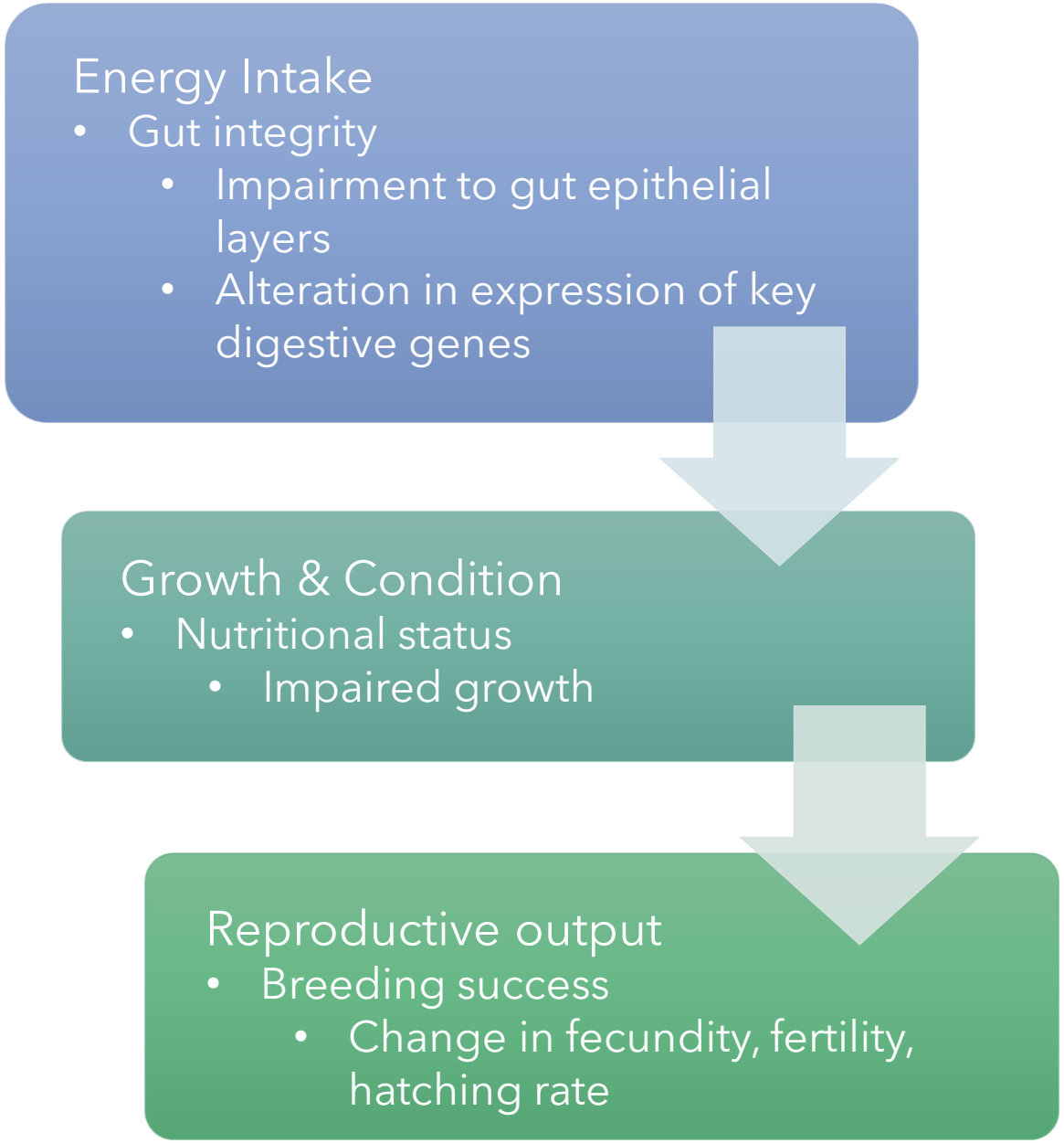
Polyethylene fibers (PE)

Adverse health effects due to consumption

Concentrations 2x – 7x higher
(Cole et al., 2011; Choi et al., 2018; Lenz et al., 2016)

Hypothesis:

Chronic exposure to environmentally relevant concentrations of PE fibers will impact fish health.



Methods

Microplastic Chronic Exposure

- Japanese Medaka life stages:
  - Larvae & Juveniles
- 5 replicates of 50 fish
- 21-day exposure to PE fibers
- Fibers cut into size (100µm and 400µm)
- Fibers incorporated into dry food (morning feed)

Group	Fibers per fish	Fibers per tank per day
A	0	0
B	0.5	25
C	1.5	75
D	3	150
E	6	300

Japanese Medaka (*Oryzias latipes*)

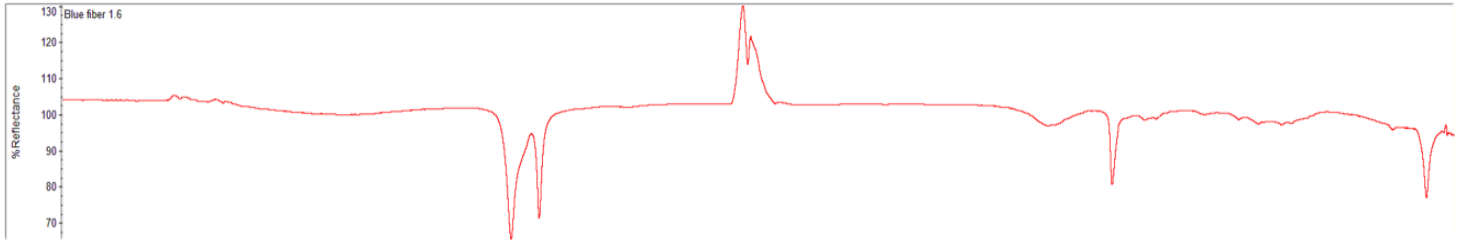
- Model organism
  - Ideal for gene study
  - Short life span
  - Quick reproductive development
  - Complete genome draft

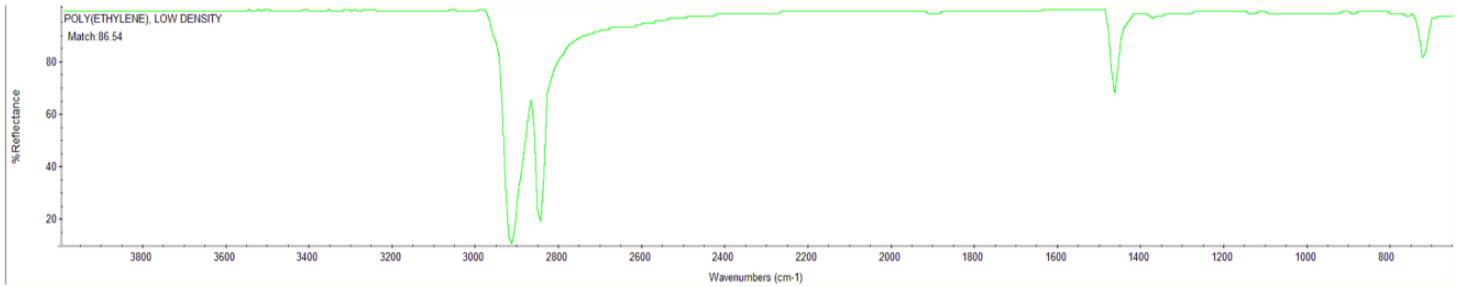
Male adult Japanese medaka

- Two life stages
  - **Larvae:** first mouth opening, crucial stage for growth
  - **Juvenile:** key stage for organ maturation including gonad development and growth

Polyethylene (PE) Fiber Preparation

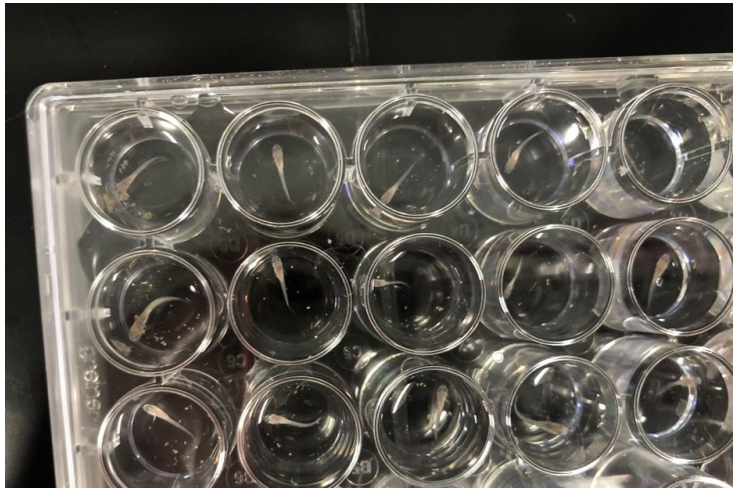
- Blue polyethylene multifilament yarn
  - Lumat (USA)
- FTIR results: 86% match to polyethylene low density
- 100µm → larvae
  - Paraffin embedded, microtome cut section, cleared overnight with Histochoice clearing agent, rinsed and filtered
- 400µm → juveniles
  - Cut with paper cutter and ruler

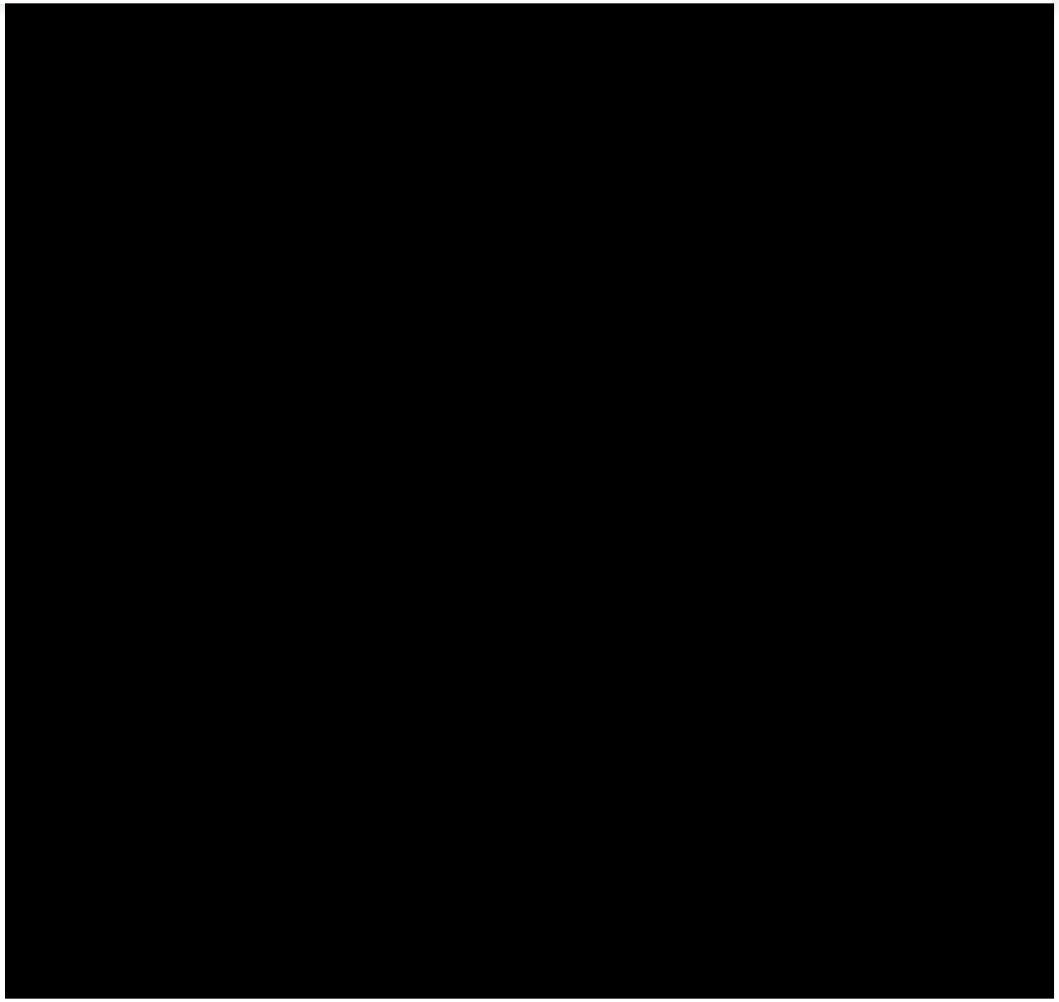




Uptake and short-term retention of PE fibers

- Medaka juveniles (1 month) fed 10 fibers per fish
- 1 hour feeding time
- Sacrificed at 2, 3, 4, 5 & 6 hours post feeding
- Medaka excreted plastics by 5-6 hours

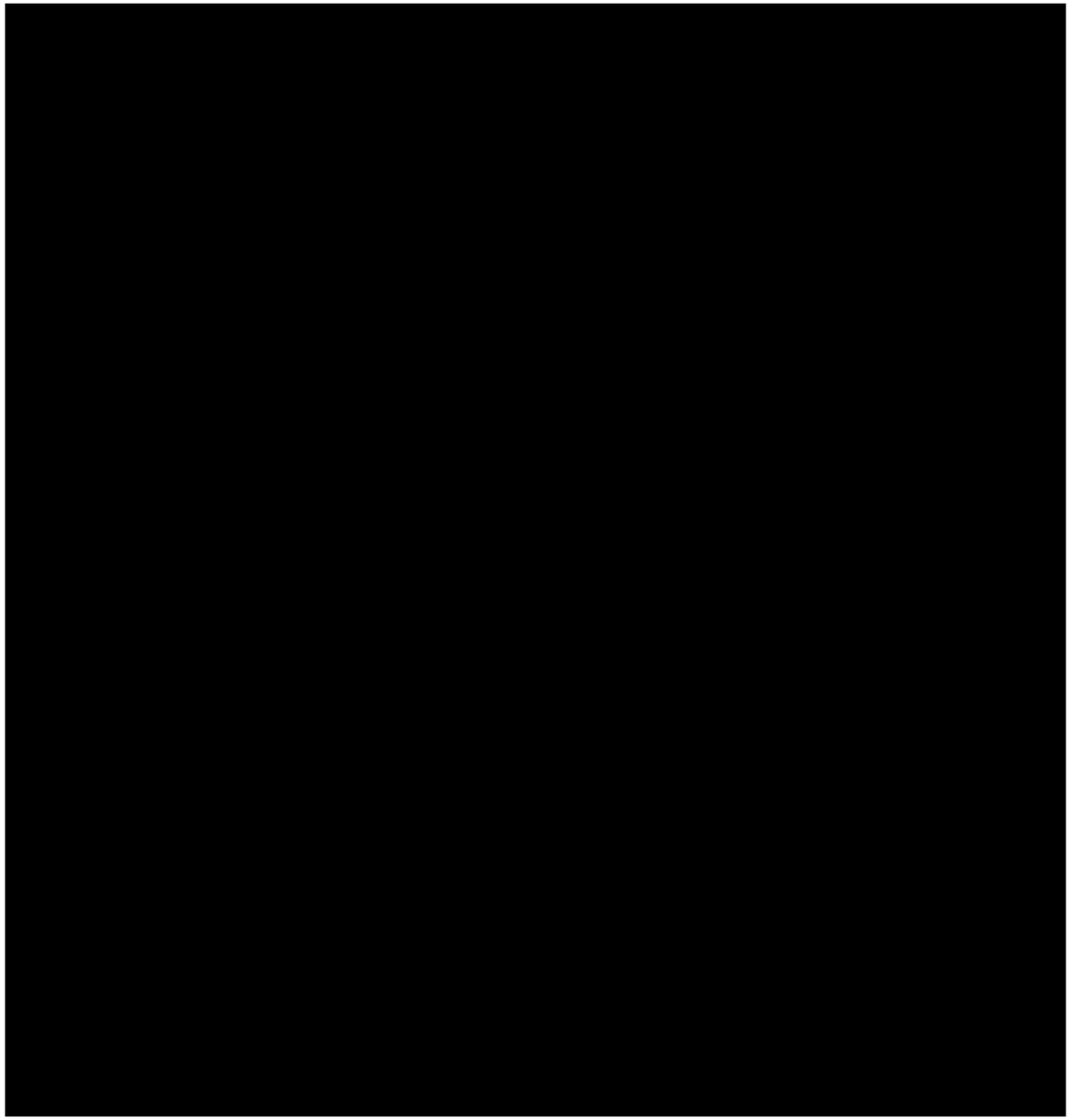




Results

Plastic Fiber Retention in Gut

- Retention time for PET fibers: 6 hours post exposure
- Sacrificed 12 hours post exposure
  - Isolated gut and dissected for plastic fibers (n=10, 5 replicates of 10)
- Larvae: Group E significantly different from control
- Juvenile: Group D and E significantly different from control

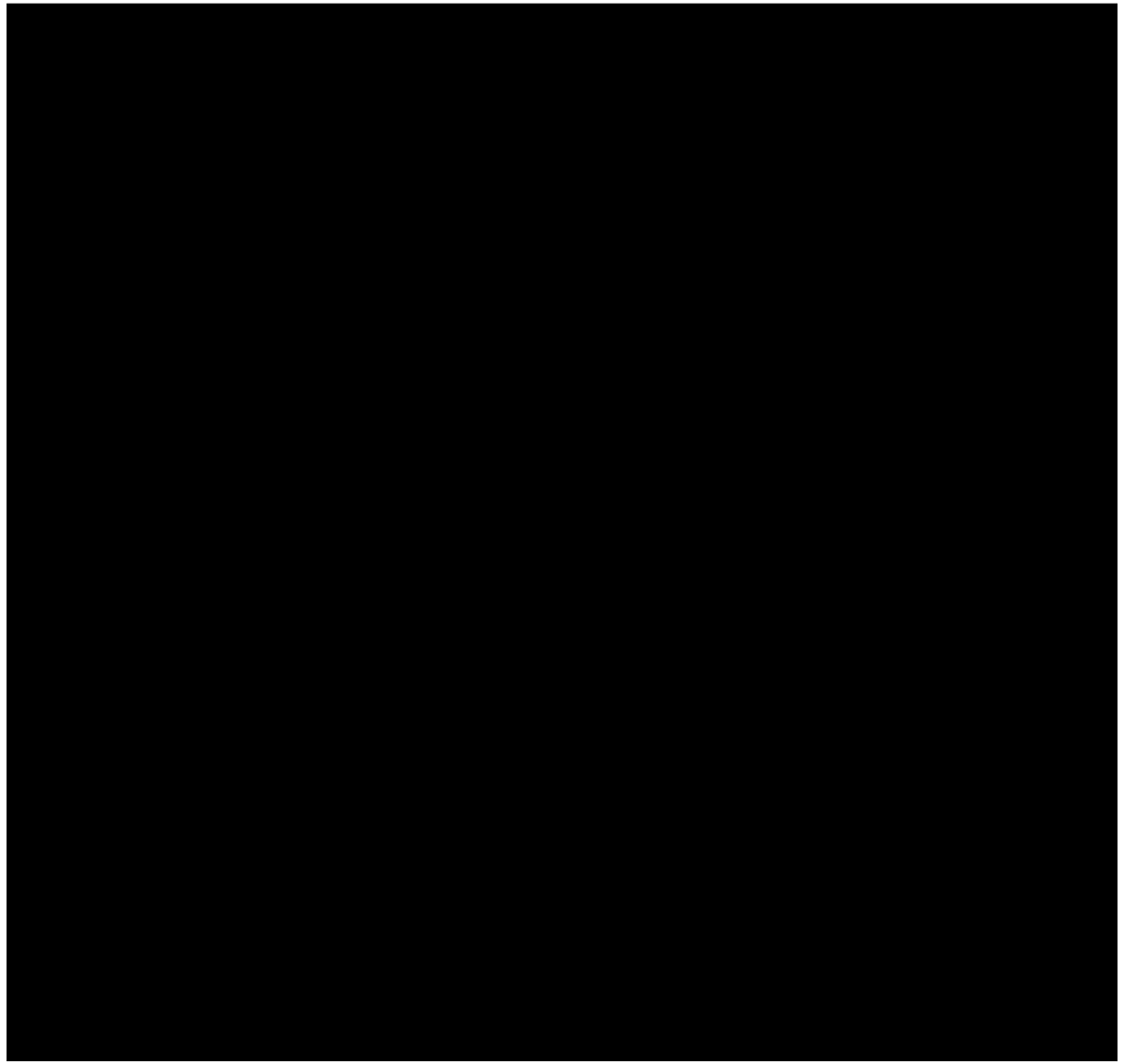


RT-qPCR:
Larvae and Juvenile

- No significant difference in Juvenile or Larvae:
  - Glucagon
  - Peptide YY
  - Trypsinogen
  - Insulin

- Significant difference for Larvae expression of **slc6a6**

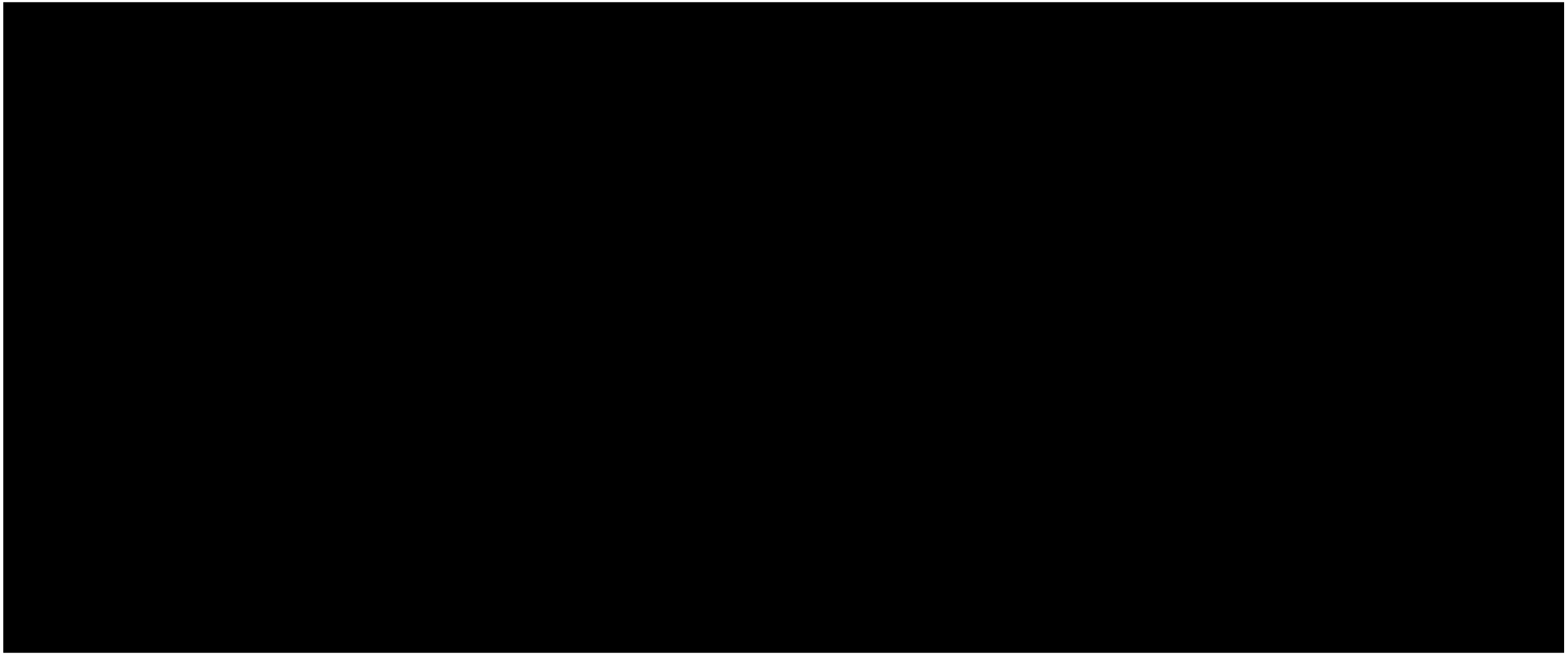
- No difference in slc6a6 for Juveniles



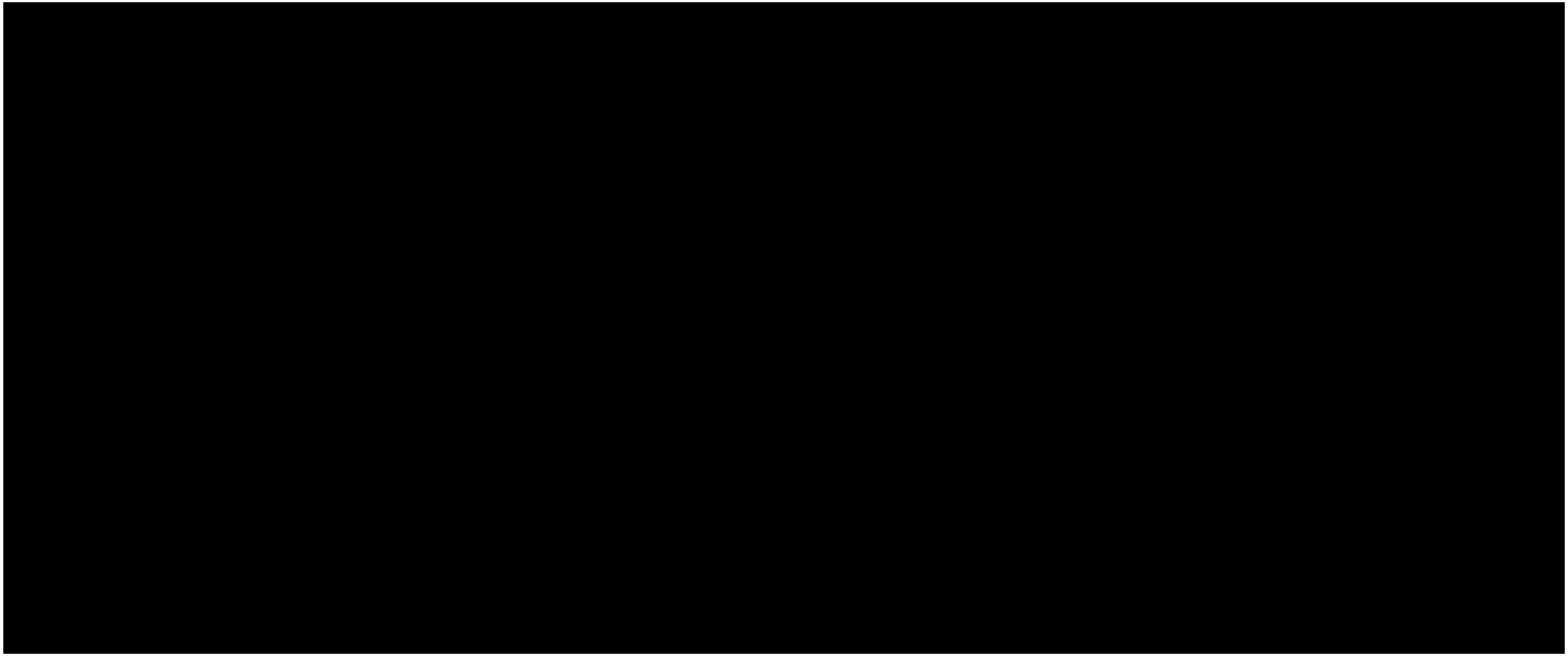
No Significant Histomorphological Changes

- No significant difference in goblet cell count, microvilli length, or microvilli width.
- No significant difference in color (pH) of mucus.
- No significant difference in inflammation.

No Change in Larval Fish Condition



No Change in Juvenile Fish Condition



Results: Juvenile Reproductive Success

- No significant difference in fertility or fecundity
- Significant difference in hatching rate
  - 2 to 3 days delayed

Summary of Results

Chronic, low dose exposure to environmentally relevant concentrations of PE fibers induced subtle changes on larval and juvenile fish health, and significantly impaired the F1 offspring development

Change in expression of slc6a6

&

Delayed hatching

Two potential causes:

Exposure to chemicals (endocrine disrupters) via PE fibers	Alteration to energy budget of medaka

Endocrine Disrupter Exposure

Potential leaching of chemicals from microplastics; some may be endocrine disrupters (Cole et al., 2011, Hamlin et al., 2015; Sax 2010).

Endocrine disrupters cause impairment of reproductive success (Lyche *et al*., 2009; Maffini *et al*., 2006; Manikkam *et al*., 2013; Sussarellu *et al*., 2016).

Recent medaka study: also saw reproductive changes; increased egg production (Hu et al., 2020)

at 100x higher concentrations

Energy Budget Alteration

- Energy budget alteration seen in other studies on microplastic exposure (Sussarellu et al., 2016; Xu et al., 2017)

- Decrease expression of slc6a6
  - Nrf2 pathway
  - Nutrient absorption

- Nrf2 pathway → antioxidant production (Hybertson et al., 2011; Ma 2013)
- Slc6a6 associated with colorectal adenocarcinomas → nutrient absorbance and fitness (Janikowska et al., 2018)

Conclusion/Recommendations

- No imminent threat to fish condition at current measured microplastic concentrations
- Close monitoring of microplastic levels in vital spawning grounds for commercially important fishes is recommended
- More studies on effects of common microplastics at relevant concentrations with both UV weathered and virgin microplastics are needed to further understand the threat microplastic pollution poses to wild fish

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Questions?