

Final Replication Project

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Paper: Turbidity interferes with foraging success of visual but not chemosensory predators; J. Lunt, D.L. Smee; 2015

This paper investigates the effect of turbidity on predation of brown shrimp and mud crabs by two predators, pin fish and blue crabs. They looked at two levels of turbidity (low and high) and tested its impact on the foraging success of pin fish and blue crabs. Thus, the independent variables in this case include predator type and turbidity, while the dependent variable includes number of prey eaten.

```
suppressWarnings(suppressMessages(library(Rmisc)))
suppressWarnings(suppressMessages(library(phia)))
suppressWarnings(suppressMessages(library(dplyr)))
suppressWarnings(suppressMessages(library(tidyr)))
suppressWarnings(suppressMessages(library(ggplot2)))
suppressWarnings(suppressMessages(library(car)))
```

Load Data

Load the data and clean the data up so that the two datasets match for variable names.

```
shrimp_data <- read.table("shrimp_data.csv", header = TRUE, sep = ",")

mud_crab_raw <- read.table("mud_crab_data.csv", header = TRUE, sep = ",")

# Remove second column of mud crab data set
mud_crab_data <- mud_crab_raw[, -2]

# Reorder by column number to match shrimp
mud_crab_data <- mud_crab_data[c(1,2,4,3,5,6,7)]

# Make column names the same between shrimp and mud crab data
colnames(mud_crab_data) <- c("Week", "Combined", "Turbidity", "Predator", "Live", "Dead", "Eaten")

# Make sure the factor variables are the same between the two data sets.
levels(shrimp_data$Predator)

[1] "Control" "Crab"    "Fish"    "Mix"

levels(mud_crab_data$Predator)

[1] "Blu" "Con" "Mix" "Pin"

lev <- with(mud_crab_data, levels(Predator))
lev[lev == "Blu"] <- "Crab"
lev[lev == "Pin"] <- "Fish"
lev[lev == "Con"] <- "Control"
mud_crab_data <- within(mud_crab_data, levels(Predator) <- lev)
```

Table 1, Diagram of Experimental Setup

Here is the chunk that handles the replication of Table 1, the diagram of the experimental setup. The table includes the number of replications for each individual experiment. Since this is a 4 x 2 factorial design with two prey types, there are a total of 16 experiments. Note that the number of replications are not the same across the experiments resulting in an unbalanced design.

```
shrimp_table <- shrimp_data %>% group_by(Turbidity,Predator) %>% tally()
mc_table <- mud_crab_data %>% group_by(Turbidity,Predator) %>% tally()

#Add column for prey type to both tables
shrimp_table$Prey <- c("S")
mc_table$Prey <- c("MC")

# Combine the two tables into one table
prey_table <- rbind(shrimp_table, mc_table)
prey_table <- prey_table[c(1,2,4,3)]

# Change the column names to match Table 1 in paper
colnames(prey_table) <- c("Turbidity", "Predator", "Prey", "Replication")

# Change the descriptions of turbidity to match diagram
lev_t <- with(prey_table, levels(Turbidity))
lev_t[lev_t == "Turbid"] <- "High (100 NTU)"
lev_t[lev_t == "Clear"] <- "Low (0 NTU)"
prey_table <- within(prey_table, levels(Turbidity) <- lev_t)

# Order the table by Turbidity
prey_table_fin <- prey_table[order(prey_table$Turbidity),]
kable(prey_table_fin, format = "markdown")
```

Turbidity	Predator	Prey	Replication
Low (0 NTU)	Control	S	8
Low (0 NTU)	Crab	S	5
Low (0 NTU)	Fish	S	12
Low (0 NTU)	Mix	S	9
Low (0 NTU)	Crab	MC	6
Low (0 NTU)	Control	MC	4
Low (0 NTU)	Mix	MC	7
Low (0 NTU)	Fish	MC	10
High (100 NTU)	Control	S	6
High (100 NTU)	Crab	S	5
High (100 NTU)	Fish	S	12
High (100 NTU)	Mix	S	8
High (100 NTU)	Crab	MC	7
High (100 NTU)	Control	MC	4
High (100 NTU)	Mix	MC	6
High (100 NTU)	Fish	MC	10

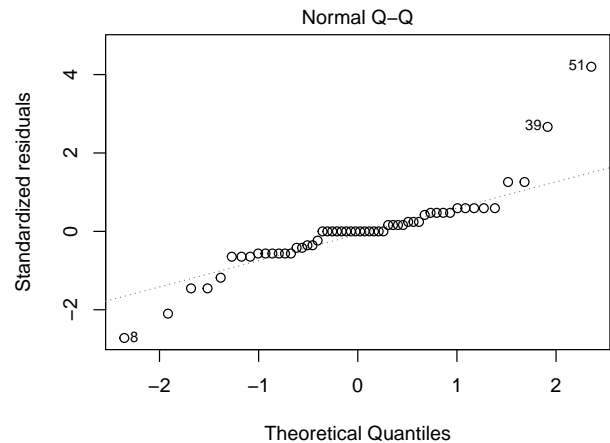
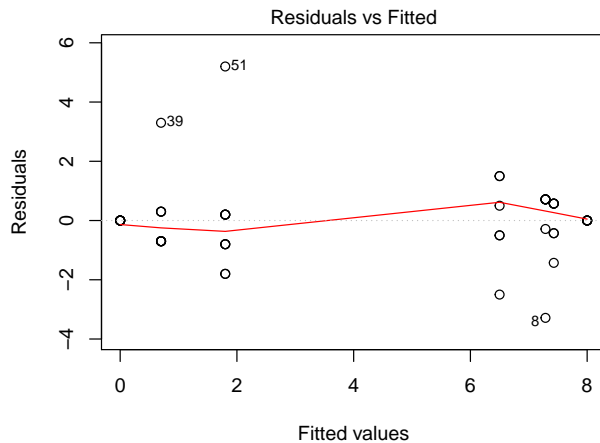
Basic Diagnostic Plots

Note: We plan on including this as part of our extension, so technically this is not part of the replication. These figures are not in the paper but may be informative when conducting further analysis with ANOVA, particularly with regards to the behavior of potential outliers. We will expand on this in our extension.

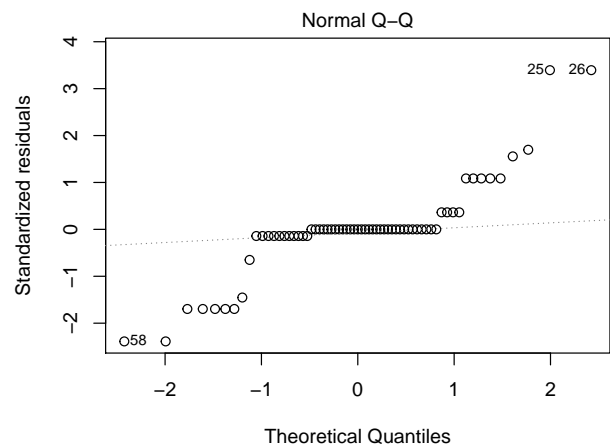
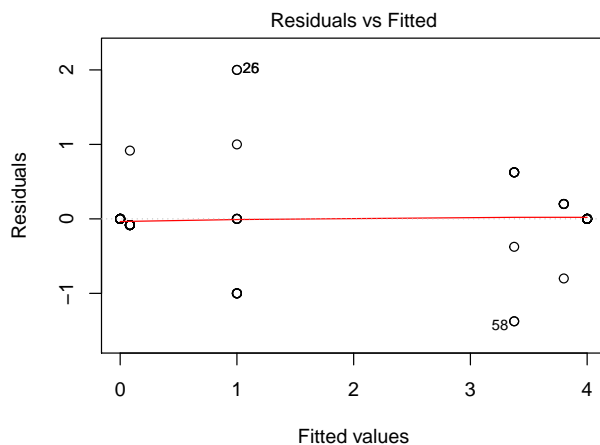
These diagnostic plots require the creation of linear models for both the shrimp and the mud crab data sets.

```
mcrab.model <- lm(Eaten ~ Predator * Turbidity, data = mud_crab_data) # mud crab linear model
shrimp.model <- lm(Eaten ~ Predator * Turbidity, data = shrimp_data) # shrimp linear model

# Plot diagnostics for the mud crab model
par(mfcol=c(1,2))
plot(mcrab.model, 1:2)
```



```
# Plot diagnostics for the shrimp model
par(mfcol=c(1,2))
plot(shrimp.model, 1:2)
```



Two-Way ANOVA

Using the function `anova` to conduct two-way ANOVAs on the data set leads to incorrect results that do not match with the results in the paper. It is important to note that since there are multiple predictors (turbidity and predator type), this method for conducting ANOVA is **not** appropriate.

```
# This is not what should be done. This is just to show differences in ANOVA methods.
mcrab_anova_0 <- anova(lm(Eaten ~ Turbidity * Predator, mud_crab_data))
shrimp_anova_0 <- anova(lm(Eaten ~ Turbidity * Predator, shrimp_data))

mcrab_anova_0
```

Analysis of Variance Table

Response: Eaten

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Turbidity	1	8.17	8.167	4.7952	0.03365 *
Predator	3	566.66	188.888	110.9082	< 2e-16 ***
Turbidity:Predator	3	1.81	0.602	0.3537	0.78668
Residuals	46	78.34	1.703		

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

```
shrimp_anova_0
```

Analysis of Variance Table

Response: Eaten

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Turbidity	1	3.797	3.797	10.0225	0.002483 **
Predator	3	189.980	63.327	167.1766	< 2.2e-16 ***
Turbidity:Predator	3	2.170	0.723	1.9097	0.138258
Residuals	57	21.592	0.379		

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

Since there are multiple predictors, we must use Anova from the car package. This allows for ANOVA in which it is possible to specify Type III sums of squares.

```
#library(car)
mcrab.Anova <- Anova(lm(Eaten ~ Turbidity * Predator, data = mud_crab_data,
                      contrasts = list(Turbidity='contr.sum',
                                      Predator = 'contr.sum')),
                   type = 'III')

shrimp.Anova <- Anova(lm(Eaten ~ Turbidity * Predator, shrimp_data,
                       contrasts = list(Turbidity='contr.sum',
                                       Predator = 'contr.sum')),
                    type = 'III')

mcrab.Anova
```

Anova Table (Type III tests)

Response: Eaten

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	762.52	1	447.7214	< 2e-16 ***
Turbidity	5.70	1	3.3489	0.07373 .
Predator	564.25	3	110.4357	< 2e-16 ***
Turbidity:Predator	1.81	3	0.3537	0.78668
Residuals	78.34	46		

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

```
shrimp.Anova
```

Anova Table (Type III tests)

Response: Eaten

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	241.523	1	637.5981	< 2.2e-16 ***
Turbidity	2.772	1	7.3169	0.008992 **
Predator	186.835	3	164.4092	< 2.2e-16 ***
Turbidity:Predator	2.170	3	1.9097	0.138258
Residuals	21.592	57		

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

The above should generate the correct statistics, and it does for the shrimp dataset but not for the mud crab data set. Inspection of the `mcrab.Anova` output also shows different degrees of freedom from what the paper reports (46 vs. 45). This suggests that they dropped an observation. Although not reported in the paper, it does appear to be the case that an observation was removed.

After going through multiple iterations of `Anova()` to determine which observation was dropped, it was determined that the 39th observation was dropped from the mud crab data (see residual plot above).

The output from the ANOVA below now matches with the paper's results.

```
mud_crab_data_fix <- mud_crab_data[-39,] # Removal of an "outlier"

# fixed mud crab linear model
mcrab.model_fix <- lm(Eaten ~ Predator * Turbidity, data = mud_crab_data_fix)

# fixed mud crab ANOVA
mcrab.Anova_fix <- Anova(lm(Eaten ~ Turbidity * Predator,
                           data = mud_crab_data_fix,
                           contrasts = list(Turbidity='contr.sum',
                                           Predator = 'contr.sum')),
                        type = 'III')

mcrab.Anova_fix
```

Anova Table (Type III tests)

Response: Eaten

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	738.76	1	501.8560	< 2e-16 ***
Turbidity	7.27	1	4.9381	0.03134 *
Predator	575.99	3	130.4269	< 2e-16 ***
Turbidity:Predator	3.24	3	0.7347	0.53683
Residuals	66.24	45		

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

Now, the results are the same as the paper. Predation on mud crabs was affected by predator type ($F_{7,45} = 130.4$, $p < 0.001$) and by turbidity ($F_{7,45} = 4.94$, $p = 0.03$). The interaction between turbidity and predator type was not significant ($F_{7,45} = 0.73$, $p = 0.54$).

Similarly, for the two-way ANOVA conducted for the shrimp data, predation on shrimp was affected by predator type ($F_{7,57} = 164.4$, $p < 0.001$) and by turbidity ($F_{7,57} = 7.32$, $p < 0.01$). The interaction between turbidity and predator type was not significant ($F_{7,45} = 1.91$, $p = 0.14$). These F-statistics match up with the

F-statistics reported in the text of the paper, and the p-values shown in the figures. There are inconsistencies in the p-values reported in the figure and p-values in the text.

Pairwise Differences

Pairwise differences for all possible predator and turbidity combinations are generated below.

In the `phia` package, the `testInteractions` function calculates and tests types of contrasts for factor interactions in linear, generalized and mixed linear models: simple main effects, interaction contrasts, etc (from `?testInteractions`). It takes a `lm` object, and these were created previously when generating the diagnostic plots and when creating the “fixed” mud crab dataset.

```
# Possible predator and turbidity combinations
idata <- expand.grid(Turbidity = c("Turbid","Clear"),
                    Predator = c("Control","Crab","Fish","Mix"))
idata
```

```
  Turbidity Predator
1   Turbid   Control
2   Clear   Control
3   Turbid     Crab
4   Clear     Crab
5   Turbid     Fish
6   Clear     Fish
7   Turbid     Mix
8   Clear     Mix
```

```
# Pairwise differences across turbidity
testInteractions(mcrab.model_fix, fixed = "Predator", across = "Turbidity",
                 idata = idata)
```

F Test:

P-value adjustment method: holm

	Value	Df	Sum of Sq	F	Pr(>F)
Crab	0.71429	1	1.648	1.1198	0.59124
Control	0.00000	1	0.000	0.0000	1.00000
Mix	0.92857	1	2.786	1.8924	0.52723
Fish	1.46667	1	10.189	6.9219	0.04647 *
Residuals		45	66.243		

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

```
#Pairwise differences
```

```
testInteractions(mcrab.model_fix, pairwise = c("Predator"),
                 fixed = c("Turbidity"), adjustment="none", idata = idata)
```

F Test:

P-value adjustment method: none

	Value	Df	Sum of Sq	F	Pr(>F)
Crab-Control : Clear	8.0000	1	153.600	104.3433	2.661e-13 ***
Crab-Mix : Clear	0.5714	1	1.055	0.7166	0.40173
Crab-Fish : Clear	6.2000	1	144.150	97.9238	7.216e-13 ***
Control-Mix : Clear	-7.4286	1	140.468	95.4222	1.078e-12 ***
Control-Fish : Clear	-1.8000	1	9.257	6.2885	0.01583 *
Mix-Fish : Clear	5.6286	1	130.450	88.6174	3.333e-12 ***
Crab-Control : Turbid	7.2857	1	135.117	91.7874	1.956e-12 ***

```

      Crab-Mix : Turbid  0.7857  1    1.995  1.3549  0.25056
      Crab-Fish : Turbid  6.9524  1   190.321 129.2889 8.004e-15 ***
      Control-Mix : Turbid -6.5000  1   101.400  68.8829 1.268e-10 ***
      Control-Fish : Turbid -0.3333  1    0.308  0.2090  0.64974
      Mix-Fish : Turbid  6.1667  1   136.900  92.9987 1.601e-12 ***
Residuals                                45    66.243

```

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

The first output using `testInteractions`, that keeps `Predator` as fixed and tests across `Turbidity` shows that turbidity only has a significant effect on pinfish foraging of mud crabs. This is the result reported in the text of the **Results** section.

Further analysis of the pairwise differences shows significant pairwise differences across some of the predator types. There were no significant pairwise differences in mud crab predation between blue crab and mix at clear and turbid conditions and no significant pairwise differences between the control and fish at turbid conditions. However, there are significant pairwise differences between the control and fish at clear conditions. Significant pairwise differences are indicated in the figure below. Letters denoting significant differences in the figures are generated from interpretation of these results.

Here we do the same but for the shrimp data:

```
testInteractions(shrimp.model, fixed = "Predator", across = "Turbidity",
                 idata = idata)
```

F Test:

P-value adjustment method: holm

```

      Value Df Sum of Sq      F Pr(>F)
Control  0.00000  1    0.0000  0.0000 1.000000
  Crab   0.20000  1    0.1000  0.2640 1.000000
  Fish   0.91667  1    5.0417 13.3095 0.002295 **
  Mix    0.62500  1    1.6544  4.3675 0.123311
Residuals          57   21.5917

```

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

Pairwise differences

```
testInteractions(shrimp.model, pairwise = c("Predator"),
                 fixed = c("Turbidity"), adjustment="none",
                 idata = idata)
```

F Test:

P-value adjustment method: none

```

      Value Df Sum of Sq      F Pr(>F)
Control-Crab : Clear -4.0000  1   49.231 129.9647 2.484e-16 ***
Control-Fish : Clear -1.0000  1    4.800  12.6716 0.0007569 ***
Control-Mix : Clear -4.0000  1   67.765 178.8925 < 2.2e-16 ***
Crab-Fish : Clear  3.0000  1   31.765  83.8559 8.554e-13 ***
Crab-Mix : Clear  0.0000  1    0.000  0.0000 1.0000000
Fish-Mix : Clear -3.0000  1   46.286 122.1900 8.411e-16 ***
Control-Crab : Turbid -3.8000  1   39.382 103.9644 1.835e-14 ***
Control-Fish : Turbid -0.0833  1    0.028  0.0733 0.7875257
Control-Mix : Turbid -3.3750  1   39.054 103.0978 2.144e-14 ***
Crab-Fish : Turbid  3.7167  1   48.754 128.7058 3.016e-16 ***
Crab-Mix : Turbid  0.4250  1    0.556  1.4672 0.2307893
Fish-Mix : Turbid -3.2917  1   52.008 137.2972 < 2.2e-16 ***
Residuals                                57   21.592

```

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

Again, the first output using `testInteractions` for the shrimp data show that turbidity only has a significant effect on pinfish foraging of brown shrimp.

In addition, there are no significant differences in shrimp predation between blue crab and mix, and no significant differences between the control and fish at turbid conditions. There are significant differences between the control and fish at clear conditions. Significant pairwise differences are indicated in the figure below. Letters denoting significant differences in the figures are generated from interpretation of these results.

Recreating Figures

The `Rmisc` package is used to summarize the data with the function `summarySE`. This gives the count, mean, standard deviation, standard error of the mean, and confidence interval. The new object created from this function is then used to generate barplots that depict the mean number of prey eaten and standard error bars as seen in Figures 2 and 3. Both figures show mean number of prey eaten for the four different predator treatments under the two different turbidity treatments. Significant pairwise differences calculated above are also plotted here, and are denoted by letters A - C. As described in the paper, letters denote significant pairwise differences

Creation of Figure 2, mud crabs eaten.

```
sum_crab = summarySE(mud_crab_data_fix,measurevar = "Eaten",
                    groupvars=c("Turbidity","Predator"),
                    conf.interval = 0.95)

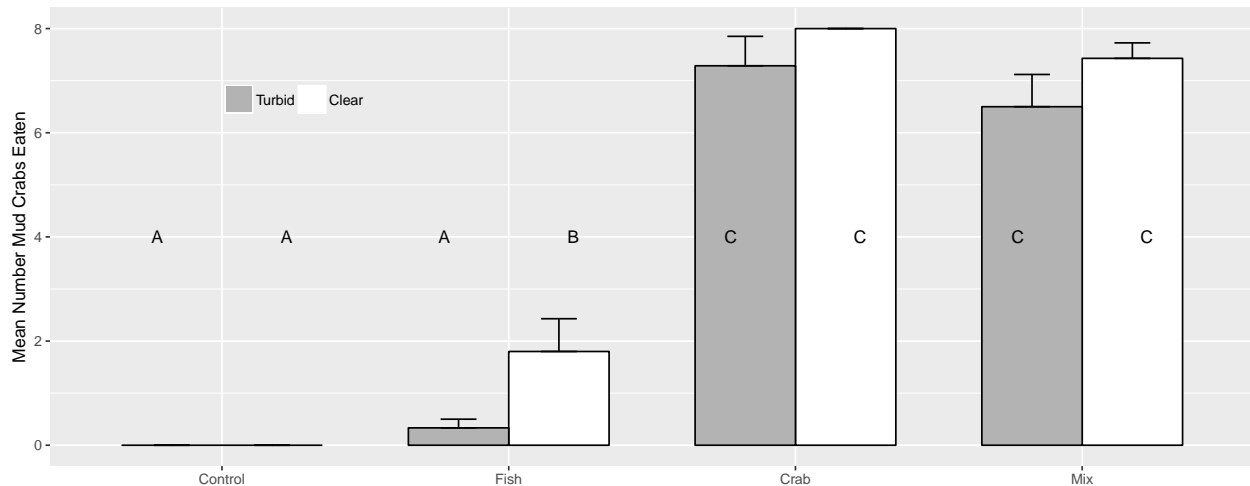
# Requires human interpretation from pairwise differences and correct assignment
sum_crab$pw_label <- c("C","A","C","B","C","A","C","A")

# Set the order here so that the plot is in the same order as the paper
sum_crab$Predator <- factor(sum_crab$Predator, levels = c("Control","Fish","Crab","Mix"))
sum_crab$Turbidity <- factor(sum_crab$Turbidity, levels = c("Turbid","Clear"))

limits <- aes(ymax = Eaten + se, ymin = Eaten)
mcrab_plot <- ggplot(sum_crab,aes(x=Predator, y = Eaten, fill = Turbidity)) +
  geom_bar(stat = "identity", position = "dodge", width = 0.7) +
  geom_bar(stat = "identity", position = "dodge", colour = "black",
          width = 0.7, show_guide = FALSE) +
  geom_errorbar(limits, position = position_dodge(width=0.7),
               width = 0.25, size = 0.5, color = "black") +
  labs(x="",y="Mean Number Mud Crabs Eaten") +
  scale_fill_grey(start = 0.7, end = 1.0) +
  theme(legend.position = c(0.2,0.8), legend.direction = "horizontal",
        legend.background = element_rect(fill = "transparent")) +
  labs(fill="") +
  geom_text(aes(label = sum_crab$pw_label,y = 4), position = position_dodge(width = 0.9))
```

Warning: `show_guide` has been deprecated. Please use `show.legend` instead.

```
suppressMessages(print(mcrab_plot))
```

Creation of Figure 3, shrimp eaten.

```
#library(Rmisc)
sum_shrimp <- summarySE(shrimp_data, measurevar = "Eaten",
  groupvars=c("Turbidity","Predator"),
  conf.interval = 0.95)

# Requires human interpretation from pairwise differences and correct assignment
sum_shrimp$pw_label <- c("A","C","B","C","A","C","A","C")

# Set the order here so that the plot is in the same order as the paper
sum_shrimp$Predator <- factor(sum_shrimp$Predator,
  levels = c("Control","Fish","Crab","Mix"))

sum_shrimp$Turbidity <- factor(sum_shrimp$Turbidity,
  levels = c("Turbid","Clear"))

limits <- aes(ymax = Eaten + se, ymin = Eaten)
shrimp_plot <- ggplot(sum_shrimp,aes(x=Predator, y = Eaten, fill = Turbidity)) +
  geom_bar(stat = "identity", position = "dodge", width = 0.7) +
  geom_bar(stat = "identity", position = "dodge", colour = "black",
    width = 0.7, show_guide = FALSE) +
  geom_errorbar(limits, position = position_dodge(width=0.7),
    width = 0.25, size = 0.5, color = "black") +
  labs(x="",y="Mean Number Shrimp Eaten") +
  scale_fill_grey(start = 0.7, end = 1.0) +
  theme(legend.position = c(0.2,0.8), legend.direction = "horizontal",
    legend.background = element_rect(fill = "transparent")) +
  labs(fill="") +
  geom_text(aes(label = sum_shrimp$pw_label,y = 1.8),
    position = position_dodge(width = 0.9))
```

Warning: `show_guide` has been deprecated. Please use `show.legend` instead.

```
suppressMessages(print(shrimp_plot))
```

