

rfu-calculation-vignette

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Introduction

Covertng between chlorophyll-a (chl-a) raw units (rfu) and mg/m^2 for the Hubbard Brook Experimental Forest long-term algal record

To do this calculation, you need three five things:

- The sample list file (sample ID, sampling date, substrate, and watershed, or weir)
- The raw chl-a units (rfu) file with: a) rfu, b) volume of etoh, c) sample ID, and d) chla run number
- The standard curve slope
- The blanks measured for each run
- the surface areas of the substrates

In general, the caculation will first, merge the raw data with the sampling listing. Second, subtract average blank values from each run. Third, calculate chl-a in mg/m^2 using the slope of the standard curve, the volume of EtOH, and the surface area of the substrates.

Step 1: clean and merge data

First, load the data into your workspace

```
rfu_data <- read.csv(paste0(projdir, "/raw data/hbef_chla_rfu.csv"))
samplinglist <- read.csv(paste0(projdir, "/raw data/samplinglist.csv"), skip = 1)
```

In the next steps, we will:

- 1) check that your sample ID's match between rfu_data and samplinglist
- 2) create factors for weir and substrate
- 3) assign a substrate code (see substrate_surfaceareas.xlsx)

```
rfu_data[rfu_data$Sample.ID %in% samplinglist$Sample.ID == F,]
```

```
## [1] Sample.ID short_id Flr_sample run vol_Etoh value_rfu
## <0 rows> (or 0-length row.names)
```

```
# if the data's sample ID's do not match, they will show up here on the rfu_data file, check for typos
```

```
chla_data <- merge(rfu_data, samplinglist, by = "Sample.ID", all.x = T) #merge two dataframes by Sample
```

```
#create factors for weir and substrate
```

```
chla_data$weir <- as.factor(substr(chla_data$WEIR.REP, 1,2))
chla_data$substrate <- as.factor(substr(chla_data$WEIR.REP,4,4))
```

```
#assign substrate code (NOTE ONLY FOR 2019+ SAMPLES)
```

```
chla_data$subs_code <- as.factor(ifelse(chla_data$substrate == "M", "M_b", "T"))

#only run for 2018 samples
#chla_data$subs_code <- ifelse(chla_data$substrate == "M", "M_s", chla_data$substrate)

#change to date format
chla_data$Date <- as.Date(chla_data$Date)
```

Now your data should have the required columns of a) rfu value, b) substrate, c) date, d) weir, and e) sampling ID which will give you substrate, date, and weir

Step 2: convert from rfu to mg/m²

To covert from rfu to mg/m2 we use the following equation:

$$RFU_{corrected} \cdot \frac{Slope}{1000} \cdot \frac{V_{EtOH}}{1000} \cdot \frac{1}{SA}$$

where RFU is the raw units (corrected by subtracting the average of the blanks), $Slope$ is the standard slope ($\frac{rfu}{\mu g/L}$), V is the volume of ethanol (mL) and SA is the surface area (m^2).

To do this calculation, we will source the function `rfu-to-mgm2-chla.R`

Please pay attention to formatting! The formatting of the sheet must be identical to this example for this function to work.

```
surfaceareas <- read_excel(path = paste0(projdir, "/raw data/substrate_surfaceareas.xlsx"), sheet = 1)

## New names:
## * `` -> ...5

blanks_df <- read.csv(paste0(projdir, "/raw data/slope_and_blanks.csv"))[1:2]
slope <- as.numeric(read.csv(paste0(projdir, "/raw data/slope_and_blanks.csv"))[1,3])

source("rfu-to-mgm2-chla.R")

chla_data2 <- rfu_mgm2_chla(chla_df = chla_data, sa_df = surfaceareas, slp = slope, blank_df = blanks_d

head(chla_data2) #view result data
```

```
##   Sample.ID short_id Flr_sample  run vol_Etoh value_rfu WEIR.REP      Date
## 1  CH190001      1 SAMPLE-008 run1      10      81.91    W1-T 2019-04-29
## 2  CH190002      2 SAMPLE-009 run1      20     284.07    W1-M 2019-04-29
## 3  CH190003      3 SAMPLE-010 run1      10      18.07    W2-T 2019-04-29
## 4  CH190004      4 AMPLE-011 run1      20      88.47    W2-M 2019-04-29
## 5  CH190005      5 SAMPLE-012 run1      10     105.27    W3-T 2019-04-29
## 6  CH190006      6 SAMPLE-013 run1      20     279.11    W3-M 2019-04-29
##   weir substrate subs_code value_mgm2
## 1   W1         T         T 0.16078204
## 2   W1         M        M_b 0.43118900
## 3   W2         T         T 0.02358289
## 4   W2         M        M_b 0.12668110
## 5   W3         T         T 0.21098524
## 6   W3         M        M_b 0.42346733
```

Now, we can save a “tidy” output version of this data, keeping only the parts that we need:

```
chla_data3 <- (chla_data2)[c(1,4,5,6,8,9,10,12)]
```

```
write.csv(chla_data3, row.names = F)
```

```
## "Sample.ID","run","vol_Etoh","value_rfu","Date","weir","substrate","value_mgm2"
## "CH190001","run1",10,81.91,2019-04-29,"W1","T",0.16078203961574
## "CH190002","run1",20,284.07,2019-04-29,"W1","M",0.431189000572834
## "CH190003","run1",10,18.07,2019-04-29,"W2","T",0.0235828940652867
## "CH190004","run1",20,88.47,2019-04-29,"W2","M",0.126681099046281
## "CH190005","run1",10,105.27,2019-04-29,"W3","T",0.210985235731946
## "CH190006","run1",20,279.11,2019-04-29,"W3","M",0.423467327814288
## "CH190007","run1",10,112.91,2019-04-29,"W4","T",0.227404431722006
## "CH190008","run1",20,587.57,2019-04-29,"W4","M",0.903674420375028
## "CH190009","run1",10,40.11,2019-04-29,"W5","T",0.0709492657434193
## "CH190010","run1",20,286.54,2019-04-29,"W5","M",0.435034269063482
## "CH190011","run1",10,213.86,2019-04-29,"W6","T",0.444357027951928
## "CH190012","run1",20,114.86,2019-04-29,"W6","M",0.167764757130564
## "CH190015","run1",10,81.05,2019-05-06,"W1","T",0.158933805512147
## "CH190016","run1",20,245.43,2019-05-06,"W1","M",0.371034678921577
## "CH190017","run1",10,1884.83,2019-05-06,"W2","T",4.03545440013928
## "CH190018","run1",20,152.34,2019-05-06,"W2","M",0.226113203701194
## "CH190019","run1",10,31.23,2019-05-06,"W3","T",0.0518651740691082
## "CH190020","run1",20,391.9,2019-05-06,"W3","M",0.599057543628091
## "CH190021","run1",10,91.25,2019-05-06,"W4","T",0.180854721624531
## "CH190022","run1",20,820.75,2019-05-06,"W4","M",1.26668644735847
## "CH190023","run1",10,17.53,2019-05-06,"W5","T",0.022422374976984
## "CH190024","run1",20,447.1,2019-05-06,"W5","M",0.684992288844174
## "CH190025","run1",10,64.34,2019-05-06,"W6","T",0.123022187057446
## "CH190026","run1",20,305.76,2019-05-06,"W6","M",0.46495575100285
## "CH190029","run1",10,142.29,2019-05-13,"W1","T",0.290545266563364
## "CH190030","run1",20,737.74,2019-05-13,"W1","M",1.1374574038732
## "CH190031","run1",10,37.94,2019-05-13,"W2","T",0.0662856982959807
## "CH190032","run1",20,219.26,2019-05-13,"W2","M",0.330293514387068
## "CH190033","run1",10,13.7,2019-05-13,"W3","T",0.014191285887726
## "CH190034","run1",20,460.78,2019-05-13,"W3","M",0.706289160484681
## "CH190035","run1",10,178.35,2019-05-13,"W4","T",0.368042152348912
## "CH190036","run1",20,1268.42,2019-05-13,"W4","M",1.96361411748318
## "CH190037","run1",10,51.51,2019-05-13,"W5","T",0.0954491131631431
## "CH190038","run1",20,716.06,2019-05-13,"W5","M",1.10370622133181
## "CH190039","run1",10,268.04,2019-05-13,"W6","T",0.5607957764783
## "CH190040","run1",20,1080,2019-05-13,"W6","M",1.67028395999016
## "CH190041","run1",10,82.5,2019-05-13,"W9","T",0.162050014175182
## "CH190042","run1",20,463.03,2019-05-13,"W9","M",0.709791935425554
## "CH190043","run1",10,96.92,2019-05-21,"W1","T",0.193040172051709
## "CH190044","run1",20,823.67,2019-05-21,"W1","M",1.27123227083729
## "CH190045","run1",10,157.43,2019-05-21,"W2","T",0.323082783224296
## "CH190046","run1",20,208.74,2019-05-21,"W2","M",0.313916095552409
## "CH190047","run1",10,26.66,2019-05-21,"W3","T",0.04204374440069909
## "CH190048","run1",20,2233.48,2019-05-21,"W3","M",3.46600877723377
## "CH190049","run1",10,169.01,2019-05-21,"W4","T",0.34796947034012
## "CH190050","run1",20,1163.86,2019-05-21,"W4","M",1.80083627400865
## "CH190051","run1",10,55.43,2019-05-21,"W5","T",0.103873622100452
## "CH190052","run1",20,915.13,2019-05-21,"W5","M",1.41361618021162
## "CH190053","run1",10,235.16,2019-05-21,"W6","T",0.490133058657202
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## "CH190054","run1",20,1440.14,2019-05-21,"W6","M",2.2309459009706
## "CH190055","run1",10,92.53,2019-05-21,"W9","T",0.183605581685693
## "CH190056","run1",20,700.3,2019-05-21,"W9","M",1.07917122885707
## "CH190057","run1",10,567.8,2019-05-28,"W1","T",1.20501281705167
## "CH190058","run1",20,2365.46,2019-05-28,"W1","M",3.67147377132106
## "CH190059","run1",10,140.77,2019-05-28,"W2","T",0.287278620240735
## "CH190060","run1",20,437.43,2019-05-28,"W2","M",0.669938140542733
## "CH190061","run1",10,312.3,2019-05-28,"W3","T",0.655915359530666
## "CH190062","run1",20,4126.33,2019-05-28,"W3","M",6.41277657582547
## "CH190063","run1",10,388.46,2019-05-28,"W4","T",0.819591533169803
## "CH190064","run1",20,1488.5,2019-05-28,"W4","M",2.30623221036643
## "CH190065","run1",10,68.53,2019-05-28,"W5","T",0.132026955538906
## "CH190066","run1",20,1458.24,2019-05-28,"W5","M",2.25912377938384
## "CH190067","run1",10,628.57,2019-05-28,"W6","T",1.33561419667418
## "CH190068","run1",20,2389.19,2019-05-28,"W6","M",3.7084163710308
## "CH190069","run1",10,469.57,2019-05-28,"W9","T",0.993905798451715
## "CH190070","run1",20,2364.69,2019-05-28,"W9","M",3.67027504389685
## "CH190071","run2",10,1169.57,2019-06-02,"W1","T",2.45949813302439
## "CH190072","run2",20,2732.37,2019-06-02,"W1","M",4.21458032323317
## "CH190073","run2",10,191.3,2019-06-02,"W2","T",0.357088857998676
## "CH190074","run2",20,955.19,2019-06-02,"W2","M",1.44788629237959
## "CH190075","run2",10,457.06,2019-06-02,"W3","T",0.928236178197429
## "CH190076","run2",20,4828.51,2019-06-02,"W3","M",7.4778277297049
## "CH190077","run2",10,303.84,2019-06-02,"W4","T",0.59894963243865
## "CH190078","run2",20,1550.27,2019-06-02,"W4","M",2.37430020874166
## "CH190079","run2",10,111.08,2019-06-02,"W5","T",0.18468730010304
## "CH190080","run2",20,2031.51,2019-06-02,"W5","M",3.12348928098419
## "CH190081","run2",10,405.56,2019-06-02,"W6","T",0.817557042924115
## "CH190082","run2",20,4372.68,2019-06-02,"W6","M",6.76819666246129
## "CH190083","run2",10,518.89,2019-06-02,"W9","T",1.06111561380809
## "CH190084","run2",20,2724.51,2019-06-02,"W9","M",4.20234396277305
## "CH190085","run2",10,1336.91,2019-06-10,"W1","T",2.81913010383286
## "CH190086","run2",20,3282.71,2019-06-10,"W1","M",5.07134350588206
## "CH190087","run2",10,238.73,2019-06-10,"W2","T",0.459021117921263
## "CH190088","run2",20,1371.16,2019-06-10,"W2","M",2.09546375555955
## "CH190089","run2",10,758.01,2019-06-10,"W3","T",1.57501065898391
## "CH190090","run2",20,5199.57,2019-06-10,"W3","M",8.05548980506503
## "CH190091","run2",10,589.53,2019-06-10,"W4","T",1.21292870343346
## "CH190092","run2",20,2345.7,2019-06-10,"W4","M",3.61261677372769
## "CH190093","run2",10,213.53,2019-06-10,"W5","T",0.404863560467137
## "CH190094","run2",20,1909.34,2019-06-10,"W5","M",2.9332963856391
## "CH190095","run2",10,304.44,2019-06-10,"W6","T",0.60023909809232
## "CH190096","run2",20,3046.5,2019-06-10,"W6","M",4.70361440864491
## "CH190097","run2",10,367.34,2019-06-10,"W9","T",0.735418080785357
## "CH190098","run2",20,1713.22,2019-06-10,"W9","M",2.62797895390399
## "CH190099","run2",10,1201.82,2019-06-17,"W1","T",2.52880691190914
## "CH190100","run2",20,4013.93,2019-06-17,"W1","M",6.20969865799989
## "CH190101","run2",10,331.52,2019-06-17,"W2","T",0.658436981261278
## "CH190102","run2",20,1783.8,2019-06-17,"W2","M",2.73785711182702
## "CH190103","run2",10,609.81,2019-06-17,"W3","T",1.2565126425275
## "CH190104","run2",20,5918.77,2019-06-17,"W3","M",9.17513235505428
## "CH190105","run2",10,837.99,2019-06-17,"W4","T",1.74689643061808
## "CH190106","run2",20,2160.32,2019-06-17,"W4","M",3.32401925437701
## "CH190107","run2",10,215.83,2019-06-17,"W5","T",0.409806512139537

```

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## "CH190108","run2",20,2650.02,2019-06-17,"W5","M",4.08637876039722
## "CH190109","run2",10,598.98,2019-06-17,"W6","T",1.23323778747876
## "CH190110","run2",20,3365.4,2019-06-17,"W6","M",5.2000743769313
## "CH190111","run2",10,332.04,2019-06-17,"W9","T",0.659554518161125
## "CH190112","run2",20,3442.07,2019-06-17,"W9","M",5.31943337902762
## "CH190113","run2",10,1823.64,2019-06-24,"W1","T",3.86516613318393
## "CH190114","run2",20,5041.57,2019-06-24,"W1","M",7.80951716477262
## "CH190115","run2",10,377.5,2019-06-24,"W2","T",0.75725303252083
## "CH190116","run2",20,1835.64,2019-06-24,"W2","M",2.81856104646473
## "CH190117","run2",10,1005.42,2019-06-24,"W3","T",2.1067218212746
## "CH190118","run2",20,6974.29,2019-06-24,"W3","M",10.8183541353166
## "CH190119","run2",10,1219.3,2019-06-24,"W4","T",2.56637334461938
## "CH190120","run2",20,2814.03,2019-06-24,"W4","M",4.34170770175392
## "CH190121","run2",10,521.64,2019-06-24,"W5","T",1.06702566472074
## "CH190122","run2",20,3797.15,2019-06-24,"W5","M",5.87221796836325
## "CH190123","run2",10,512.5,2019-06-24,"W6","T",1.04738280459651
## "CH190124","run2",20,5926.96,2019-06-24,"W6","M",9.18788245583906
## "CH190125","run2",10,493.5,2019-06-24,"W9","T",1.00654972556363
## "CH190126","run2",20,4945.01,2019-06-24,"W9","M",7.65919363219898
## "CH190127","run2",10,1547.05,2019-07-01,"W1","T",3.27074395793644
## "CH190128","run2",20,6648.09,2019-07-01,"W1","M",10.3105296083331
## "CH190129","run2",10,309.85,2019-07-01,"W2","T",0.611865780069575
## "CH190130","run2",20,1741.82,2019-07-01,"W2","M",2.67250311537464
## "CH190131","run2",10,1523.9,2019-07-01,"W3","T",3.22099207479902
## "CH190132","run2",20,8441.93,2019-07-01,"W3","M",13.1031597416378
## "CH190133","run2",10,783.35,2019-07-01,"W4","T",1.62946909175722
## "CH190134","run2",20,2899.53,2019-07-01,"W4","M",4.47481314950709
## "CH190135","run2",10,863.85,2019-07-01,"W5","T",1.80247240029124
## "CH190136","run2",20,3851.86,2019-07-01,"W5","M",5.95738988703665

```