Fish Example: Three-way Factorial Analysis

In this example, the growth of a certain species of fish was studied in a 3 x 2 x 2 factorial design. 36 tanks were randomly assigned in a CRD (n=3) considering water temperature (temp = cold, lukewarn or warm), water movement (move = still or flowing) and light level (light = high or low).

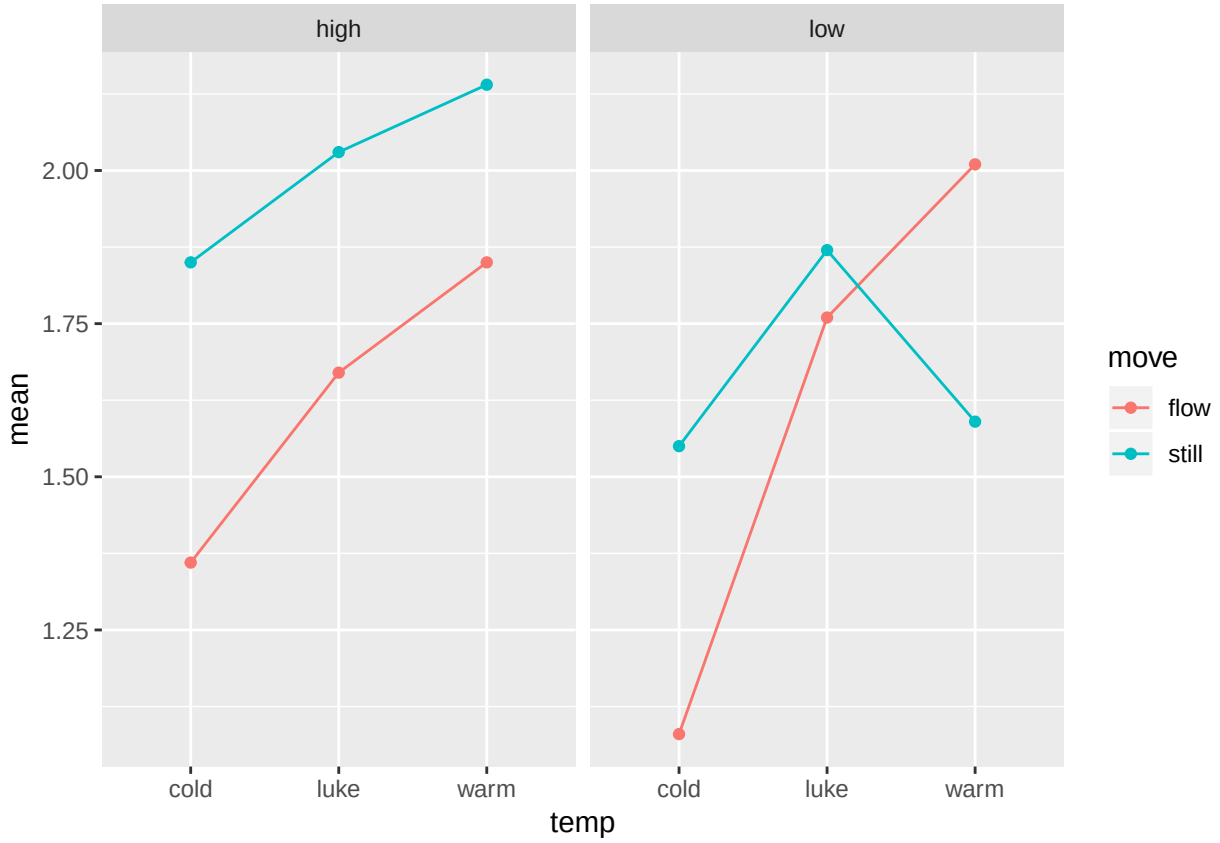
```
library(dplyr)
library(ggplot2)
library(car)
library(emmeans)
Fish <- read.csv("C:/hess/STAT512/RNotes/ExpDesign2/ED2_Fish.csv")
str(Fish)
```

```
## 'data.frame':    36 obs. of  6 variables:
##  $ obs  : int  1 2 3 4 5 6 7 8 9 10 ...
##  $ trt  : int  1 1 1 2 2 2 3 3 3 4 ...
##  $ light: Factor w/ 2 levels "high","low": 2 2 2 2 2 2 2 2 2 2 ...
##  $ temp : Factor w/ 3 levels "cold","luke",..: 1 1 1 1 1 1 2 2 2 2 ...
##  $ move : Factor w/ 2 levels "flow","still": 2 2 2 1 1 1 2 2 2 1 ...
##  $ gain : num  1.281 1.66 1.709 1.245 0.799 ...
```

```
#Make sure things are defined as factors!
SumStats <- summarize(group_by(Fish, light, temp, move),
                n    = n(),
                mean = mean(gain),
                sd   = sd(gain),
                SE   = sd/sqrt(n))
SumStats
```

```
## # A tibble: 12 x 7
## # Groups:   light, temp [?]
##    light temp  move      n  mean     sd     SE
##    <fct> <fct> <fct> <int> <dbl>  <dbl>  <dbl>
##  1 high  cold  flow      3  1.36 0.161  0.0929
##  2 high  cold  still     3  1.85 0.0714 0.0412
##  3 high  luke  flow      3  1.67 0.0380 0.0220
##  4 high  luke  still     3  2.03 0.191  0.110
##  5 high  warm  flow      3  1.85 0.182  0.105
##  6 high  warm  still     3  2.14 0.0668 0.0385
##  7 low   cold  flow      3  1.08 0.244  0.141
##  8 low   cold  still     3  1.55 0.234  0.135
##  9 low   luke  flow      3  1.76 0.130  0.0752
## 10 low   luke  still     3  1.87 0.0756 0.0436
## 11 low   warm  flow      3  2.01 0.103  0.0595
## 12 low   warm  still     3  1.59 0.147  0.0847
```

```
qplot(x = temp, y = mean, colour = move, group = move, data = SumStats) +
  geom_line() +
  facet_grid(. ~ light)
```



Three way analysis

Typical research questions are addressed using Type 3 tests (using Anova() from the car package) and pairwise comparisons (using emmeans() from the emmeans package). Important: Change contrasts options to get meaningful Type 3 tests!

```
#Change contrasts options to get meaningful Type 3 tests!
options(contrasts = c("contr.sum", "contr.poly"))
Model1 <- lm(gain ~ light*temp*move, data = Fish)
Anova(Model1, type = 3)
```

```
## Anova Table (Type III tests)
##
## Response: gain
##                  Sum Sq Df   F value    Pr(>F)
## (Intercept)     107.745  1 4684.5786 < 2.2e-16 ***
## light             0.270  1   11.7567 0.0021963 **
## temp              1.338  2   29.0776 3.863e-07 ***
## move              0.423  1   18.3698 0.0002552 ***
## light:temp        0.100  2    2.1663 0.1364942
## light:move        0.240  1   10.4391 0.0035630 **
## temp:move         0.447  2    9.7185 0.0008095 ***
## light:temp:move   0.185  2    4.0250 0.0310887 *
## Residuals         0.552 24
## ---
```

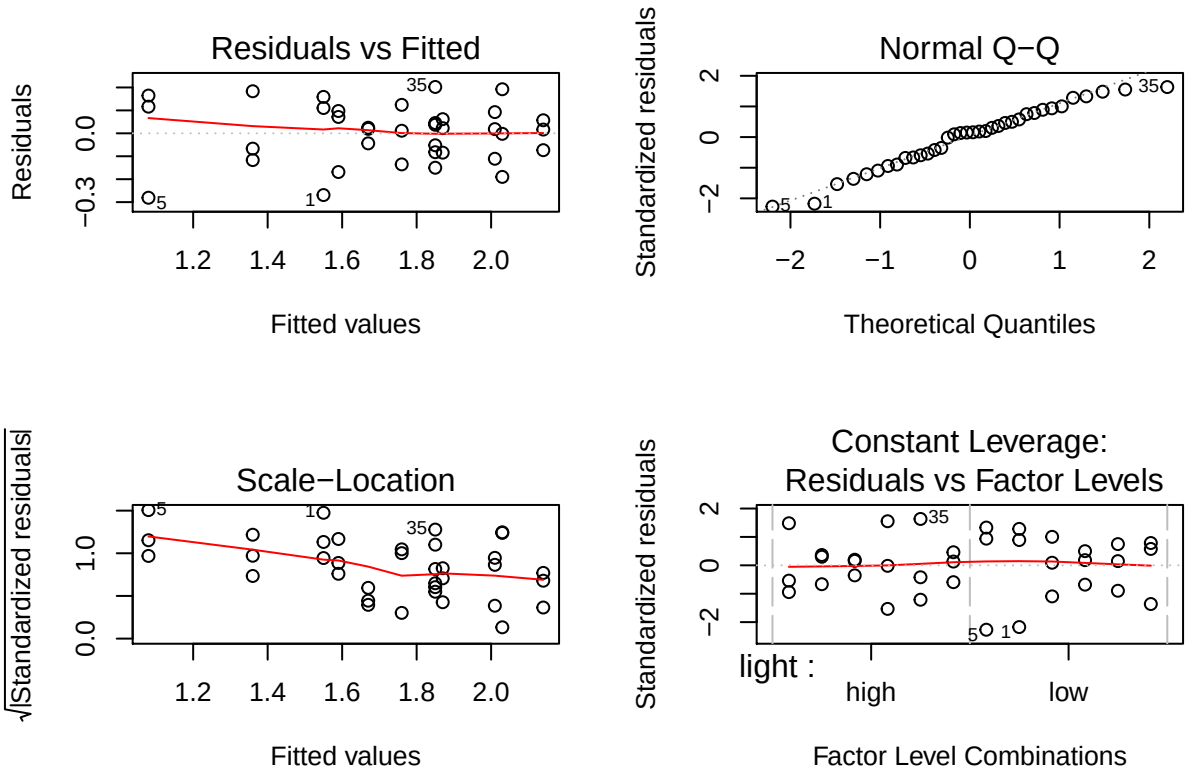
```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans(Model1, pairwise ~ temp:move|light)
```

```
## $emmeans
## light = high:
##  temp move  emmean     SE df lower.CL upper.CL
##  cold flow    1.36 0.0876 24    1.179     1.54
##  luke flow    1.67 0.0876 24    1.489     1.85
##  warm flow    1.85 0.0876 24    1.669     2.03
##  cold still   1.85 0.0876 24    1.669     2.03
##  luke still   2.03 0.0876 24    1.849     2.21
##  warm still   2.14 0.0876 24    1.959     2.32
##
## light = low:
##  temp move  emmean     SE df lower.CL upper.CL
##  cold flow    1.08 0.0876 24    0.899     1.26
##  luke flow    1.76 0.0876 24    1.579     1.94
##  warm flow    2.01 0.0876 24    1.829     2.19
##  cold still   1.55 0.0876 24    1.369     1.73
##  luke still   1.87 0.0876 24    1.689     2.05
##  warm still   1.59 0.0876 24    1.409     1.77
##
## Confidence level used: 0.95
##
## $contrasts
## light = high:
##  contrast                estimate    SE df t.ratio p.value
##  cold,flow - luke,flow      -0.31 0.124 24 -2.504  0.1626
##  cold,flow - warm,flow      -0.49 0.124 24 -3.957  0.0069
##  cold,flow - cold,still     -0.49 0.124 24 -3.957  0.0069
##  cold,flow - luke,still     -0.67 0.124 24 -5.411  0.0002
##  cold,flow - warm,still     -0.78 0.124 24 -6.299  <.0001
##  luke,flow - warm,flow      -0.18 0.124 24 -1.454  0.6953
##  luke,flow - cold,still     -0.18 0.124 24 -1.454  0.6953
##  luke,flow - luke,still     -0.36 0.124 24 -2.907  0.0738
##  luke,flow - warm,still     -0.47 0.124 24 -3.796  0.0101
##  warm,flow - cold,still      0.00 0.124 24  0.000  1.0000
##  warm,flow - luke,still     -0.18 0.124 24 -1.454  0.6953
##  warm,flow - warm,still     -0.29 0.124 24 -2.342  0.2166
##  cold,still - luke,still    -0.18 0.124 24 -1.454  0.6953
##  cold,still - warm,still    -0.29 0.124 24 -2.342  0.2166
##  luke,still - warm,still    -0.11 0.124 24 -0.888  0.9457
##
## light = low:
##  contrast                estimate    SE df t.ratio p.value
##  cold flow - luke flow      -0.68 0.124 24 -5.492  0.0002
##  cold flow - warm flow      -0.93 0.124 24 -7.510  <.0001
##  cold flow - cold still     -0.47 0.124 24 -3.796  0.0101
##  cold flow - luke still     -0.79 0.124 24 -6.380  <.0001
##  cold flow - warm still     -0.51 0.124 24 -4.119  0.0047
##  luke flow - warm flow      -0.25 0.124 24 -2.019  0.3613
##  luke flow - cold still      0.21 0.124 24  1.696  0.5473
##  luke flow - luke still     -0.11 0.124 24 -0.888  0.9457
##  luke flow - warm still      0.17 0.124 24  1.373  0.7421
```

```
##  warm flow - cold still      0.46 0.124 24  3.715  0.0122
##  warm flow - luke still      0.14 0.124 24  1.131  0.8638
##  warm flow - warm still      0.42 0.124 24  3.392  0.0258
##  cold still - luke still    -0.32 0.124 24 -2.584  0.1400
##  cold still - warm still    -0.04 0.124 24 -0.323  0.9995
##  luke still - warm still     0.28 0.124 24  2.261  0.2482
##
## P value adjustment: tukey method for comparing a family of 6 estimates
```

```
par(mfrow = c(2, 2))
plot(Model1)
```



Two way analysis, separately at High and Low light

For simplicity, we consider running a separate two-way ANOVA analysis at High and Low light.

```
#Note: contrasts specified above still hold.
Model2 <- lm(gain ~ temp*move, data = Fish[Fish$light == "high",])
Anova(Model2, type = 3)
```

```
## Anova Table (Type III tests)
##
## Response: gain
##             Sum Sq Df   F value    Pr(>F)
## (Intercept) 59.405  1 3347.2829 4.694e-16 ***
## temp         0.466  2   13.1373 0.0009498 ***
```

```
## move         0.650  1   36.6141 5.750e-05 ***
## temp:move    0.031  2    0.8706 0.4435634
## Residuals    0.213 12
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans(Model2, pairwise ~ move)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
## $emmeans
##  move  emmean     SE df lower.CL upper.CL
##  flow    1.63 0.0444 12     1.53     1.72
##  still   2.01 0.0444 12     1.91     2.10
##
## Results are averaged over the levels of: temp
## Confidence level used: 0.95
##
## $contrasts
##  contrast     estimate     SE df t.ratio p.value
##  flow - still    -0.38 0.0628 12 -6.051  0.0001
##
## Results are averaged over the levels of: temp
```

```
emmeans(Model2, pairwise ~ temp)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
## $emmeans
##  temp emmean     SE df lower.CL upper.CL
##  cold   1.60 0.0544 12     1.49     1.72
##  luke   1.85 0.0544 12     1.73     1.97
##  warm   2.00 0.0544 12     1.88     2.11
##
## Results are averaged over the levels of: move
## Confidence level used: 0.95
##
## $contrasts
##  contrast    estimate     SE df t.ratio p.value
##  cold - luke   -0.245 0.0769 12 -3.185  0.0199
##  cold - warm   -0.390 0.0769 12 -5.071  0.0007
##  luke - warm   -0.145 0.0769 12 -1.885  0.1852
##
## Results are averaged over the levels of: move
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
Model3 <- lm(gain ~ temp*move, data = Fish[Fish$light == "low",])
Anova(Model3, type = 3)
```

```
## Anova Table (Type III tests)
##
## Response: gain
##             Sum Sq Df   F value    Pr(>F)
## (Intercept) 48.610  1 1720.5556 2.498e-14 ***
## temp         0.971  2   17.1827 0.0003006 ***
## move         0.013  1    0.4531 0.5136317
## temp:move    0.601  2   10.6415 0.0021966 **
```

```
## Residuals    0.339 12
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans(Model3, pairwise ~ move|temp)
```

```
## $emmeans
## temp = cold:
##  move  emmean    SE df lower.CL upper.CL
##  flow    1.08 0.097 12    0.869     1.29
##  still   1.55 0.097 12    1.339     1.76
##
## temp = luke:
##  move  emmean    SE df lower.CL upper.CL
##  flow    1.76 0.097 12    1.549     1.97
##  still   1.87 0.097 12    1.659     2.08
##
## temp = warm:
##  move  emmean    SE df lower.CL upper.CL
##  flow    2.01 0.097 12    1.799     2.22
##  still   1.59 0.097 12    1.379     1.80
##
## Confidence level used: 0.95
##
## $contrasts
## temp = cold:
##  contrast     estimate    SE df t.ratio p.value
##  flow - still    -0.47 0.137 12 -3.425  0.0050
##
## temp = luke:
##  contrast     estimate    SE df t.ratio p.value
##  flow - still    -0.11 0.137 12 -0.802  0.4384
##
## temp = warm:
##  contrast     estimate    SE df t.ratio p.value
##  flow - still     0.42 0.137 12  3.060  0.0099
```