

# Morro Bay Trawl Analyses

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This document contains analyses of trawl data from O’Leary et al., “Effects of estuary-wide seagrass loss on fish populations”, submitted to the journal *Estuaries and Coasts* and in review as of September 2020. Not all code is shown in document output, but code for plots or analyses which are not shown below can be viewed in the R markdown source code.

## Metadata

### Data Description

Trawl surveys were conducted before (2006-2007) and after (2016-2017) the eelgrass decline at the same seven sites in Morro Bay (Figure 1a) during high tide (post-decline: intertidal flats  $0.93 \pm 0.08$  m SE MLLW, channel  $1.17 \pm 0.06$  m SE MLLW; unrecorded during pre-decline period). We surveyed four sites in the back-bay intertidal flats using a beam trawl (2 m wide at the mouth, 4 m long, with a 1 m reinforced cod end and a 4 mm mesh size). The mean depth of the beam trawls was  $1.0 \pm 0.06$  m (SE). The remaining three sites surveyed were in the channel running from the mid-bay to the bay mouth. The channel is dredged annually and is too deep for eelgrass to survive, but has persistent eelgrass along the margins. We surveyed the channel sites using an otter trawl (4.6 m wide at the mouth, 7.2 m long with a mesh size of 14 mm in the wings and 8 mm in the code end). The mean depth of channel trawls was  $5.3 \pm 0.2$  m (SE). The size of the trawls used was based on the equipment used in the early period, and we replicated prior methods for the 2016-2017 surveys. Though trawl size between the two habitats varied, we do not explicitly compare the trawl types, but rather compare results between seasons and period for each respective trawl type.

In both pre- and post-decline periods, the trawls (beam and otter) were 10-11 minutes long at a speed (over ground) of 0.8 – 1.8 knots. In both periods, trawls were conducted during two seasons: October-November (fall) and June-August (summer). In the pre-decline period (2006-2007), we conducted three replicate trawls per site in six sites per season per year, with data collected in the summer and fall of both years. In the intertidal flats during the pre-decline period, sites B1, B2, and B4 were consistently surveyed using a beam trawl except in the summer of 2006, when site B3 was surveyed instead of site B4 (Figure 1b). In the post-decline period (2016-2017), we conducted three replicate trawls per site per season per year at all seven sites (Figure 1b), with surveys conducted in the fall of 2016 and the summer of 2017. The only exceptions to replication level in the post-decline period were that in the fall of 2016 where we were only able to conduct two replicate trawls at channel site O3, and we conducted four replicate trawls at intertidal site 2. At the end of each trawl (otter and beam), all organisms were removed from the trawl nets, fish were identified to species, and the total length of each fish was measured to the nearest mm. Fish were returned alive to the water at their collection site.

### Sample Size Breakdown

Below is a table of sample sizes for each site, broken down by period (pre-decline, 2006-2007, or post-decline, 2016-2017) and season (Fall or Summer). “Beam” sites are those conducted in the mudflats, while “otter” sites were conducted in the channel. The data are unbalanced, with between 2 and 6 observations per site in each period and season. Site Beam 3 was not sampled in the pre-decline period in the Fall, and we therefore exclude it from analyses.

This yields a total of 52 observations across three sites in the channel, and 53 observations across three sites in the mudflats.

```
trawl_info <- read.csv(here("data", "trawl_information.csv"), stringsAsFactors = FALSE)

trawl_info %>%
  group_by(Period, Season, Site) %>%
  filter(Year != 2008 & Season != "Spring") %>% tally() %>%
  pivot_wider(names_from = Site, values_from = n) %>%
  knitr::kable()
```

Period	Season	Beam 1	Beam 2	Beam 3	Beam 4	Otter 1	Otter 2	Otter 3
Post-Decline	Fall	3	4	3	3	3	3	2
Post-Decline	Summer	3	3	3	3	3	3	3
Pre-Decline	Fall	6	6		6	6	6	6
Pre-Decline	Summer	6	6		3	6	6	6

## Tide Level Estimates

During both time periods, trawls were conducted during high tide. However, trawl start and end times were not recorded for trawls conducted before decline. Just as a check, here are estimated tidal heights for the fall and summer trawls following Eelgrass decline, in both habitats, confirming that trawls were conducted during high tide.

```
trawl_tides <- trawl_info %>%
  filter(nchar(time_start) > 0 & Season != "Spring") %>%
  mutate(time_start = as.POSIXct(time_start, format = "%Y-%m-%d %H:%M:%S"))

tide_data <- data.frame(Station = "Port San Luis, Pacific Ocean, California",
  DateTime = trawl_tides$time_start,
  stringsAsFactors = FALSE)

tide_data <- rtide::tide_height_data(tide_data)
trawl_tides$tide <- tide_data$TideHeight

trawl_tides %>%
  group_by(Period, `trawl type` = TrawlType, Season) %>%
  summarize(mean = round(mean(tide), 3),
    `standard error` = round(sd(tide)/sqrt(n()), 3)) %>%
  knitr::kable()
```

Period	trawl type	Season	mean	standard error
Post-Decline	Beam	Fall	1.303	0.056
Post-Decline	Beam	Summer	0.614	0.111
Post-Decline	Otter	Fall	1.228	0.068
Post-Decline	Otter	Summer	1.029	0.069
Pre-Decline	Beam	Fall	0.744	0.109
Pre-Decline	Beam	Summer	1.074	0.034
Pre-Decline	Otter	Fall	0.889	0.051
Pre-Decline	Otter	Summer	1.103	0.021

## Trawl Depths

### By Period, Trawl Type, and Season

Likewise, these data only exist for trawls conducted between 2016 and 2017.

```
trawl_tides %>%  
  filter(!is.na(depth_m)) %>%  
  group_by(Period, TrawlType, Season) %>%  
  summarize(`Mean Depth (m)` = mean(depth_m),  
            `Depth SE` = sd(depth_m)/sqrt(n())) %>%  
  knitr::kable()
```

Period	TrawlType	Season	Mean Depth (m)	Depth SE
Post-Divine	Beam	Fall	1.0653846	0.0602411
Post-Divine	Beam	Summer	0.9181818	0.1081398
Post-Divine	Otter	Fall	5.4642857	0.4236961
Post-Divine	Otter	Summer	5.1744444	0.2829267
Pre-Divine	Beam	Fall	1.0000000	0.0000000
Pre-Divine	Beam	Summer	1.1111111	0.1059932
Pre-Divine	Otter	Fall	4.2083333	0.5917947
Pre-Divine	Otter	Summer	4.9944444	0.3781164

### By Period and Trawl Type

```
trawl_tides %>%  
  filter(!is.na(depth_m)) %>%  
  group_by(Period, TrawlType) %>%  
  summarize(`Mean Depth (m)` = mean(depth_m),  
            `Depth SE` = sd(depth_m)/sqrt(n())) %>%  
  knitr::kable()
```

Period	TrawlType	Mean Depth (m)	Depth SE
Post-Divine	Beam	0.9979167	0.0599153
Post-Divine	Otter	5.3012500	0.2383448
Pre-Divine	Beam	1.0476190	0.0455752
Pre-Divine	Otter	4.6800000	0.3294510

## Abundance

### Bar Chart

The error bars on this plot are estimated via a two-stage bootstrap, by taking the standard deviation of the mean, sampling with replacement from both sites and trawl samples within sites. Unless otherwise stated, this applies to all other plots with error bars.

```
filter_trawls <- function(data) {  
  data %>%  
  filter(Year %in% c("2006", "2007", "2016", "2017")) %>%  
  filter(Season != "Spring") %>%  
  filter(Site != "Beam 3") %>%  
  mutate(Period = ifelse(grepl("200", Year), "Pre-Divine", "Post-Divine") %>%  
    factor(levels = c("Pre-Divine", "Post-Divine"),
```

```

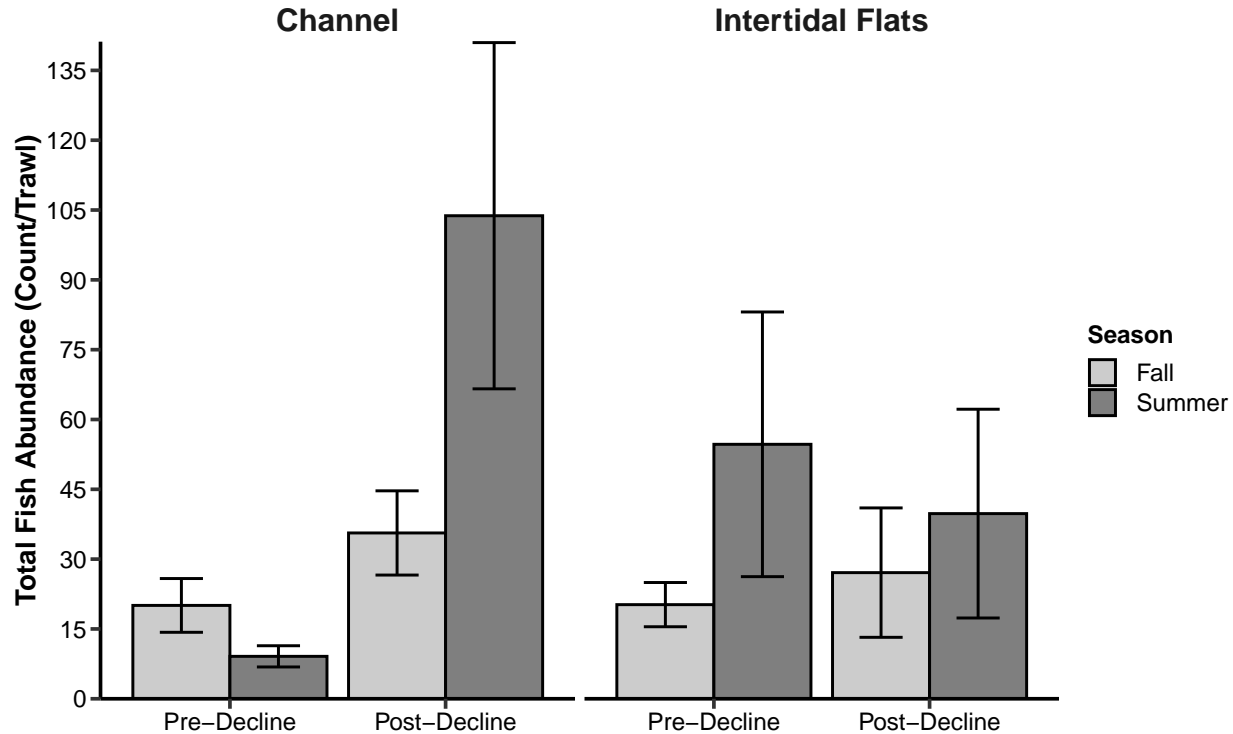
        ordered = TRUE),
    Site = dplyr::recode(Site, "Otter 1" = "Otter 3", "Otter 3" = "Otter 1"))
}

abundance <-
  read.csv(here("data", "trawl_fish_counts.csv"), stringsAsFactors = FALSE) %>%
  filter_trawls %>%
  group_by(Period, Season, Year, TrawlType, Site, Replicate) %>%
  summarize(Count = sum(Count)) %>%
  ungroup()

abundance_summary <- abundance %>%
  group_by(Period, Season, TrawlType) %>%
  cluster_bootstrap(Count, Site) %>% ungroup() %>%
  mutate(Period = factor(Period, levels = c("Pre-Divide", "Post-Divide"),
    ordered = TRUE),
    TrawlType = dplyr::recode(TrawlType, "Beam" = "Intertidal Flats",
      "Otter" = "Channel"))

abundance_summary %>%
  ggplot(aes(Period, mean, fill = Season)) +
  geom_bar(stat = "identity", position = "dodge",
    color = "black", size = 0.8) +
  scale_y_continuous(expand = c(0,0), breaks = seq(0, 140, 15),
    limits = c(0, max(abundance_summary$upper + 0.2))) +
  scale_fill_manual(values = c("grey80", "grey50")) +
  facet_wrap(~TrawlType, ncol = 2) +
  geom_errorbar(aes(x = Period, ymin = lower, ymax = upper),
    position = position_dodge(width = 0.9),
    width = 0.4, size = 0.8) +
  theme_trawls +
  theme(axis.title.x = element_blank()) +
  ylab("Total Fish Abundance (Count/Trawl)")

```



Here, I fit separate generalized linear models for Otter and Beam trawls, where the response is total fish abundance (the total number of fish per trawl replicate), and the predictors are period, season, trawl site, and an interaction between period and season. The model uses a negative binomial error distribution and a log link. As for all analyses, site Beam 3 was excluded, and only data from Fall and Summer trawls were used.

**How to Interpret this Table:** These are likelihood ratio tests, which are generated by dropping each term (and all higher order interactions it's involved in, if any) from the full model to produce a “reduced” model, which is then compared to the full model using the log-likelihood of both models. The “Df” column is the degrees of freedom of a  $\chi^2$  distribution; it is the difference between the number of parameters in the full and reduced models. The “LR Chisq” column is the associated test statistic, which by Wilk’s theorem is twice the difference in the log-likelihoods of the full and reduced model. The provided p-values, which are compared to a “significance” threshold of  $\alpha = 0.05$ , are the probability of observing a value as or more extreme than the given test statistic under a  $\chi^2$  distribution with the given degrees of freedom.

## Channel Model

In the channel, we find a significant difference in abundance before and after eelgrass decline, as well as a significant interaction between period and season.

```
otter_glm_nbinom <- MASS::glm.nb(
  Count ~ Period*Season + Site,
  data = abundance[abundance$TrawlType == "Otter",]
)

otter_glm_nbinom %>%
  car::Anova(test.statistic = "LR") %>% as.data.frame() %>%
  rownames_to_column("term") %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

term	LR Chisq	Df	Pr(>Chisq)
Period	27.142	1	0.000
Season	0.489	1	0.484
Site	0.080	2	0.961
Period:Season	8.752	1	0.003

## Pairwise Comparisons

Provided are pairwise comparisons for trawls conducted during each period and season in the channel. Abundance does not differ significantly between the Fall of the pre-decline and post-decline periods, but otherwise differs between all period/season combinations, including seasons within the same sampling period.

```
otter_glm_pairwise <- emmeans(
  otter_glm_nbinom,
  pairwise ~ Period:Season,
  data = abundance[abundance$TrawlType == "Otter",],
  adjust = "fdr"
)

otter_glm_nbinom %<>% summary()

otter_glm_pairwise$contrasts %>%
  as.data.frame() %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

contrast	estimate	SE	df	z.ratio	p.value
(Pre-Decline Fall) - (Post-Decline Fall)	-0.570	0.441	Inf	-1.292	0.196
(Pre-Decline Fall) - (Pre-Decline Summer)	0.784	0.353	Inf	2.222	0.035
(Pre-Decline Fall) - (Post-Decline Summer)	-1.660	0.421	Inf	-3.944	0.000
(Post-Decline Fall) - (Pre-Decline Summer)	1.354	0.445	Inf	3.043	0.005
(Post-Decline Fall) - (Post-Decline Summer)	-1.090	0.501	Inf	-2.177	0.035
(Pre-Decline Summer) - (Post-Decline Summer)	-2.445	0.425	Inf	-5.753	0.000

## Mudflats Model

Model structure is identical to the channel abundance model presented above. Only the season term is significant, so no pairwise comparisons were fitted.

```
beam_glm_nbinom <- MASS::glm.nb(
  Count ~ Period*Season + Site,
  data = abundance[abundance$TrawlType == "Beam",]
)

beam_glm_nbinom %>%
  car::Anova(test.statistic = "LR") %>% as.data.frame() %>%
  rownames_to_column("term") %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

term	LR Chisq	Df	Pr(>Chisq)
Period	0.075	1	0.784

term	LR Chisq	Df	Pr(>Chisq)
Season	4.182	1	0.041
Site	1.997	2	0.368
Period:Season	1.283	1	0.257

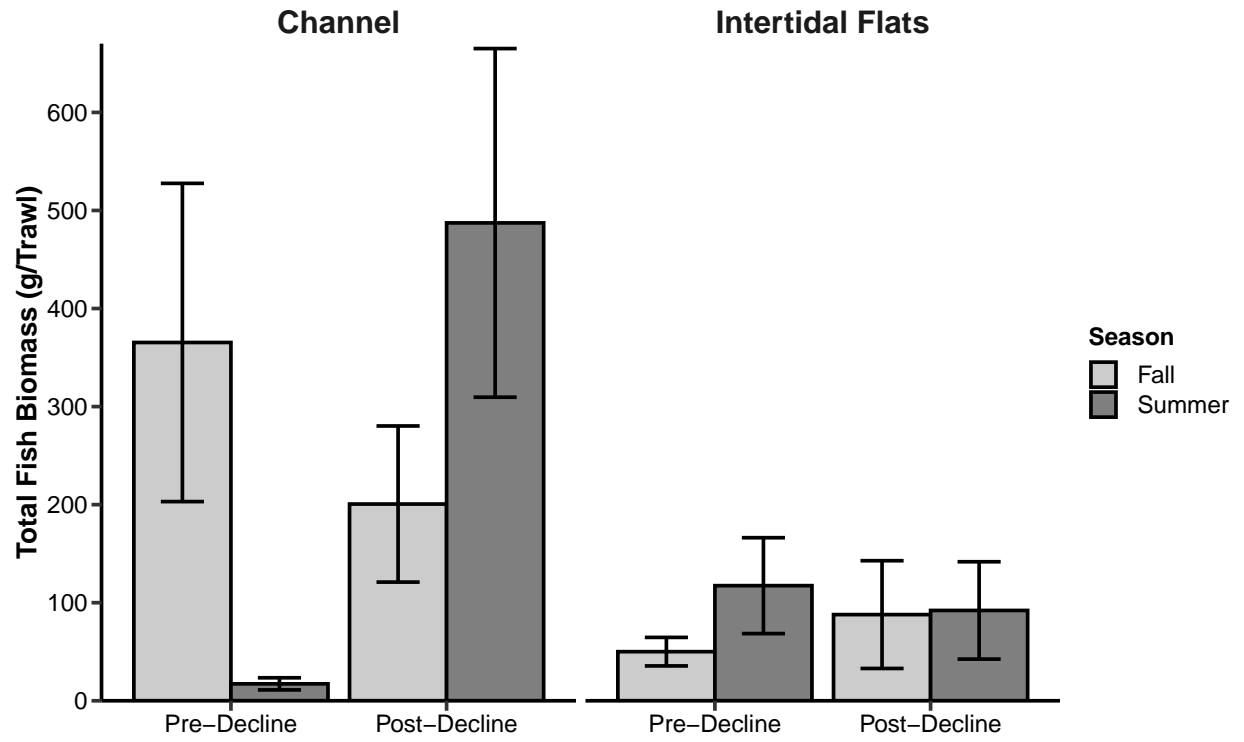
## Biomass

### Bar Chart

```
biomass <-
  read.csv(here("data", "trawl_fish_lengths.csv"), stringsAsFactors = FALSE) %>%
  filter_trawls() %>%
  mutate(spec_binomial = paste(Genus, Species)) %>%
  group_by(Period, Season, Year, TrawlType, Site, Replicate) %>%
  summarize(biomass = sum(weight_g)) %>%
  right_join(
    abundance %>%
      select(Period, Season, Year, TrawlType, Site, Replicate)
  ) %>%
  mutate(biomass = ifelse(is.na(biomass), 0, biomass))

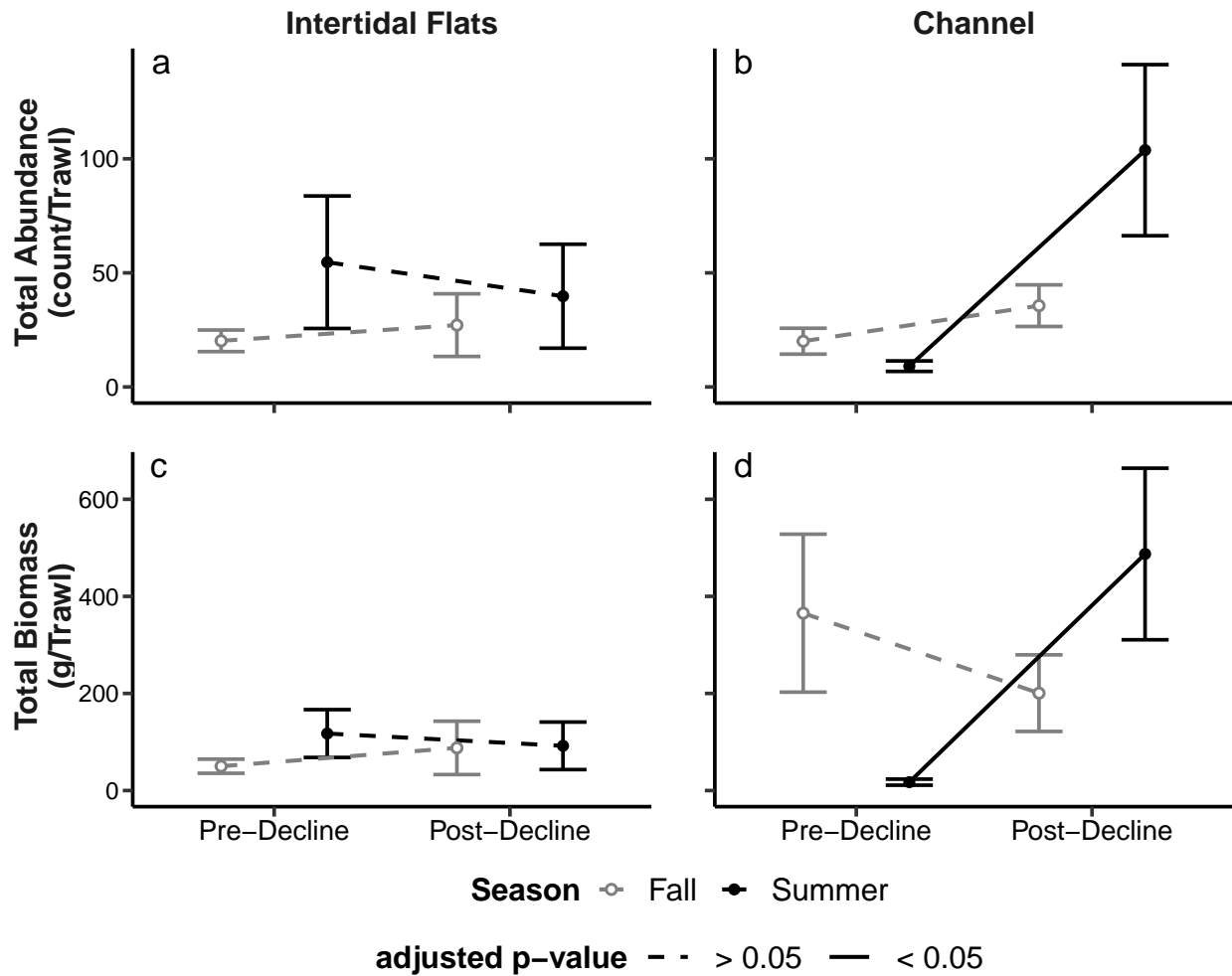
biomass_summary <- biomass %>%
  group_by(Period, Season, TrawlType) %>%
  cluster_bootstrap(biomass, Site) %>% ungroup() %>%
  mutate(Period = factor(Period, levels = c("Pre-Divide", "Post-Divide"),
    ordered = TRUE),
    TrawlType = dplyr::recode(TrawlType, "Beam" = "Intertidal Flats",
      "Otter" = "Channel"))

biomass_summary %>%
  ggplot(aes(Period, mean, fill = Season)) +
  geom_bar(stat = "identity", position = "dodge",
    color = "black", size = 1) +
  scale_y_continuous(expand = c(0,0), breaks = seq(0, 1000, 100),
    limits = c(0, max(biomass_summary$upper + 5))) +
  scale_fill_manual(values = c("grey80", "grey50")) +
  facet_wrap(~TrawlType, ncol = 2) +
  geom_errorbar(aes(x = Period, ymin = lower, ymax = upper),
    position = position_dodge(width = 0.9),
    width = 0.4, size = 1) +
  theme_trawls +
  theme(axis.title.x = element_blank()) +
  ylab("Total Fish Biomass (g/Trawl)")
```





## Plot: Biomass & Abundance



## Channel Model

Model structure is the same as for abundance models, but with a Gamma error distribution. Likelihood ratio tests are calculated as above.

```
otter_biomass_glm <- glm(
  (biomass + 1) ~ Period*Season + Site,
  family = Gamma(link = "log"),
  data = biomass[biomass$TrawlType == "Otter", ]
)

otter_biomass_glm %>%
  car::Anova(test.statistic = "LR") %>%
  rownames_to_column("term") %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

term	LR Chisq	Df	Pr(>Chisq)
Period	18.516	1	0.00
Season	18.320	1	0.00

term	LR Chisq	Df	Pr(>Chisq)
Site	0.862	2	0.65
Period:Season	21.176	1	0.00

## Pairwise Comparisons

Because there was a significant period by season interaction, pairwise comparisons were fit. P-values are adjusted using the Benjamini & Hochberg method, as above. Biomass is significantly different between the summer of the pre-decline and post-decline periods, as well as between the fall and summer in the pre-decline period, but it otherwise unchanged.

```
otter_biomass_pairwise <- emmeans(
  otter_biomass_glm,
  pairwise ~ Period:Season,
  data = biomass[biomass$TrawlType == "Otter",],
  adjust = "fdr"
)

otter_biomass_pairwise %<>% summary()

otter_biomass_pairwise$contrasts %>% as.data.frame() %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

contrast	estimate	SE	df	z.ratio	p.value
(Pre-Decline Fall) - (Post-Decline Fall)	0.567	0.547	Inf	1.037	0.360
(Pre-Decline Fall) - (Pre-Decline Summer)	3.052	0.428	Inf	7.125	0.000
(Pre-Decline Fall) - (Post-Decline Summer)	-0.289	0.525	Inf	-0.551	0.581
(Post-Decline Fall) - (Pre-Decline Summer)	2.485	0.547	Inf	4.543	0.000
(Post-Decline Fall) - (Post-Decline Summer)	-0.857	0.625	Inf	-1.370	0.256
(Pre-Decline Summer) - (Post-Decline Summer)	-3.341	0.525	Inf	-6.369	0.000

## Mudflats Model

As for the abundance model, only season is significant, and thus no pairwise comparisons are fitted.

```
beam_biomass_glm <- glm(
  (biomass + 1) ~ Period*Season + Site,
  family = Gamma(link = "log"),
  data = biomass[biomass$TrawlType == "Beam",]
)

beam_biomass_glm %>%
  car::Anova(test.statistic = "LR") %>% as.data.frame() %>%
  rownames_to_column("term") %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

term	LR Chisq	Df	Pr(>Chisq)
Period	0.341	1	0.559
Season	2.888	1	0.089
Site	0.174	2	0.917

term	LR Chisq	Df	Pr(>Chisq)
Period:Season	1.752	1	0.186

## Species Composition

### NMDS Plot

Separate non-metric multidimensional scaling (NMDS) plots were made for each of the habits (channel and mudflats). Stress for the channel NMDS was 0.16, stress for the mudflats NMDS was 0.19. One extreme outlier was removed from the Channel plot. It contained only one individual of *Paralichthys californicus*, of which only two other individuals were observed in the study. The observation was not dropped from the PERMANOVA analysis, but the qualitative (significance test) results do not change if it is removed.

```
species_data <-
  read.csv(here("data", "trawl_fish_counts.csv")) %>%
  filter_trawls() %>%
  mutate(species = paste(Genus, Species, sep = " ")) %>%
  dplyr::select(Period, Year, Season, TrawlType, Site, Replicate, species, Count) %>%
  spread(species, Count) ## transform to wide format

## Columns containing species abundances
species_columns <- which(
  !(names(species_data) %in%
    c("Period", "Year", "Season", "Site", "TrawlType", "Replicate"))
)

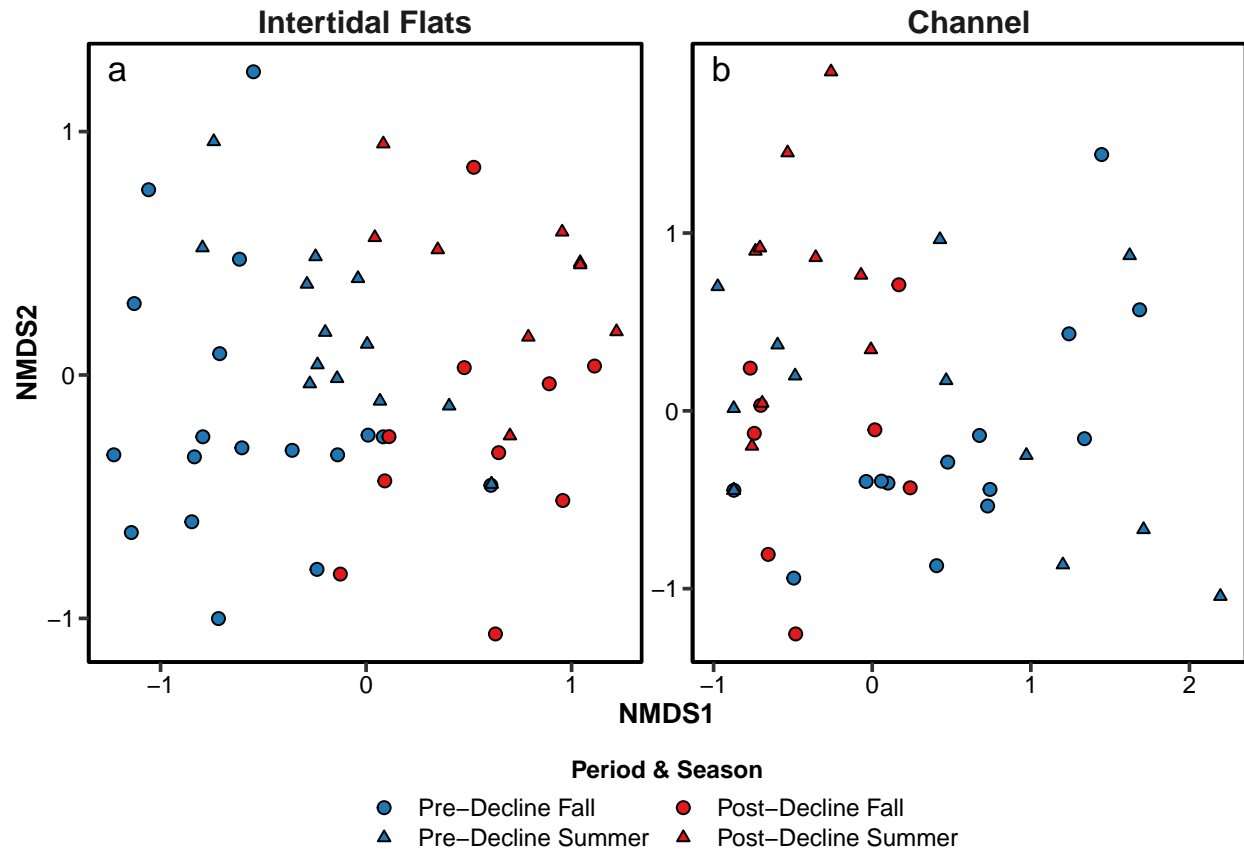
## Remove observations where no individuals were recorded
species_data <- species_data[rowSums(species_data[,species_columns]) > 0,]

## Channel trawls species matrix
otter_species <- species_data[species_data$TrawlType == "Otter",]
otter_matrix <- as.matrix(otter_species[,species_columns])
rownames(otter_matrix) <- with(otter_species, paste(TrawlType, Period, Season))
otter_matrix <- otter_matrix[,colSums(otter_matrix) > 0]

## Mudflat trawls species matrix
beam_species <- species_data[species_data$TrawlType == "Beam",]
beam_matrix <- as.matrix(beam_species[,species_columns])
rownames(beam_matrix) <- with(beam_species, paste(TrawlType, Period, Season))
beam_matrix <- beam_matrix[,colSums(beam_matrix) > 0]

## set random number generator seed, for reproducibility
set.seed(200)

## Fit NMDS for channel and mudflats
otter_nmnds <- metaMDS(otter_matrix[-18,], distance = "bray", k = 2, trymax = 1000)
beam_nmnds <- metaMDS(beam_matrix, distance = "bray", k = 2, trymax = 1000)
```



## PERMANOVA

For each habitat type, we fit a permutational multivariate analysis of variance (PERMANOVA) on a Bray-Curtis dissimilarity matrix of species abundance with 10,000 permutations. We used period, season, site, and the interaction between period and season as predictors, and sites as strata (although p-values for period, season, and their interaction are near-identical regardless of how sites are included in the model). We used marginal pseudo-F tests to evaluate the significance of model terms.

### Channel Model

All model terms are significant, except site.

```
set.seed(200)

## Fit PERMANOVA
adonis_otter <- adonis2(
  otter_matrix ~ Period*Season + Site,
  data = otter_species,
  strata = Site,
  method = "bray", # Bray-Curtis dissimilarity
  perm = 10000
)

adonis_otter %>% as.data.frame() %>%
  rownames_to_column("term") %>%
  mutate_if(is.numeric, round, digits = 3) %>%
```

```
knitr::kable()
```

term	Df	SumOfSqs	R2	F	Pr(>F)
Period	1	1.801	0.117	6.972	0.000
Season	1	0.755	0.049	2.922	0.012
Site	2	0.834	0.054	1.615	0.078
Period:Season	1	0.611	0.040	2.365	0.032
Residual	44	11.369	0.740		
Total	49	15.371	1.000		

**Pairwise Comparisons** Pairwise PERMANOVA models are fit using a wrapper function from the `RVAideMemoire` package; when the response provided is a distance matrix (in this case, Bray-Curtis dissimilarity), each comparison is fit using the `adonis` function. Pseudo-F tests are calculated for each comparison. P-values are adjusted using the Benjamini & Hochberg method.

All pairwise comparisons are significant, except Fall pre-decline vs. post-decline.

```
## Function to fit pairwise PERMANOVA models
source(here("R", "pairwise_permanova.R"))

otter_species$group <- with(otter_species, paste(Period, Season))

set.seed(200)

## Fit pairwise PERMANOVA models
otter_permanova_pairs <- pairwise_permanova(
  sp_matrix = otter_matrix,
  group_var = otter_species$group
)

knitr::kable(otter_permanova_pairs$contrasts)
```

group1	group2	R2	F_value	df1	df2	p_value
Pre-Decline Fall	Pre-Decline Summer	0.077	2.591	1	32	0.039
Pre-Decline Fall	Post-Decline Fall	0.082	1.881	1	22	0.083
Pre-Decline Fall	Post-Decline Summer	0.163	4.299	1	23	0.001
Pre-Decline Summer	Post-Decline Fall	0.167	4.814	1	25	0.007
Pre-Decline Summer	Post-Decline Summer	0.222	7.153	1	26	0.001
Post-Decline Fall	Post-Decline Summer	0.152	2.689	1	16	0.042

**SIMPER** For each significant pairwise comparison of interest, we fit a similarity percentages (SIMPER) analysis, reporting abundance for species identified by the SIMPER as contributing to observed differences in community composition more than would be expected at random. For each SIMPER output, relevant species are listed, and an abundance plot is provided.

**How to interpret this output:** This output is ordered in terms of each species contribution to the difference between groups examined. The **average** variable is the average contribution to the overall dissimilarity, and the **sd** is the standard deviation of that contribution. The **cumsum** variable is the total percent of the variation accounted for by the species and all other species above it.

Code is shown in output for the first SIMPER only, as the code is near-identical for each SIMPER.

```
## functions for formatting and plotting simper results
source(here("R", "simper_functions.R"))

## Split Channel data by Period
otter_simper <- split(otter_species, otter_species$Period)
otter_simper %<>% append(split(otter_species, otter_species$Season))

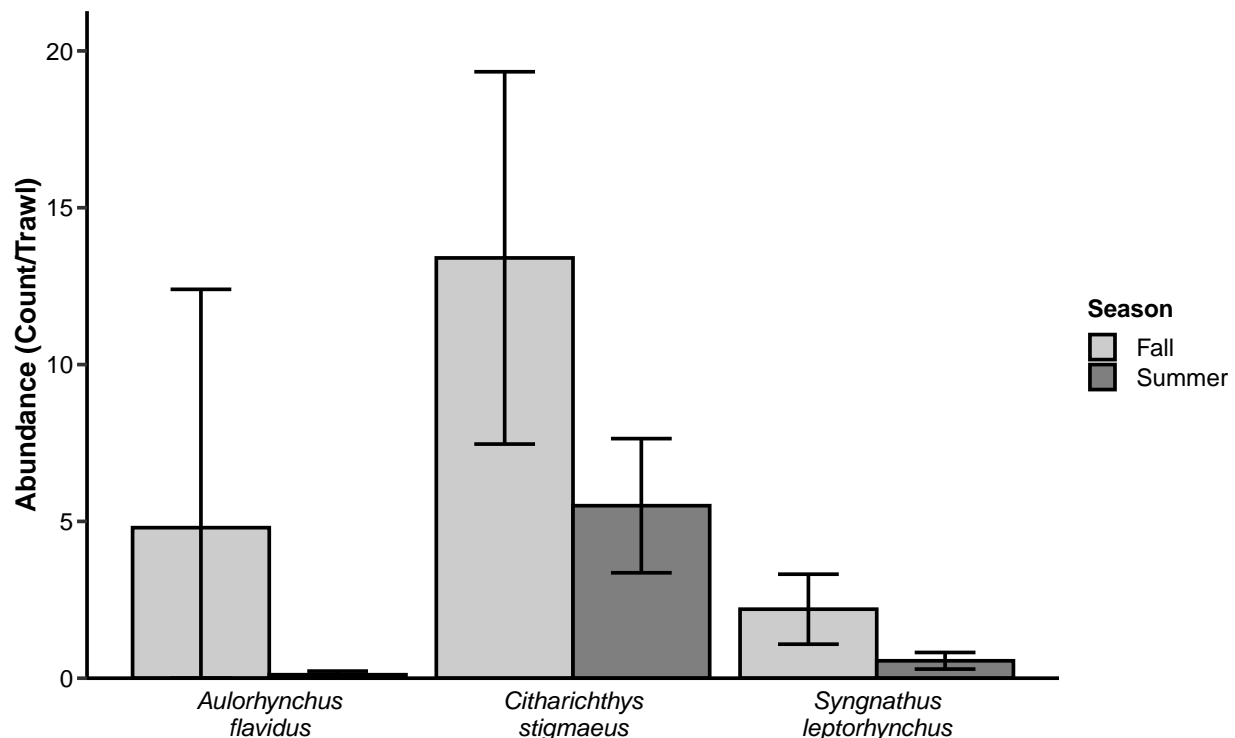
otter_simper$`Pre-Decline_simper` <-
  otter_simper$`Pre-Decline`[,species_columns] %>%
  filter_sp() %>%
  simper(otter_simper$`Pre-Decline`$Season) %>%
  summary %$% Fall_Summer %>%
  format_simper() %>%
  rename(Fall = ava, Summer = avb)

knitr::kable(otter_simper$`Pre-Decline_simper`)
```

#### Pre-Decline Fall vs. Summer

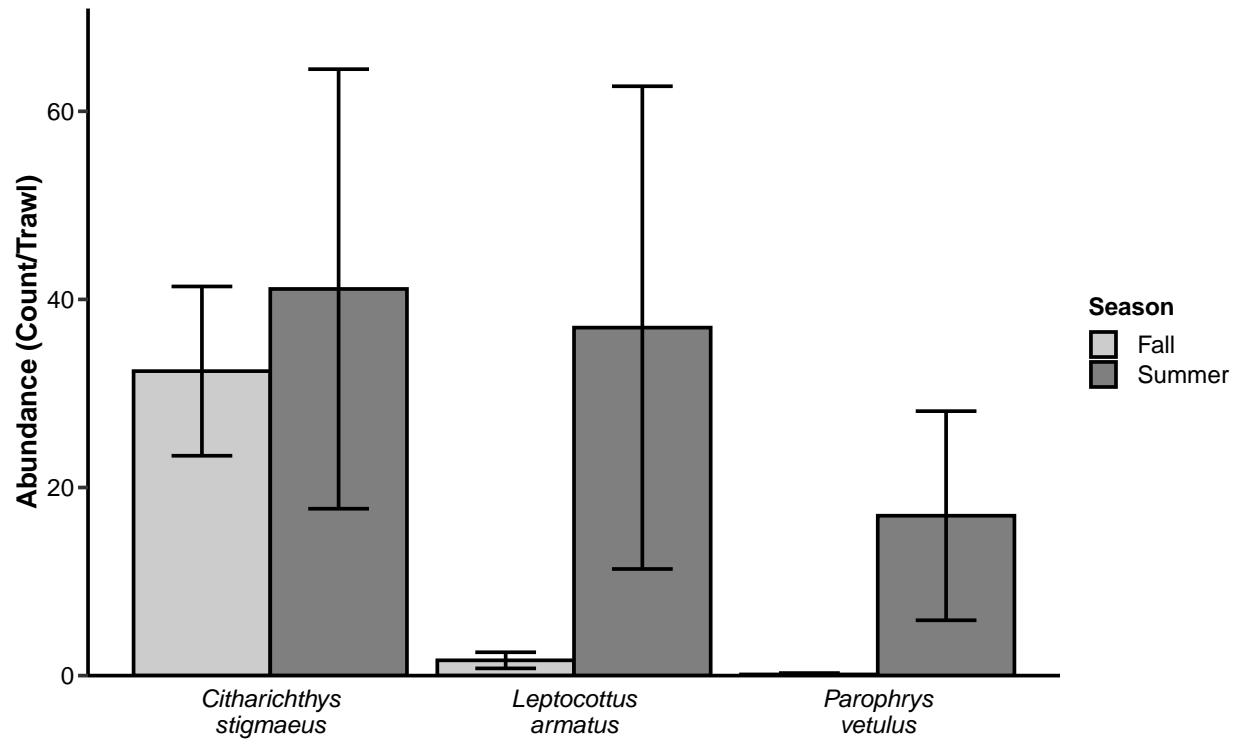
Species	average	sd	ratio	Fall	Summer	cumsum	n
Citharichthys stigmaeus	0.38	0.30	1.25	13.4	5.50	0.50	21
Syngnathus leptorhynchus	0.10	0.12	0.79	2.2	0.56	0.63	21
Aulorhynchus flavidus	0.05	0.18	0.29	4.8	0.11	0.70	21

```
with(otter_simper,
  sum_abundance(`Pre-Decline`, `Pre-Decline_simper`$Species, "Season")) %>%
  plot_abundance("Season")
```



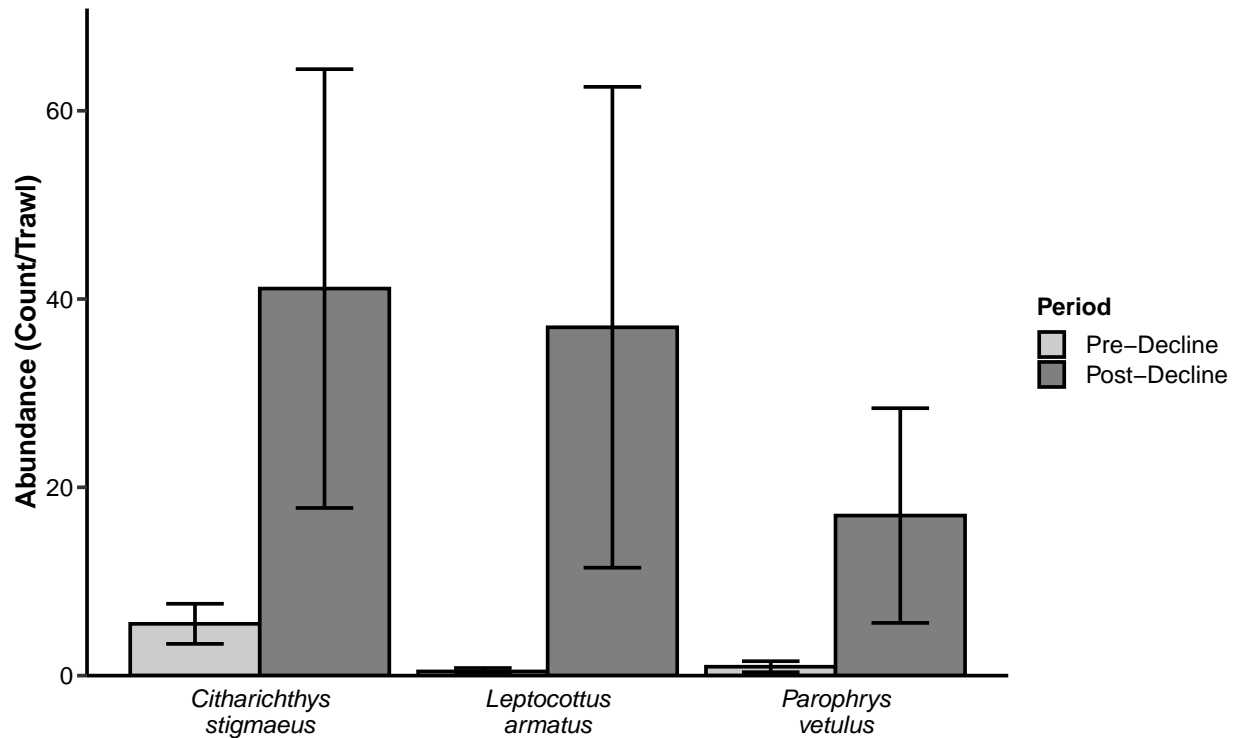
# Post-Dedline Fall vs. Summer

Species	average	sd	ratio	Fall	Summer	cumsum	n
<i>Citharichthys stigmaeus</i>	0.33	0.25	1.35	32.38	41.11	0.47	13
<i>Leptocottus armatus</i>	0.21	0.21	1.01	1.62	37.00	0.78	13
<i>Parophrys vetulus</i>	0.11	0.11	0.98	0.12	17.00	0.93	13



## Summer Pre-Dcline vs. Post-Dcline

Species	average	sd	ratio	Pre-Dcline	Post-Dcline	cumsum	n
<i>Citharichthys stigmaeus</i>	0.39	0.34	1.13	5.50	41.11	0.44	21
<i>Leptocottus armatus</i>	0.29	0.27	1.06	0.44	37.00	0.77	21
<i>Parophrys vetulus</i>	0.13	0.13	1.02	0.94	17.00	0.92	21



## Mudflats Model

Model structure is as described above. All model terms are significant.

```
set.seed(200)

## Fit PERMANOVA
adonis_beam <- adonis2(
  beam_matrix ~ Period*Season + Site,
  data = beam_species,
  strata = Site,
  method = "bray", # Bray-Curtis dissimilarity
  perm = 10000
)

adonis_beam %>% as.data.frame() %>%
  rownames_to_column("term") %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```



term	Df	SumOfSqs	R2	F	Pr(>F)
Period	1	1.481	0.099	6.408	0.000
Season	1	1.045	0.070	4.523	0.000
Site	2	1.194	0.080	2.583	0.003
Period:Season	1	0.873	0.058	3.777	0.002
Residual	45	10.400	0.694		
Total	50	14.993	1.000		

**Pairwise Comparisons** Pairwise comparisons are as described above. All pairwise comparisons are significant.

```
beam_species$group <- with(beam_species, paste(Period, Season))

set.seed(200)

## Fit pairwise PERMANOVA models
beam_permanova_pairs <- pairwise_permanova(
  sp_matrix = beam_matrix,
  group_var = beam_species$group
)

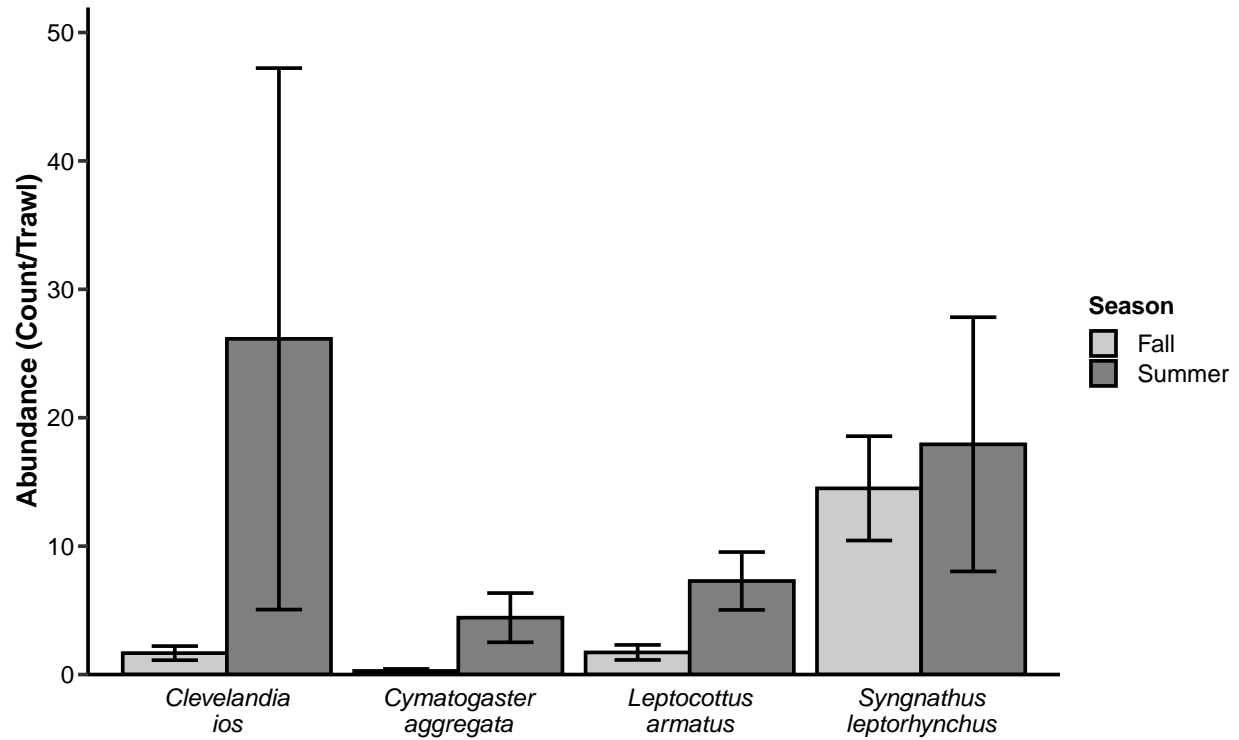
knitr::kable(beam_permanova_pairs$contrasts)
```

group1	group2	R2	F_value	df1	df2	p_value
Pre-Decline Fall	Pre-Decline Summer	0.138	4.784	1	31	0.002
Pre-Decline Fall	Post-Decline Fall	0.196	6.345	1	27	0.002
Pre-Decline Fall	Post-Decline Summer	0.211	6.677	1	26	0.001
Pre-Decline Summer	Post-Decline Fall	0.118	2.948	1	23	0.010
Pre-Decline Summer	Post-Decline Summer	0.129	3.105	1	22	0.010
Post-Decline Fall	Post-Decline Summer	0.146	2.898	1	18	0.013

## SIMPER

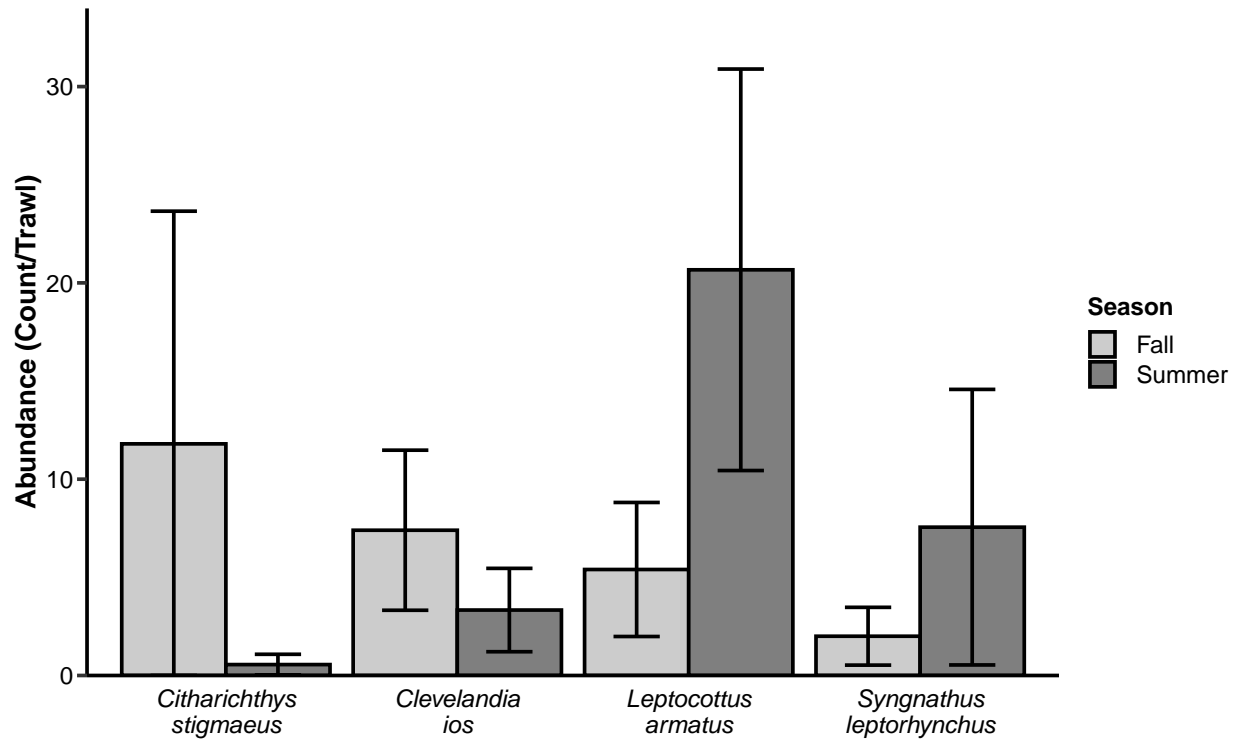
### Pre-Dcline Fall vs. Summer

Species	average	sd	ratio	Fall	Summer	cumsum	n
<i>Syngnathus leptorhynchus</i>	0.28	0.23	1.20	14.50	17.93	0.38	18
<i>Clevelandia ios</i>	0.26	0.23	1.12	1.67	26.14	0.72	18
<i>Leptocottus armatus</i>	0.09	0.06	1.42	1.72	7.29	0.84	18
<i>Cymatogaster aggregata</i>	0.06	0.08	0.84	0.28	4.43	0.92	18



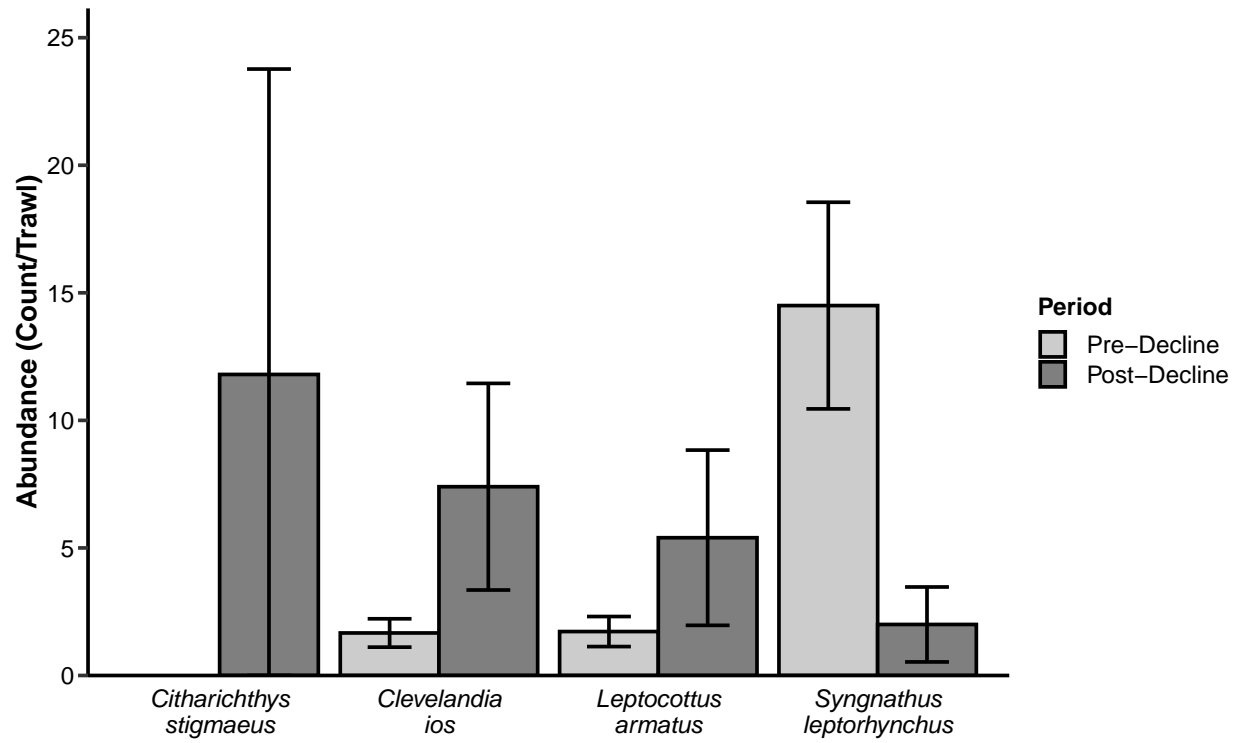
# Post-Degradation Fall vs. Summer

Species	average	sd	ratio	Fall	Summer	cumsum	n
<i>Leptocottus armatus</i>	0.28	0.20	1.37	5.4	20.67	0.35	10
<i>Clevelandia ios</i>	0.18	0.20	0.87	7.4	3.33	0.58	10
<i>Citharichthys stigmaeus</i>	0.13	0.20	0.69	11.8	0.56	0.75	10
<i>Syngnathus leptorhynchus</i>	0.10	0.11	0.91	2.0	7.56	0.88	10



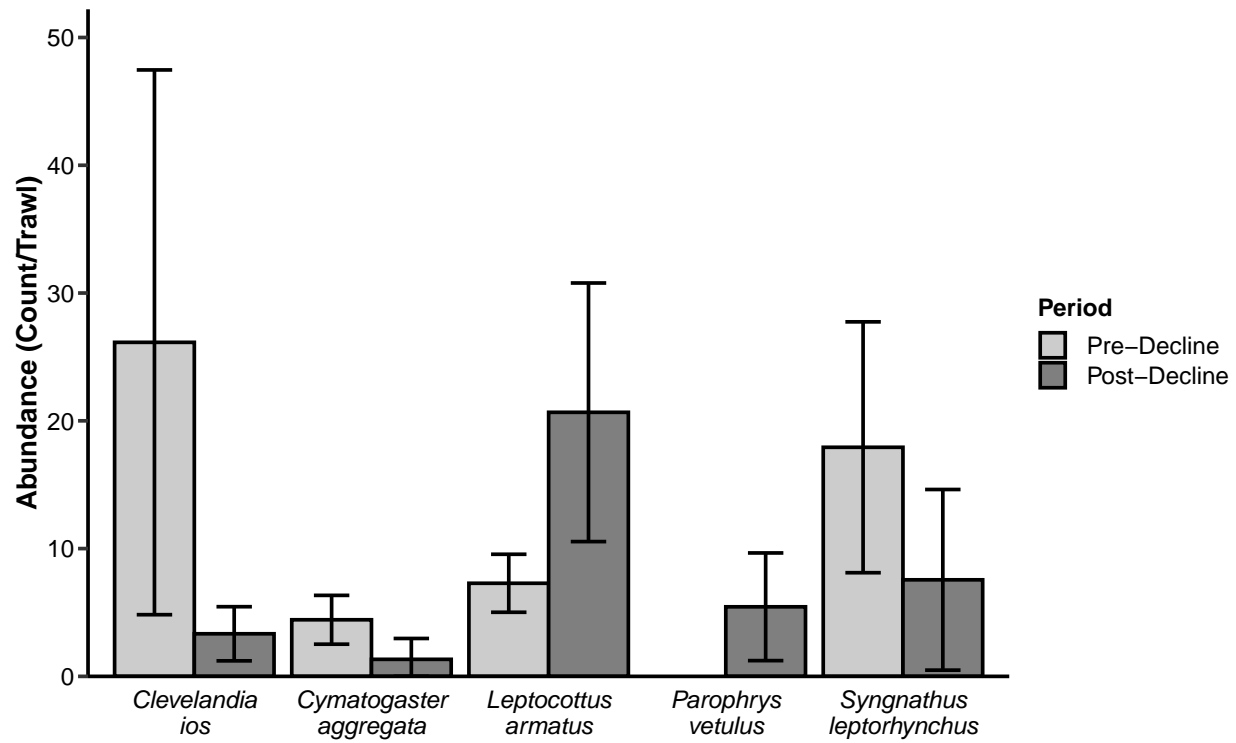
### Fall Pre-Dcline vs. Post-Dcline

Species	average	sd	ratio	Pre-Dcline	Post-Dcline	cumsum	n
<i>Syngnathus leptorhynchus</i>	0.28	0.23	1.22	14.50	2.0	0.35	17
<i>Clevelandia ios</i>	0.18	0.18	0.99	1.67	7.4	0.57	17
<i>Citharichthys stigmaeus</i>	0.15	0.20	0.75	0.00	11.8	0.76	17
<i>Leptocottus armatus</i>	0.13	0.14	0.88	1.72	5.4	0.92	17



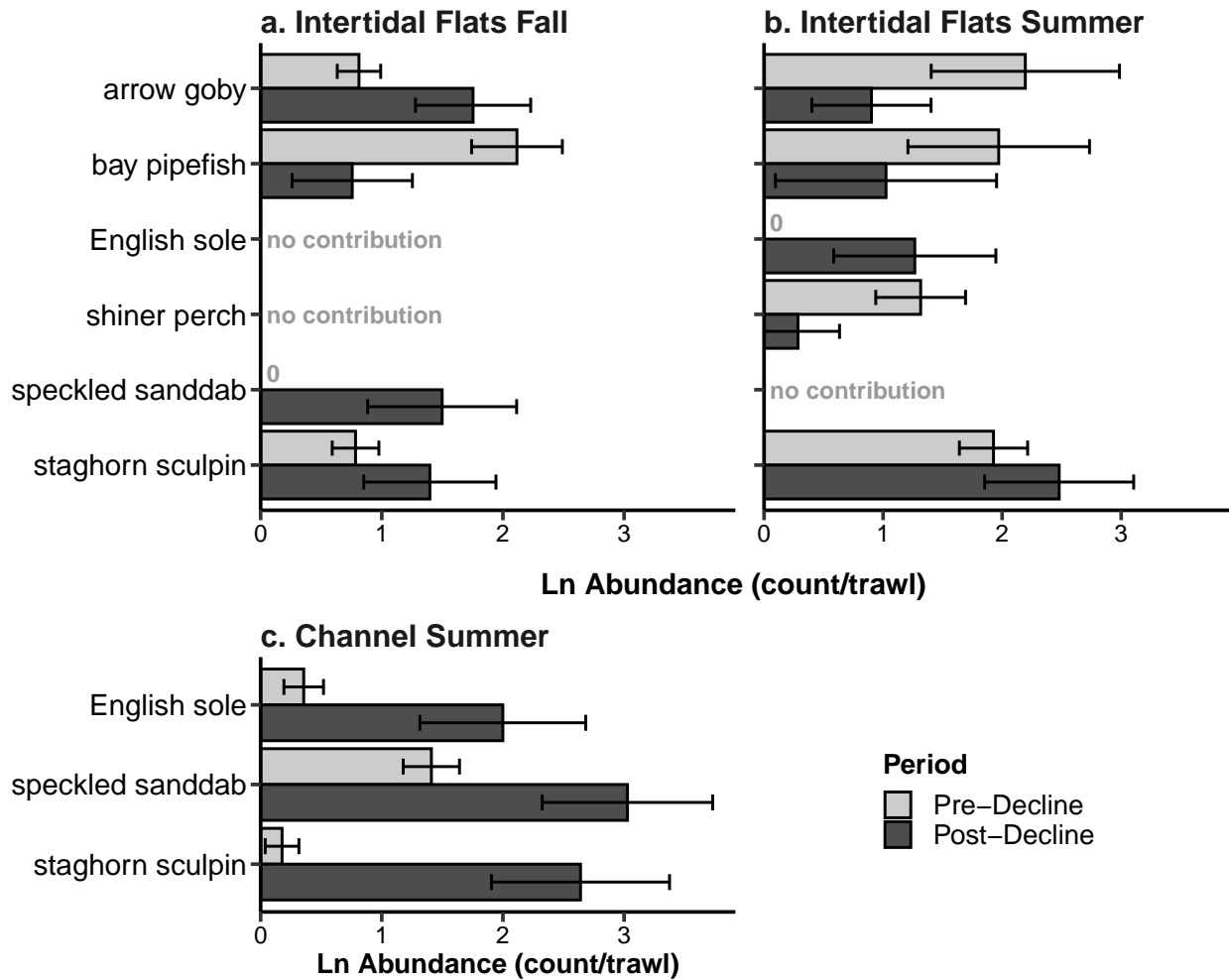
### Summer Pre-Degradation vs. Post-Degradation

Species	average	sd	ratio	Pre-Degradation	Post-Degradation	cumsum	n
<i>Clevelandia ios</i>	0.25	0.24	1.04	26.14	3.33	0.32	16
<i>Leptocottus armatus</i>	0.20	0.17	1.18	7.29	20.67	0.57	16
<i>Syngnathus leptorhynchus</i>	0.18	0.19	0.94	17.93	7.56	0.80	16
<i>Cymatogaster aggregata</i>	0.06	0.08	0.80	4.43	1.33	0.88	16
<i>Parophrys vetulus</i>	0.05	0.06	0.93	0.00	5.44	0.95	16



## Plot: SIMPER Species

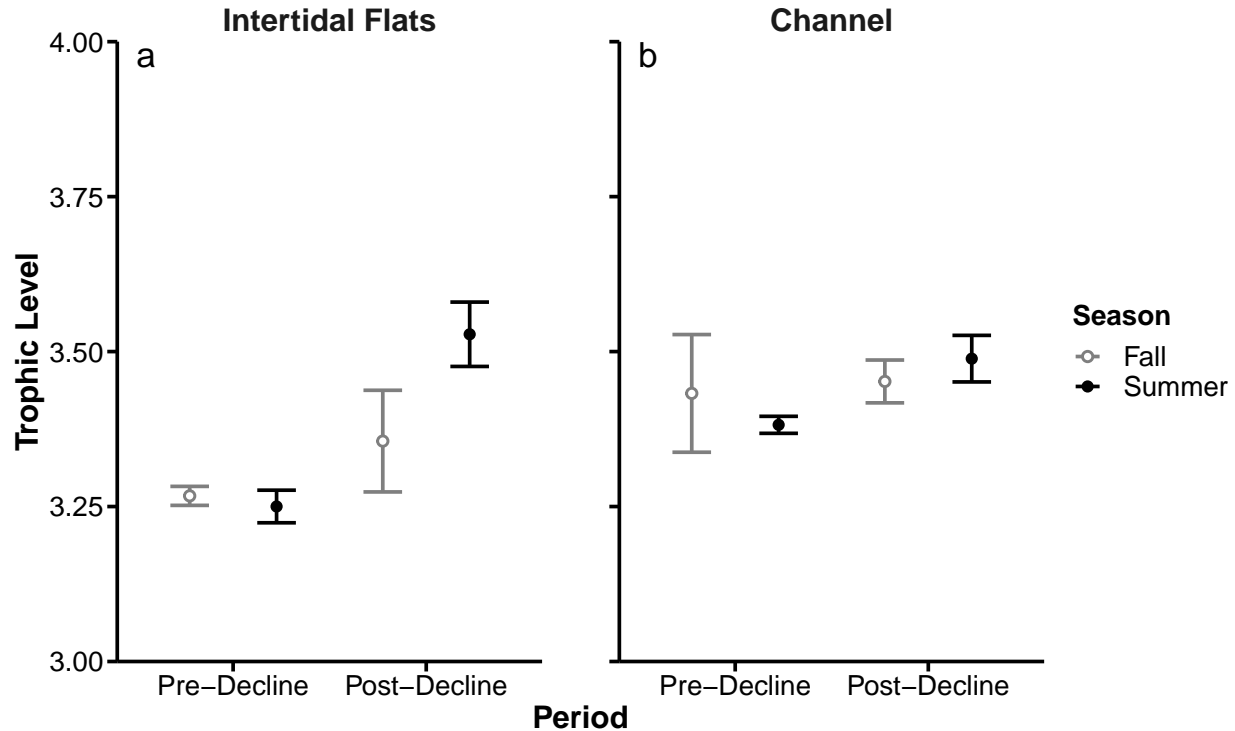
Species abundances (natural log (+1) before (light gray) and after (dark gray) eelgrass decline for (a) intertidal flats in the fall, (b) intertidal flats in the summer, and (c) the channel in the summer. Species composition did not differ significantly between pre-decline and post-decline periods for the channel in the fall based on a pairwise PERMANOVA. Species shown are those identified by a SIMPER analysis as contributing most to observed differences in species composition; standard errors are estimated using a two-stage bootstrap. Where there were no individuals of a species for a particular period and habitat, this is indicated with a zero.



## Trophic Level

### Plot: Mean & SE

Mean trophic level  $\pm$  standard error, for a) intertidal flats, and b) channel, in the fall and summer of the pre-decline and post-decline periods.



## Channel Model

We assessed changes in average sample trophic level with linear regression, weighted by the number of individuals in each trawl. Trophic level estimates were gathered from FishBase; most species estimates were from diet studies, but some were Bayesian estimates based on nearest relatives.

In the channel, we find a general increase in trophic level across both fall and summer samples following eelgrass decline ( $p < 0.001$ ), with no interaction ( $p = 0.65$ ) between period and season, suggesting the magnitude of increase was similar across seasons.

```
## Fit linear model
otter_trophic_lm <- lm(
  trophic_level ~ Period*Season + Site,
  weights = count,
  data = trophic_lm_data[trophic_lm_data$TrawlType == "Otter",]
)

## F-tests
otter_trophic_lm %>% anova() %>%
  rownames_to_column("term") %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

term	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Period	1	1.943	1.943	14.272	0.000
Season	1	0.161	0.161	1.186	0.282
Site	2	0.798	0.399	2.932	0.064
Period:Season	1	0.029	0.029	0.211	0.648
Residuals	44	5.989	0.136		

## Mudflats Model

We also find a significant increase in mean-sample trophic level following eelgrass decline in the mudflats ( $p < 0.001$ ), but with an interaction between period and season ( $p = 0.001$ ), suggesting the magnitude of the increase differed between fall and summer.

```
## Fit linear model
beam_trophic_lm <- lm(
  trophic_level ~ Period*Season + Site,
  weights = count,
  data = trophic_lm_data[trophic_lm_data$TrawlType == "Beam",]
)

## F-tests
beam_trophic_lm %>% anova() %>%
  rownames_to_column("term") %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

term	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Period	1	14.560	14.560	88.221	0.000
Season	1	0.043	0.043	0.258	0.614
Site	2	1.807	0.903	5.473	0.007
Period:Season	1	2.036	2.036	12.334	0.001
Residuals	45	7.427	0.165		

## Pairwise Comparisons

While the average difference between pre-decline and post-decline samples was greater in the summer, we find significant increases in trophic level during both the fall ( $p = 0.014$ ) and summer ( $p < 0.001$ ) following eelgrass decline.

```
beam_trophic_pairwise <- emmeans(
  beam_trophic_lm,
  pairwise ~ Period:Season,
  data = trophic_lm_data[trophic_lm_data$TrawlType == "Beam",],
  adjust = "fdr"
)

beam_trophic_pairwise %<>% summary()

beam_trophic_pairwise$contrasts %>%
  as.data.frame() %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

contrast	estimate	SE	df	t.ratio	p.value
(Pre-Decline Fall) - (Post-Decline Fall)	-0.087	0.033	45	-2.622	0.014
(Pre-Decline Fall) - (Pre-Decline Summer)	0.022	0.027	45	0.816	0.419
(Pre-Decline Fall) - (Post-Decline Summer)	-0.214	0.033	45	-6.547	0.000
(Post-Decline Fall) - (Pre-Decline Summer)	0.109	0.032	45	3.418	0.002
(Post-Decline Fall) - (Post-Decline Summer)	-0.127	0.037	45	-3.450	0.002
(Pre-Decline Summer) - (Post-Decline Summer)	-0.236	0.026	45	-8.915	0.000



# Species Richness

## Per-sample Richness

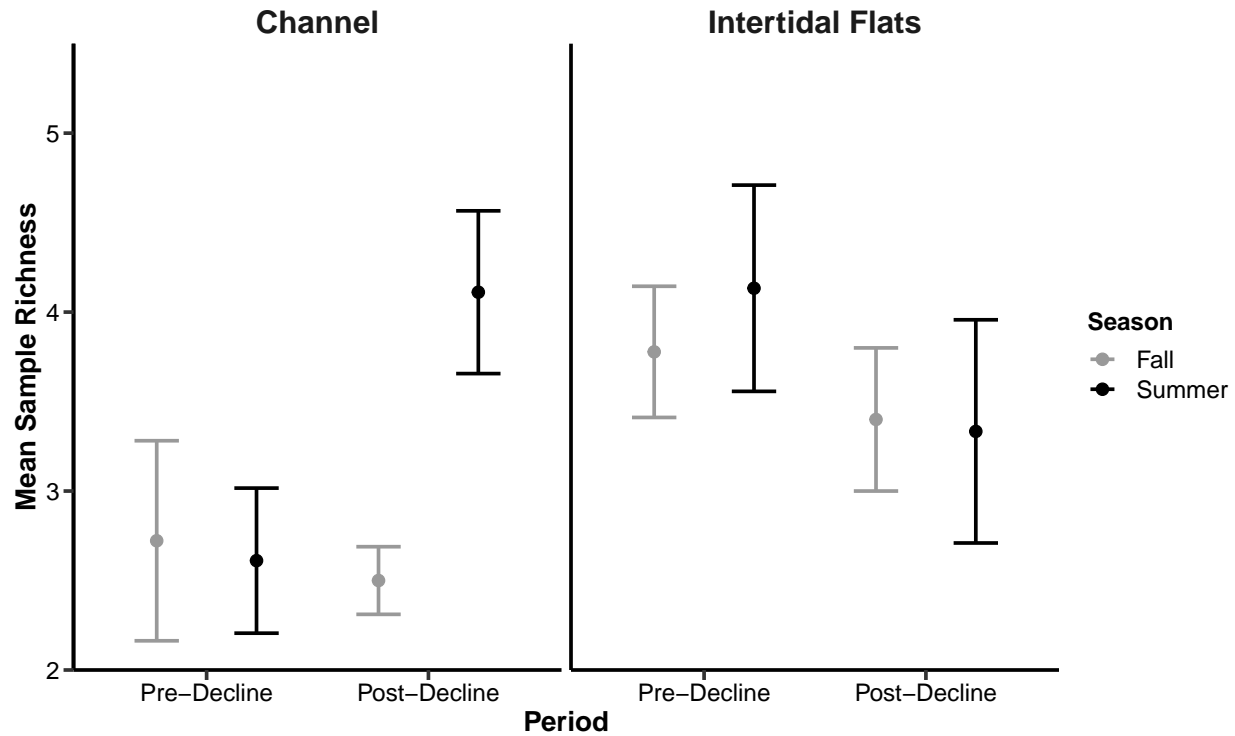
Plot: Mean & SE

```
## Function to Calculate Shannon index for species abundance vector
shannon_H <- function(x) {
  x <- x[x>0]
  p <- x/sum(x)
  -sum(p*log(p))
}

## Summarize richness and shannon per sample
diversity <-
  read.csv(here("data", "trawl_fish_counts.csv"), stringsAsFactors = FALSE) %>%
  filter_trawls() %>%
  mutate(species = paste(Genus, Species)) %>%
  group_by(TrawlType, Period, Season, Site, Replicate) %>%
  summarize(richness = sum(Count > 0),
            shannon = shannon_H(Count))

## Cluster-bootstrap summary of species richness
richness_summary <- diversity %>%
  group_by(TrawlType, Period, Season) %>%
  cluster_bootstrap(richness, Site) %>% ungroup() %>%
  mutate(TrawlType = dplyr::recode(TrawlType,
                                   "Beam" = "Intertidal Flats",
                                   "Otter" = "Channel"))

diversity %>%
  group_by(TrawlType, Period, Season) %>%
  summarize(mean = mean(richness), se = sd(richness)/sqrt(n())) %>%
  mutate(TrawlType = dplyr::recode(TrawlType,
                                   "Beam" = "Intertidal Flats",
                                   "Otter" = "Channel")) %>%
  ggplot(aes(Period, mean, color = Season)) +
  geom_point(position = position_dodge(width = 0.9), size = 3) +
  scale_y_continuous(expand = c(0,0), limits = c(2, 5.5)) +
  scale_color_manual(values = c("grey60", "black")) +
  facet_wrap(~TrawlType, ncol = 2) +
  geom_errorbar(aes(x = Period, ymin = mean - se, ymax = mean + se),
               position = position_dodge(width = 0.9),
               width = 0.4, size = 1) +
  theme_trawls +
  ylab("Mean Sample Richness") +
  annotate("segment", x = -Inf, xend = -Inf, y = -Inf, yend = Inf, size = 1) +
  coord_cartesian(clip = "off")
```



#### Channel Model

```
richness_lm_otter <- lm(
  richness ~ Period*Season + Site,
  data = diversity[diversity$TrawlType == "Otter",]
)

richness_lm_otter %>% anova() %>%
  rownames_to_column("term") %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

term	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Period	1	5.438	5.438	1.599	0.212
Season	1	2.562	2.562	0.753	0.390
Site	2	3.320	1.660	0.488	0.617
Period:Season	1	8.126	8.126	2.389	0.129
Residuals	47	159.874	3.402		

#### Mudflats Model

```
richness_lm_beam <- lm(
  richness ~ Period*Season + Site,
  data = diversity[diversity$TrawlType == "Beam",]
)

richness_lm_beam %>% anova() %>%
  rownames_to_column("term") %>%
```

```
mutate_if(is.numeric, round, digits = 3) %>%
knitr::kable()
```

term	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Period	1	3.931	3.931	1.284	0.263
Season	1	0.521	0.521	0.170	0.682
Site	2	12.650	6.325	2.066	0.138
Period:Season	1	0.300	0.300	0.098	0.756
Residuals	46	140.829	3.062		

## Rarefaction Curves

```
## Read in trawl species counts
trawl_counts <-
  read.csv(here("data", "trawl_fish_counts.csv"), stringsAsFactors = FALSE) %>%
  filter_trawls() %>%
  mutate(species = paste(Genus, Species)) %>%
  select(Period, Year, Season, TrawlType, Site, Replicate,
         species, Count) %>%
  pivot_wider(names_from = species, values_from = Count)

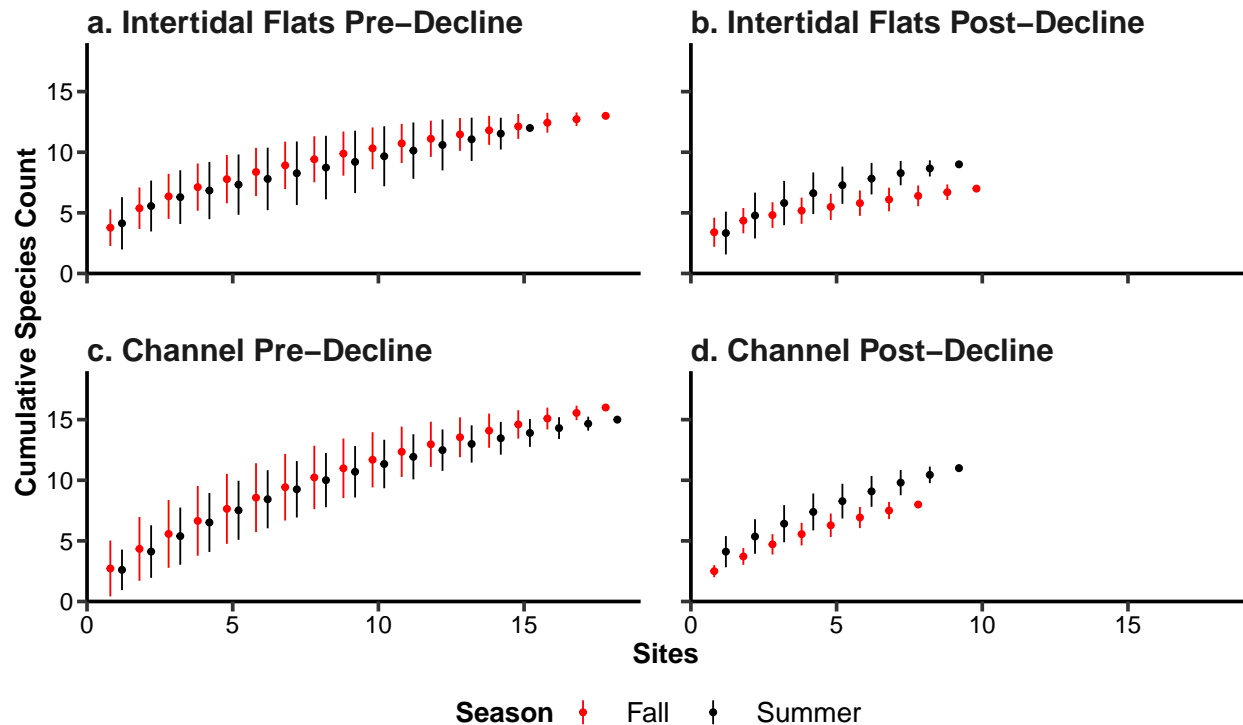
## Fit species accumulation curves for each subset
species_accum <- trawl_counts %>%
  group_by(TrawlType, Period, Season) %>%
  nest() %>%
  mutate(
    species = map(data, dplyr::select, -Year, -Site, -Replicate),
    spec_accum = map(species, specaccum),
    spec_accum = map(spec_accum, function(x) {
      tibble(site = x$site, richness = x$richness, sd = x$sd)
    })
  ) %>%
  dplyr::select(TrawlType, Period, Season, spec_accum) %>%
  unnest(cols = c(spec_accum)) %>%
  ungroup()

## Plot species accumulation curves
species_accum %>%
  mutate(
    site = ifelse(Season == "Summer", site + 0.2, site - 0.2), ## x-offset
    Season = paste0(" ", Season, " "), ## for legend
    TrawlType = dplyr::recode(TrawlType,
                             "Beam" = "Intertidal Flats",
                             "Otter" = "Channel"),
    group = fct_rev(factor(paste(TrawlType, Period))),
    group = paste(letters[group], group, sep = ". ")
  ) %>%
  ggplot(aes(site, richness, color = Season, fill = Season)) +
  geom_point() +
  lemon::facet_rep_wrap(~group, ncol = 2) +
  geom_linerange(aes(ymin = richness - sd, ymax = richness + sd)) +
  scale_color_manual(values = c("red", "black")) +
```

```

labs(x = "Sites", y = "Cumulative Species Count") +
scale_y_continuous(expand = c(0,0), breaks = seq(0, 15, 5), limits = c(0, 19)) +
scale_x_continuous(expand = c(0,0), breaks = seq(0, 15, 5), limits = c(0, 19)) +
theme_trawls +
theme(strip.text = element_text(hjust = 0),
      legend.position = "bottom",
      legend.text = element_text(size = 16),
      legend.title = element_text(size = 16, face = "bold"),
      panel.spacing.y = unit(1, "lines")) +
coord_cartesian(clip = "off")

```



## Asymptotic Richness

### Plot

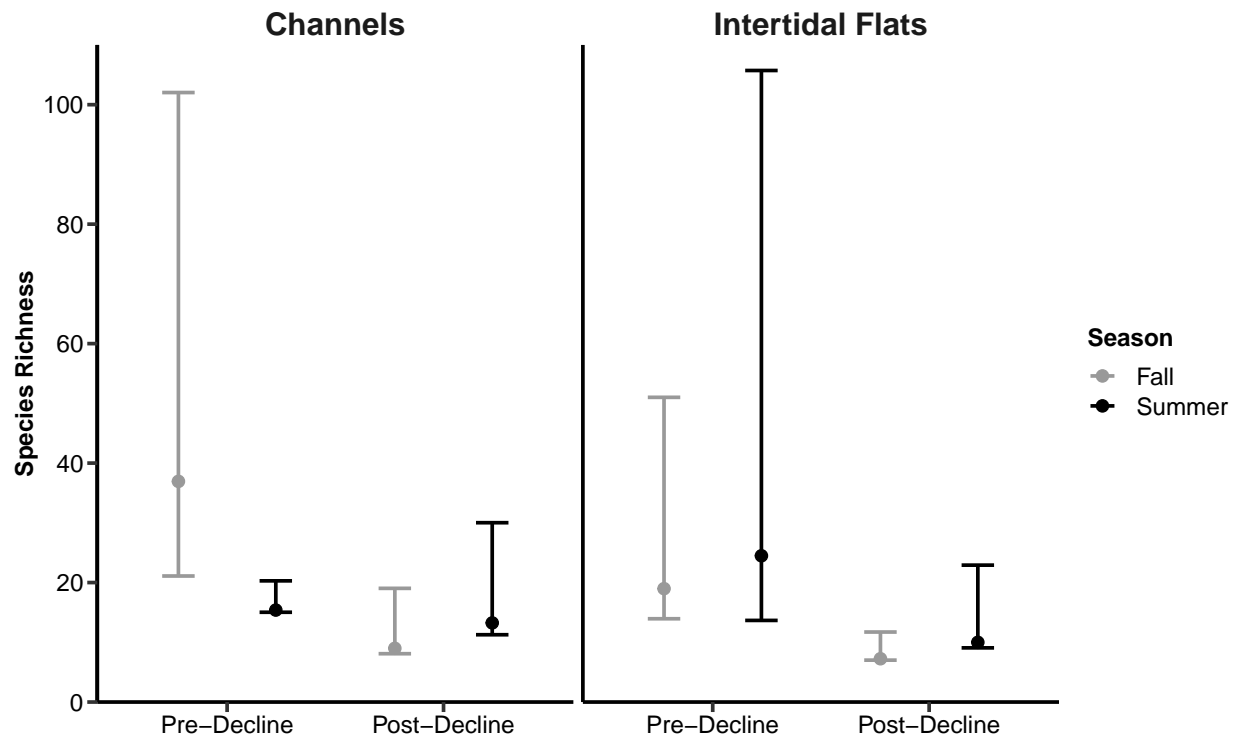
```

trawl_counts_nested <- trawl_counts %>%
  select(-Year, -Year, -Replicate, -Site) %>%
  group_by(TrawlType, Period, Season) %>%
  nest_by(.key = "sp_matrix") %>%
  rowwise() %>%
  mutate(Chao_data = list(as.abucount(t(sp_matrix))),
         richness = list(ChaoRichness(Chao_data, datatype = "abundance")),
         shannon = list(ChaoShannon(Chao_data, datatype = "abundance")))

richness_asymp <- trawl_counts_nested %>%
  dplyr::select(TrawlType, Period, Season, richness) %>%
  unnest(cols = c(richness)) %>%
  mutate(TrawlType = dplyr::recode(TrawlType, "Beam" = "Intertidal Flats",
                                   "Otter" = "Channels"))

```

```
richness_asymp %>%
  ggplot(aes(Period, Estimator, color = Season, group = Season)) +
  geom_point(position = position_dodge(width = 0.9), size = 3) +
  geom_errorbar(aes(ymin = `95% Lower`, ymax = `95% Upper`), width = 0.3,
    position = position_dodge(width = 0.9),
    size = 1) +
  facet_wrap(~TrawlType) +
  scale_y_continuous(limits = c(0, 110), breaks = seq(0, 100, 20), expand = c(0,0)) +
  scale_color_manual(values = c("grey60", "black")) +
  ylab("Species Richness") +
  theme_trawls +
  theme(axis.title.x = element_blank(),
    axis.title = element_text(size = 14, face = "bold")) +
  annotate("segment", x = -Inf, xend = -Inf, y = -Inf, yend = Inf, size = 1) +
  coord_cartesian(clip = "off")
```



Table

```
knitr::kable(richness_asymp)
```

TrawlType	Period	Season	Observed	Estimator	Est_s.e.	95% Lower	95% Upper
Intertidal Flats	Pre-Dcline	Fall	13	18.984	7.167	13.942	51.008
Intertidal Flats	Pre-Dcline	Summer	12	24.485	17.119	13.663	105.723
Intertidal Flats	Post-Dcline	Fall	7	7.249	0.727	7.013	11.720
Intertidal Flats	Post-Dcline	Summer	9	9.997	2.253	9.071	22.917
Channels	Pre-Dcline	Fall	16	36.942	17.289	21.098	102.031
Channels	Pre-Dcline	Summer	15	15.398	0.863	15.030	20.287
Channels	Post-Dcline	Fall	8	8.996	1.865	8.090	19.032

TrawlType	Period	Season	Observed	Estimator	Est_s.e.	95% Lower	95% Upper
Channels	Post-Decline	Summer	11	13.248	3.392	11.266	30.020

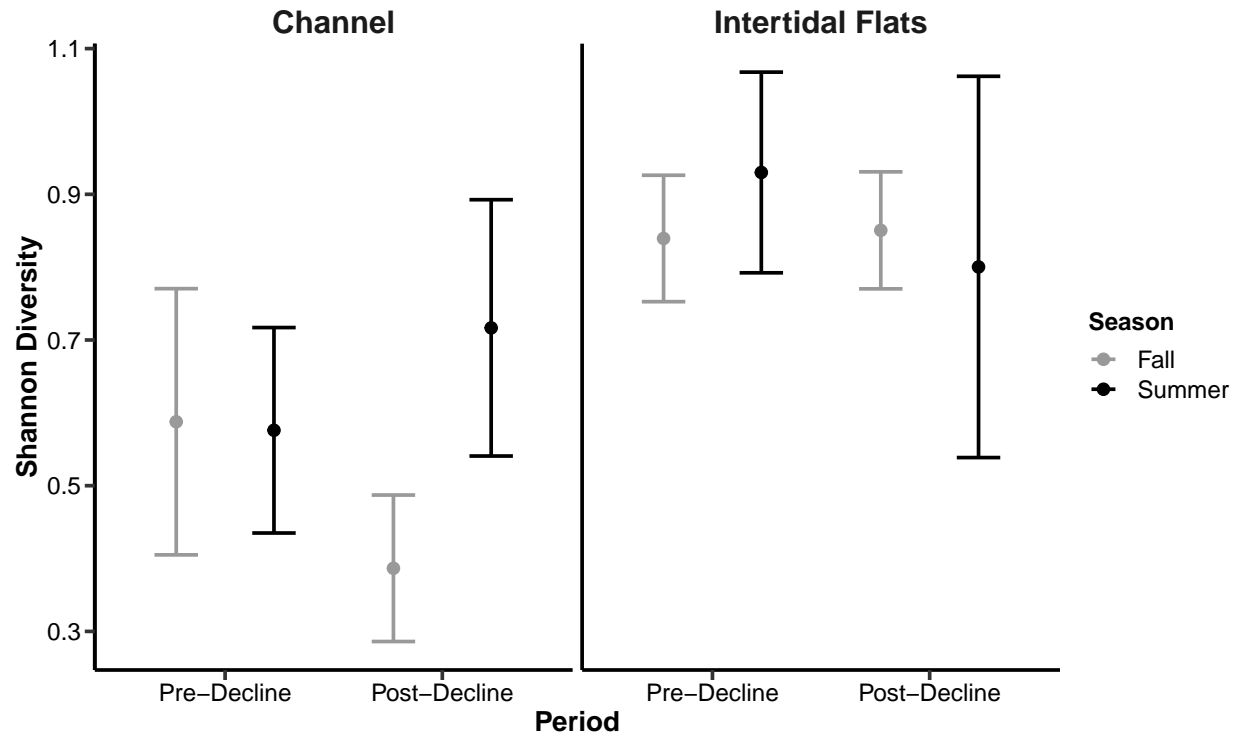
## Shannon Diversity

### Per-Sample Diversity

Plot: Mean & SE

```
shannon_summary <- diversity %>%
  group_by(TrawlType, Period, Season) %>%
  cluster_bootstrap(shannon, Site) %>% ungroup() %>%
  mutate(TrawlType = dplyr::recode(TrawlType,
                                   "Beam" = "Intertidal Flats",
                                   "Otter" = "Channel"))

shannon_summary %>%
  ggplot(aes(Period, mean, color = Season)) +
  geom_point(position = position_dodge(width = 0.9), size = 3) +
  #scale_y_continuous(expand = c(0,0), limits = c(2, 5)) +
  scale_color_manual(values = c("grey60", "black")) +
  facet_wrap(~TrawlType, ncol = 2) +
  geom_errorbar(aes(x = Period, ymin = lower, ymax = upper),
               position = position_dodge(width = 0.9),
               width = 0.4, size = 1) +
  theme_trawls +
  ylab("Shannon Diversity") +
  annotate("segment", x = -Inf, xend = -Inf, y = -Inf, yend = Inf, size = 1) +
  coord_cartesian(clip = "off")
```



#### Channel Model

```
shannon_lm_otter <- lm(
  shannon ~ Period*Season + Site,
  data = diversity[diversity$TrawlType == "Otter",]
)

shannon_lm_otter %>% anova() %>%
  rownames_to_column("term") %>%
  mutate_if(is.numeric, round, digits = 3) %>%
  knitr::kable()
```

term	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Period	1	0.005	0.005	0.018	0.895
Season	1	0.126	0.126	0.459	0.501
Site	2	0.289	0.144	0.525	0.595
Period:Season	1	0.324	0.324	1.178	0.283
Residuals	47	12.919	0.275		

#### Mudflats Model

```
shannon_lm_beam <- lm(
  shannon ~ Period*Season + Site,
  data = diversity[diversity$TrawlType == "Beam",]
)

shannon_lm_beam %>% anova() %>%
  rownames_to_column("term") %>%
```

```
mutate_if(is.numeric, round, digits = 3) %>%
knitr::kable()
```

term	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Period	1	0.035	0.035	0.286	0.595
Season	1	0.020	0.020	0.161	0.690
Site	2	0.897	0.448	3.674	0.033
Period:Season	1	0.031	0.031	0.251	0.619
Residuals	46	5.614	0.122		

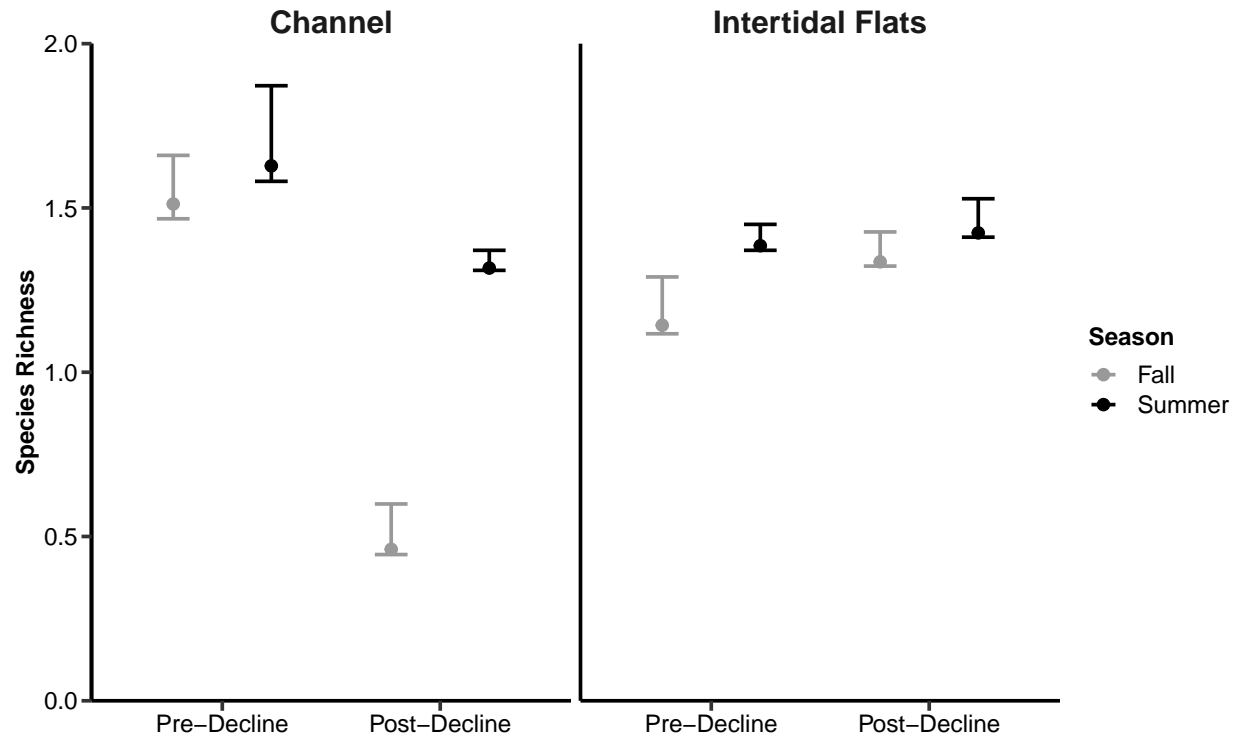
## Assymptotic Diversity

### Plot

```
shannon_asymp <- trawl_counts_nested %>%
  dplyr::select(TrawlType, Period, Season, shannon) %>%
  unnest(cols = c(shannon)) %>%
  mutate(TrawlType = dplyr::recode(TrawlType, "Beam" = "Intertidal Flats",
                                   "Otter" = "Channel"))

shannon_asymp %>%
  ggplot(aes(Period, Estimator, color = Season, group = Season)) +
  geom_point(position = position_dodge(width = 0.9), size = 3) +
  geom_errorbar(aes(ymin = `95% Lower`, ymax = `95% Upper`, width = 0.3,
                    position = position_dodge(width = 0.9),
                    size = 1) +
  facet_wrap(~TrawlType) +
  scale_y_continuous(limits = c(0, 2), breaks = seq(0, 2, 0.5), expand = c(0,0)) +
  scale_color_manual(values = c("grey60", "black")) +
  ylab("Species Richness") +
  theme_trawls +
  theme(axis.title.x = element_blank(),
        axis.title = element_text(size = 14, face = "bold")) +
  annotate("segment", x = -Inf, xend = -Inf, y = -Inf, yend = Inf, size = 1) +
  coord_cartesian(clip = "off")
```





Table

```
knitr::kable(shannon_asymp)
```

TrawlType	Period	Season	Observed	Estimator	Est_s.e	95% Lower	95% Upper
Intertidal Flats	Pre-Dcline	Fall	1.117	1.143	0.075	1.117	1.290
Intertidal Flats	Pre-Dcline	Summer	1.371	1.385	0.033	1.371	1.450
Intertidal Flats	Post-Dcline	Fall	1.323	1.336	0.047	1.323	1.427
Intertidal Flats	Post-Dcline	Summer	1.411	1.424	0.053	1.411	1.528
Channel	Pre-Dcline	Fall	1.467	1.512	0.075	1.467	1.660
Channel	Pre-Dcline	Summer	1.581	1.628	0.124	1.581	1.872
Channel	Post-Dcline	Fall	0.445	0.461	0.071	0.445	0.599
Channel	Post-Dcline	Summer	1.310	1.317	0.027	1.310	1.371

Plot: Richness & Diversity

