Chlorophyll analysis

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Import data

```
# Define function to read in chl data
read_chl <- function(file) {
  chl_data <- read_csv(file, skip = 24, n_max = 24) %>%
    select(-1) %>%
    magrittr::set_colnames(c("row", 1:12, "wavelength")) %>%
    fill(row) %>%
    gather("col", "absorbance", -wavelength, -row) %>%
    unite("well", c(row, col), sep = "")
}

# List chlorophyll data files
chl_path <- "June_2022/data/7_chlorophyll/"                                        # Path to chlorophyl
all_chl_files <- list.files(path = chl_path, pattern = "*.csv")          # List all files in directory
chl_platemaps <- list.files(path = chl_path, pattern = "platemap")       # List platemap files
chl_data_files <- setdiff(all_chl_files, chl_platemaps)                  # List absorbance data files

# Read in all files into tibble
df <- tibble(file = chl_data_files) %>%
  mutate(platemap = map(file, ~ read_csv(paste0(chl_path, tools::file_path_sans_ext(.), "_platemap.csv"
         chl_data = map(file, ~ read_chl(paste0(chl_path, .))))

# Merge platemap and data for each plate
df <- df %>%
  mutate(merged = map2(platemap, chl_data, ~ right_join(.x, .y)))
```

Calculate chlorophyll concentrations

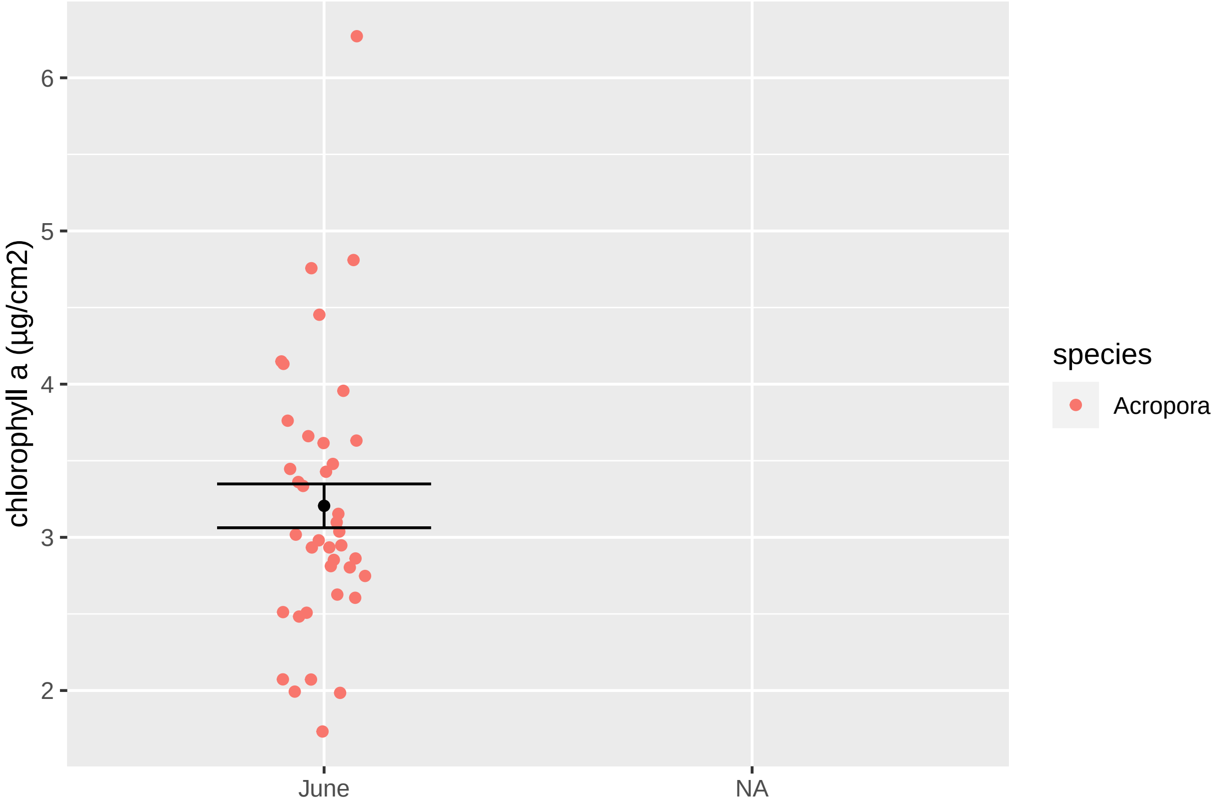
```
# average all technical replicates for each plate/sample/wavelength, including all acetone blanks toget
df <- df %>%
  unnest(merged) %>%
  filter(!is.na(colony_id)) %>%                         # remove empty wells (colony_id is NA)
  group_by(file, colony_id, wavelength) %>%
  summarise(n = n(), mean_abs = mean(absorbance)) %>%
  spread(wavelength, mean_abs)

# get the acetone blank 750 absorbace for each file (i.e., plate), and subtract from 630 and 663 values
df <- df %>%
  group_by(file) %>%
  mutate(blank750 = `750`[colony_id == "BK"]) %>%
  ungroup() %>%
  mutate(adj630 = `630` - blank750,
         adj663 = `663` - blank750)

# calculate chla and chlc2 values based on equations from Jeffrey and Humphrey 1975
# units µg/ml
#path length adjustment = 0.6

df <- df %>%
  mutate(chla.ug.ml = (11.43 * adj663)/0.6 - (0.64 * adj630)/0.6,
        chlc2.ug.ml = (27.09 * adj630)/0.6 - (3.63 * adj663)/0.6)

#previous, with no pathlength adjustment
#df <- df %>%
  #mutate(chla.ug.ml = (11.43 * adj663) - (0.64 * adj630),
        #chlc2.ug.ml = (27.09 * adj630) - (3.63 * adj663))
```

Normalize to surface area

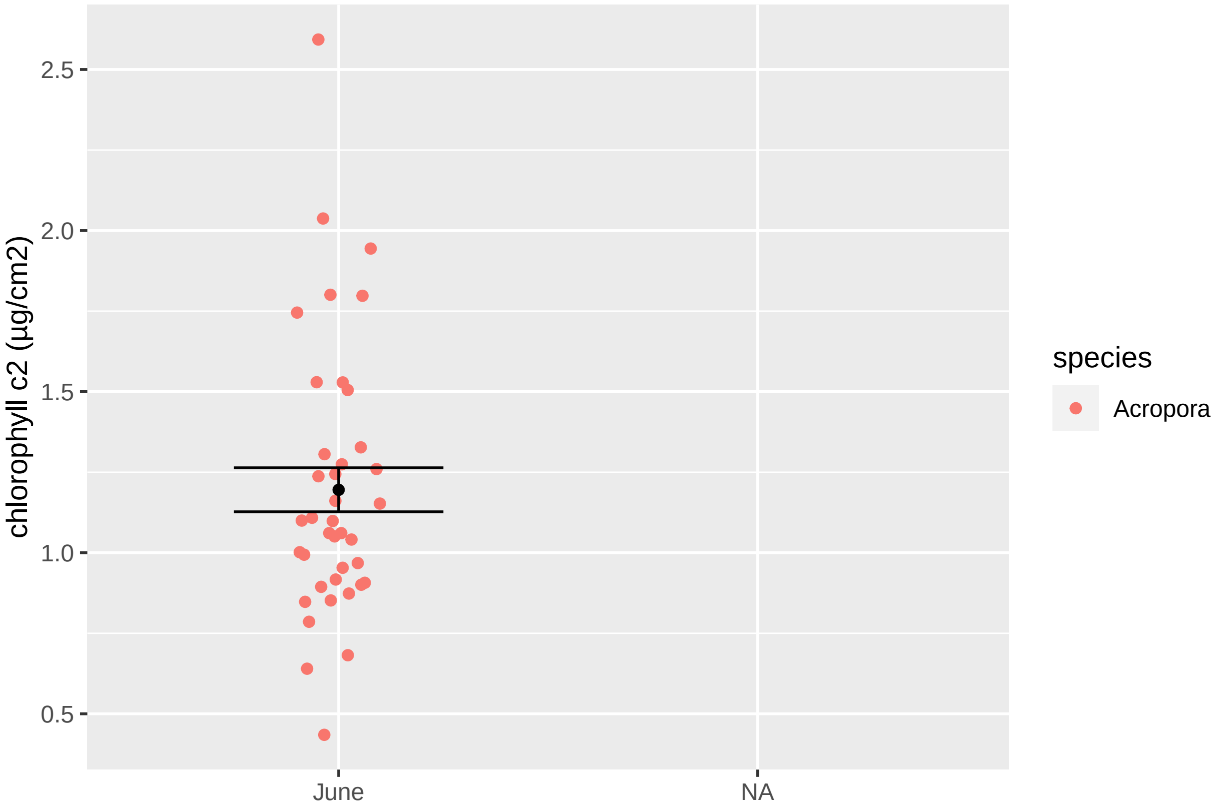
```
# Load homogenate volume
homog.vol <- read_csv("June_2022/data/7_homogenate_vols/7_homogenate_vols.csv") %>%
  select(colony_id, homog_vol_ml)
chl <- full_join(df, homog.vol)

# Load surface area
sa <- read_csv("June_2022/output/7_surface_area.calc.csv")
chl <- full_join(chl, sa)

# Multiply chlorophyll by the homogenate volume and divide by surface area
chl <- chl %>%
  mutate(chla.ug.cm2 = chla.ug.ml * homog_vol_ml / surface.area.cm2,
         chlc2.ug.cm2 = chlc2.ug.ml * homog_vol_ml / surface.area.cm2)

# remove blanks and NAs
chl <- filter(chl, !colony_id %in% c("NA", "BK"))

# write chlorophyll data to file
chl %>%
  select(colony_id, chla.ug.cm2, chlc2.ug.cm2) %>%
  mutate(timepoint="June")%>%
  filter(!is.na(chla.ug.cm2))%>%
  filter(!is.na(chlc2.ug.cm2))%>%
  write_csv(path = "June_2022/output/7_chlorophyll.csv")
```

Plot results by species and site

```
# Join with sample metadata
meta <- read_csv("June_2022/7_corals_sampled.csv")
chl <- right_join(chl, meta, by = "colony_id")

# Plot chlorophyll a
chl %>%
  ggplot(aes(x = timepoint , y = chla.ug.cm2, color = species)) +
  #facet_wrap(~species) +
  labs(x = "", y = "chlorophyll a (µg/cm2)") +
  geom_jitter(width = 0.1) +                                        # Plot all points
  stat_summary(fun.data = mean_cl_normal, fun.args = list(mult = 1),    # Plot standard error
               geom = "errorbar", color = "black", width = 0.5) +
  stat_summary(fun.y = mean, geom = "point", color = "black")          # Plot mean
```



```
# Plot chlorophyll c2
chl %>%
  ggplot(aes(x = timepoint, y = chlc2.ug.cm2, color = species)) +
  #facet_wrap(~species) +
  labs(x = "", y = "chlorophyll c2 (µg/cm2)") +
  geom_jitter(width = 0.1) +                                        # Plot all points
  stat_summary(fun.data = mean_cl_normal, fun.args = list(mult = 1),    # Plot standard error
               geom = "errorbar", color = "black", width = 0.5) +
  stat_summary(fun.y = mean, geom = "point", color = "black")          # Plot mean
```


chlorophyll c2 (µg/cm2)
2.5
2.0
1.5
1.0
0.5
June
NA
species
Acropora