EDE Final Project

Plastic Additives Adversely Affecting Aquatic Life

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1 Rationale and Research Questions

We wanted to look into the differences in the NOEC concentration of Di-ethyl- hexyl-phthalate (DEHP), an abundant plasticizer in plastics, across species of fish. NOEC stands for "No Observed Effect Concentration" which means it is the highest tested concentration of a substance where no statistically significant adverse effect is observed. It's used in ecotoxicology studies to determine the lowest concentration at which a chemical might be considered safe for a particular species or ecosystem.

If we had found out that the NOEC concentration across all the species was the same, we could have provided a generic threshold limit for all the species. This analysis would be most helpful from a risk assessment perspective. If we could not find the same NOEC concentration from multiple studies for various fish species, then we could not give a generic threshold. Also, the life stage is important for all the organisms. We are looking to see if the NOEC concentration of DEHP is the same for embryonic, larval, and adult life stage or not.

We want to look to answer specific questions such as: -Which fish species is most sensitive to DEHP? -Which life stage of the fish species is most sensitive to DEHP? -For our Anova analysis, our Null Hypothesis is: there is no significant difference between the fish species and their life stage. -And the Alternate Hypothesis: there will be a significant difference between the fish species and their life stage.

2 Dataset Information

We extracted data from the eco-toxicolgy knowledge-base resource hub. This dataset is regulated by the U.S. EPA and contains over 13,000 chemicals, and 14,000 species. We chose phthalate esters as the chemical to inspect the concentrations of. We downloaded data for phthalates for 16 classes of species, such as fish, crustaceans, amphibians, insects/spiders and more. See the link here for more details: https://cfpub.epa.gov/ecotox/. We wrangled to look at Fish, Amphibians and Crustaceans, and then decided to deep dive into Fish. We also filtered to only look at DEHP at the NOEC levels of concentration.

3 Exploratory Analysis

```
#Raw data dimensions and column names
dim(ET.Pht)
```

```
## [1] 8963   88
```

```
colnames(ET.Pht)
```

```
##  [1] "CAS.Number"                          "Chemical.Name"
##  [3] "Chemical.abbreviation"               "Chemical.Grade"
##  [5] "Chemical.Analysis"                   "Chemical.Purity.Mean.Op"
##  [7] "Chemical.Purity.Mean..."             "Chemical.Purity.Min.Op"
##  [9] "Chemical.Purity.Min..."              "Chemical.Purity.Max.Op"
## [11] "Chemical.Purity.Max..."              "Species.Scientific.Name"
## [13] "Species.Common.Name"                 "Species.Group"
## [15] "Organism.Lifestage"                  "Organism.Age.Mean.Op"
## [17] "Organism.Age.Mean"                   "Organism.Age.Min.Op"
## [19] "Organism.Age.Min"                    "Organism.Age.Max.Op"
## [21] "Organism.Age.Max"                    "Age.Units"
## [23] "Exposure.Type"                       "Media.Type"
## [25] "Test.Location"                       "Number.of.Doses"
## [27] "Conc.1.Type..Standardized."          "Conc.1.Mean.Op..Standardized."
## [29] "Conc.1.Mean..Standardized."          "Conc.1.Min.Op..Standardized."
## [31] "Conc.Min.1..Standardized."           "Conc.1.Max.Op..Standardized."
## [33] "Conc.1.Max..Standardized."           "Conc.1.Units..Standardized."
## [35] "Conc.2.Type..Standardized."          "Conc.2.Mean.Op..Standardized."
## [37] "Conc.2.Mean..Standardized."          "Conc.2.Min.Op..Standardized."
## [39] "Conc.Min.2..Standardized."           "Conc.2.Max.Op..Standardized."
## [41] "Conc.2.Max..Standardized."           "Conc.2.Units..Standardized."
## [43] "Conc.3.Type..Standardized."          "Conc.3.Mean.Op..Standardized."
## [45] "Conc.3.Mean..Standardized."          "Conc.3.Min.Op..Standardized."
## [47] "Conc.Min.3..Standardized."           "Conc.3.Max.Op..Standardized."
## [49] "Conc.3.Max..Standardized."           "Conc.3.Units..Standardized."
## [51] "Effect"                              "Effect.Measurement"
## [53] "Endpoint"                            "Response.Site"
## [55] "Observed.Duration.Mean.Op..Days."    "Observed.Duration.Mean..Days."
## [57] "Observed.Duration.Min.Op..Days."     "Observed.Duration.Min..Days."
## [59] "Observed.Duration.Max.Op..Days."     "Observed.Duration.Max..Days."
## [61] "Observed.Duration.Units..Days."      "BCF.1.Value.Op"
## [63] "BCF.1.Value"                         "BCF.1.Min.Op"
## [65] "BCF.1.Min"                           "BCF.1.Max.Op"
## [67] "BCF.1.Max"                           "BCF.1.Unit"
## [69] "BCF.2.Value.Op"                      "BCF.2.Value"
## [71] "BCF.2.Min.Op"                        "BCF.2.Min"
## [73] "BCF.2.Max.Op"                        "BCF.2.Max"
## [75] "BCF.2.Unit"                          "BCF.3.Value.Op"
## [77] "BCF.3.Value"                         "BCF.3.Min.Op"
## [79] "BCF.3.Min"                           "BCF.3.Max.Op"
## [81] "BCF.3.Max"                           "BCF.3.Unit"
## [83] "Author"                              "Reference.Number"
## [85] "Title"                               "Source"
## [87] "Publication.Year"                    "Citation"
```

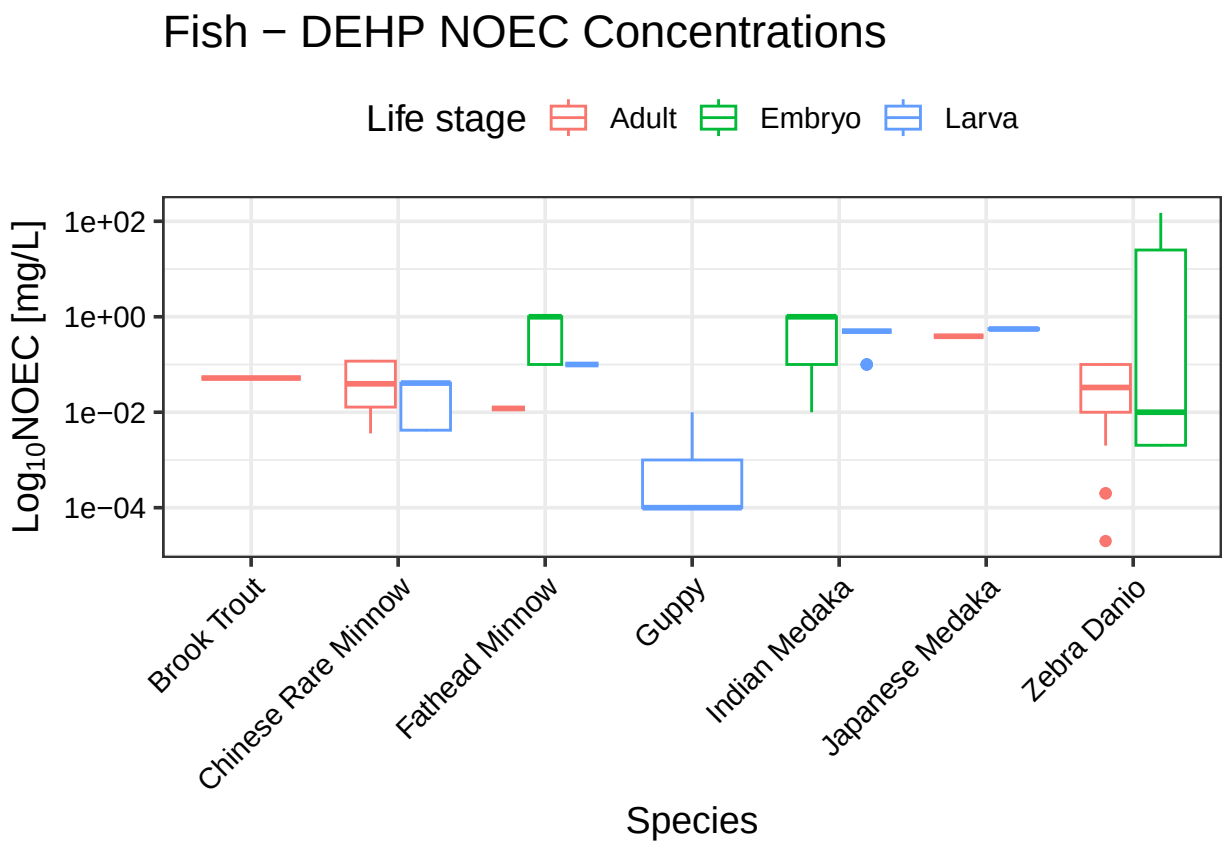
```
#We are interested only in DEHP, fish, NOEC, and life stages.
#Selected the following columns: Chemical.abbreviation, Species.Common.Name,
#Organism.Lifestage, Endpoint, and Conc.1.Mean..Standardized.
```

4 Visualizations

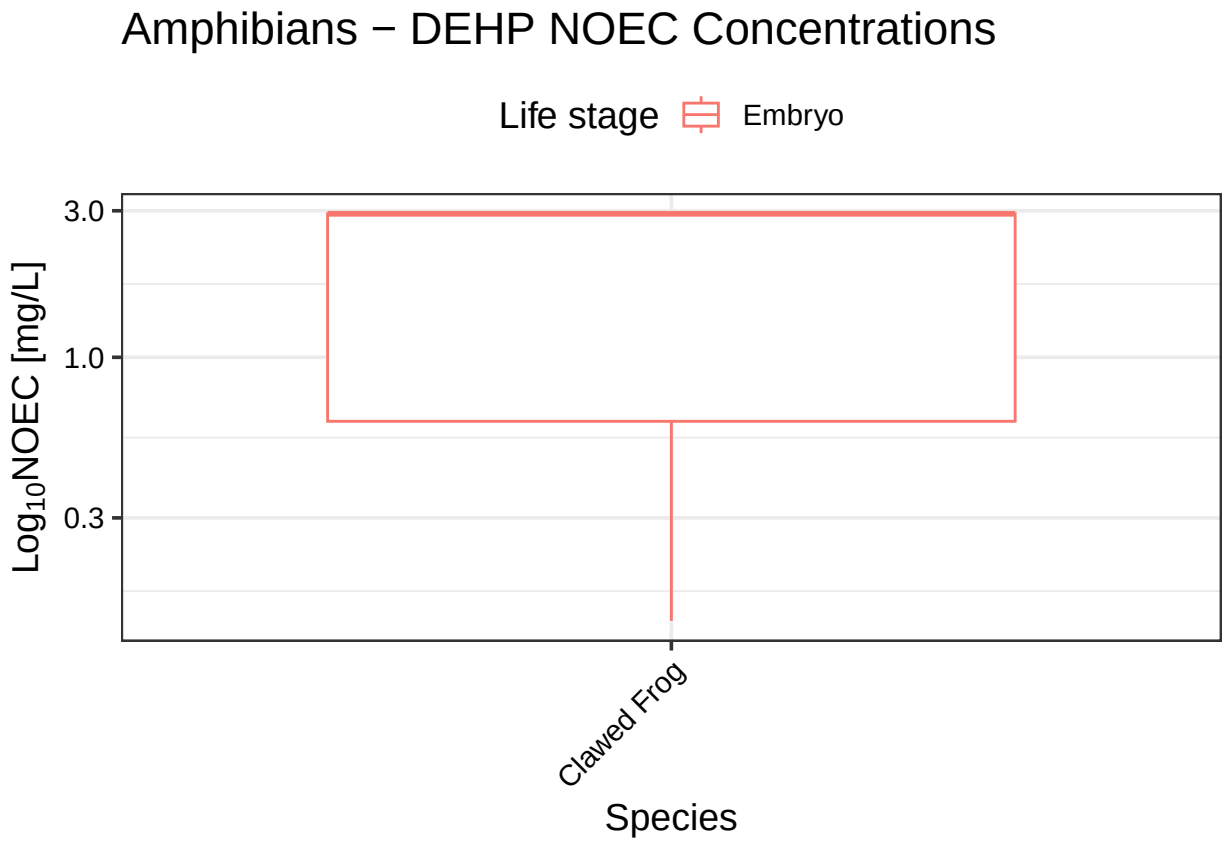
```
#Data Exploration and Visualization

#Fish Boxplot
ggplot(fish_tidy, aes(x = species, y = concentration, color = life_stage)) +
  geom_boxplot() +
  scale_y_log10() +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle("Fish - DEHP NOEC Concentrations") +
  labs(x = "Species",
       y = expression(Log[10] * NOEC * " [mg/L]"),
       color = "Life stage")
```

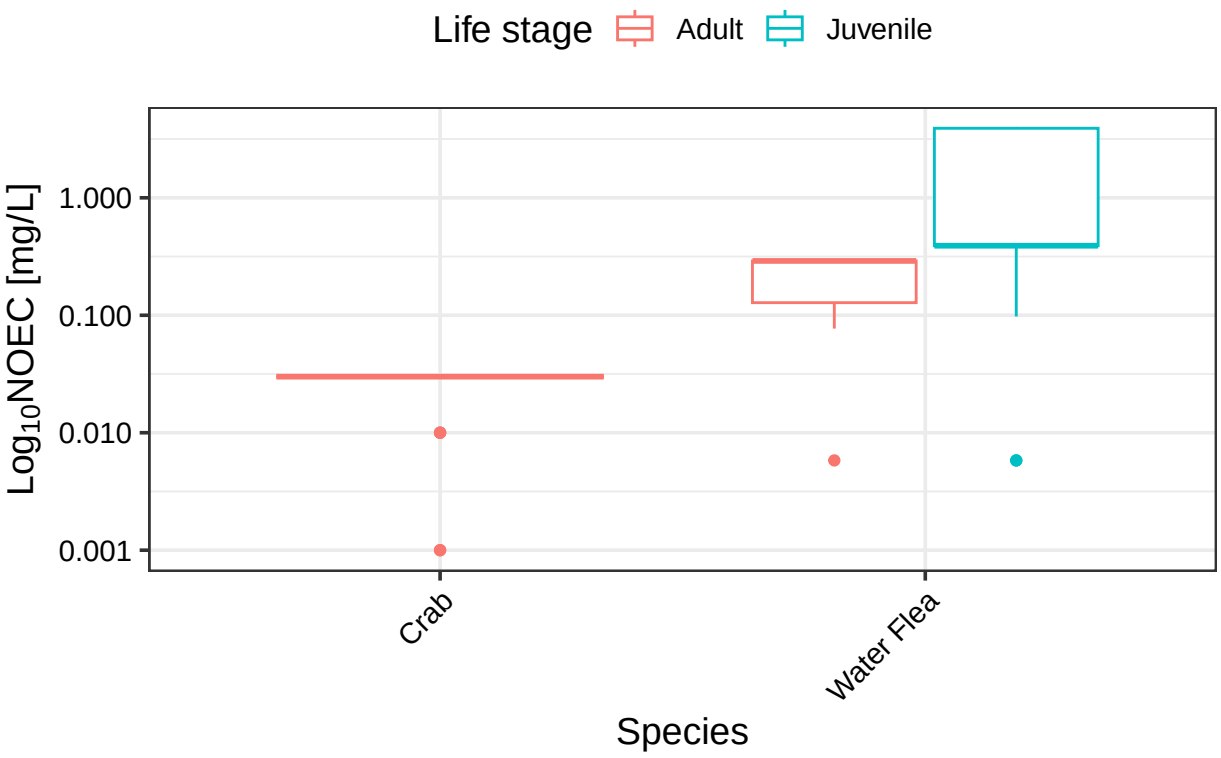
```
## Warning: Removed 21 rows containing non-finite outside the scale range
## (‘stat_boxplot()‘).
```



```
#Amphibians Boxplot
ggplot(amphibians_tidy, aes(x = species, y = concentration, color = life_stage))+
  geom_boxplot() +
  scale_y_log10() +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle("Amphibians - DEHP NOEC Concentrations") +
  labs(x = "Species",
       y = expression(Log[10] * NOEC * " [mg/L]"),
       color = "Life stage")
```




```
#Crustaceans Boxplot
ggplot(crustaceans_tidy, aes(x = species, y = concentration, color =
                               life_stage)) +
  geom_boxplot() +
  scale_y_log10() +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle("Crustaceans - DEHP NOEC Concentrations") +
  labs(x = "Species",
       y = expression(Log[10] * NOEC * " [mg/L]"),
       color = "Life stage")
```


Crustaceans − DEHP NOEC Concentrations
Life stage
Adult
Juvenile
Log10NOEC [mg/L]
1.000
0.100
0.010
0.001
Crab
Water Flea
Species

5 Analysis

Ho: The mean NOEC concentration is the same across the fish species and their life stages.

Ha: There is a significant difference between the fish species and their life stages.

```
#ANOVA DEHP
anova <- aov(data = fish_tidy, concentration ~ life_stage + species)
summary(anova)
```

```
##              Df Sum Sq Mean Sq F value   Pr(>F)
## life_stage    2   4856  2428.0  12.773 4.46e-06 ***
## species       6   3504   584.0   3.072  0.00608 **
## Residuals   344  65389   190.1
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 21 observations deleted due to missingness
```

```
#These anova results show that life stage and species are both significantly
#different with life stage having a stronger p value than species.

#HSD DEHP Species
hsd.test.species <- HSD.test(anova, "species",
group = TRUE)
hsd.test.species
```

```
## $statistics
##    MSerror  Df     Mean       CV
##   190.0832 344 3.035125 454.2503
##
## $parameters
##    test  name.t ntr StudentizedRange alpha
##   Tukey species   7         4.194391  0.05
##
## $means
##                      concentration          std   r       se       Min      Max
## Brook Trout           0.052000000  0.000000000   2 9.748927 0.0520000   0.0520
## Chinese Rare Minnow   0.037636765  0.036504843  68 1.671927 0.0036000   0.1176
## Fathead Minnow        0.251900000  0.409664265  40 2.179926 0.0120000   1.0000
## Guppy                 0.002582759  0.004266738  29 2.560194 0.0001000   0.0100
## Indian Medaka         0.600985915  0.382036479  71 1.636224 0.0100000   1.0000
## Japanese Medaka       0.512765825  0.073720239  16 3.446766 0.3905633   0.5660
## Zebra Danio           7.934729981 23.393640087 127 1.223404 0.0000200 150.0000
##                             Q25    Q50    Q75
## Brook Trout          0.052000000 0.0520 0.0520
## Chinese Rare Minnow  0.012800000 0.0394 0.0408
## Fathead Minnow       0.012000000 0.0120 0.1000
## Guppy                0.000100000 0.0001 0.0010
## Indian Medaka        0.300000000 0.5000 1.0000
## Japanese Medaka      0.503390825 0.5410 0.5660
## Zebra Danio          0.002265464 0.0330 0.2000
##
## $comparison
```

```
## NULL
##
## $groups
##                      concentration groups
## Zebra Danio           7.934729981      a
## Indian Medaka         0.600985915      b
## Japanese Medaka       0.512765825      b
## Fathead Minnow        0.251900000      b
## Brook Trout           0.052000000      b
## Chinese Rare Minnow   0.037636765      b
## Guppy                 0.002582759      b
##
## attr(,"class")
## [1] "group"
```

```
#The output of the HSD test for species specifically shows that the Zebra Danio
#was in its own group compared to the rest of the species.

#HSD DEHP Life Stage
hsd.test.lifestage <- HSD.test(anova, "life_stage",
group = TRUE)
hsd.test.lifestage
```

```
## $statistics
##    MSerror  Df     Mean       CV
##   190.0832 344 3.035125 454.2503
##
## $parameters
##    test     name.t ntr StudentizedRange alpha
##   Tukey life_stage   3         3.328922  0.05
##
## $means
##        concentration         std   r       se         Min         Max    Q25
## Adult     0.05038695  0.07465608 117 1.274615 0.000020000   0.3905633 0.0120
## Embryo    7.74776509 22.67324403 135 1.186602 0.002030929 150.0000000 0.0025
## Larva     0.19361980  0.23924006 101 1.371864 0.000100000   0.5660000 0.0042
##           Q50   Q75
## Adult  0.0200 0.052
## Embryo 0.4000 1.000
## Larva  0.0408 0.500
##
## $comparison
## NULL
##
## $groups
##        concentration groups
## Embryo    7.74776509      a
## Larva     0.19361980      b
## Adult     0.05038695      b
##
## attr(,"class")
## [1] "group"
```

```r
#The output of the HSD test for life stage specifically shows that the Embryo
#was in its own group compared with the Larva and Adult life stages.

#For two-way anova
TukeyHSD(anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = concentration ~ life_stage + species, data = fish_tidy)
##
## $life_stage
##                     diff        lwr       upr     p adj
## Embryo-Adult  7.6973781   3.598161 11.796595 0.0000393
## Larva-Adult   0.1432329  -4.264701  4.551167 0.9967797
## Larva-Embryo -7.5541453 -11.823762 -3.284529 0.0001167
##
## $species
##                                           diff        lwr       upr     p adj
## Chinese Rare Minnow-Brook Trout     -0.08176693 -29.418065 29.254531 1.0000000
## Fathead Minnow-Brook Trout          -2.69377845 -32.321987 26.934430 0.9999682
## Guppy-Brook Trout                   -0.19265009 -30.087239 29.701939 1.0000000
## Indian Medaka-Brook Trout           -4.38208550 -33.700670 24.936499 0.9994201
## Japanese Medaka-Brook Trout          0.35334119 -30.314768 31.021450 1.0000000
## Zebra Danio-Brook Trout              3.33703423 -25.803918 32.477986 0.9998764
## Fathead Minnow-Chinese Rare Minnow  -2.61201152 -10.760052  5.536029 0.9638050
## Guppy-Chinese Rare Minnow           -0.11088316  -9.179857  8.958091 1.0000000
## Indian Medaka-Chinese Rare Minnow   -4.30031857 -11.238562  2.637924 0.5230907
## Japanese Medaka-Chinese Rare Minnow  0.43510812 -10.926791 11.797007 0.9999998
## Zebra Danio-Chinese Rare Minnow      3.41880116  -2.725701  9.563304 0.6496797
## Guppy-Fathead Minnow                 2.50112835  -7.471768 12.474024 0.9896861
## Indian Medaka-Fathead Minnow        -1.68830705  -9.772340  6.395726 0.9961715
## Japanese Medaka-Fathead Minnow       3.04711963  -9.048546 15.142785 0.9894383
## Zebra Danio-Fathead Minnow           6.03081267  -1.383179 13.444805 0.1964248
## Indian Medaka-Guppy                 -4.18943541 -13.200945  4.822075 0.8128735
## Japanese Medaka-Guppy                0.54599128 -12.188248 13.280231 0.9999996
## Zebra Danio-Guppy                    3.52968432  -4.885956 11.945325 0.8763611
## Japanese Medaka-Indian Medaka        4.73542669  -6.580658 16.051512 0.8775344
## Zebra Danio-Indian Medaka            7.71911973   1.659752 13.778487 0.0034903
## Zebra Danio-Japanese Medaka          2.98369304  -7.863863 13.831249 0.9832667
```

6 Summary and Conclusions

Questions: -Which fish species is most sensitive to DEHP? -Which life stage of the fish species is most sensitive to DEHP?

We found a significant difference between the fish species and their life stages, which means, the NOEC concentrations vary within these species. Also, they vary between their life stages. So, we could consider the lowest NOEC concentration that we found for Guppy for all fish species, but that would be a very risk averse option for some of the larger fish.

For future studies, life stage is an important parameter to consider for all the chemicals and all the species. It would be interesting to bring in other variables that might affect the NOEC concentration level in the future like weight or length of the fish species, for example.

7 References

<Olker, J. H., Elonen, C. M., Pilli, A., Anderson, A., Kinziger, B., Erickson, S., Skopinski, M., Pomplun, A., LaLone, C. A., Russom, C. L., & Hoff, D. (2022). The ECOTOXicology Knowledgebase: A Curated Database of Ecologically Relevant Toxicity Tests to Support Environmental Research and Risk Assessment. Environmental Toxicology and Chemistry, 41(6):1520-1539. https://doi.org/10.1002/etc.5324>