

# Script for analysing dietary data

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## Load packages

```
library("corrplot")
library("ggplot2")
library("plyr")
library("reshape2")
library("viridis")
library("scales")
library("RColorBrewer")
library("mvabund")
library("boral")
library("birpartite")
```

## Correlation plot

Load data and extract necessary information

```
buffer <- read.csv("Otter10kmVariables.csv", header = T)
summary(buffer)
colnames(buffer)
corrcheck <- buffer[,c(12,19,13,9,10)]
str(corrcheck)
```

Check correlation between landscape variables acquired from 10km buffers around each otter

```
cor1 <- cor(corrcheck)
corrplot(cor1, type = "upper", order = "hclust", tl.srt = 45, tl.col = "black")
```

## Create heat charts to showing taxonomic identifications by each method

### Compare metabarcoding 16S and COI identifications

Load in data and format for plotting

```
HeatChart <- read.csv("Combined Heat Chart.csv", header = T)
summary(HeatChart)
meltedHeatChart <- melt(HeatChart)
```

Plot heat chart

```
ggchart <- ggplot(meltedHeatChart, aes(variable, y = reorder(Taxon, desc(Taxon)))) +
  geom_tile(aes(fill = value), colour = "white") +
  scale_fill_gradientn(na.value = "white", colours=viridis(3),
    values=rescale(c(1,2,3)),
    breaks = c(1,2,3), labels = c("16S Only", "COI Only", "16S and COI"),
    guide = "legend") +
  coord_fixed(ratio = 3, xlim = NULL, ylim = NULL, expand = TRUE) +
  theme(axis.title.y=element_blank(),
    axis.title.x=element_blank(),
    axis.text.x=element_blank(),
    axis.text.y = element_text(size = 9, colour = "black"),
    legend.position="none")
ggchart
```

## Compare identifications from morphological analysis of prey remains and metabarcoding

Load in data and format for plotting

```
HeatMethods <- read.csv("HTSvsHP_HeatChart.csv", header = T)
HeatMethods$Group <- as.character(HeatMethods$Group)
HeatMethods$Group <- factor(HeatMethods$Group, levels=unique(HeatMethods$Group))
HeatMethods$Group <- factor(HeatMethods$Group, levels = rev(levels(HeatMethods$Group)))
summary(HeatMethods)
meltedHeatMethods <- melt(HeatMethods)
```

Plot heat chart with all data

```
ggplot(meltedHeatMethods, aes(variable, Group)) +
  geom_tile(stat = "identity", aes(fill = value), colour = "white") +
  scale_fill_gradientn(na.value = "white", colours=viridis(4, direction = -1),
    values=rescale(c(1,2,3,4)),
    breaks = c(1,2,3,4),
    labels = c("Only molecular", "Only morphological",
      "Molecular and morphological at same taxonomic level",
      "Molecular and morphological at different taxonomic level"), guide = "legend") +
  theme(legend.position="none",
    axis.title.x = element_blank(),
    axis.title.y = element_blank(),
    axis.text.x = element_blank(),
    axis.text.y = element_text(size=10, colour = "black"),
    plot.margin=unit(c(0.5,0.5,1,1),"cm"))
```

Load in data for just fish taxa and reformat

```
HeatMethods <- read.csv("HTSvsHP_HeatChart_FishPrey.csv", header = T)
HeatMethods$Group <- as.character(HeatMethods$Group)
HeatMethods$Group <- factor(HeatMethods$Group, levels=unique(HeatMethods$Group))
HeatMethods$Group <- factor(HeatMethods$Group, levels = rev(levels(HeatMethods$Group)))
summary(HeatMethods)
meltedHeatMethods <- melt(HeatMethods)
```

Plot heat chart for just fish taxa

```
ggplot(meltedHeatMethods, aes(variable, Group)) +  
  geom_tile(stat = "identity", aes(fill = value), colour = "white") +  
  scale_fill_gradientn(na.value = "white", colours=viridis(4, direction = -1),  
    values=rescale(c(1,2,3,4)),  
    breaks = c(1,2,3,4),  
    labels = c("Only molecular", "Only morphological",  
      "Molecular and morphological at same taxonomic level",  
      "Molecular and morphological at different taxonomic  
level"), guide = "legend") +  
  
  theme(legend.position="none",  
    axis.title.x = element_blank(),  
    axis.title.y = element_blank(),  
    axis.text.x = element_blank(),  
    axis.text.y = element_text(size=12, colour = "black"),  
    plot.margin=unit(c(0.5,0.5,1,1),"cm"))
```

Load in data for non-fish taxa and reformat

```
HeatMethods2 <- read.csv("HTSvsHP_HeatChart_AltPrey.csv", header = T)  
HeatMethods2$Group <- as.character(HeatMethods2$Group)  
HeatMethods2$Group <- factor(HeatMethods2$Group, levels=unique(HeatMethods2$Group))  
HeatMethods2$Group <- factor(HeatMethods2$Group, levels = rev(levels(HeatMethods2$Group)))  
summary(HeatMethods2)  
meltedHHeatMethods2<- melt(HeatMethods2)
```

Plot heat chart for just fish taxa

```
ggplot(meltedHHeatMethods2, aes(variable, Group)) +  
  geom_tile(stat = "identity", aes(fill = value), colour = "white") +  
  scale_fill_gradientn(na.value = "white", colours=viridis(4, direction = -1),  
    values=rescale(c(1,2,3,4)),  
    breaks = c(1,2,3,4),  
    labels = c("Only molecular", "Only morphological",  
      "Molecular and morphological at same taxonomic level",  
      "Molecular and morphological at different taxonomic  
level"), guide = "legend") +  
  
  theme(legend.position="none",  
    axis.title.x = element_blank(),  
    axis.title.y = element_blank(),  
    axis.text.x = element_blank(),  
    axis.text.y = element_text(size=12, colour = "black"),  
    plot.margin=unit(c(0.5,0.5,1,1),"cm"))
```

## Model based analysis of dietary data

Load in data and check format

```
HTSHP2 <- read.csv("HTS+HPThreePlusNoInsectORMollusc.csv", header = T)  
summary(HTSHP2)
```

```
colnames(HTSHP2)
rownames(HTSHP2) <- HTSHP2[,1]
rownames(HTSHP2)
str(HTSHP2)
```

Create object of only species consumed

```
dietHTSHP2 <- mvabund(HTSHP2[,21:47])
Create model and check assumptions
MVdietHTSHP2 <- manyglm(dietHTSHP2 ~ Sex + Size + Size:Sex + Scored.SMI + Year2 + Season
                        + Sex:Season + Size:Season + long + lat + lat:long + KmRiverDist
                        + WaterClass + Urban + Sex:WaterClass + Size:WaterClass
                        + KmRiverDist:Sex + KmRiverDist:Size
                        , family = binomial(link="cloglog"), data = HTSHP2)
plot(MVdietHTSHP2)
```

Conduct model simplification by stepwise deletion by AIC and extract significance of variables on the general prey composition and specific prey groups

```
step(MVdietHTSHP2, test = "Chisq")
MVdietHTSHP2.2 <- manyglm(dietHTSHP2 ~ Season + long + KmRiverDist
                        , family = binomial(link="cloglog"), data = HTSHP2)
plot(MVdietHTSHP2.2)
anovaMVdietHTSHP2.2 <- anova(MVdietHTSHP2.2, resamp = "montecarlo", test = "LR",
                             p.uni="adjusted")
anovaMVdietHTSHP2.2
```

## Bayesian Ordination And Regression Analysis (BORAL)

Create an object with only significant variables from the mvabund analysis and then run BORAL with and without this object

```
X <- as.data.frame(HTSHP2[,c(9,16,20)])
dietHTSHP2Boral <- boral(dietHTSHP2,family = "binomial",lv.control=list(num.lv=2))
dietHTSHP2Boral2 <- boral(dietHTSHP2,X=X,family = "binomial",lv.control=list(num.lv=2))
```

Check BORAL objects and plot ordinations

```
summary(dietHTSHP2Boral)
plot(dietHTSHP2Boral)
lvplot(dietHTSHP2Boral, return.vals = T)
summary(dietHTSHP2Boral2)
plot(dietHTSHP2Boral2)
lvplot(dietHTSHP2Boral2, return.vals = T)
```

## Bipartite plots

Load in data and check format

```

BipartiteHTSHP <- read.csv("Combined_HTS_HP_Bipartite.csv", header = T)
summary(BipartiteHTSHP)
rownames(BipartiteHTSHP) <- BipartiteHTSHP[,1]
rownames(BipartiteHTSHP)
colnames(BipartiteHTSHP)

```

## Plot data for season

```

plotweb(BipartiteHTSHP[,3:6], text.rot=90,
        col.high = c("darkolivegreen3", "gold2", "indianred2", "steelblue3"),
        bor.col.high = c("darkolivegreen3", "gold2", "indianred2", "steelblue3"),
        col.interaction = c("darkolivegreen3", "gold2", "indianred2", "steelblue3"),
        bor.col.interaction = c("darkolivegreen3", "gold2", "indianred2", "steelblue3"),
        bor.col.low = "ivory4", col.low = "ivory4", y.width.low = 0.1, high.xoff = F,
        low.y = 0.7, high.y = 1.7, labsize = 2.3, low.spacing = 0.0175,
        high.spacing = 0.15, method = "normal", text.low.col = "black")

```

## Plot data for longitude

```

plotweb(BipartiteHTSHP[,7:272], text.rot = 90, col.high = viridis(263),
        bor.col.high = viridis(263), method = "normal", col.interaction = viridis(263),
        bor.col.interaction = viridis(263), high.lablength = 0, high.spacing = 0.00145,
        low.spacing = 0.015, low.y = 0.7, high.y = 1.7, text.low.col = "black",
        labsize = 2.3)

```

## Plot data for distance from the coast

```

plotweb(BipartiteHTSHP[,273:531], text.rot = 90, col.high = viridis(256),
        bor.col.high = viridis(256), method = "normal", col.interaction = viridis(256),
        bor.col.interaction = viridis(256), high.spacing = 0.00145, low.spacing = 0.015,
        low.y = 0.7, high.y = 1, text.low.col = "black", labsize = 2.3)

```