Ecologically Relevant Perspectives: Exploring Sublethal Effects of Endocrine Disruptors on Behavior and Morphology in Aquatic Organisms

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ABSTRACT

Estrogen-like endocrine disrupting chemicals (EDCs) attracted concern after an assortment studies in the 1980s highlighted feminized fish and amphibian populations. Even though the Environmental Protection Agency (EPA) started testing for the effects of these chemicals on the environment, the ecological and biological relevance of these substances are still largely unknown. One of the chemicals of current concern and debate is bisphenol A (BPA), which is widely used as a plasticizer. BPA is thought to act as an estrogen mimic (Staples et al. 2002). Though limits exist for lethal amounts of BPA, many scientific studies continue to find effects of BPA well below the "toxic" limit. The National Institute of Health and Environmental Sciences lists BPA as a chemical of "some concern" since we are still unsure whether or not it poses reproductive or developmental hazards to humans.

Many studies have shown an increased amount of intersex fish in populations exposed to xenoestrogens (Sumpter 1998). Our behavioral and morphology study on two species of fish seeks to elucidate whether or not BPA influences exist in organisms at real-world environmental levels, which are well below what is considered toxic. Both of these studies will express the importance of focusing on sublethal effects of BPA. Sublethal and synergistic effects of EDCs could lessen survivorship of an individual and more broadly cause population decline of a species.

The behavioral study will demonstrate whether or not ecologically relevant doses of BPA have an effect on the aggressive nature of Betta splendens at a level of 35 ng/L (Floating Island 2008). The aggressive nature of the Betta fish is important for mate selection and paternal care of the young. Additionally a morphological study using common minnows will demonstrate whether or not ecologically relevant BPA has an effect on the growth and gonadal development of local Indiana minnows at 650 µg/L, 1280 µg/L, and 3500 µg/L (Caunter 1999, Caunter 2000, & Alexander et al. 1988).

BACKGROUND

Endocrine Disrupting Chemicals (EDCs)

- Any foreign substance in the body that disrupts hormone messaging by blocking or mimicking responses
- Include certain pesticides (triazines), biphenol A (BPA) in plastics, and other synthetic chemicals

BPA

- Normally used to make epoxy and plastic resins (Figure 1).
- Originally invented in 1891 to synthesize plastics, but in the 1930s it was used as a pharmaceutical hormone. Diethystilbestrol (DES) was used instead but later found to cause reproductive cancers.
- Bisphenol A (BPA) acts as an estrogen mimic (Houlihan et al. 2011).

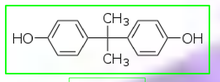


Figure 1

Sublethal Effects

- Changes in physiology, growth, reproduction, or behavior
- Example (Figure 2): The herbicide atrazine is also an estrogenic EDC and many studies have demonstrated sublethal effects on fish(Rohr et al. 2010)

Effect on amphibians/fish (studies monitored)	Studies That Found Adverse Effects	Studies That Didn't Find Adverse Effects
motor skills (13)	12	1
predation-relation risk (7)	6	1
olfactory sensitivity (5)	5	0
gonadal morphology (10)	7	3
sex ratios	not enough information	not enough information
aromatase	not enough information	not enough information

Figure 2

EXPERIMENTAL DESIGN/METHODOLOGY

Betta Fish Behavior Study

- Fish acclimated for 18 days in tanks of 700ml spring water, 165g gravel, & 7g of live plant
  - Opaque dividers between tanks to prevent male:male, female:male, female:female sight
- Male fish divided and treated on day 19 with 35ng/ml of BPA or β-estradiol (both in EtOH vehicle) with another group of EtOH vehicle control.
  - Tanks re-dosed approximately every week to account for evaporation and chemical degradation (Bell 2004)
- Fish video (via Flip Video ™) aggressive behavior recorded at days 2, 4, 6, and 9 post-treatment
  - EtOH control vs. BPA, EtOH control vs. β-estradiol, BPA vs. β-estradiol
  - Two fish tested per blind test protocol (Figure 4)
  - Recorded for 10 minutes, discarding first two minutes to account for human interaction
  - All video data was analyzed as a blind study

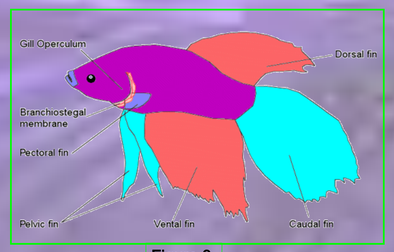


Figure 3

Figure 4

Minnow Morphology Study

- Minnows obtained from local IN bait shops ("Anglers' World", "Bait Barn")
- Minnows immediately placed in 10G tanks of 29.5L of spring water, gravel, & non-chemical sponge filter and acclimated for 3 days
- Healthy minnows dosed with the following treatments (Figure 5, Figure 6):
  - Low BPA (650µg/L), medium BPA (1280µg/L), high BPA (3500µg/L)
  - β-estradiol (500ng/L) as positive control
  - EtOH as vehicle control
  - No treatment control
- Performed water changes and redosing on tanks approximately weekly to ensure tank health & prevent chemical degradation
- Dead fish collected, recorded, and stored for dissection
- Day 15 post-treatment remaining fish were anesthetized and sacrificed for remaining dissections

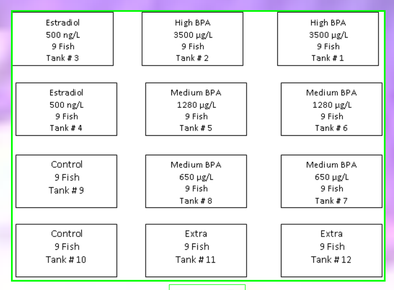


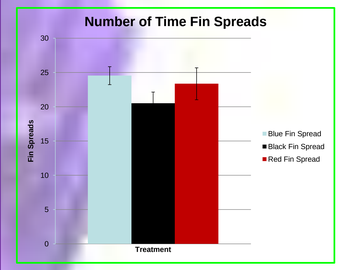
Figure 5

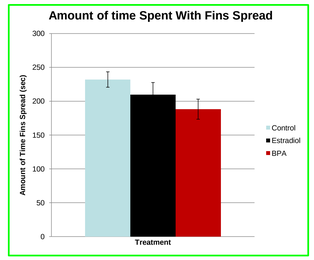
Figure 6

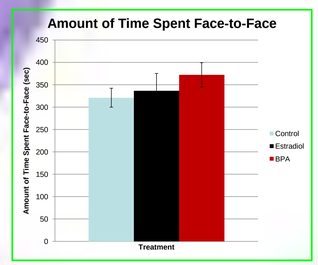
RESULTS

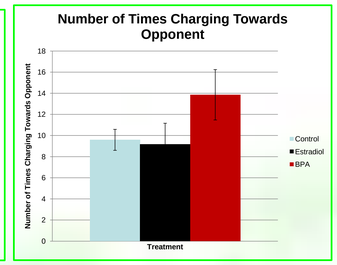
BETTA FISH BEHAVIOR

- Behavior was recorded over an 8 minute interval

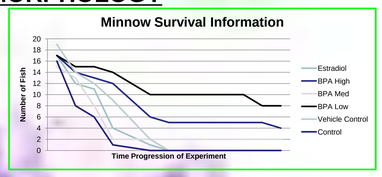








MINNOW MORPHOLOGY



Treatment	Avg. Weight (g)	Avg. Length (cm)	Avg. Girth (cm)	Number of Fish
BPA High	0.74	4.61	2.24	15
BPA Med	0.71	4.82	2.19	15
BPA Low	0.82	4.83	2.19	15
Estradiol	0.79	4.85	2.20	17
Vehicle Control	1.09	4.94	2.61	15
Control	0.85	4.78	2.40	21

Treatment	Male	Female	Intersex	Number of Fish
BPA High	2	4	0	6
BPA Med	2	2	0	4
BPA Low	2	1	0	6
Estradiol	4	0	1	5
Vehicle Control	3	3	0	6
Control	1	3	0	4

CONCLUSIONS

Betta Fish

- Analyze additional aggressive interactions including air gulps, gill flares, and swaying motions.
- 35 ng/L of estradiol/BPA may not be high enough to detect change in all behaviors, however did have influences in some behaviors

Minnows

- Study only found one intersex fish in positive control
- May need to run study longer to find greater effects