```
 [1] "channel"   "db.oocyte" "g3.g"      "g3.h"      "g3.oocyte" "g3.sf"     "my.stderr"
 [8] "sb.g"      "sb.h"      "sb.sf"     "sql"       "sr.g"      "sr.h"      "sr.sf"
[15] "stderr"
```

The project

Between 1988 and early 1992, Warwick Nash and team took monthly samples (~ 12 females) of abalone from George III Rock as part of a study on spawning periodicity. During the last year of sampling (1991), monthly samples were also taken at Shag Rock bay and Stinking Bay on the Tasman Peninsular. Morphometric data were collected from each animal, prior to the gonad being fixed. Routine histology was then done on the gonads. The section was taken approximately 1/3 of the way along the conical appendage on the basis that the gonad state was uniform throughout.

Warwick Nash and team processed the histology slides, mapping out the gonad state within the section into 8 categories. That work was never published. In 2002, I (CM) had Leigh Gurney review the 8 state- gonad classification system and re-classify the 8 states into a more typical 5 state system. Leigh also conducted detailed measureoments of oocyte diameter for the 1991 Georges III Rock samples. Thi identfied a transition from pre-vitellegenci to vitellegenic stages at around 95 microns. On the results of that exercise between 2004 and 2010 I had various people process the histology slides for stage- frequency analysis. The last person I employed casually was so efficient that I ended up getting her to do the entire 4-year George III slides and the slides from Shag Rock Bay and Stinking Bay, so that we had a consistent dataset.

The data

The stage frequency data were collected from 4 transects across the section oriented north, south, east, west. Within each transect the area of the gonad sampled (outer membrane to boundary with the digestive gland) was measured using imageJ, and a count done of the number of pre-vitellegenic and vitellegenic oocytes in each transect.

Several data frames are loaded from this .R Data file. Sites are designated as follows;

- g3.X = George III Rock
- sr.X = Shag Rock Bay
- sb.X = Stinking Bay

Different atasets are designated as follows;

- g3.g = basic morphometric information
- g3.h = histology and gonad state information
- g3.sf = stage frequency information

Examples are given below on joining the data frames to make a useable dataset.

Gonad data

```
kable(g3.g[1:10,])
```

week_no	abalone_id	sample_date	shell_length_mm	shell_width_mm	whole_wt_g	meat_wt_g	viscera_w
1	7402	1988-02-11	132	101	334.4	158.8	
1	7403	1988-02-11	140	NA	442.0	219.8	
1	7404	1988-02-11	142	111	404.7	188.4	
1	7405	1988-02-11	134	NA	363.6	179.7	
1	7406	1988-02-11	117	92	195.2	93.0	
1	7407	1988-02-11	136	115	390.4	170.7	
1	7408	1988-02-11	97	79	136.1	61.0	
1	7409	1988-02-11	132	107	380.5	189.5	
1	7410	1988-02-11	126	99	293.9	146.7	
1	7411	1988-02-11	123	93	190.5	80.7	

```
str(g3.g, digits.d = 2, vec.len = 3)
```

```
'data.frame':   3142 obs. of  16 variables:
 $ week_no        : num  1 1 1 1 1 1 1 1 ...
 $ abalone_id     : num  7402 7403 7404 7405 ...
 $ sample_date    : POSIXct, format: "1988-02-11" "1988-02-11" ...
 $ shell_length_mm: num  132 140 142 134 ...
 $ shell_width_mm : num  101 NA 111 NA ...
 $ whole_wt_g     : num  334 442 405 364 ...
 $ meat_wt_g      : num  159 220 188 180 ...
 $ viscera_wt_g   : num  83 85 96 86 ...
 $ sex            : Factor w/ 4 levels "F","I","M","T": 1 3 1 3 2 1 2 1 ...
 $ shell_ht_mm    : num  42 NA 47 NA 30 41 25 33 ...
 $ shell_wt_g     : num  80 NA 93 NA ...
 $ no_major_rings : num  13 NA 13 NA 13 14 6 14 ...
 $ no_minor_rings : num  NA NA NA NA NA NA NA NA ...
 $ total_no_rings : num  13 NA 13 NA 13 14 6 14 ...
 $ samp_year      : num  1988 1988 1988 1988 ...
 $ samp_month     : num  2 2 2 2 2 2 2 2 ...
```

Histology data

```
kable(g3.h[1:10,])
```

week_no	abalone_id	shell_length_mm	whole_wt_g	no_major_rings	stage_1_mm2	stage_2_mm2	stage_
1	7402	132	334.4	13	0.00	0.00	
1	7404	142	404.7	13	0.00	0.00	
1	7407	136	390.4	14	0.00	0.00	
1	7409	132	380.5	14	0.00	30.37	
1	7410	126	293.9	10	0.00	0.00	
1	7414	147	504.1	12	0.00	0.00	
1	7415	138	348.2	12	17.04	0.00	
1	7416	125	278.8	9	0.00	0.00	
1	7417	158	649.6	14	0.00	0.00	
1	7418	134	384.7	14	0.00	0.00	

```
str(g3.h, digits.d = 2, vec.len = 3)
```

```
'data.frame':   746 obs. of  30 variables:
 $ week_no        : num  1 1 1 1 1 1 1 1 ...
 $ abalone_id     : num  7402 7404 7407 7409 ...
 $ shell_length_mm: num  132 142 136 132 ...
```

```
 $ whole_wt_g     : num  334 405 390 380 ...
 $ no_major_rings : num  13 13 14 14 10 12 12 9 ...
 $ stage_1_mm2    : num  0 0 0 0 ...
 $ stage_2_mm2    : num  0 0 0 30 ...
 $ stage_3_mm2    : num  21 0 0 0 ...
 $ stage_4_mm2    : num  1.4 103.6 86.3 0 ...
 $ stage_5_mm2    : num  0 0 0 0 0 0 0 0 ...
 $ stage_6_mm2    : num  0.84 2.27 6.92 0 ...
 $ stage_7_mm2    : num  16.7 9.8 25.3 6.9 ...
 $ stage_8_mm2    : num  0 0 0 2.7 ...
 $ liver_area_mm2 : num  114 141 80 106 ...
 $ gonad_area_mm2 : num  40 116 119 40 ...
 $ gonad_index    : num  0.26 0.45 0.6 0.27 ...
 $ tafi_index     : num  0 0 0 0 0 0 0 0 ...
 $ pc_developing  : num  0 0 0 76 ...
 $ pc_ripe        : num  56 90 73 0 ...
 $ pc_spent       : num  44 10 27 24 ...
 $ rec_dev        : int  0 0 0 33 0 0 17 0 ...
 $ locules        : int  21 0 0 0 42 0 0 62 ...
 $ mature         : int  1 104 86 0 0 215 0 0 ...
 $ spawning       : int  17 10 25 7 7 0 0 6 ...
 $ necrotic       : int  1 2 7 0 0 0 0 0 ...
 $ pc_rec_dev     : int  0 0 0 83 0 0 100 0 ...
 $ pc_locules     : int  53 0 0 0 86 0 0 91 ...
 $ pc_mature      : int  3 90 73 0 0 100 0 0 ...
 $ pc_spawning    : int  43 9 21 18 14 0 0 9 ...
 $ pc_necrotic    : int  3 2 6 0 0 0 0 0 ...
```

Stage Frequency data

```
kable(g3.sf[1:10,])
```

site	sample_date	samp_year	samp_month	abalone_id	quadrant	previt	vit	qarea
G3R	1988-09-09	1988	9	36022	E	102	1	0.359
G3R	1988-09-09	1988	9	36022	N	0	0	0.000
G3R	1988-09-09	1988	9	36022	S	68	2	0.577
G3R	1988-09-09	1988	9	36022	W	72	4	0.324
G3R	1988-09-09	1988	9	36032	E	37	10	0.954
G3R	1988-09-09	1988	9	36032	N	13	4	1.287
G3R	1988-09-09	1988	9	36032	S	79	5	2.832
G3R	1988-09-09	1988	9	36032	W	141	17	3.498
G3R	1988-09-09	1988	9	36033	E	83	41	1.908
G3R	1988-09-09	1988	9	36033	N	35	33	2.280

```
str(g3.sf, digits.d = 2, vec.len = 3)
```

```
'data.frame':   2223 obs. of  9 variables:
 $ site       : Factor w/ 1 level "G3R": 1 1 1 1 1 1 1 1 ...
 $ sample_date: POSIXct, format: "1988-09-09" "1988-09-09" ...
 $ samp_year  : num  1988 1988 1988 1988 ...
 $ samp_month : num  9 9 9 9 9 9 9 9 ...
 $ abalone_id : num  36022 36022 36022 36022 ...
 $ quadrant   : Factor w/ 4 levels "E","N","S","W": 1 2 3 4 1 2 3 4 ...
```

```
 $ previt     : num  102 0 68 72 ...
 $ vit        : num  1 0 2 4 10 4 5 17 ...
 $ qarea      : num  0.36 0 0.58 0.32 ...
```

Joining the tables

Morphometrics and Histology

```
## Add histology data to morpomeric data
histodat <- left_join(g3.g, g3.h ) %>%
 filter(!is.na(tafi_index)) %>%
 ungroup()
```

```
Joining, by = c("week_no", "abalone_id", "shell_length_mm", "whole_wt_g", "no_major_rings")
```

```
histodat %>% filter(sex == "F" ) %>%
 group_by(samp_year, samp_month) %>%
 summarise(reps = n()) %>% kable()
```

samp_year	samp_month	reps
1988	2	15
1988	3	13
1988	4	9
1988	5	9
1988	7	13
1988	8	13
1988	9	13
1988	10	12
1988	11	13
1988	12	11
1989	1	14
1989	2	12
1989	5	7
1989	6	13
1989	7	14
1989	9	18
1989	10	4
1989	12	2
1990	1	1
1990	2	12
1990	3	6
1990	4	14
1990	5	11
1990	6	11
1990	7	15
1990	8	19
1990	9	15
1990	10	17
1990	11	16
1990	12	13
1991	1	15
1991	2	15
1991	3	16
1991	4	14
1991	5	14
1991	6	12
1991	7	12
1991	8	16
1991	9	13
1991	10	18
1991	11	17
1991	12	19
1992	1	16

Morphometrics and Histology

Not all histology slides were processed for stage frequency. Some sections were too fragmented or of poor quality, and were skipped.

```
## first step is to summarise the data in each transect
g3sfdat <- g3.sf %>% filter(qarea > 0) %>%
```

```
  group_by(abalone_id) %>%
  summarise(vits = sum(vit, na.rm=T),
            previts = sum(previt, na.rm=T),
            sum_area = sum(qarea, na.rm=T),
            ntrans = n())

## Add summarised stage frequency data to histo and morphometric data
stagedat <- left_join(histodat, g3sfdat ) %>%
 ungroup()
```

```
Joining, by = "abalone_id"
```

```
## add month.yr variable to dataframe
stagedat <- stagedat %>%
 mutate(sample_month = lubridate::month(sample_date, label = TRUE, abbr = TRUE),
        sample_year = year(sample_date),
        month.yr = interaction(sample_month, sample_year))

## summarise the stagedat dataframe for each gonad stage recorded in each month.yr
stagedat.summ <- stagedat %>%
 group_by(sample_year, sample_month, month.yr) %>%
 summarise_each(funs(mean), pc_rec_dev:pc_necrotic)
```

```
Warning: funs() is soft deprecated as of dplyr 0.8.0
please use list() instead

  # Before:
  funs(name = f(.))

  # After:
  list(name = ~ f(.))
This warning is displayed once per session.
```

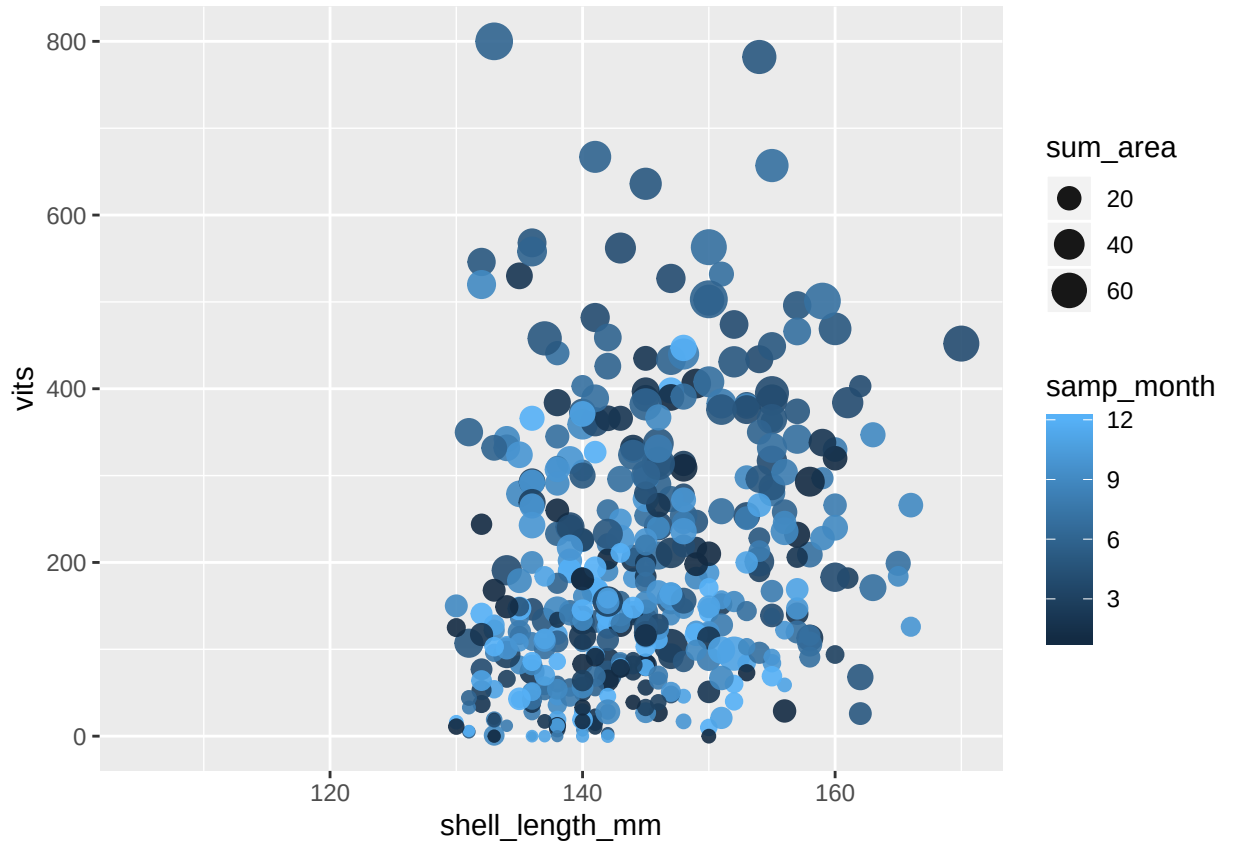
```
## ensure month.yr is a factor
stagedat.summ$month.yr <- as.factor(stagedat.summ$month.yr)

## transform the stagedat summary from wide to long format
stagedat.summ.long <- melt(stagedat.summ, id.vars = c('sample_year', 'sample_month', 'month.yr'))
```

Simple stage frequency plots

Some basic plots of the relationship between shell length and the number of vits and previts, colour coded by month, and with a bubble size set by the section area sampled.

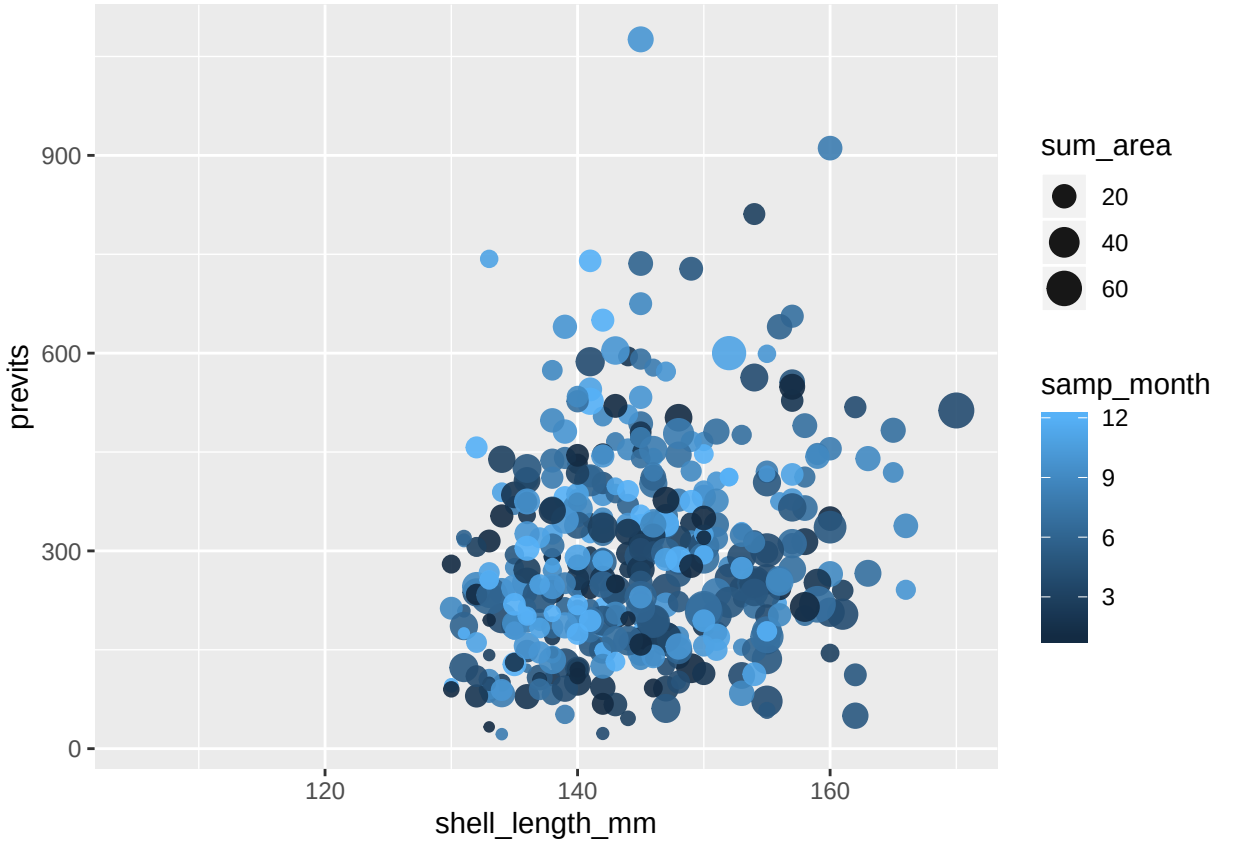
```
stagedat %>% ggplot(aes(x=shell_length_mm, y=vits, colour=samp_month, size = sum_area)) +
geom_point(alpha = 0.9)
```

```
Warning: Removed 138 rows containing missing values (geom_point).
```




```
stagedat %>% ggplot(aes(x=shell_length_mm, y=previts, colour=samp_month, size = sum_area)) +
geom_point(alpha = 0.9)
```

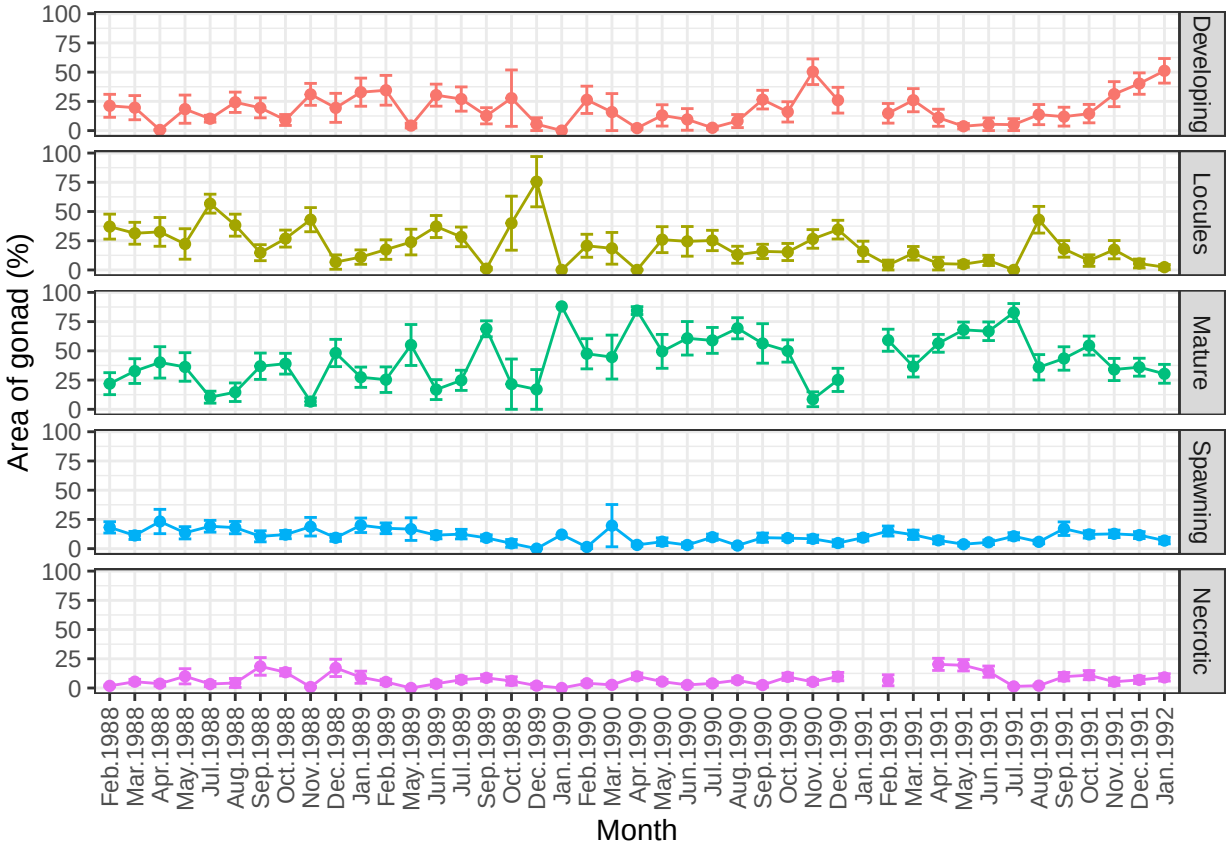
```
Warning: Removed 138 rows containing missing values (geom_point).
```



Plot mean area of gonad for each gonad stage for month and year with error bars

```
## select gonad pc stage data and convert to long format
stagedat.1 <- stagedat %>%
 select(abalone_id,sample_year, sample_month, month.yr,
        pc_rec_dev, pc_locules, pc_mature, pc_spawning, pc_necrotic) %>%
 melt(id.vars = c('abalone_id', 'sample_year', 'sample_month', 'month.yr'))

## summarise gonad pc stage data for mean and se
stagedat.summ.long.1 <- stagedat.1 %>%
 group_by(sample_year, sample_month, month.yr, variable) %>%
 summarise(mean.pc = mean(value),
           se.pc = stderr(value))

## change the variable names for plotting in facet grid
levels(stagedat.summ.long.1$variable) <- c('Developing', 'Locules', 'Mature', 'Spawning', 'Necrotic')

## plot mean for each gonad stage for month and year including error bars
stagedat.summ.long.1 %>%
 ggplot(aes(x = month.yr, y = mean.pc, colour = variable, group = 1)) +
 geom_point()+
 geom_line() +
 geom_errorbar(aes(ymin = mean.pc - se.pc, ymax = mean.pc + se.pc), width = 0.5)+
```

```
facet_grid(variable ~ .) +
ylab('Area of gonad (%)') +
xlab('Month') +
theme_bw() +
theme(legend.position = 'none')+
theme(axis.text.x = element_text(angle = 90, hjust = 1, vjust = 0.5))
```



No clear seasonal pattern in gonad state that would indicate a defined or consistent spawning period between years.

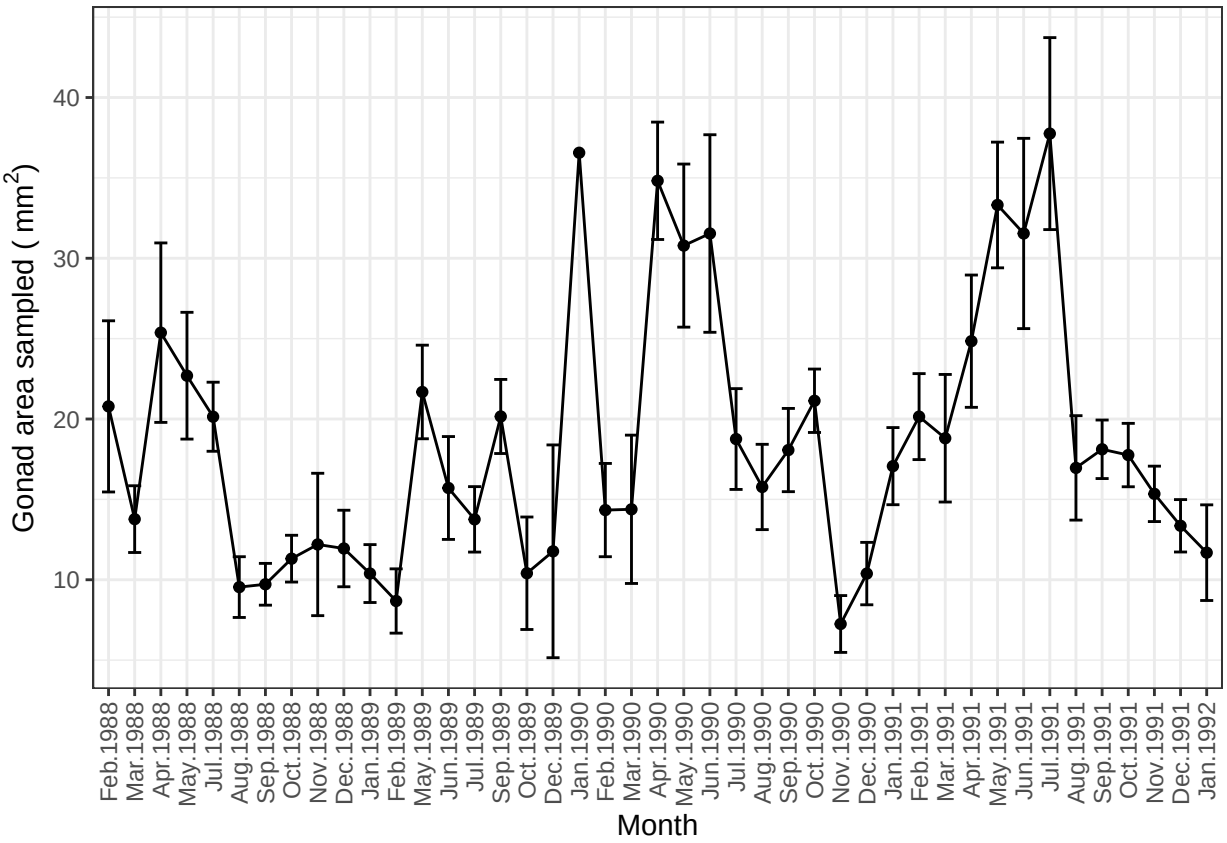
Spawning or necrotic gonad tissue was uncommon (i.e. <25 %) and remained relatively stable across the entire sampling period with no evident peaks to indicate a spawning event had recently occured.

Pre-mature and mature gonad tissue were generally more common (25-50%) however fluctuated between months and displayed no clear seasonal pattern throughout the sampling period. Some evidence of less mature tissue between October to December of 1989 and 1990 following more elevated levels in the months prior (June to August).

Interestingly, there appears to be some inverse relationship between mature and locule tissue between October and December of 1989 and to a lesser extent in the same months of 1990 and 1991. These patterns may be relative of a recent partial spawning event and the start of developing new folucules in the gonad, however the absence of a relationship between more reproductive gonad tissue (i.e. elevated presence of spawning or necrotic tissue) woud suggest that a major spawning event was unlikely to have occured.

Plot mean area of gonad sampled with error bars

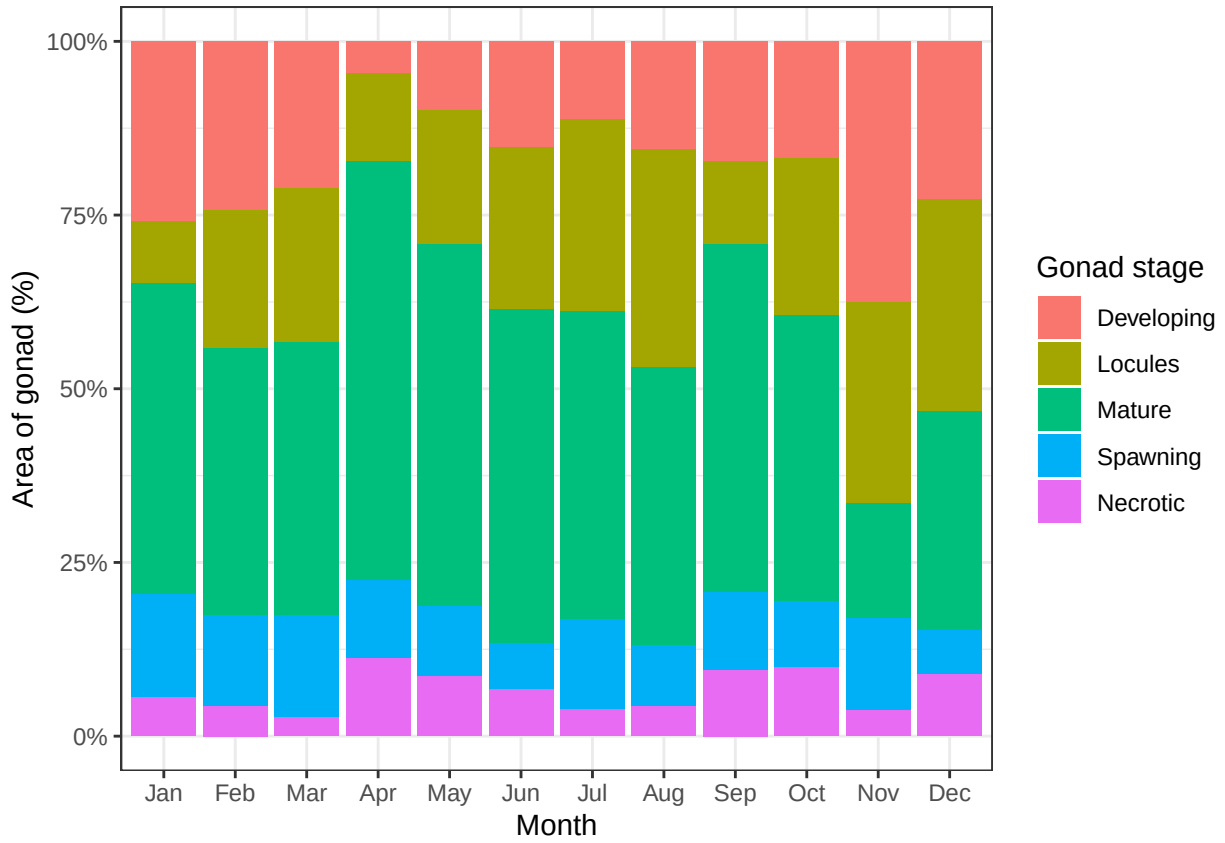
```
## select gonad area sampled data and convert to long format
nadarea.dat <- stagedat %>%
 select(abalone_id,sample_year, sample_month, month.yr,
        sum_area) %>%
 melt(id.vars = c('abalone_id', 'sample_year', 'sample_month', 'month.yr'))

## summarise gonad area sampled data for mean and se
nadarea.dat.summ <- nadarea.dat %>%
  filter(!is.na(value)) %>%
 group_by(sample_year, sample_month, month.yr, variable) %>%
 summarise(mean.size = mean(value),
           se.size = stderr(value))

## plot mean gonad area sampled for month and year including error bars
nadarea.dat.summ %>%
 ggplot(aes(x = month.yr, y = mean.size, group = 1)) +
 geom_point()+
 geom_line() +
 geom_errorbar(aes(ymin = mean.size - se.size, ymax = mean.size + se.size), width = 0.5)+
 ylab(bquote('Gonad area sampled ('*~mm^2*')')) +
 xlab('Month') +
 theme_bw() +
 theme(legend.position = 'none')+
 theme(axis.text.x = element_text(angle = 90, hjust = 1, vjust = 0.5))
```



Assuming the gonad area sampled in the sum of the area of the four cross-section quandrants. Therefore a larger area sampled would suggests a larger proportion of reproductive gonad tissue in cross-section relative to digestive gland. The results indicate a clear seasonal trend in cross-sectional gonad area are present across all years, peaking from March to Jun/July (Autumn) and declining through winter leading into summer months. These peaks are particualry evident from March to Jun/July in 1990 and 1991 suggesting increased reproductive activity likely occurs during autumn.

Plot mean pc of each gonad stage in each month (all years)

```
stagedat.summ.long.1 %>%
 ggplot(aes(x = sample_month, y = mean.pc, fill = variable)) +
 geom_bar(position = 'fill', stat = 'identity') +
 scale_y_continuous(labels = scales::percent_format()) +
 ylab('Area of gonad (%)') +
 xlab('Month') +
  scale_fill_discrete(name = 'Gonad stage', labels = c('Developing', 'Locules', 'Mature', 'Spawning', '
  theme_bw()
```



Pooling the gonad state data across years for each month indicates that developing and locules gonad tissue is most common through the warmer months, October to March. From April to July, developing tissue falls below 20% and is replaced with a higher proportion of more reproductively active tissue, particualry in April.

plot mean pc of each gonand stage in each yr.month

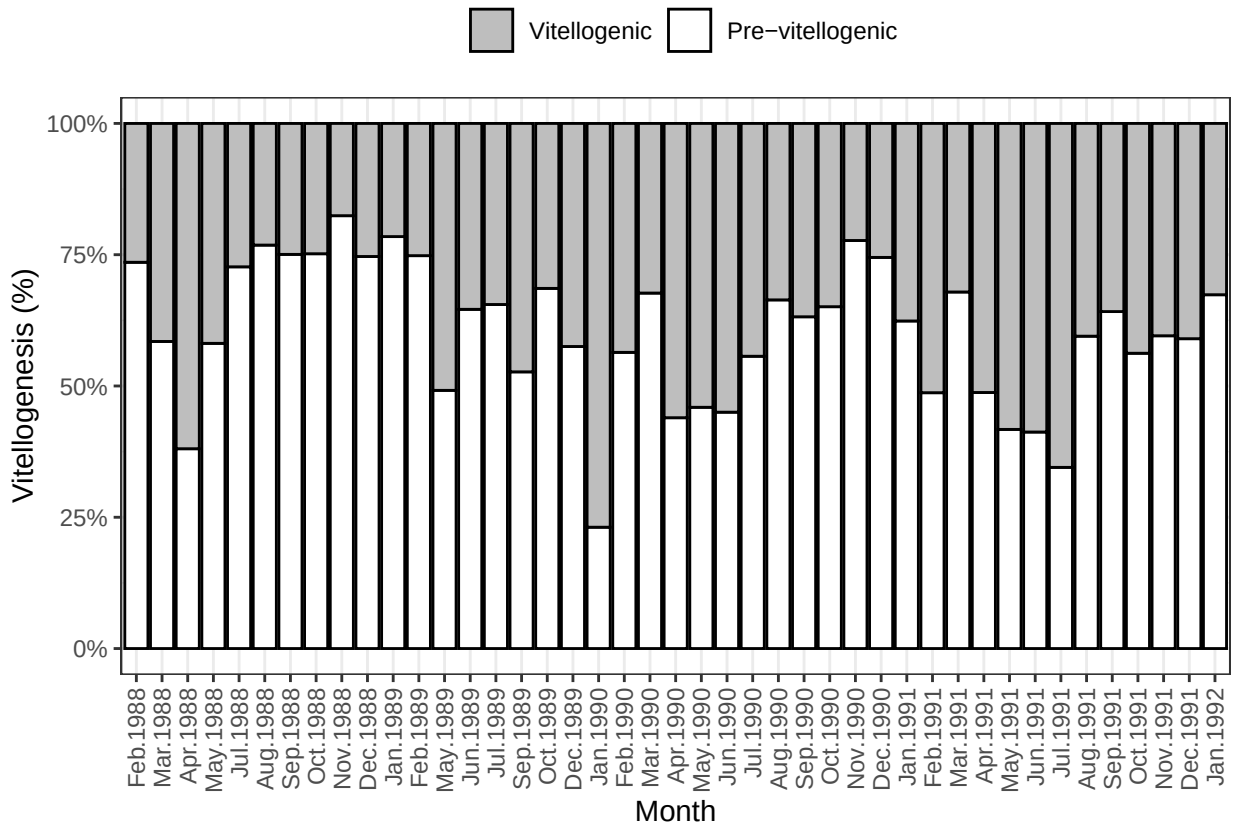
```
stagedat.summ.long.1 %>%
 ggplot(aes(x = month.yr, y = mean.pc, fill = variable)) +
 geom_bar(position = 'fill', stat = 'identity') +
 scale_y_continuous(labels = scales::percent_format()) +
 ylab('Area of gonad (%)') +
 xlab('Month') +
  scale_fill_discrete(name = 'Gonad stage', labels = c('Developing', 'Locules', 'Mature', 'Spawning', '
  theme_bw() +
  theme(axis.text.x = element_text(angle = 90, hjust = 1, vjust = 0.5))
```

Splitting the gonad state data into year and month reveals some seasonal pattern particualry in 1990 and 1991. In both years developing and locules tissue is more apparent in the warmer months of October to March, compared to autumn (April to June) where mature and necrotic tissue is more common. These data suggest peak spawning occurs during these cooling months of April to June.

plot mean pc of vitellogenesis stage in each yr.month

```
# select vitellegenic data and convert to long format
vitsdat <- stagedat %>%
        select(abalone_id,sample_year, sample_month, month.yr,
               vits, previts) %>%
        melt(id.vars = c('abalone_id', 'sample_year', 'sample_month', 'month.yr'))

# summarise vitellegenic data for mean and se
vitsdat.summ <- vitsdat %>%
        filter(!is.na(value)) %>%
        group_by(sample_year, sample_month, month.yr, variable) %>%
        summarise(mean.vit = mean(value),
                  se.vit = stderr(value))

vitsdat.summ %>%
        ggplot(aes(x = month.yr, y = mean.vit, fill = variable)) +
        geom_bar(position = 'fill', stat = 'identity', colour = 'black') +
        scale_y_continuous(labels = scales::percent_format()) +
        ylab('Vitellogenesis (%)') +
```

```
        xlab('Month') +
        theme_bw() +
        theme(axis.text.x = element_text(angle = 90, hjust = 1, vjust = 0.5)) +
        theme(legend.title = element_blank(), legend.position = 'top') +
        scale_fill_manual(values = c('grey', 'white'), labels = c('Vitellogenic', 'Pre-vitellogenic'))
```



Examining the revised gonad staging data also reveals some evidence of a seasonality in condition across years. Typically, vitellogenic tissue progressively increases from November/December onwards, peaking between April and July, and in most cases declines rapidly in the months following.

In January 1990 the sudden peak in vitellogenic tissue seems abnormal compared to other occurances where the peak has typically followed a more gradual increase in vitellogenic tissue in the months prior. Whilst the January 1990 data may represent an abnormal spawning event, in most years the vitellogenesis data would suggest the peak spawning period occurs between April and July.