

# Cal\_Clean: A Script for Tidying Sable Indirect Calorimetry Data

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## Inputs:

- Cohort and rundate of calorimetry run
- List of animal ID numbers to be excluded (NA if none)

## Outputs:

- .Rda file containing cleaned dataframe at original sampling rate (typically, 1-3 minutes), 1h-binned dataframe, and photoperiod-binned dataframes for further plotting and statistical analyses.

## Requirements:

- .exp data files processed with MI v2.46 in 1-minute bins
- TimeSeries sheet as a .csv saved in your default working directory (run `library(here)` to find out where this is!)
- Group decoding sheet with same prefix as TimeSeries sheet (e.g., `expID_date_code.csv`), saved in same directory

## Known bugs:

- EnviroLightlux\_M is inconsistent in numbering (sometimes has 0s and 1s, or 1s and 3s, or 3s and 7s), therefore photoperiod assignment is done based on ZT/clock time and NOT from sensor. This will need to be updated for DD or other light schedule experiments

## Load required packages

```
library(plyr, include.only = 'mapvalues')
library(tidyverse)
library(magrittr)
library(lubridate)
library(here)
```

## Define inputs to script for analysis

If everything is working properly, these variables are the only ones a user should have to change to clean a given file's calorimetry data. Eventually, this entire script will be converted to a function, such that the user would type these in-line when calling that function (e.g., `Cal_Clean(cohort = 'mon001', rundate = '2021-10-18', remove_animals = 274, trim.short.days = FALSE)`).

```
# Run-specific user-defined parameters
cohort      <- "mon001"
rundate     <- "2021-10-18"
remove_animals <- 274

# Default parameters
trim.short.days <- FALSE
diet.kcal      <- 4.2
cols2excl     <- c('Age', 'Cage', 'Stilltime_M',
                  'Sleeptime_M', 'XBreak_R', 'YBreak_R',
                  'Mass_g', 'AllMeters_M')
ext           <- ".csv"
```

## Load data

To load your data, please ensure that it is located in the repository that the `here()` function points to. Otherwise, you will get an error.

A workaround, if you do not wish to load your data using the `here()` package, is to type the entire file path in the call to `read_csv()`.

```
filename     <- paste(rundate, cohort, sep = "_")
code         <- paste(rundate, cohort, "DECODED", sep = "_")
```

```
# Load csv with run metadata
df_code      <- read_csv(here::here(paste(code, ".csv", sep = "")))
df_code
```

Animal	Mass_g	Sex	Age	ID_Code	Group	Cohort	Treatment	Diet
1	9.40	M	NA	271	mHFD	1	HIchABC	NA
2	9.30	M	NA	272	mHFD	1	HIchABC	NA
3	9.25	F	NA	273	mHFD	1	HIchABC	NA
4	8.60	F	NA	274	mHFD	1	HIchABC	NA
5	9.30	M	NA	275	mHFD	1	HIchABC	NA
6	7.60	F	NA	276	mHFD	1	HIchABC	NA
9	9.65	M	NA	264	mHFD	1	chABC	NA
10	10.00	M	NA	265	mHFD	1	chABC	NA
11	8.75	F	NA	266	mHFD	1	chABC	NA
12	8.85	F	NA	267	mHFD	1	chABC	NA
13	9.70	M	NA	268	mHFD	1	chABC	NA
14	9.80	F	NA	269	mHFD	1	chABC	NA
15	9.30	F	NA	270	mHFD	1	chABC	NA

```
# Load cal.csv and merge metadata
df      <- read_csv(here::here(paste(filename, ".csv", sep = ""))) %>%
  left_join(df_code, by = "Animal") %>% #unblind by merging with decoding
  mutate(across(.cols = everything()), na_if(., ".")) %>% #replace "." with "NA"
  rename(Cage = Animal)%>%
  select(!(starts_with("Enviro")
    | starts_with("Ped")
    | all_of(cols2excl)))
head(df)
```

Date	Time	VO2	MCO2	VN2O	kcal	IR	REM	Mod	Water	MB	Body	Mass	Level	Sex	ID	Cage	Group	Cohort	Treatm	Diet
2021/10/18	12:47:24	18599407.17	55296-	0.1746342	634267790	0	10.599240230902	271	mHFD	1	HIchABC	NA								
2021/10/18	12:50:24	1857792530	15539-	0.1699336	855570	0	10.599240000000	271	mHFD	1	HIchABC	NA								
2021/10/18	12:53:24	1850855644	198845-	0.15010738	51830	0	10.599240180902	271	mHFD	1	HIchABC	NA								
2021/10/18	12:56:24	18576102356512	2956726845.78	449407		0	10.599240135375	271	mHFD	1	HIchABC	NA								

DateTime	VO2	MCO2	VN2O	kcal	REM	Mod	Water	Body	Mass	Sex	ID	Group	Cohort	Treatment	Int
2021/10/18 12:59:24	1854690.115849	254004115974	8390020	0	10.59924	0180902	271	mHFD	HIchABC						
2021/10/18 13:02:24	1853974.345350	703687657723	405670	0	10.59924	0085375	271	mHFD	HIchABC						

## Tidy data

Here, we remove any animals that we had previously specified, and perform some general data cleaning - most importantly, extraction of time of day information from the DateTime column.

```
# Remove dead/sick animals
df %<>%
  filter(ID_Code != remove_animals)

# Parse date-times and sort by animal ID (ID_Code) and date-time
df %<>%
  relocate(c(DateTime, Sex, Group, Treatment, Cohort, ID_Code)) %>%
  mutate(DateTime = round_date(ymd_hms(df$DateTime), unit = "minute")) %>%
  mutate(month = as.numeric(month(DateTime)),
         day = as.numeric(day(DateTime)),
         hour = as.numeric(hour(DateTime)),
         minute = as.numeric(minute(DateTime)))

df <- df[order(df$DateTime),] %>%
  arrange(ID_Code)

head(df)
```

DateTime	Sex	Group	Treatment	Cohort	ID	VO2	MCO2	VN2O	kcal	REM	Mod	Water	Body	Mass	Sex	ID	Group	Cohort	Treatment	Int
2021-10-18 12:47:00	M	mHFD	ABC	264	0.797095	0.08689	0.231721	0.0161321	0.0802	0	12.82739	655902	10	18	12	47				
2021-10-18 12:50:00	M	mHFD	ABC	264	0.872637	0.10709	0.248250	0.01483	0.083	0	12.82739	619619	10	18	12	50				

DateTime	Sex	Group	Treat	Code	ED	VCO2	VO2	VH2O	kcal	RER	Food	Water	BodyMass	Day	hour	minute
2021-10-18 12:53:00	M	mHE	ABC	264	0.870835	0.017884	255686	16011	0	12.820337	40236	10	18	12	53	
2021-10-18 12:56:00	M	mHE	ABC	264	0.849056	0.015340	0.250492	99883	0	12.820337	40236	10	18	12	56	
2021-10-18 12:59:00	M	mHE	ABC	264	0.848077	0.016004	0.325106	9853653	0	12.820337	40236	10	18	12	59	
2021-10-18 13:02:00	M	mHE	ABC	264	0.711054	0.012679	540321	84678085	0	12.820337	40236	10	18	13	2	

## Transform data

Here, we compute a handful of new variables from existing ones, including Zeitgeber time, Photoperiod, and experimental day from DateTime, etc.

```
# Compute experimental day and ZT time, rename some variables
df %<>%
  mutate(ZT = plyr::mapvalues(hour, from = (0:23), to = c(18:23,0:17)),.before = Sex) %>%
  mutate(Photoperiod = as.factor(plyr::mapvalues(ZT, from = c(0:23), to = c(rep(1,12),rep(0,11))))
  mutate(exp_day = plyr::mapvalues(day, from = unique(day), to = 1:length(unique(day))),.after = Sex) %>%
  rename(VO2 = VO2_M,
         Animal = ID_Code,
         VCO2 = VCO2_M,
         EE = kcal_hr_M,
         RER = RER_M,
         FoodIn.cum = FoodInA_M,
         WaterIn.cum = WaterInA_M,
         AllMeters = AllMeters_R,
         BodyMass = BodyMass_M,
         VH2O = VH2O_M)
```

For plotting purposes, here we create a new column containing the time from which the recording started, in hours.

```
# Compute hours from recording start for plots
start_time <- df$DateTime[1]
df %<>%
  mutate(Time = as.numeric(difftime(DateTime,start_time),units = "hours"),.after = DateTime)

head(df$Time)
```

```
[1] 0.00 0.05 0.10 0.15 0.20 0.25
```

Next, we will compute some more parameters of interest, including binned food and water intake (from the original cumulative values), food intake in kcal (from grams), binned and cumulative energy expenditure (from the instantaneous estimate of hourly rate), cumulative distance traveled, and energy balance. The binned energy expenditure is questionable currently - please let me know if you find any issues.

```
# Compute time interval (in hours) for estimating binned energy expenditure
int <- df$Time[2] - df$Time[1]

# Compute new columns
df %<>%
  group_by(Animal) %>%
  mutate(FoodIn.g = c(diff(FoodIn.cum),0),.before = FoodIn.cum) %>% # convert cumulative to g
  mutate(FoodIn.cum.kcal = FoodIn.cum * diet.kcal,.after = FoodIn.cum) %>%
  mutate(WaterIn.g = c(diff(WaterIn.cum),0),.before = WaterIn.cum) %>% # convert cumulative to g
  mutate(FoodIn.kcal = FoodIn.g * diet.kcal, .before = FoodIn.g) %>% # convert g to kcal
  mutate(EE.kcal.bin = EE * int, .before = EE) %>% # EE is kcal/hr, multiply by int (x hours)
  mutate(EE.cum = cumsum(EE.kcal.bin), .before = EE.kcal.bin) %>%
  mutate(EBalance = FoodIn.kcal - EE.kcal.bin, .before = V02) %>% # compute energy balance
  mutate(EB.cum = cumsum(EBalance), .after = EBalance) %>%
  mutate(AllMeters.cum = cumsum(AllMeters),.before = AllMeters) %>% # compute cumulative distance
  ungroup()
```

Next, we remove rows/columns with missing values (NAs) across entire row/column.

```
# Remove any rows/columns with only NAs
df <- df[rowSums(is.na(df)) != ncol(df), ]
df <- df[, colSums(is.na(df)) != nrow(df)]
```

Here we can optionally remove data points that are not part of a full day's recording. I only recommend this if your first and last days of recording are not important to your analysis.

By default, this will not run. If you want to trim the incomplete days, run `trim.short.days <- TRUE` (default is `FALSE`).

```
# Remove days with <24h
if (trim.short.days) {
  trim.df <- df %>%
    group_by(exp_day) %>%
    mutate(n = n()) %>%
    ungroup() %>%
    filter(!(n < max(n)))
}
```

## Bin to hourly

Here, we compute hourly bins for each variable. Depending on variable, this is done by either summing all values within the hour (`cols2sum`: binned, non-cumulative measures), taking the mean of all values within the hour (`cols2avg`: rates), taking the maximum value within the hour (`cols4cum`: binned, cumulative measures), and taking the median value for assigning the new bin time (`cols2keep`: dates and times only).

```
cols2sum <- c('FoodIn.g', 'FoodIn.kcal', 'WaterIn.g', 'EBalance', 'AllMeters')
cols2avg <- c('VO2', 'VC02', 'VH20', 'EE', 'RER', 'BodyMass')
cols4cum <- c('AllMeters.cum', 'FoodIn.cum', 'WaterIn.cum', 'EE.cum', 'EB.cum', 'FoodIn.cum.kcal')
cols2keep <- c('DateTime', 'Time', 'minute')

df.hourly <- df %>%
  group_by(Animal, exp_day, hour) %>%
  select(!EE.kcal.bin) %>% # don't need the 1-3" bin anymore
  mutate(
    across(all_of(cols2keep), median)) %>% # assign middle of time bin to new bin
  mutate(across(
    all_of(cols2avg), mean)) %>% # rates get averaged
  mutate(across(
    all_of(cols2sum), sum)) %>% # intake, distances get summed
  mutate(across(
    all_of(cols4cum), max)) %>% # cumulative values just keep the maximum (total for the hour)
  ungroup() %>%
  distinct() %>% # squashes down to one observation per hour
  select(!c(hour, minute, month, day)) %>%
  mutate(Animal = as.factor(Animal)) %>%
  group_by(Animal) %>%
```

```
slice(2:(n()-1)) %>% # trim incomplete hours at start and end
mutate(Time = as.numeric(
  difftime(DateTime,DateTime[1]),units = "hours"),.after = DateTime) %>% # starts clock
ungroup()
```

## Photoperiod and daily means

Next, we compute the cumulative totals or averages of all variables for each experimental day.

*Note that the experimental day is derived from the calendar day.*

If incomplete days were not trimmed above, then the daily and photoperiod averages will be affected by the start and end of recording where <24-hours are included. Working on a fix for this, where each day starts from the start of the recording (day 1 = first 24 hours, and so on).

```
# Compute average for each day
total.avg.daily <- df %>%
  group_by(exp_day,Group,Treatment,Sex,Animal) %>%
  summarize(
    across(all_of(cols2sum),sum),
    across(all_of(cols2avg),mean)) %>%
  mutate(Photoperiod = "Total") %>%
  ungroup()

total.avg.daily
```

exp_day	Group	Treatment	Sex	Animal	FoodIn	FoodInWater	FB	Balance	Adipose	VO2	VCO2	VH2O	EE	RER	BodyMass	Photoperiod
1	mHR	DAB	F	266	0.5748	3894	3232	538862	46.968	60250	9746	65038	10370	28684	474084	560
								0.6264031								
1	mHR	DAB	F	267	1.1352	6768	9092	5832	20928	34375	55447	49072	22320	64339	13696	6577
1	mHR	DAB	F	269	1.6775	6045	7032	6614	30208	57841	87024	70663	50921	83283	39035	195045
1	mHR	DAB	F	270	1.4772	3130	4421	5905	5390	5983	9769	29147	8719	19333	128230	876792010568
1	mHR	DAB	M	264	1.5098	3030	1168	7727	3626	2858	6106	7248	8928	01058	7508	36288887796763
1	mHR	DAB	M	265	1.5487	1504	6039	5443	8411	3627	5080	3701	7537	041208	23675	892550684532
1	mHR	DAB	M	268	1.7384	6201	5401	6464	6031	6398	8279	6135	2407	3174	7396	30908537913470
1	mHF	HchABC	273	1.4280	0997	6342	8049	6729	3583	9516	7861	0202	2938	15020	16290	33618855
1	mHF	HchABC	276	1.0751	6205	6808	1836	7731	4263	1088	2763	7245	0512	7301	24378	870027650136
1	mHF	HchABC	271	1.7242	7400	1808	6360	5208	8066	9123	6757	0188	3386	5175	6238	78854386463194
1	mHF	HchABC	272	1.7588	2387	0655	0723	2052	7490	6890	5531	2873	2106	4013	3939	688325075110



exp	Group	Treatment	Sex	Animal	Food	IF	Topd	In	Water	FB	Balance	Ad	Me	VO2	VCO2	VH2O	EE	RER	Body	Wt	Post	period
1	mHFD	chABC	275	1.913381036029412458287571516158204307297071524820242288350192763dal																		
2	mHFD	ABC	266	3.335714701001223318299792.41551088806168853529916891242973B1dal																		
2	mHFD	ABC	267	2.49665608505575871373972.4805066682283898806686892009377528tal																		
2	mHFD	ABC	269	2.120589006486356413853365.975661816892880863225878982066479896dal																		
2	mHFD	ABC	270	3.23783969892311302024082.32303761924677805864277.8695127976528dal																		
2	mHFD	ABM	264	3.599929019702225816842354.5429686018800518701.88981.893114003306dal																		
2	mHFD	ABM	265	3.97907671211566252647739.697.8631627676633249082688937204026150dal																		
2	mHFD	ABM	268	4.25986748912295122708564901708035939917.1910503770188740046022dal																		
2	mHFD	chABC	273	3.247897641167201550478733.87795810752027484862838889605848183tal																		
2	mHFD	chABC	276	3.5632300652606685022748.8459014760832796462868089178610501dal																		
2	mHFD	chABC	271	3.18789008923854785077390.5668205327393032401203390005411816050dal																		
2	mHFD	chABC	272	4.3446681047372685531463120631571386217017305012935904226079B1dal																		
2	mHFD	chABC	275	3.166313629842426728271566.461888757793169707.8871089123194056dal																		
3	mHFD	ABC	266	3.392614504898344384010243.50602257089360821043253397893078656dal																		
3	mHFD	ABC	267	1.23722806358521819032831.52466002829970986079721938006388576dal																		
3	mHFD	ABC	269	2.510910645863661418976309.29920621973645003182395096792682063dal																		
3	mHFD	ABC	270	3.81917904051924423079886.26358367967473744932177967086807586dal																		
3	mHFD	ABM	264	2.776993066337867302067456.4050813293254177890399401269442813860dal																		
3	mHFD	ABM	265	4.682729667462885002036337879906938026884323126932686467629H70dal																		
3	mHFD	ABM	268	3.360244101301018470867026.57088378732951428918317807412850657dal																		
3	mHFD	chABC	273	3.59008907837049015993632.53796722096375423222829224770484803dal																		
3	mHFD	chABC	276	3.041272073340738614877709.6102984278813539797264398853942547dal																		
3	mHFD	chABC	271	4.0547173029495017808214712637687936800376673825868265562769H70dal																		
3	mHFD	chABC	272	4.4005581082327759864653839.958238964189744482090390165205602dal																		
3	mHFD	chABC	275	2.984682063545579705668822.21896650186873061960261989209799759dal																		
4	mHFD	ABC	266	4.73091986986079445900259398298350890039388662060760184032576dal																		
4	mHFD	ABC	267	2.613889097883570833895187.34528710127545416266820396177140975dal																		
4	mHFD	ABC	269	3.0214128689958637614731103.4601868788877651166926903925042508306dal																		
4	mHFD	ABC	270	3.540599870310659538606185.406727480917582499823701692432446206dal																		
4	mHFD	ABM	264	3.690686600881108960308230.19040439002621204373387919062067417B3dal																		
4	mHFD	ABM	265	4.29884805512828969043651591969358655125540387977073451846537dal																		
4	mHFD	ABM	268	3.74925374686353723094027.11090577.884476639288822890037.85354dal																		
4	mHFD	chABC	273	3.677326044369257760750989.69023319662208642804456967136510228dal																		
4	mHFD	chABC	276	3.54354688289815360421127.60094785894984768628086895694321E36dal																		
4	mHFD	chABC	271	3.70260765094757308074203.08304128780744189040028097923381234296dal																		
4	mHFD	chABC	272	3.50558072333650193062509.835784704704414522823363972014731282dal																		
4	mHFD	chABC	275	3.3650580332451090905429.85196970895416843329340189702091770dal																		
5	mHFD	ABC	266	1.71703201526779146012083979775688007162271884039863845780824dal																		
5	mHFD	ABC	267	2.51628666800940419855767.78204468679926356922253934728304180dal																		
5	mHFD	ABC	269	1.4995289800844104955989514591195814486722238969884489047636dal																		
5	mHFD	ABC	270	2.18630082460908473628702.47592189986062091037283877685122381dal																	</	

exp_Group	Treatment	Sex	Animal	Food	Food	InWater	FB	Balance	MeV	VO2	VCO2	VH2O	EE	RER	BodyMass	Photoperiod	
5	mHFD	D	A	B	C	264	2.2917	200520	478478	2785593	82572550	09387	00680	133209	87600	63271	total
5	mHFD	D	A	B	C	265	2.9071	1000982	1186959	4286958	8970286	62014	0936236	078803	61898	0361	total
5	mHFD	D	A	B	C	268	1.7431	600122	217642	190488	656919	2868885	1585651	123368	885744	1849	total
5	mHFD	I	ch	A	B	C273	1.4201	500479	868681	653528	586651	630128	6895400	360824	30036	2871	total
5	mHFD	I	ch	A	B	C276	1.4119	510010	854202	0480508	620796	0158120	891722	08708705	55206	2001	total
5	mHFD	I	ch	A	B	C271	3.1679	200052	600901	0878502	1208254	179231	0484106	195882	851215	7361	total
5	mHFD	I	ch	A	B	C272	2.3526	888123	622562	0018968	421276	738854	293612	389158	827818	5061	total
5	mHFD	I	ch	A	B	C275	1.9475	800798	365440	206973	0968052	193390	9936645	21286	827122	0706	total

Next, we compute cumulative totals or averages of all variables within each photoperiod per day. This is merged with the `total.avg.daily` data so that plots containing dark, light, and total can be obtained from the same data frame.

```
# Compute average within each photoperiod, append to total.avg.daily
pp.avg.daily <- df %>%
  group_by(exp_day, Photoperiod, Group, Treatment, Sex, Animal) %>%
  summarize(
    across(all_of(cols2sum), sum),
    across(all_of(cols2avg), mean)) %>%
  ungroup() %>%
  bind_rows(., total.avg.daily)
rm(total.avg.daily)
pp.avg.daily
```

exp_Phy	Photoperiod	Group	Treatment	Sex	Animal	Food	Food	InWater	FB	Balance	MeV	VO2	VCO2	VH2O	EE	RER	BodyMass
1	0	mHFD	D	A	B	C	266	0.3598	4231	13877	50593	28.6181	196939	281573	468392	323354	657697
																	0.1887897
1	0	mHFD	D	A	B	C	267	0.4912	003070	027633	038092	3.1187	576279	208292	805570	829203	1829304
1	0	mHFD	D	A	B	C	269	1.2283	663918	833773	077912	261546	3750970	406084	8222999	905826	63861
1	0	mHFD	D	A	B	C	270	1.2116	598905	1319745	369826	526902	285624	173581	130319	281532	7106588
1	0	mHFD	D	A	B	C	264	0.6125	787282	860891	843230	564696	793694	965933	738826	821437	019402
1	0	mHFD	D	A	B	C	265	0.8983	687310	780772	287239	657383	706309	161390	624761	694120	919458
1	0	mHFD	D	A	B	C	268	0.8950	273910	259162	214038	705286	851835	757616	523751	190236	3023929
1	0	mHFD	I	ch	A	B	C273	0.8674	654233	096367	734533	833171	229366	609733	948206	320836	1306443
1	0	mHFD	I	ch	A	B	C276	0.5706	569675	813012	899126	702708	607927	694148	740854	608908	21884354
1	0	mHFD	I	ch	A	B	C271	1.1790	693206	594761	834932	676598	568145	718226	922628	187680	1692473
1	0	mHFD	I	ch	A	B	C272	1.3078	912931	598278	829699	832236	704288	753186	501289	360993	116242561
1	0	mHFD	I	ch	A	B	C275	1.4244	508248	107122	840283	345968	890018	959724	126263	278890	28783482



exp	Phyto	Grain	Treat	Sex	Anim	Ed	Mod	IF	Ed	In	Water	FB	gland	Ad	Me	VO	2	VCO	2	VH	2	OEE	RER	Body	Mass											
3	0	mHR	DAB	C	270	2.7023	1100	49	70	267	96	68	37	87	23	82	53	105	05	56	68	66	76	39	88	29	48	85	94	06	67	8				
3	0	mHR	DAB	C	264	1.5518	750	78	83	20	22	56	54	99	93	10	98	96	53	74	90	22	91	92	37	28	28	28	07	65	78	0	0			
3	0	mHR	DAB	C	265	2.6841	144	27	34	02	89	90	82	37	88	79	80	07	96	05	09	49	25	77	07	08	74	69	76	21	47	12	4			
3	0	mHR	DAB	C	268	2.7235	310	38	82	26	14	80	89	39	66	39	94	93	77	89	16	72	89	28	41	57	25	64	29	96	39	74	0	0		
3	0	mHFI	ch	A	B	C	273	2.0596	565	05	34	21	02	51	80	97	94	04	29	81	75	23	73	73	85	94	53	44	23	98	52	07	8			
3	0	mHFI	ch	A	B	C	276	2.0364	836	29	84	84	19	09	43	69	15	48	96	32	95	10	75	74	24	46	28	21	87	43	60	38	05	9		
3	0	mHFI	ch	A	B	C	271	2.2800	547	62	26	85	23	20	13	70	3.67	19	07	16	68	23	07	09	06	37	04	02	39	05	81	11	28	1		
3	0	mHFI	ch	A	B	C	272	3.3224	131	95	42	38	24	32	04	29	56	66	88	29	77	10	64	43	25	88	43	29	43	55	29	77	10	0		
3	0	mHFI	ch	A	B	C	275	1.9330	813	08	78	06	25	06	42	02	83	77	11	03	21	09	67	59	47	61	38	21	93	44	35	19	64	32	0	
3	1	mHR	DAB	C	266	0.9673	386	27	98	57	77	88	80	93	89	46	32	08	93	70	15	63	87	06	22	90	30	01	22	42	33	0	0			
3	1	mHR	DAB	C	267	0.4294	580	36	98	11	01	991	66.64	89	62	68	95	73	48	89	28	82	86	29	92	35	51	45	60	48	0.43	17	95	4		
3	1	mHR	DAB	C	269	0.6541	270	76	06	00	00	09	44	36	24	32	35	19	18	52	37	98	01	03	10	85	51	18	02	98	99	6	0			
3	1	mHR	DAB	C	270	1.1168	699	08	09	85	64	57	03	42	41	02	53	03	62	98	99	27	76	22	80	47	18	65	31	38	08	49	4	0		
3	1	mHR	DAB	C	264	1.2251	560	54	87	25	07	27	13	38	84	74	19	83	68	83	76	04	65	03	88	95	18	60	57	05	11	41	0	0		
3	1	mHR	DAB	C	265	1.9985	839	40	57	29	85	59	87	98	49	79	85	85	33	77	04	43	38	92	74	68	91	18	86	67	23	19	11	8		
3	1	mHR	DAB	C	268	0.6367	167	41	86	25	40	12	07	73	22	15	19	58	20	04	67	89	74	82	22	20	89	15	21	73	33	39	0	0		
3	1	mHFI	ch	A	B	C	273	1.5304	382	78	99	63	76	87	96	35	09	49	71	69	18	53	73	85	19	29	23	18	04	33	08	21	05	5	0	
3	1	mHFI	ch	A	B	C	276	1.0048	520	04	07	83	18	59	34	60	51	81	88	53	21	87	23	50	33	48	20	05	78	06	42	89	21	36	6	0
3	1	mHFI	ch	A	B	C	271	1.7746	596	35	67	83	65	89	00	68	70	20	83	15	57	38	41	50	57	12	76	96	90	72	99	27	66	3	0	
3	1	mHFI	ch	A	B	C	272	1.0781	462	81	88	80	35	32	30	37	58	91	62	48	15	32	73	09	47	12	63	75	85	89	49	21	61	34	0	0
3	1	mHFI	ch	A	B	C	275	1.0516	870	68	75	25	46	12	06	63	44	76	90	09	52	70	70	15	63	02	24	18	84	97	59	79	89	49	0	0
4	0	mHR	DAB	C	266	3.0258	557	08	57	02	07	60	11	09	2.69	63	42	69	99	39	27	06	56	35	06	29	32	83	50	04	22	27	0	0		
4	0	mHR	DAB	C	267	1.5953	397	06	50	68	18	10	79	99	99	06	25	34	14	82	44	03	82	08	23	39	07	64	07	44	72	18	0	0		
4	0	mHR	DAB	C	269	1.2985	816	40	00	29	64	80	18	73	83	08	92	96	68	96	89	63	07	77	29	31	98	37	28	60	02	85	0	0		
4	0	mHR	DAB	C	270	2.1065	880	75	68	84	34	46	67	87	09	29	50	86	35	96	88	93	56	90	18	47	40	93	22	05	44	44	02	0	0	
4	0	mHR	DAB	C	264	2.1183	199	70	65	87	67	45	86	18	73	60	37	67	36	73	85	78	23	51	83	50	90	09	10	02	39	01	51	6	0	
4	0	mHR	DAB	C	265	2.7036	703	55	01	80	17	07	50	93	82	35	52	44	69	07	12	76	70	05	98	70	00	59	68	87	98	19	0	0		
4	0	mHR	DAB	C	268	2.4406	103	20	50	27	48	77	72	44	89	40	09	98	15	93	18	19	37	31	86	38	12	16	73	62	32	12	5	0		
4	0	mHFI	ch	A	B	C	273	2.4163	101	04	82	64	27	56	69	66	73	1.26	89	07	18	29	09	61	19	06	82	09	82	35	25	26	28	50	1	0
4	0	mHFI	ch	A	B	C	276	2.1554	096	28	85	87	79	16	77	88	8.40	07	23	18	99	31	20	17	82	69	59	94	96	37	98	27	49	0	0	
4	0	mHFI	ch	A	B	C	271	2.5242	217	60	13	53	20	98	07	00	89	8.98	19	82	75	93	10	34	66	50	94	30	93	72	95	27	24	33	0	0
4	0	mHFI	ch	A	B	C	272	1.9925	810	68	62	80	71	52	66	89	35	75	86	27	82	11	79	49	72	25	85	09	91	12	98	23	47	1	0	
4	0	mHFI	ch	A	B	C	275	2.3532	588	36	68	42	10	06	90	83	77	28	63	68	19	82	76	46	83	16	06	03	89	02	70	22	48	66	5	0
4	1	mHR	DAB	C	266	1.7050	764	61	26	82	77	37	39	91	46	72	86	66	24	89	38	61	56	12	13	35	08	89	20	15	06	09	22	0	0	
4	1	mHR	DAB	C	267	1.0184	007	76	83	28	90	18	69	59	82	27	57	28	36	26	50	44	04	20	06	79	27	13	62	37	22	90	0	0		
4	1	mHR	DAB	C	269	1.7228	329	59	05	06	72	83	71	23	74	63	32	80	78	94	18	03	38	20	02	68	72	88	63	02	26	76	37	0	0	
4	1	mHR	DAB	C	270	1.4340	352	29	07	01	60	27	18	19	18	01	16	09	13	79	34	58	18	09	01	82	00	86	54	26	59	84	80	08	0	0
4	1	mHR	DAB	C	264	1.5723	360	38	05	43	12	94	46	35	58	66	03	51	20	67	04	83	96	36	49	86	23	91	43	31	50	13	21	0	0	
4	1	mHR	DAB	C	265	1.5951	670	09	97	04	22	88	50	06	86	58	76	81	01	42	57	29	74	65	38	32	89	25	86	87	15	41	32	16	0	0
4	1	mHR	DAB	C	268	1.3086	109	62	88	76	49	28	88	49	37	35	09	83	03	85	69	71	02	90	53	80	26	12	83	33	38	07	51	58	0	0

exp_	Phytop	Grain	Treat	Sex	Anim	Flood	Fog	In Water	EB	Balance	Adm	Me	VO2	VCO2	VH2O	OEE	RER	Body Mass
4	1	mHFD	chABC	273	1.2610523963050782206784288425373921033454984927810679021691955													
4	1	mHFD	chABC	276	1.3880528300072374206437639642823398606520191047210831651660123													
4	1	mHFD	chABC	271	1.17838949196036320773925101090298845754341046765809467396153													
4	1	mHFD	chABC	272	1.513061664768243088096012781470671637650248120876862899339093													
4	1	mHFD	chABC	275	1.01170204953580899001539278704236888068955027650841338934875													
5	0	mHRD	DABCE	266	0.8251300654906919120425354068625275510124390137510909975306913													
5	0	mHRD	DABCE	267	1.34675205640059560809222973279942667930165250209532961318283765													
5	0	mHRD	DABCE	269	1.2284680950538281302635545114017969070222499660503926222730095													
5	0	mHRD	DABCE	270	1.7547787008940787080702823247235849695079365674050952020903003													
5	0	mHRD	DABCM	264	1.55609603550868388208460359803105766610032180780996537045549													
5	0	mHRD	DABCM	265	1.640878016540161429868080789406300996317694501747931940331937													
5	0	mHRD	DABCM	268	0.60423603776672810006595371987035011019800082010941365891131													
5	0	mHFD	chABC	273	0.420347054287010379224283635670110623500915328710686957075753													
5	0	mHFD	chABC	276	0.4449468674080128236064558230485508385850034505301590648606200													
5	0	mHFD	chABC	271	1.740980021160779660734934659007209486969489299107981822348457													
5	0	mHFD	chABC	272	1.25068062836064086012038249597945884060045912802682746660244													
5	0	mHFD	chABC	275	1.270640366880006360442577510965058867926441028701973986085738													
5	1	mHRD	DABCE	266	0.89193704610608723506940543900941029492580129249701896445427846													
5	1	mHRD	DABCE	267	1.1695290200084483606353204925152386270605712124007051316800													
5	1	mHRD	DABCE	269	0.27106068462058231032.003184898537930976234839801876998646													
					1.3703169													
5	1	mHRD	DABCE	270	0.43153802426029764044.151181795964911245183639833519036184													
					1.4841478													
5	1	mHRD	DABCM	264	0.73563089606000905043.465886280807109901107330818000571334959													
					0.0499253													
5	1	mHRD	DABCM	265	1.266238081660025580748616901915207232348525268388097251996708													
5	1	mHRD	DABCM	268	1.138930835060483020982793197284452028728025520606													
5	1	mHFD	chABC	273	0.99985099370076423501150823115659643236681709851782656086117													
5	1	mHFD	chABC	276	0.96700006140022926307362499735290046092280992334258986198606200													
5	1	mHFD	chABC	271	1.4269510931480316560705708541813102737229586126986671549830949													
5	1	mHFD	chABC	272	1.1020006284000615208064087107556315674276060502801668064575993													
5	1	mHFD	chABC	275	0.6769280431482433966535022917369183840081034601845222061711													
1	Total	mHRD	DABCE	266	0.57483894303253886246.9686025096766503810370286844721084560													
					0.6264031													
1	Total	mHRD	DABCE	267	1.13526768096258320928943759544759072202320643389136766577													
1	Total	mHRD	DABCE	269	1.67756504570326614302085741870247066350921830833907351080457													
1	Total	mHRD	DABCE	270	1.477213044206903590595209769291478719019333128230876792010568													
1	Total	mHRD	DABCM	264	1.509803011687273626285806106724808980010587508362888872967631													
1	Total	mHRD	DABCM	265	1.548715046030543864113327508037075370412082367872550684532													
1	Total	mHRD	DABCM	268	1.73846201540164646031039882796135340731747306309085377913479													
1	Total	mHFD	chABC	273	1.428099763328049602935839516786102029381502010290356188554													

exp	Phyto	Grain	Treat	Sex	Anim	Ed	Mod	IF	Pod	In	Watr	FB	gland	Ad	Me	VO	CO2	VCO2	VH2O	EE	RER	Body	Mass
1	Total	mHFD	chABC	276	1.07516	20568081	183607	131436	3188	276362	450512	730104	3788	170027	050136								
1	Total	mHFD	chABC	271	1.72427	10018086	605268	0669	1236	775701	283386	17562	3878	854386	463194								
1	Total	mHFD	chABC	272	1.75882	387065	50723	2052	4906	890553	128732	18641	3939	88832	5075110								
1	Total	mHFD	chABC	275	1.91338	103602	98124	5808	7575	6158	2043	72970	7124	8242	2283	5019	27612						
2	Total	mHFD	ABC	266	3.33571	470100	1323	3188	9979	2.415	5108	8806	1688	5352	9916	8012	2973	316					
2	Total	mHFD	ABC	267	2.49665	608505	5287	1373	972	4.80	5666	8228	3808	8066	8920	0037	7528						
2	Total	mHFD	ABC	269	2.12058	906486	8564	1385	3305	9.75	6618	1689	2837	8632	2587	8982	6647	9893					
2	Total	mHFD	ABC	270	3.23783	96989	3281	3009	2405	3.32	3703	7619	2467	7805	3842	7786	9512	7976	628				
2	Total	mHFD	ABC	264	3.59992	901970	1225	8168	4235	4.54	2968	6188	0051	8701	8898	1893	1140	0339	64				
2	Total	mHFD	ABC	265	3.97907	671211	3625	1264	7730	4.69	7863	1626	7663	3249	0826	8937	2042	6159					
2	Total	mHFD	ABC	268	4.25986	748912	2885	1270	8564	9.17	8035	3939	1719	1052	3770	1887	4004	6022	1				
2	Total	mHFD	chABC	273	3.24789	764116	7201	5570	7873	3.87	7058	1752	0274	8486	2938	8896	0584	8183					
2	Total	mHFD	chABC	276	3.56323	006526	0068	5922	748	8.45	9214	7608	3279	6462	8680	8917	8650	5016					
2	Total	mHFD	chABC	271	3.18780	008912	3804	7850	7730	9.56	8203	2743	9303	2402	3390	0054	1816	959					
2	Total	mHFD	chABC	272	4.34461	824737	2825	5310	6312	4.63	1671	3862	1701	7303	0129	3590	4226	279	315				
2	Total	mHFD	chABC	275	3.16631	629842	2726	7280	7156	6.46	1388	7577	9316	9707	8871	0891	2319	4056	4				
3	Total	mHFD	ABC	266	3.39261	50489	8304	3840	1024	1.50	6092	5768	9360	8210	3253	9789	3078	6561					
3	Total	mHFD	ABC	267	1.23722	89635	7621	8190	3281	1.52	4400	2829	9709	8047	9721	9380	1638	8576					
3	Total	mHFD	ABC	269	2.51091	004585	3861	1879	7630	9.29	8662	1736	4500	3182	3950	9679	2682	0634					
3	Total	mHFD	ABC	270	3.81917	040512	2442	3079	836	2.63	5836	7967	4737	4493	2177	9670	8680	7586					
3	Total	mHFD	ABC	264	2.77699	36633	7867	3020	6718	6.40	5081	3293	2517	7899	3994	1269	4428	13860					
3	Total	mHFD	ABC	265	4.68272	066746	3885	0020	3633	7.79	9069	3892	6884	3231	2693	3208	6467	0294	70				
3	Total	mHFD	ABC	268	3.36024	101302	1284	8667	026	5.79	8537	7329	5142	8928	3178	9740	2850	657					
3	Total	mHFD	chABC	273	3.59008	007837	3801	5093	652	5.37	7672	2706	3754	2322	2829	2147	0184	8931					
3	Total	mHFD	chABC	276	3.04127	20773	3427	3814	8777	704	6.12	9842	7881	5539	7972	6439	8853	9942	5471	3			
3	Total	mHFD	chABC	271	4.05471	302979	4061	7808	2117	12.37	6369	3680	0376	6738	2586	8265	1076	6947	2				
3	Total	mHFD	chABC	272	4.40058	108232	6585	9860	6533	9.81	9580	3896	3189	7444	8309	0890	1652	2569	22				
3	Total	mHFD	chABC	275	2.98468	063565	7670	7056	6882	2.21	8766	5186	8730	6196	0286	1089	2097	9976	91				
4	Total	mHFD	ABC	266	4.73091	08698	5989	4450	0025	9898	2983	5089	0052	3886	6206	0760	1803	2575					
4	Total	mHFD	ABC	267	2.61388	09780	3380	8388	9518	4.34	5287	1027	5451	6268	2030	6177	1409	754					
4	Total	mHFD	ABC	269	3.02141	86899	5363	7147	3110	3.46	1868	7888	7765	1609	2690	8392	5042	8396	1				
4	Total	mHFD	ABC	270	3.54059	087021	6895	3860	6185	4.06	7274	8791	7582	4998	3237	1692	4324	4620	5				
4	Total	mHFD	ABC	264	3.69068	66008	8110	8960	0820	1.90	4043	9806	2120	4373	8791	1906	2074	1733					
4	Total	mHFD	ABC	265	4.29881	005512	8289	6904	4367	1.59	1769	3586	5512	5541	2879	7707	3451	8465	17				
4	Total	mHFD	ABC	268	3.74925	374686	2637	2309	4027	1.10	9057	7788	4476	6339	2888	2289	0037	8536	42				
4	Total	mHFD	chABC	273	3.67732	604443	6925	7767	5098	9.69	1823	1966	2208	6428	0445	6967	1365	10228					
4	Total	mHFD	chABC	276	3.54354	68828	9325	3600	2112	7.60	1937	8589	4984	7686	2808	8689	5694	3214	36				
4	Total	mHFD	chABC	271	3.70261	765094	4737	3080	7420	3.08	3912	8787	4418	9404	0080	9792	3381	2342	93				
4	Total	mHFD	chABC	272	3.50558	072343	6601	3062	505	8.35	7347	1704	4415	2238	3336	8972	0147	1312	82				
4	Total	mHFD	chABC	275	3.36505	803322	2610	0900	0519	8.51	9697	0805	4168	4332	0341	8770	2091	770					
5	Total	mHFD	ABC	266	1.71703	001506	7914	6012	9809	7.71	5688	0071	6227	1880	3926	3845	8082	43					

exp_	Photo	Group	Treat	Sex	Animal	Food	In	Water	FB	Balance	Ad	Me	VO2	VCO2	VH2O	EE	RER	BodyMass
5	Total	mHF	DABC	F	267	2.5162	8666800	140461	9855	767.782	0446867	9926356	9225389	3472830	4489			
5	Total	mHF	DABC	F	269	1.4995	3289800	764104	95598	95145	91195	814486	7223896	9824489	947633			
5	Total	mHF	DABC	F	270	2.1863	3008246	00847	26287	92.475	9818988	6063891	372838	176851	23818			
5	Total	mHF	DABC	M	264	2.2917	2605204	78478	27855	593.825	7355069	3870068	3432098	76006	227104			
5	Total	mHF	DABC	M	265	2.9071	1000982	20869	59428	69588	9702866	2614079	363866	788036	1898036			
5	Total	mHF	DABC	M	268	1.7431	6001222	07642	90486	65691	928688	515856	513736	8885744	849397			
5	Total	mHF	HChABC	273	1.4201	9064798	68681	66352	35866	61631	288689	5400360	823300	3828714				
5	Total	mHF	HChABC	276	1.4119	5193014	85420	20480	508.620	7960158	1208917	226870	870555	306200				
5	Total	mHF	HChABC	271	3.1679	2300526	30961	08785	42.120	8854198	2310784	106195	882851	215735				
5	Total	mHF	HChABC	272	2.3526	8881236	25620	01806	3.421	767388	5429361	238915	88278	1885030				
5	Total	mHF	HChABC	275	1.9475	8079836	64402	06973	769.680	52193	790993	664524	286827	7122270	665			

Finally, we compute cumulative totals and averages across the entire experiment, for each day...

```
# Compute average across variables for entire experiment
total.avg <- df %>%
  group_by(Group,Treatment,Sex,Animal) %>%
  summarize(
    across(all_of(cols2sum),sum),
    across(all_of(cols2avg),mean)) %>%
  mutate(Photoperiod = "Total") %>%
  ungroup()
```

... and within each photoperiod.

```
# Compute the overall average daily value for every requested variable across the whole ex
pp.avg.total <- pp.avg.daily %>%
  group_by(Photoperiod,Group,Treatment,Sex,Animal) %>%
  summarize(
    across(all_of(c(cols2sum,cols2avg)),mean)) %>%
  ungroup()
rm(total.avg)
pp.avg.total
```

Photo	Group	Treat	Sex	Animal	Food	In	Water	FB	Balance	Ad	Me	VO2	VCO2	VH2O	EE	RER	BodyMass
0	mHF	DABC	F	266	1.7060	76695	397144	53959	47534	8081230	8371640	22840233	300539	74837	799641		
0	mHF	DABC	F	267	1.2276	518559	28362	63219	273218	924568	2462519	0642477	3046336	50848	502718		
0	mHF	DABC	F	269	1.3232	55773	39317	63703	34288	165238	2005841	0924061	2639036	14213	51147		



Photop	Geno	Freat	Sex	Anim	Food	Food	In	Water	EB	Balance	Ad	Met	CO2	VCO2	VH2O	EE	RER	Body	Mass	
0	mHF	D	A	B	C	F	270	1.97178	231320	371557	035167	251351	1320330	579601	370092	388751	10074	72493		
0	mHF	D	A	B	C	M	264	1.56106	256209	871738	011095	99657	000368	100720	668632	856373	441561	22205		
0	mHF	D	A	B	C	M	265	2.03738	155670	854058	710463	655495	565849	166516	582285	364028	59463	4653		
0	mHF	D	A	B	C	M	268	1.82485	964410	818382	261727	506049	521408	900080	504028	420403	11829	59235		
0	mHF	D	I	ch	A	B	C	273	1.43904	804400	237793	578404	196657	725471	367847	170231	110931	17933	5284	
0	mHF	D	I	ch	A	B	C	276	1.60586	434450	178324	217406	187639	192634	286807	720247	330111	23928	50264	
0	mHF	D	I	ch	A	M	C	271	1.94008	614827	822691	167749	381760	922834	710551	710274	440341	12527	85493	
0	mHF	D	I	ch	A	M	C	272	2.17799	112173	118057	684559	822966	811007	534075	662623	23177	22643	1963060	
0	mHF	D	I	ch	A	M	C	275	1.68874	409272	555784	129236	381162	968298	956310	648212	883076	97528	62751	
1	mHF	D	A	B	C	F	266	1.04414	738554	127147	129573	250519	057048	180616	166529	630337	20265	21190		
1	mHF	D	A	B	C	F	267	0.77228	224350	113082	624625	072616	861410	961561	109656	138248	27132	20387	148	
1	mHF	D	A	B	C	F	269	0.84273	099470	041022	193284	93387	450036	508786	080027	93687	20452	75339		
1	mHF	D	A	B	C	F	270	0.88048	960805	158728	534866	275870	419288	970485	490360	540985	17237	51853		
1	mHF	D	A	B	C	M	264	1.21282	409386	131992	111354	409184	277107	261450	406090	287104	85902	51655	533	
1	mHF	D	A	B	C	M	265	1.44596	007311	821733	147564	616049	485138	993646	117128	350101	85915	26247	49	
1	mHF	D	A	B	C	M	268	1.14533	651041	543320	147073	357925	724816	624074	426907	32662	26477	264	2480	
1	mHF	D	I	ch	A	B	C	273	1.23365	408134	175344	495462	282210	557602	583903	870063	94260	88049	803	223
1	mHF	D	I	ch	A	B	C	276	0.92119	586902	234799	308636	558607	837107	269629	344023	461843	62027	1755	187
1	mHF	D	I	ch	A	M	C	271	1.22745	795511	300498	966904	923548	802306	918768	821986	123740	13889	3032	45976
1	mHF	D	I	ch	A	M	C	272	1.09452	889702	193974	961270	180106	408956	130750	348879	46655	9960	6454	
1	mHF	D	I	ch	A	M	C	275	0.98664	961392	818491	920281	106328	282874	679091	510294	340185	94886	928	198
Total	mHF	D	A	B	C	F	266	2.75022	115509	441160	125521	130953	676076	943607	149393	313409	82903	29170	51	
Total	mHF	D	A	B	C	F	267	1.99988	409942	494934	783901	127654	631659	990378	231030	78296	22917	794	47385	
Total	mHF	D	A	B	C	F	269	2.16609	209720	835864	638283	523990	810208	836869	125830	404890	38556	62516		
Total	mHF	D	A	B	C	F	270	2.85223	109793	795885	769969	848938	440868	838470	497320	68588	70397	12961		
Total	mHF	D	A	B	C	M	264	2.77382	665006	991650	146119	911493	496932	105083	237306	50782	47291	51978		
Total	mHF	D	A	B	C	M	265	3.48329	216298	267138	928609	993260	400698	992817	443016	66683	32208	44369	43	
Total	mHF	D	A	B	C	M	268	2.97019	201748	236168	417943	208525	759396	916628	307185	38285	8982	74547	9	
Total	mHF	D	I	ch	A	B	C	273	2.67270	202253	491246	941033	241839	147010	155685	339521	18009	43937	4922	
Total	mHF	D	I	ch	A	B	C	276	2.52703	061352	631263	882670	634638	754507	821062	400235	89033	66874	807500	
Total	mHF	D	I	ch	A	M	C	271	3.16747	430332	231986	186496	081085	879175	880053	201254	76799	3427	99930	
Total	mHF	D	I	ch	A	M	C	272	3.27246	874433	245598	883882	030671	842165	016203	39681	30229	57935	745532	
Total	mHF	D	I	ch	A	M	C	275	2.67539	392366	574289	622620	387089	340300	863138	460644	39882	024	45660	