Repeability of Frog Morphometric Measurements

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Table of contents

Assessing Repeatability of Morphometric Measurements

We want to measure repeatability of our morphometric measurements, both within individual measurers and between measurers. We plan to combine data with that measured by Julio Rivera in 2015, so we want to know that the data are compatible.

The accuracy we are aiming for is 95%, or 5% measurement error (both within and between individual measurers).

Each measurer measured the same 7 frogs (*Hylophorbus sp.* from Buyetai: JR306,308,311,320,321,324), which was repeated (2 sets of measurements per measurer).

Setup

```
require( googlesheets4 )
require(dplyr)
require(magrittr)
require(ggplot2)
require(reshape2)
require(cowplot)

if(dir.exists("output")!=TRUE) dir.create("output") # check if output directory out exists
```

Read in our data from a Google Spreadsheet, and convert measurer, session and jr number to factors.

```
file <- "https://docs.google.com/spreadsheets/d/1-w62GXvKwQ868dwiMVzgPdLYbgSkXxSqasE-YoaGO

gs4_deauth() # not a private sheet, so no need for authentication
alldat <- as.data.frame(read_sheet(file)) %>%  ### INPUT DATA from googlesheet
         filter(!is.na(measurer))           # remove spacer rows
```

```
v Reading from "Frog Repeatability Measurements".

v Range 'Sheet1'.

New names:
* `` -> `...22`
* `` -> `...23`
* `` -> `...24`
* `` -> `...25`
```

```
alldat %<>% mutate_at(c("measurer", "session", "jr"), as.factor) %>%
    mutate( shape = case_when( measurer=="JR" ~ 25, measurer!="JR" ~ 19 )) %>%
    mutate( shape = as.factor(shape))

jrblock <- alldat[alldat$measurer=="JR",]  # copy JR data into all sessions (0,1,2)
jrblock3 <- jrblock2 <- jrblock0 <- jrblock
jrblock0$session <- 0
jrblock2$session <- 2
jrblock3$session <- 3
alldat <- rbind(alldat, jrblock0, jrblock2, jrblock3)
```

Repeatability

This requires two full sets of measurements, so this is still in progress.

```
mod <- with(alldat, summary(aov(lm( svl ~ jr ))))

s2_within <- ms_within <- mod[[1]][2,3]
s2_within
```

```
[1] 0.3153581
```

```
ms_among <- mod[[1]][1,3]
s2_among <- (ms_among-ms_within)/2
ME <- s2_within/(s2_within+s2_among) * 100
ME
```

```
[1] 0.3911961
```

Functions

These function creates the plots that are repeated for each morphometric variable.

```
dat <- alldat %>% filter(session==0)

p <- dat %>% ggplot(aes(svl, femur, color=measurer, label=jr))
v <- dat %>% ggplot(aes(jr, svl, group=jr, color=measurer, shape=measurer))


make_plots <- function(p, v) {
  q1 <- p + geom_point(size = 3) +
    geom_smooth( aes(group=measurer), method="lm", alpha=.1) +
    geom_text(nudge_y = .15) +
    theme_bw()

  q2 <- p + geom_point(size = 3) +
    geom_smooth( method="lm", alpha=.1) +
    geom_text(nudge_y = 1) +
    facet_grid( measurer ~ . ) +
    theme_bw()
```

```
   plot_grid(q1, q2, labels="AUTO")

 }

make_violins <- function(v) {

 v + geom_violin() +
  geom_jitter(aes(x=jr, color=measurer, shape=measurer, size=measurer), width=.2) +
  scale_shape_manual(values=c(19,19,17,19,19,19)) +
  scale_size_manual(values=c(3,3,5,3,3,3))

}
```

Set up the ggplots:

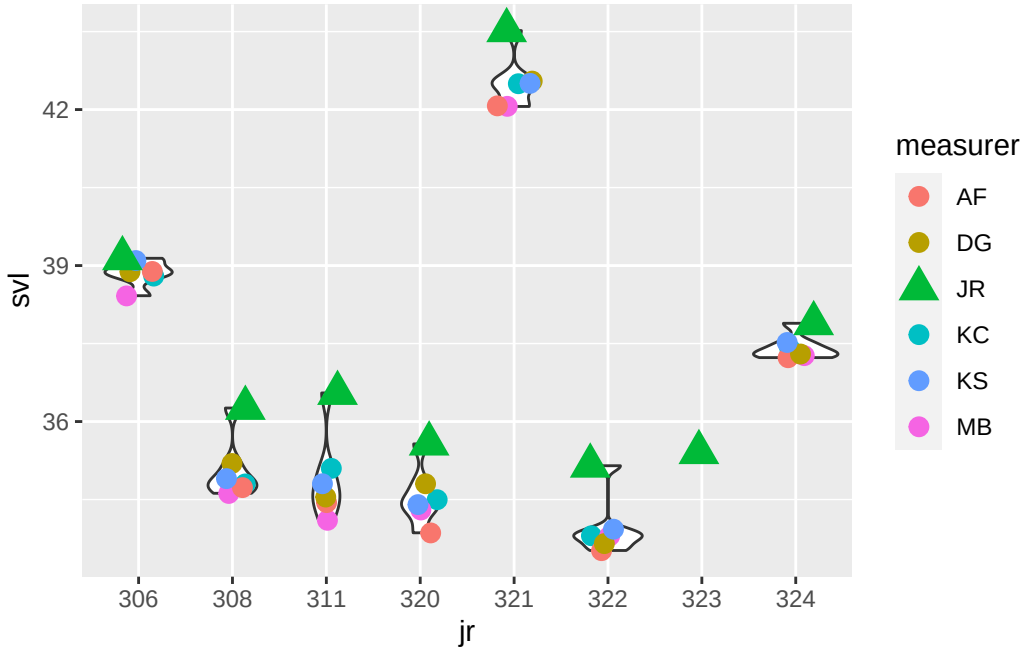
```
dat <- alldat %>% filter(session==0)  # filter just for session 0 (naive)

femp <- dat %>% ggplot(aes(svl, femur, color=measurer, label=jr))
tibp <- dat %>% ggplot(aes(svl, tibiofibula, color=measurer, label=jr))
tarp <- dat %>% ggplot(aes(svl, tarsus, color=measurer, label=jr))
footp <- dat %>% ggplot(aes(svl, foot, color=measurer, label=jr))
hwp <- dat %>% ggplot(aes(svl, headW, color=measurer, label=jr))
hlp <- dat %>% ggplot(aes(svl, headL, color=measurer, label=jr))
hump <- dat %>% ggplot(aes(svl, humerus, color=measurer, label=jr))
radp <- dat %>% ggplot(aes(svl, radioulna, color=measurer, label=jr))
handp <- dat %>% ggplot(aes(svl, hand, color=measurer, label=jr))

sv <- dat %>% ggplot(aes(jr, svl, group=jr))
femv <- dat %>% ggplot(aes(jr, femur, group=jr))
tibv <- dat %>% ggplot(aes(jr, tibiofibula, group=jr))
tarv <- dat %>% ggplot(aes(jr, tarsus, group=jr))
footv <- dat %>% ggplot(aes(jr, foot, group=jr))
hwv <- dat %>% ggplot(aes(jr, headW, group=jr))
hlv <- dat %>% ggplot(aes(jr, headL, group=jr))
humv <- dat %>% ggplot(aes(jr, humerus, group=jr))
radv <- dat %>% ggplot(aes(jr, radioulna, group=jr))
handv <- dat %>% ggplot(aes(jr, hand, group=jr))
```

Validating Landmarks (session 0)

Plot all of our data against the original dataset (JR in green), as well as faceted by individual. The goal here is to validate the landmarks.

SVL

```
make_violins(sv)
```

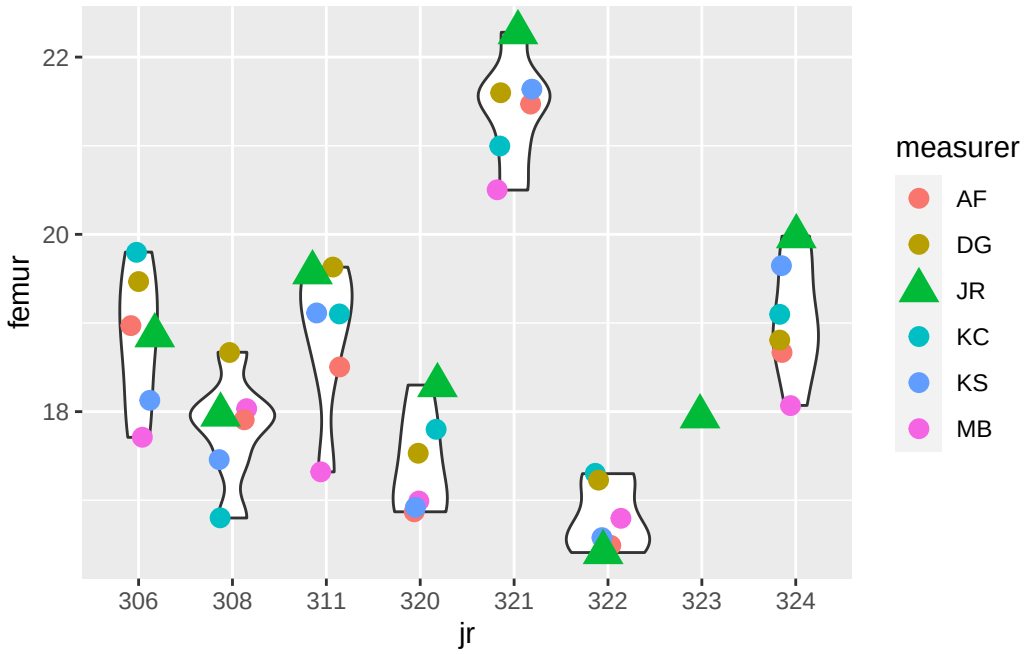


Looks like everyone s SVL are a little short. Basically JR304 and JR306 match well, but the others, JR308, JR311, JR320, JR321, JR322, JR324 are a little short. Did he press them down? Or use a ruler?

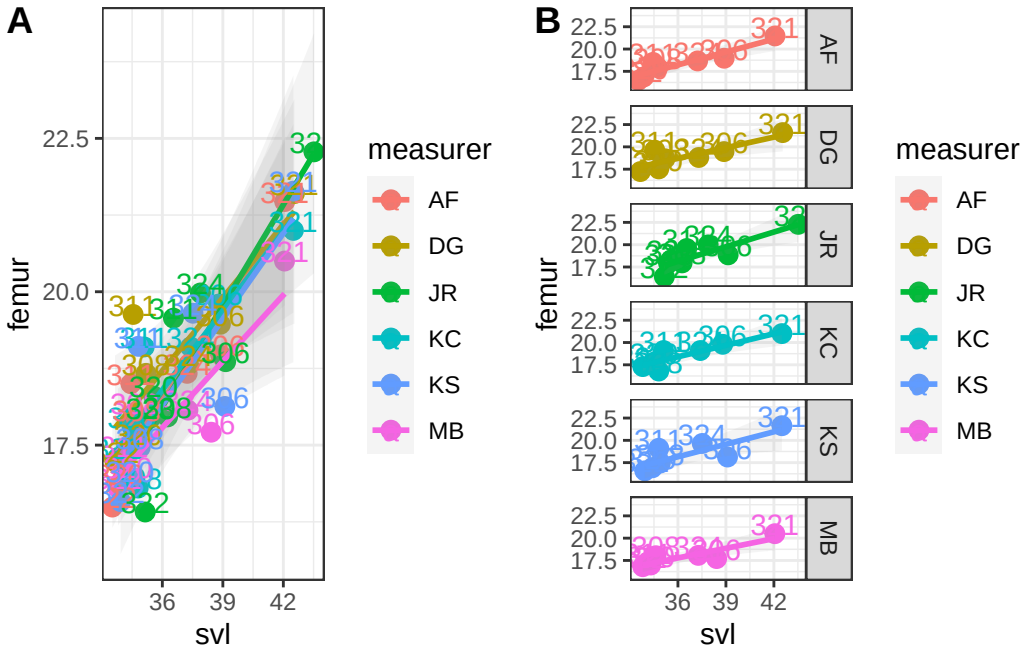
Diana and Ke investigated and found that **gently pressing on the frog** was necessary to reapeat JR s measurements. Just enough to maximize the length.

Femur

```
make_violins(femv)
```




```
make_plots( femp )
```



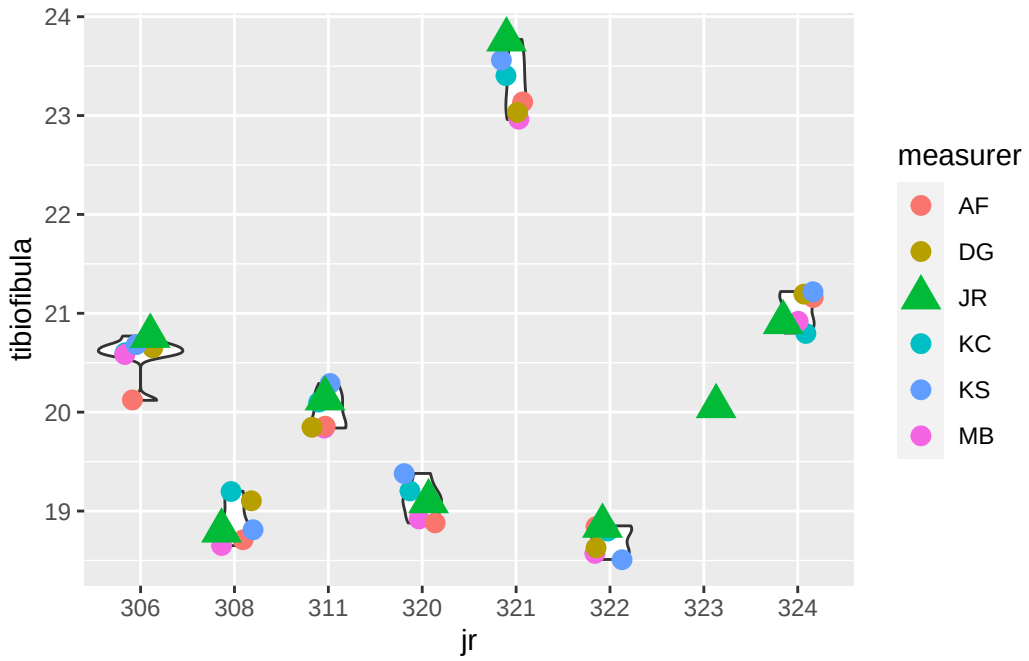
Looks like my femur measurements (MB) are a little short - JR must have measured from the

vent rather than from the midline perpendicular to the femur.

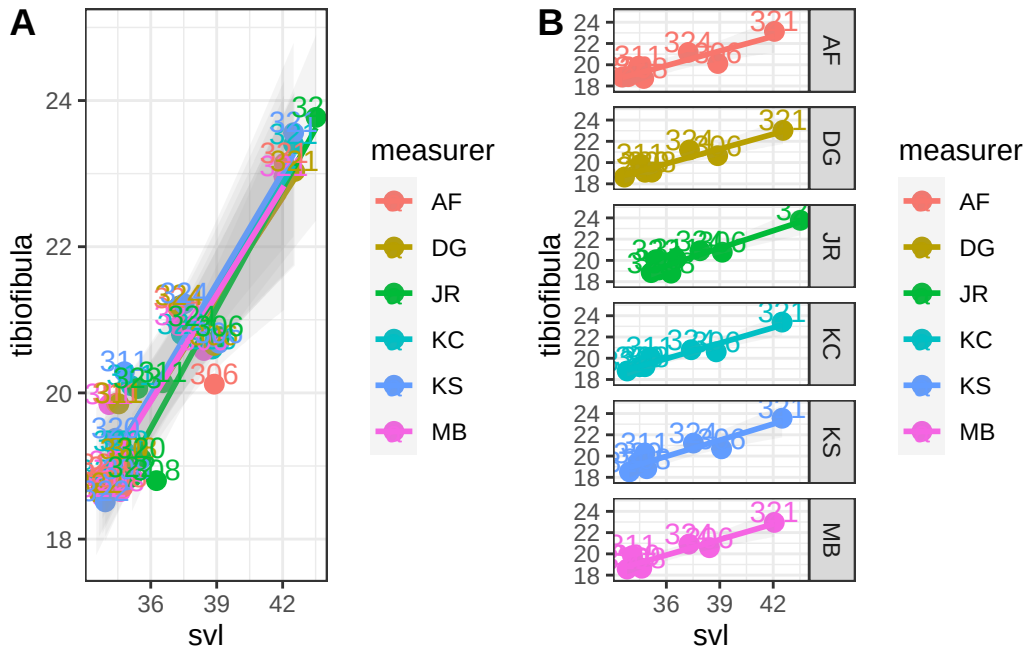
The landmarks are from **the edge of the knee to the midline at the vent**.

Tibiofibula

```
make_violins(tibv)
```




```
make_plots( tibp )
```

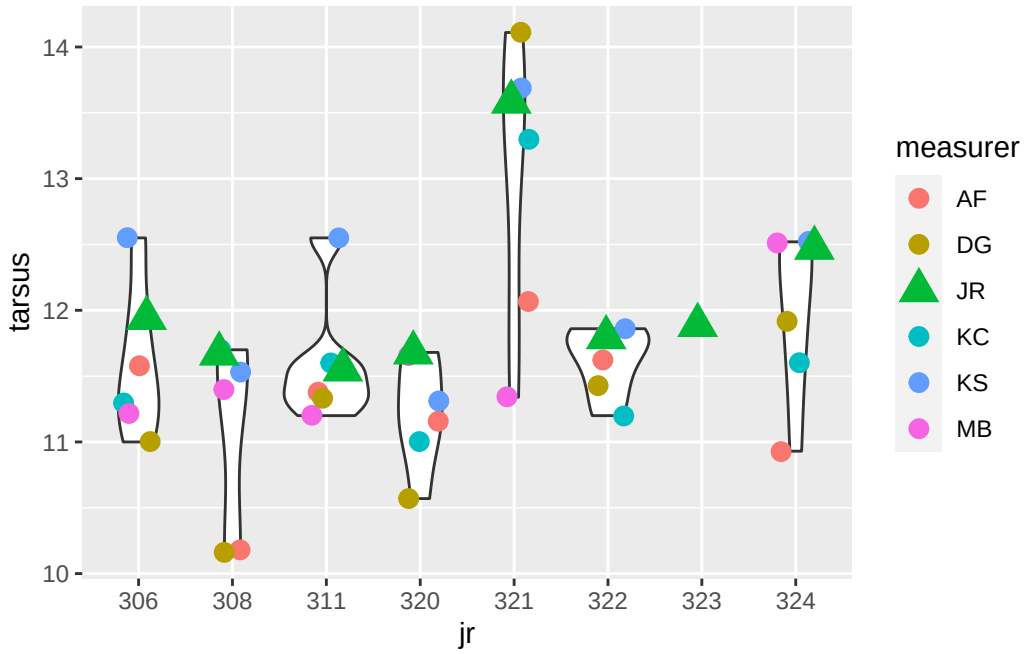


Tibiofibula looks good, except for what looks like a typo in DG s data?

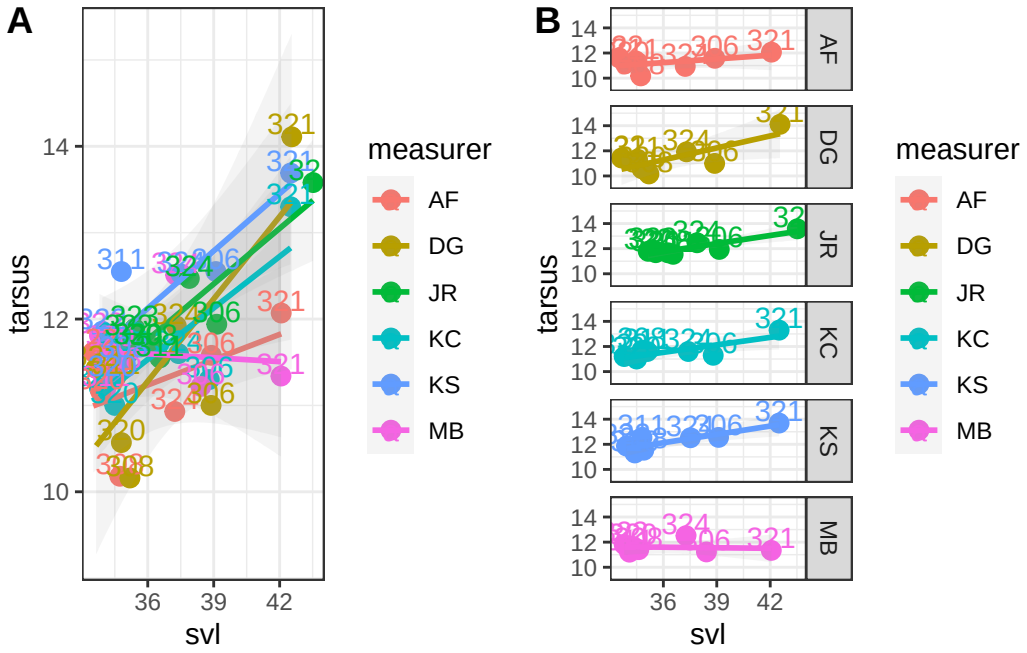
We measured the bone, from end to end.

Tarsus

```
make_violins(tarv)
```




```
make_plots( tarp )
```

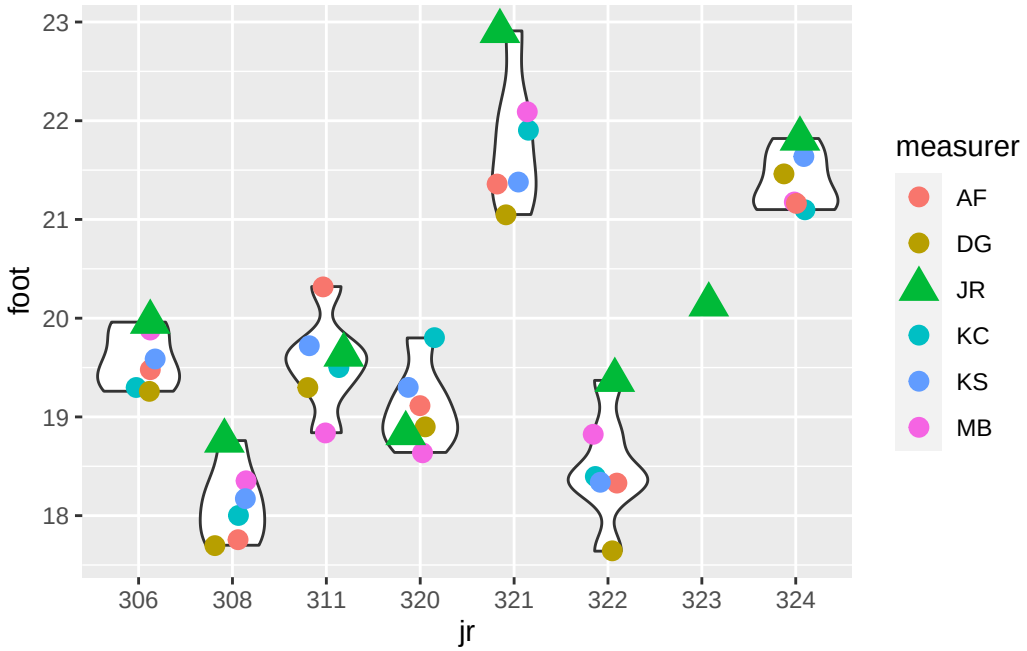


Not sure what I did! Lol. Good example of the unfortunate outlier being the largest throwing

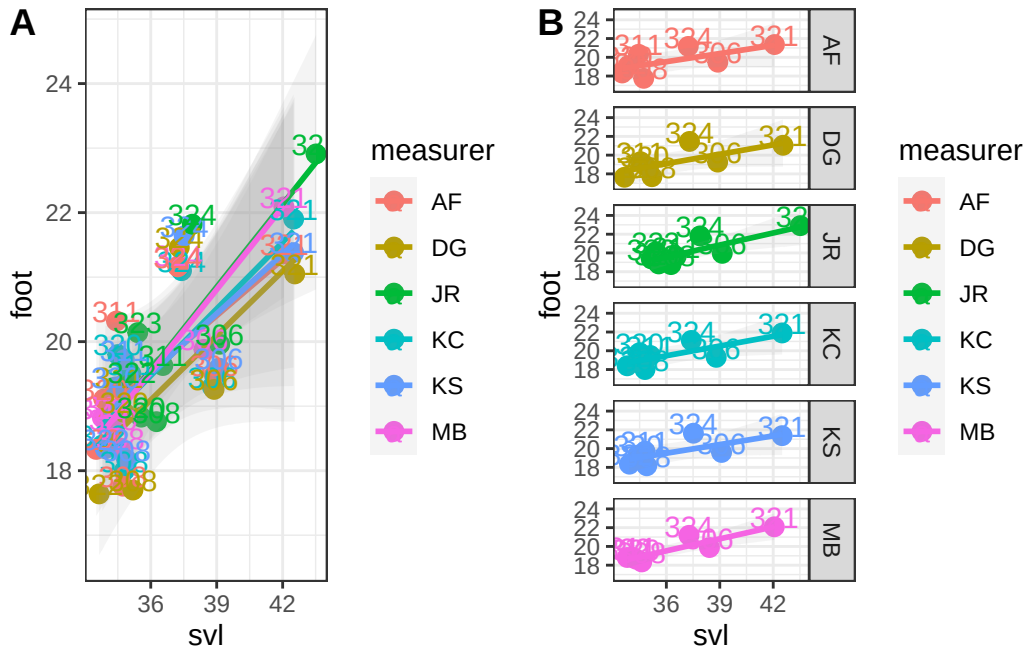
the whole trend off. A few measurers are off on some specimens.

Foot

```
make_violins(footv)
```



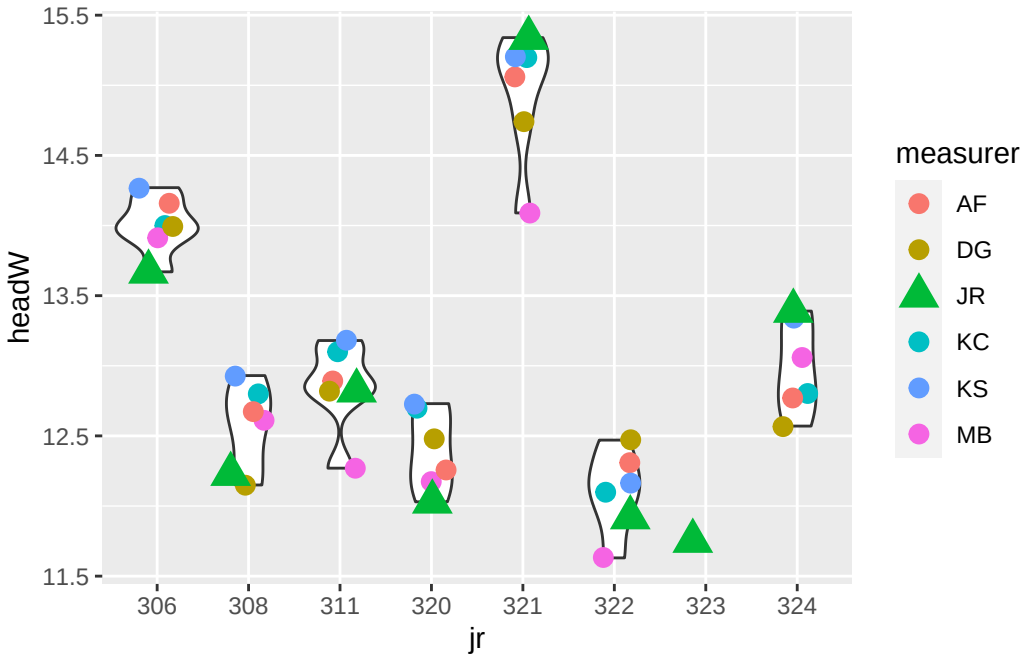

```
make_plots( footp )
```



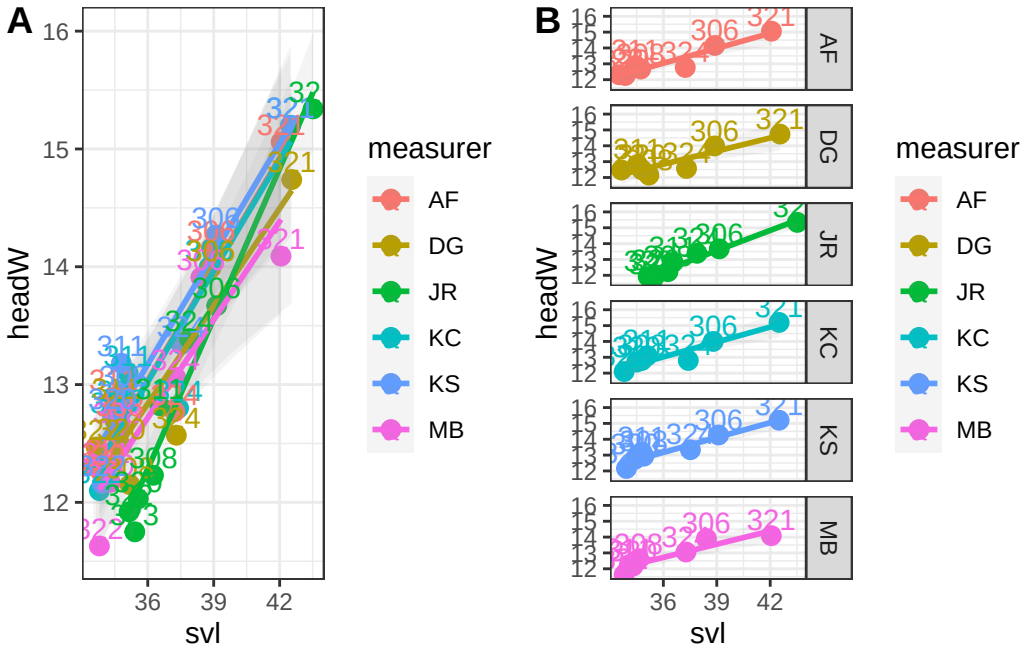
Looks good!

Head Width

```
make_violins(hwv)
```




```
make_plots( hwp )
```

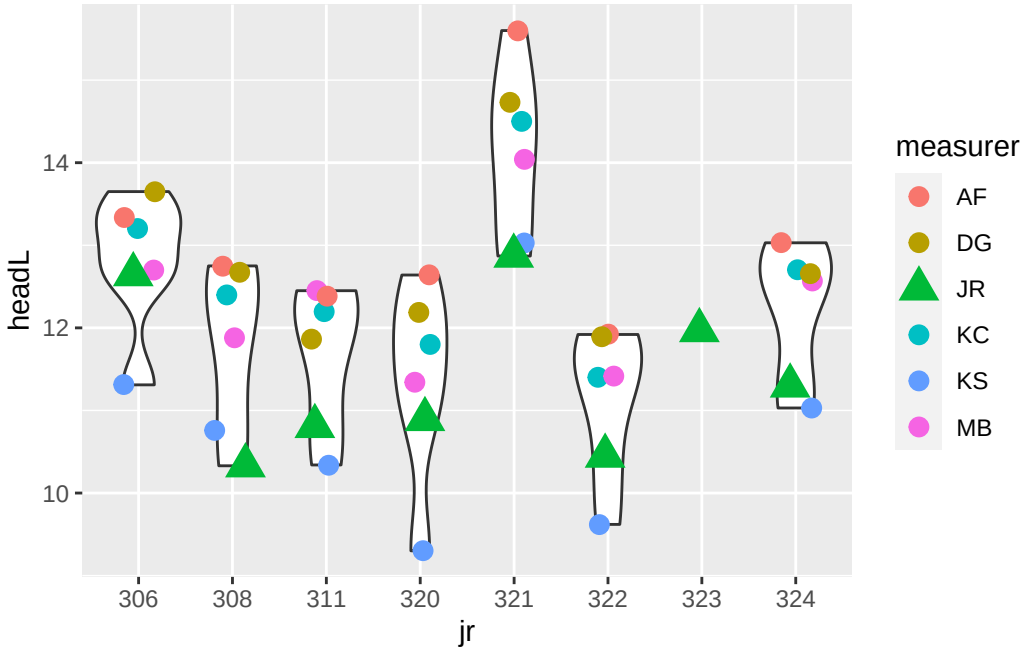


Looks good! Measured at the widest part of the head or at the center of the tympanum (no

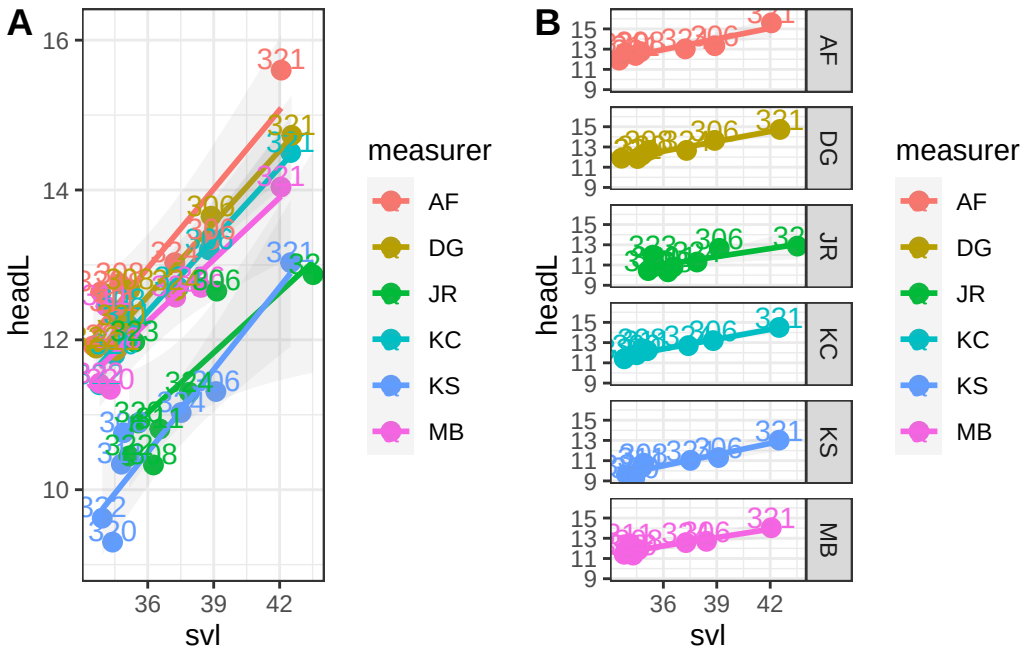
further back than the tympanum).

Head Length

```
make_violins(hlv)
```




```
make_plots( hlp )
```

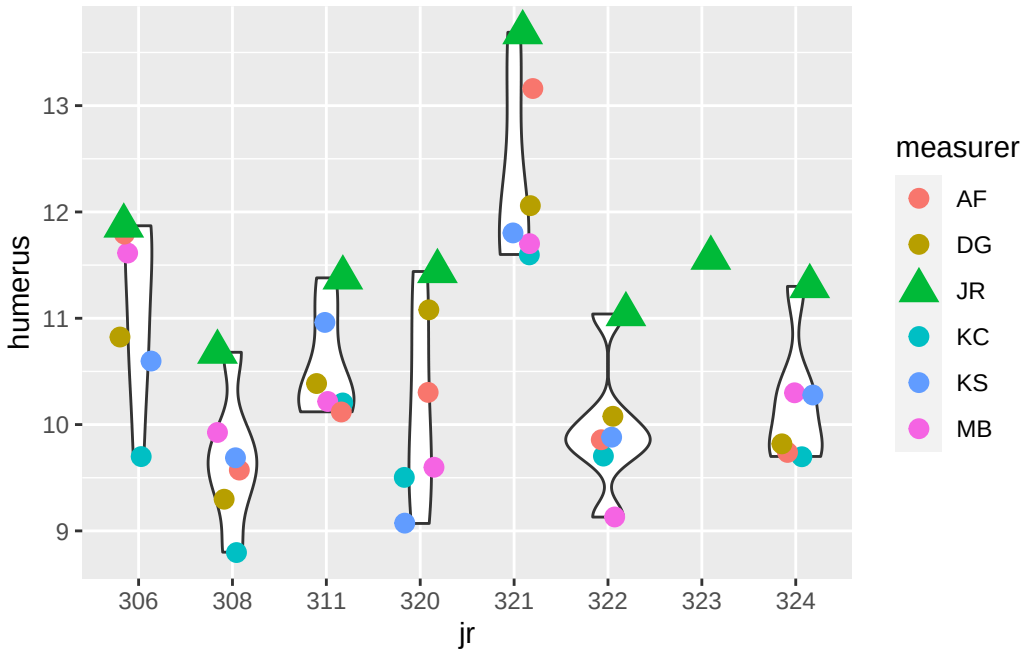


Oh oh. We are not using the right Head Length landmark.

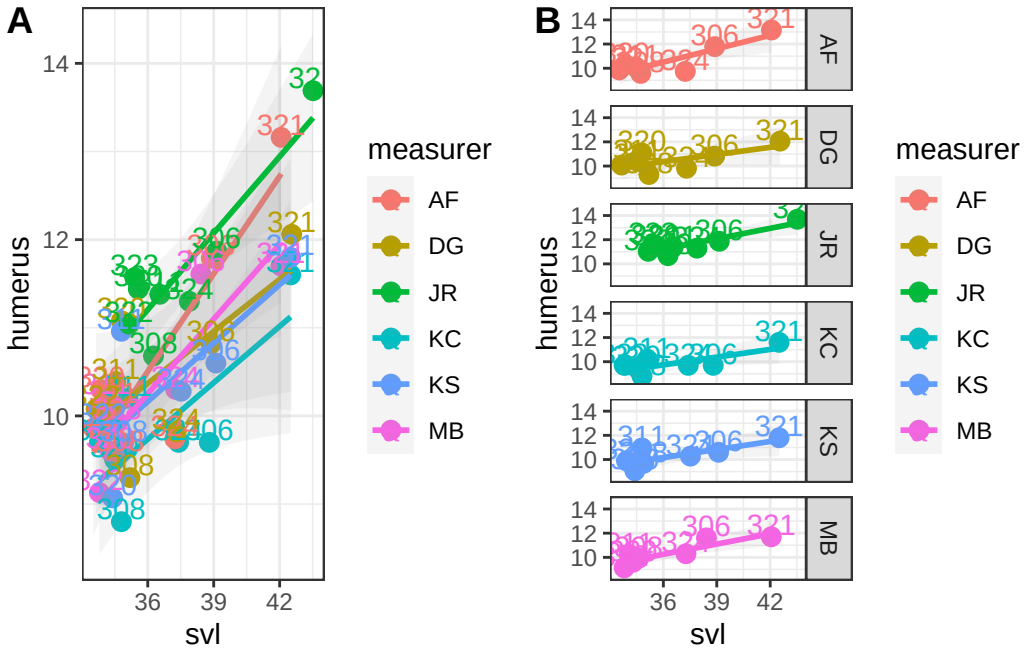
Diana and Ke discovered the the measurement is from the tip of the snout to the center of the tympanum.

Humerus

```
make_violins(humv)
```




```
make_plots( hump )
```

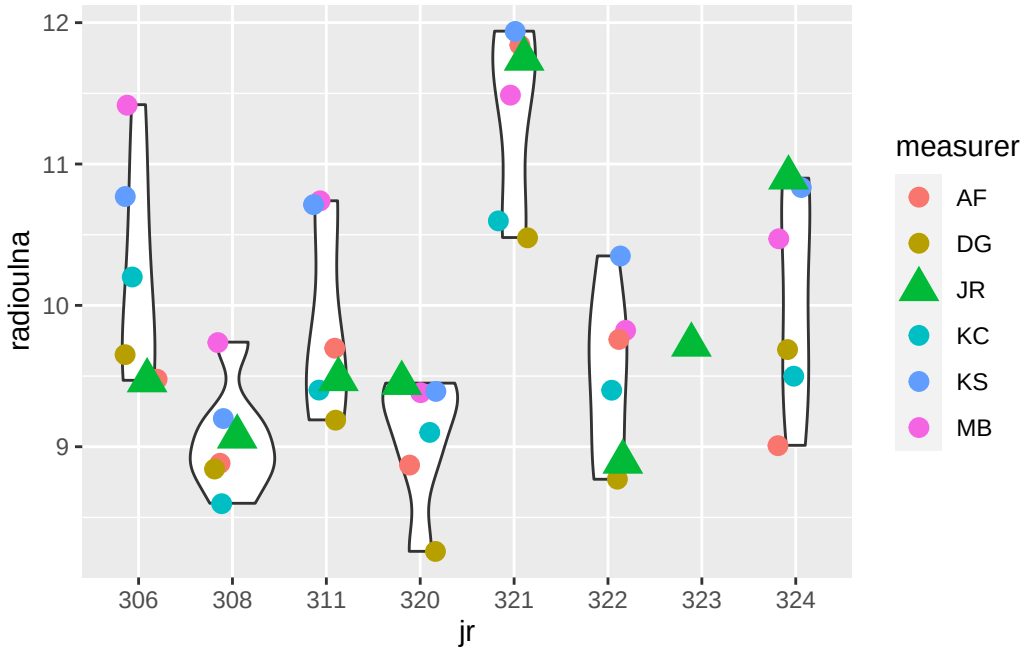


Hmm. We are all a little consistently lower than JR, some more so.

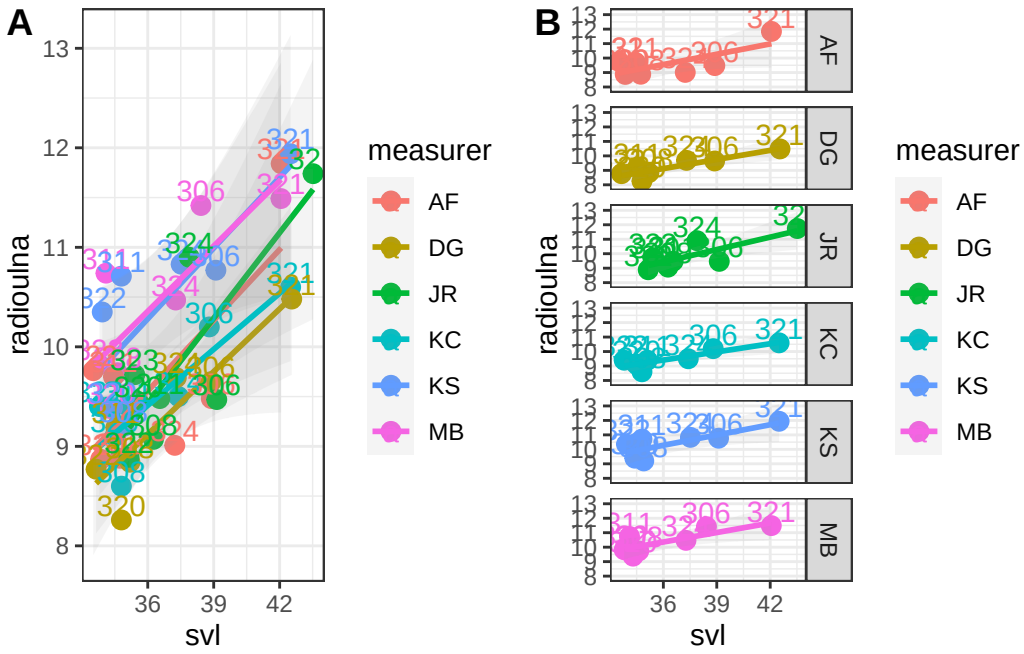
The landmark for the end of the humerus can be most easily found by gently pressing the caliper onto the chest, which will move the arm. It is from **the articulation of the humerus with the scapula to the edge of the elbow**.

Radioulna

```
make_violins(radv)
```



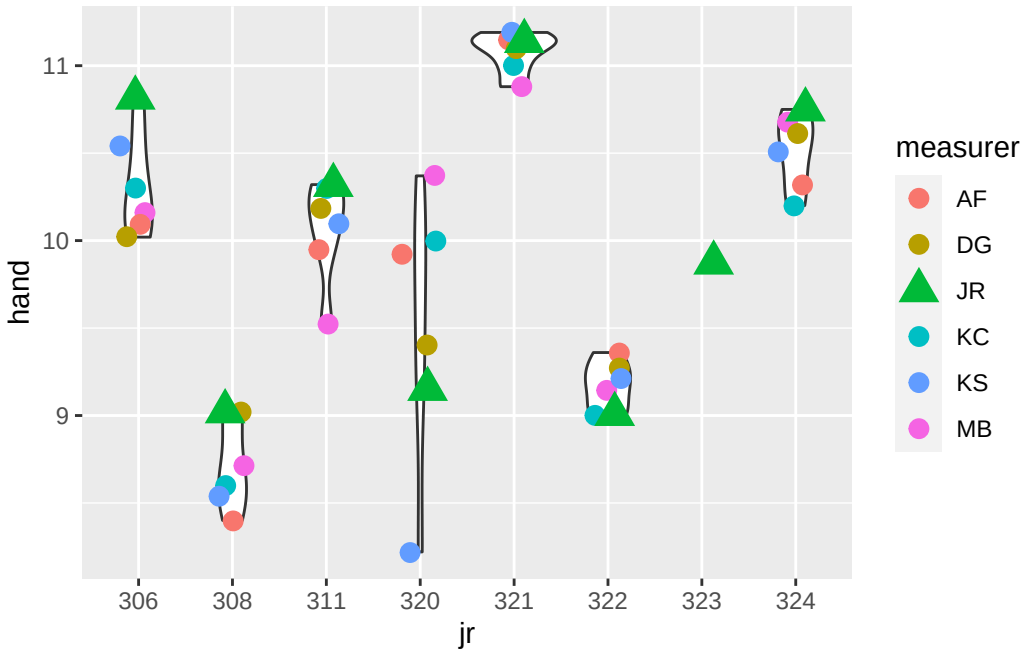

```
make_plots( radp)
```



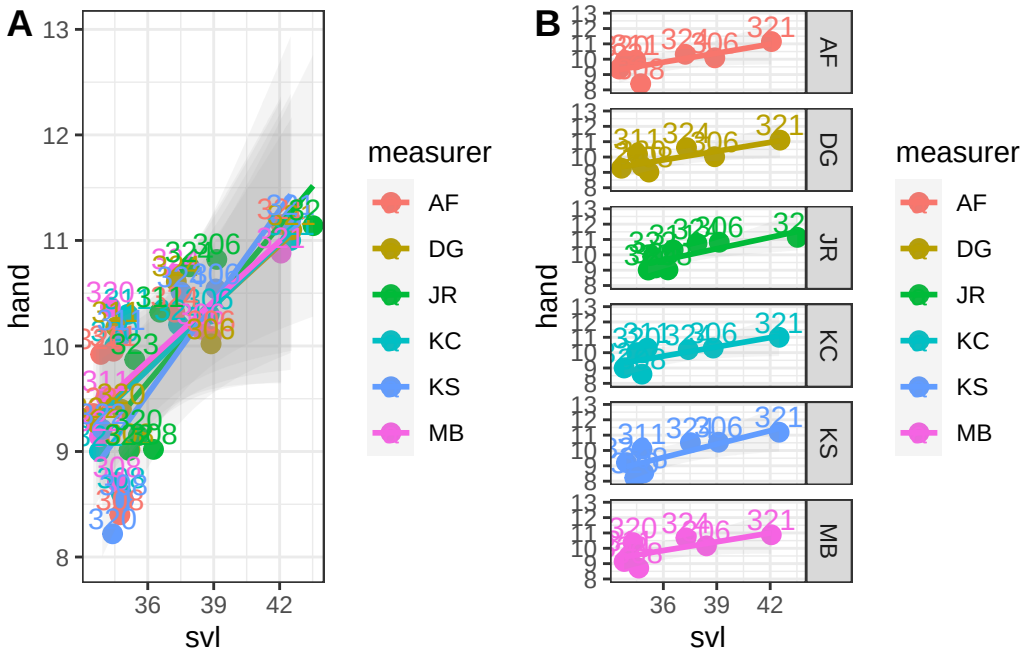
Need to work on this one too.

Hand

```
make_violins(handv)
```




```
make_plots( handp )
```



Looks good!

Conclusions

We need to confirm the landmarks JR used for the limb segment and head length measurements (foot and hand are OK). It looks promising for repeatability but we need to confirm after we get two full sets of measurements.

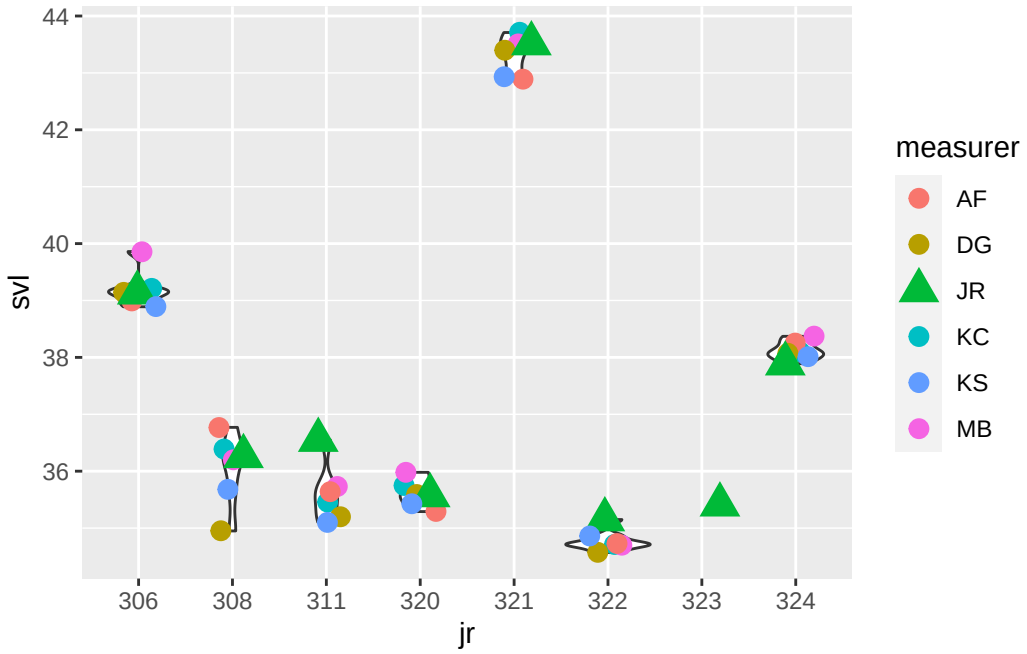
Session 1

Set up the ggplots:

```
dat <- alldat %>% filter(session==1)

femp <- dat %>% ggplot(aes(svl, femur, color=measurer, label=jr))
tibp <- dat %>% ggplot(aes(svl, tibiofibula, color=measurer, label=jr))
tarp <- dat %>% ggplot(aes(svl, tarsus, color=measurer, label=jr))
footp <- dat %>% ggplot(aes(svl, foot, color=measurer, label=jr))
hwp <- dat %>% ggplot(aes(svl, headW, color=measurer, label=jr))
hlp <- dat %>% ggplot(aes(svl, headL, color=measurer, label=jr))
hump <- dat %>% ggplot(aes(svl, humerus, color=measurer, label=jr))
radp <- dat %>% ggplot(aes(svl, radioulna, color=measurer, label=jr))
handp <- dat %>% ggplot(aes(svl, hand, color=measurer, label=jr))

sv <- dat %>% ggplot(aes(jr, svl, group=jr))
femv <- dat %>% ggplot(aes(jr, femur, group=jr))
tibv <- dat %>% ggplot(aes(jr, tibiofibula, group=jr))
tarv <- dat %>% ggplot(aes(jr, tarsus, group=jr))
footv <- dat %>% ggplot(aes(jr, foot, group=jr))
hwv <- dat %>% ggplot(aes(jr, headW, group=jr))
hlv <- dat %>% ggplot(aes(jr, headL, group=jr))
humv <- dat %>% ggplot(aes(jr, humerus, group=jr))
radv <- dat %>% ggplot(aes(jr, radioulna, group=jr))
handv <- dat %>% ggplot(aes(jr, hand, group=jr))
```

SVL

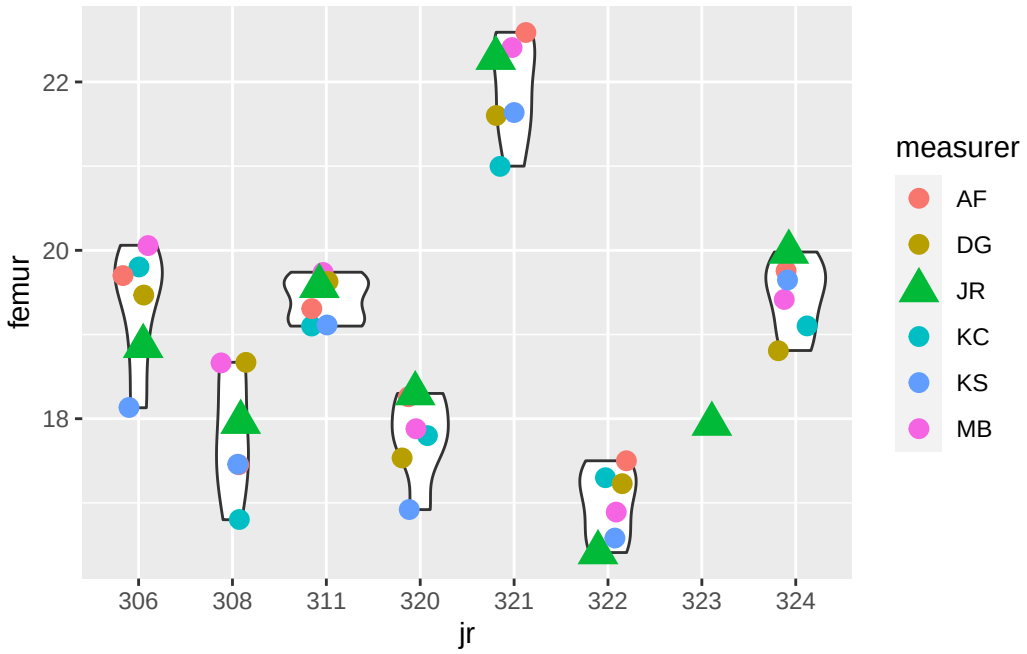
```
make_violins(sv)
```



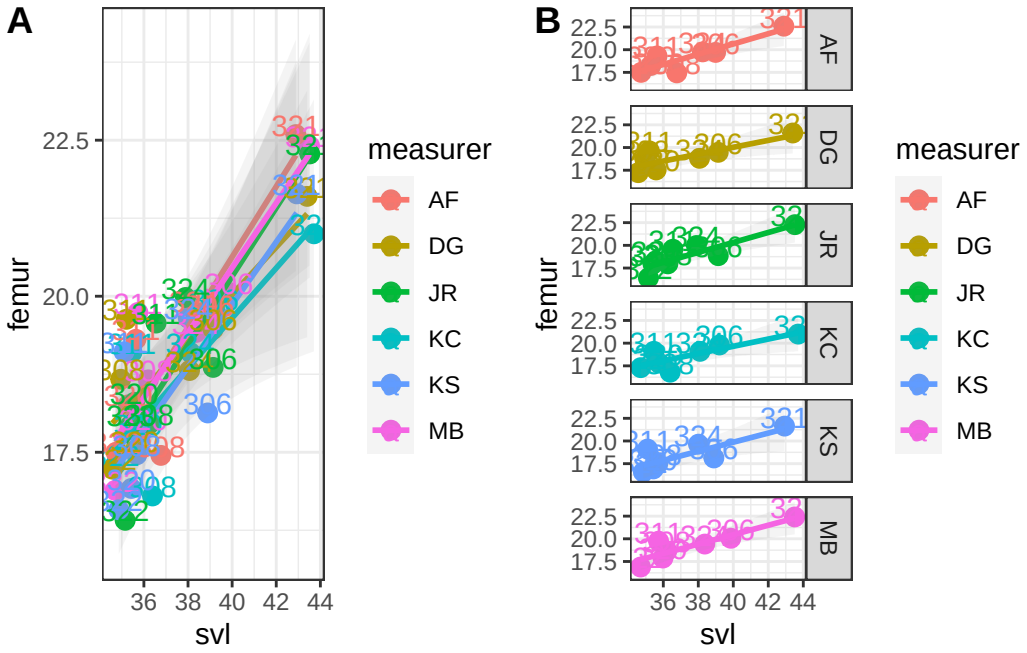
Much better!

Femur

```
make_violins(femv)
```



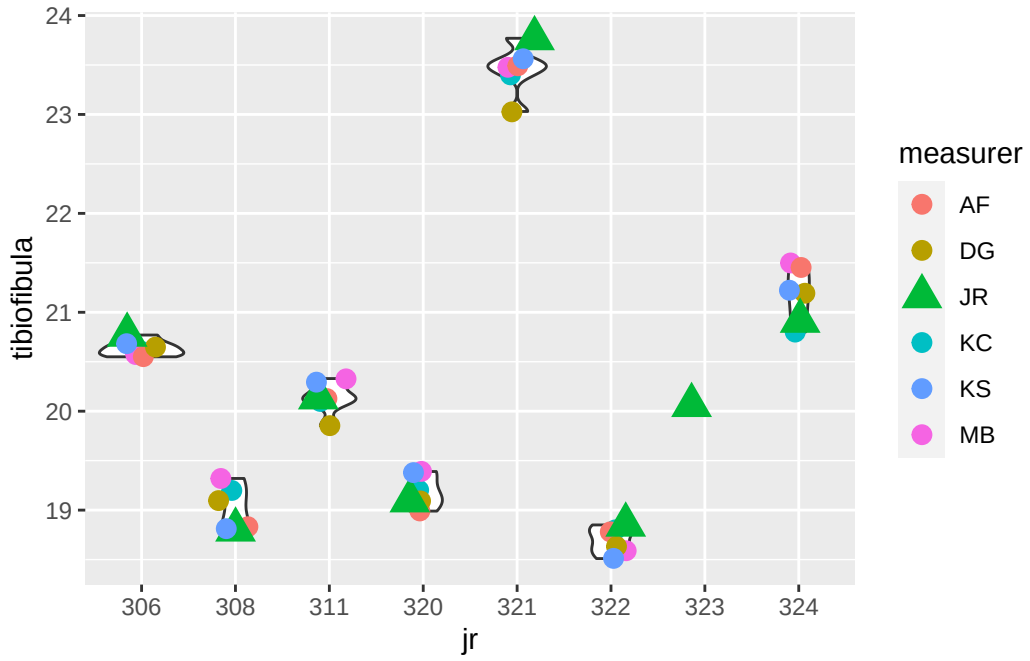

```
make_plots( femp )
```



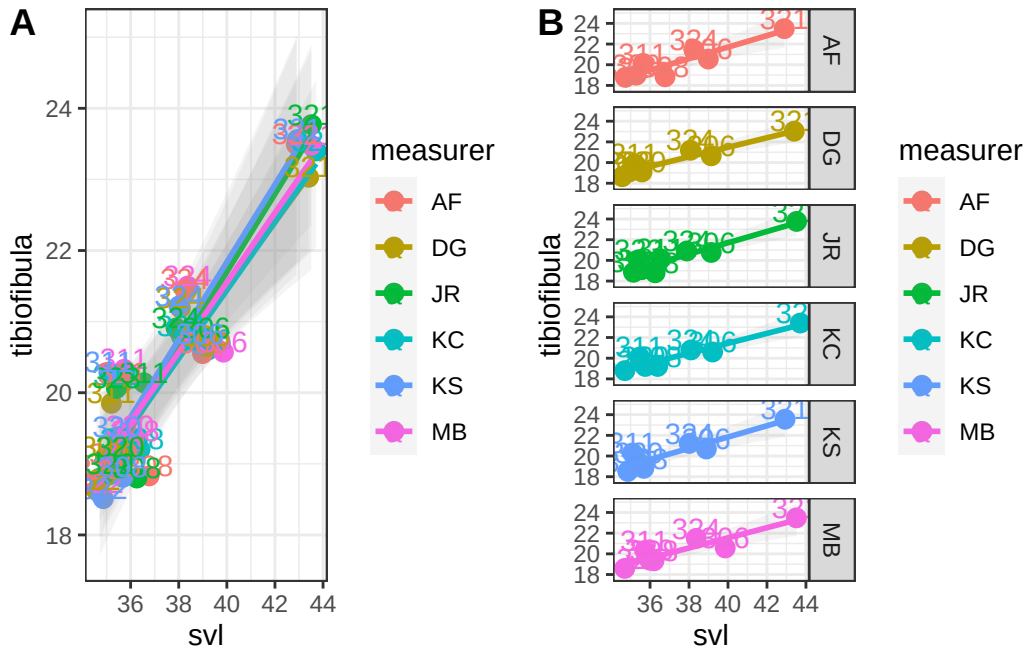
Better!

Tibiofibula

```
make_violins(tibv)
```



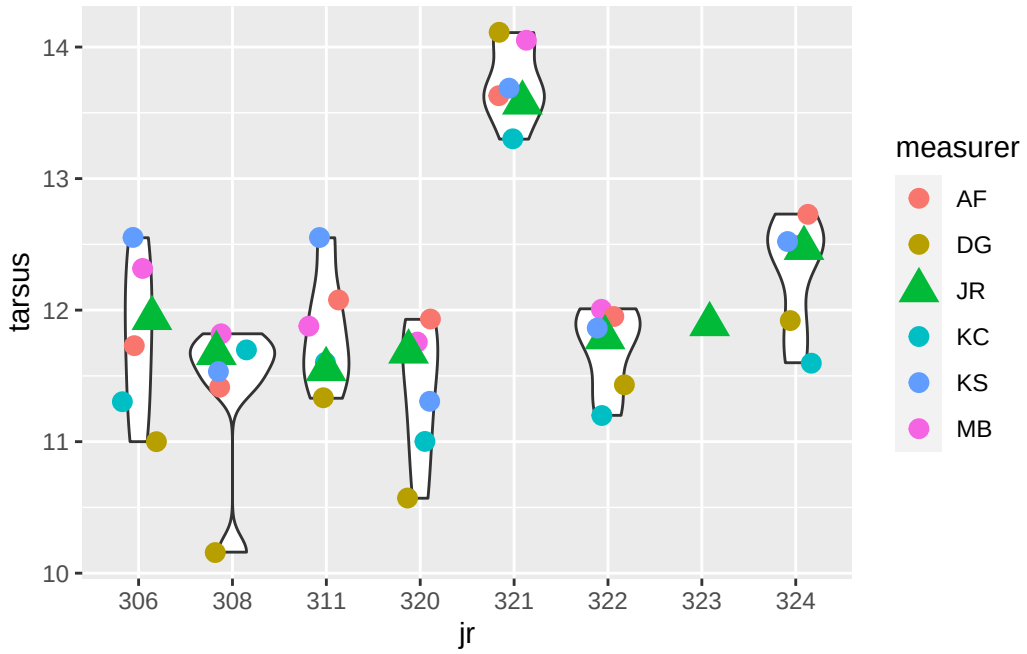

```
make_plots( tibp )
```



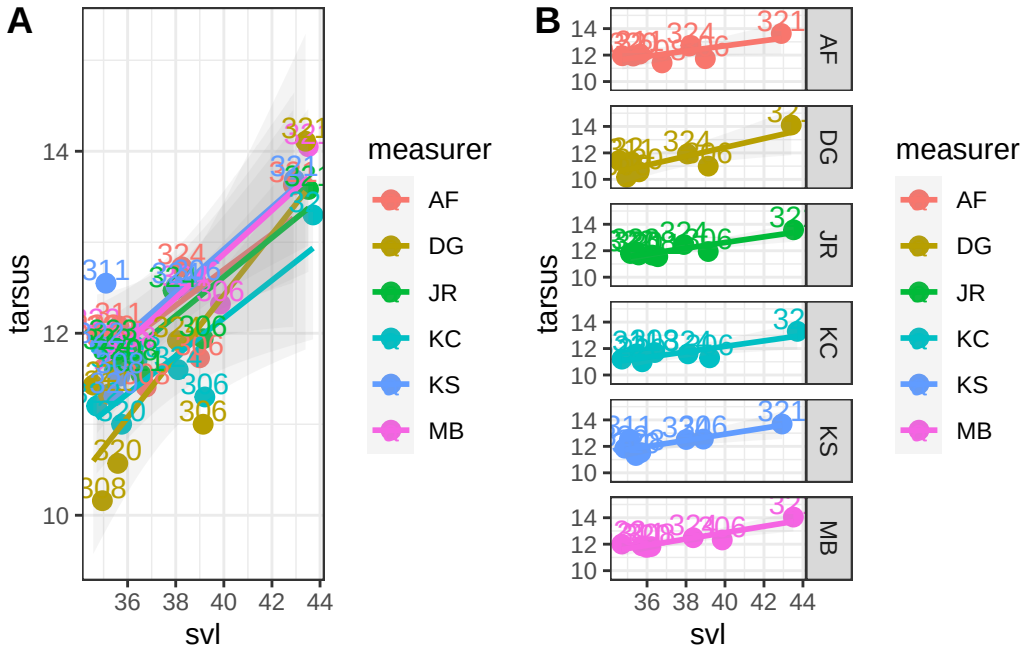
Better!

Tarsus

```
make_violins(tarv)
```



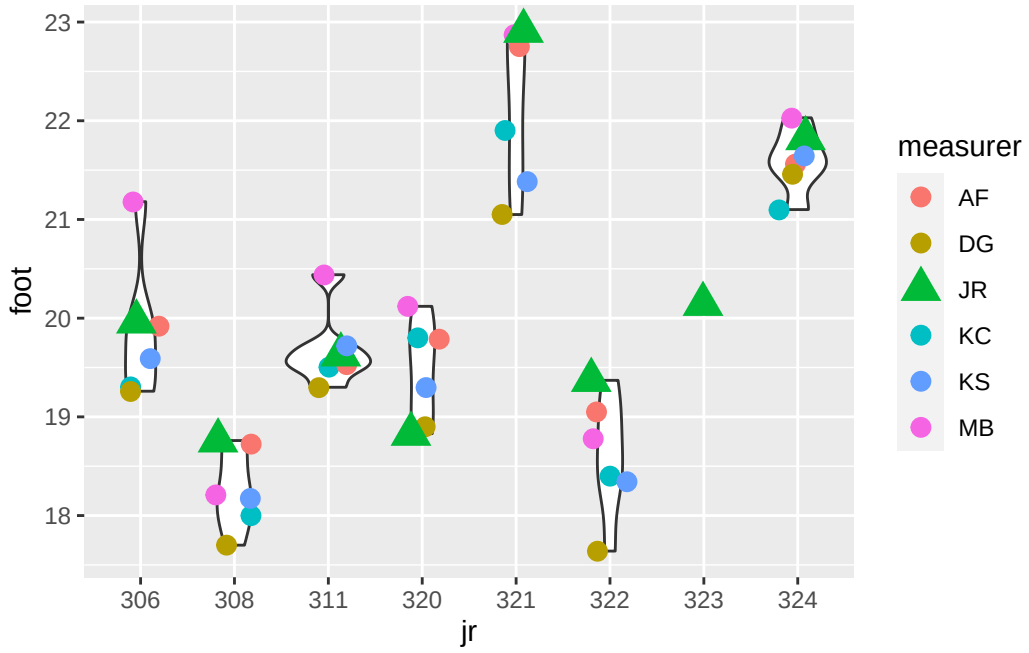

```
make_plots( tarp )
```



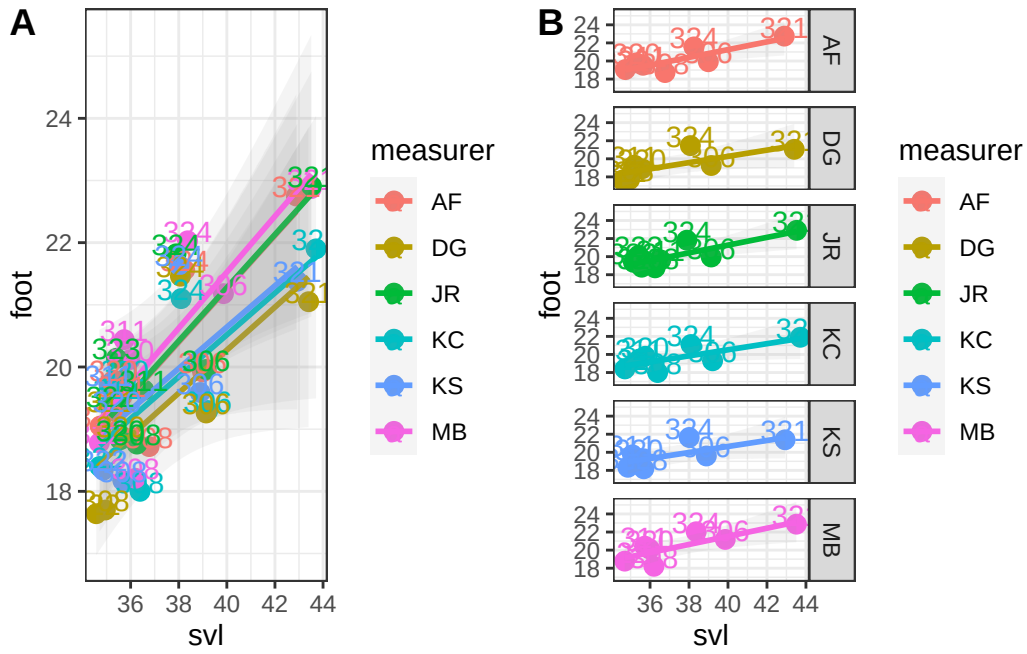
Better but Diana still a bit low and Ke a bit high

Foot

```
make_violins(footv)
```



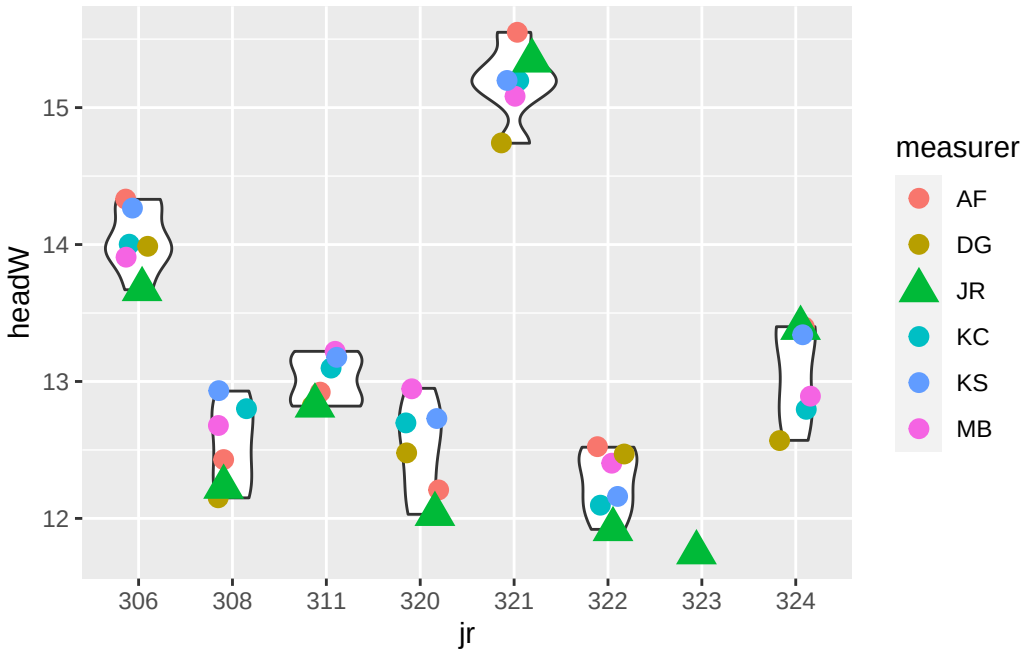

```
make_plots( footp )
```



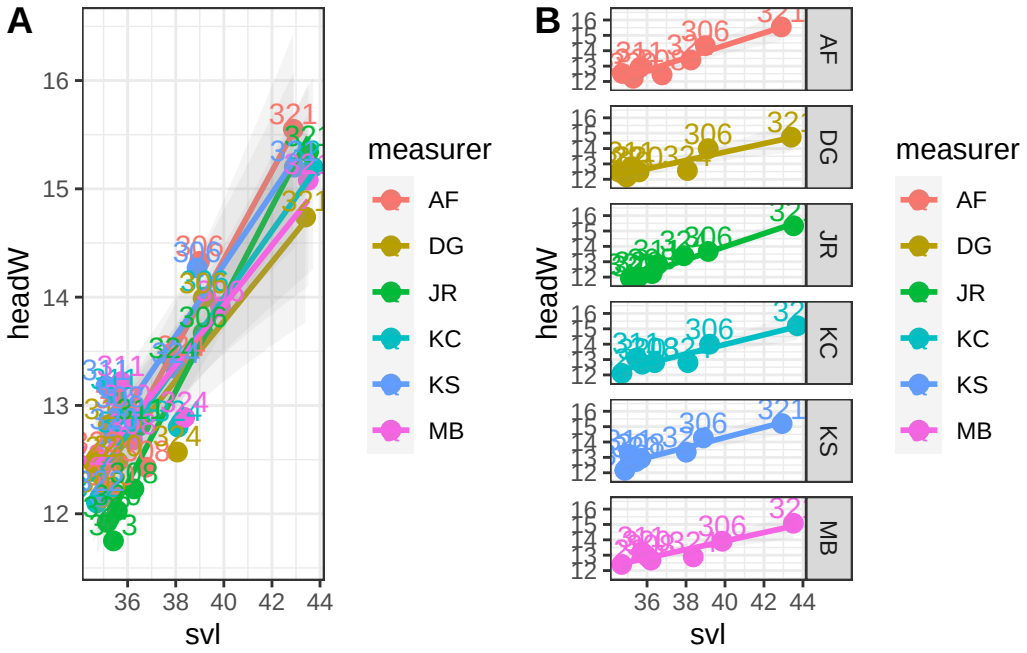
Still good!

Head Width

```
make_violins(hwv)
```



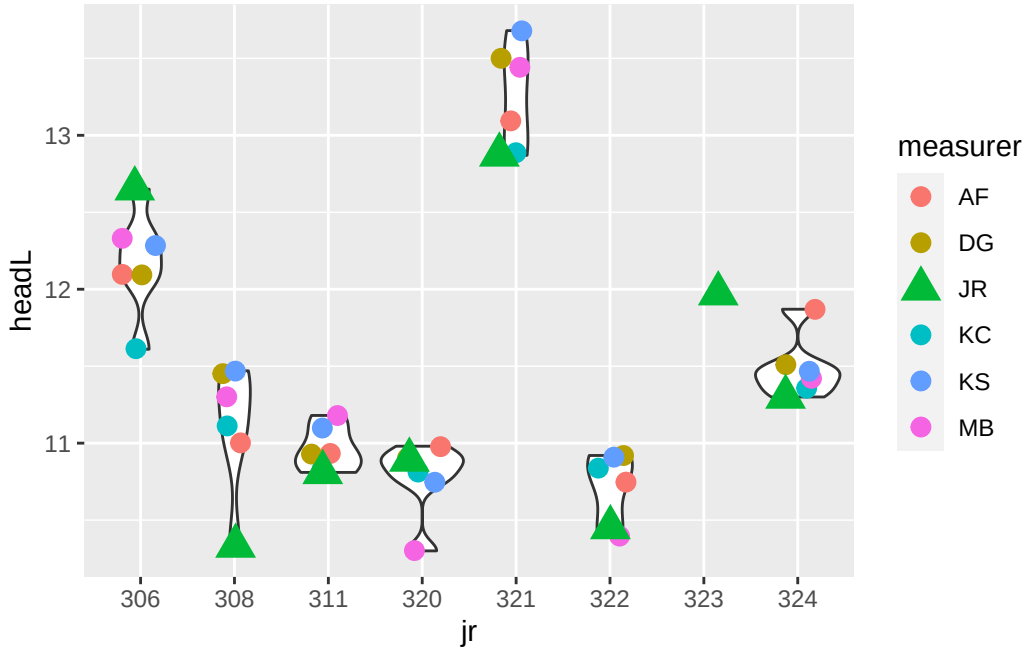

```
make_plots( hwp )
```



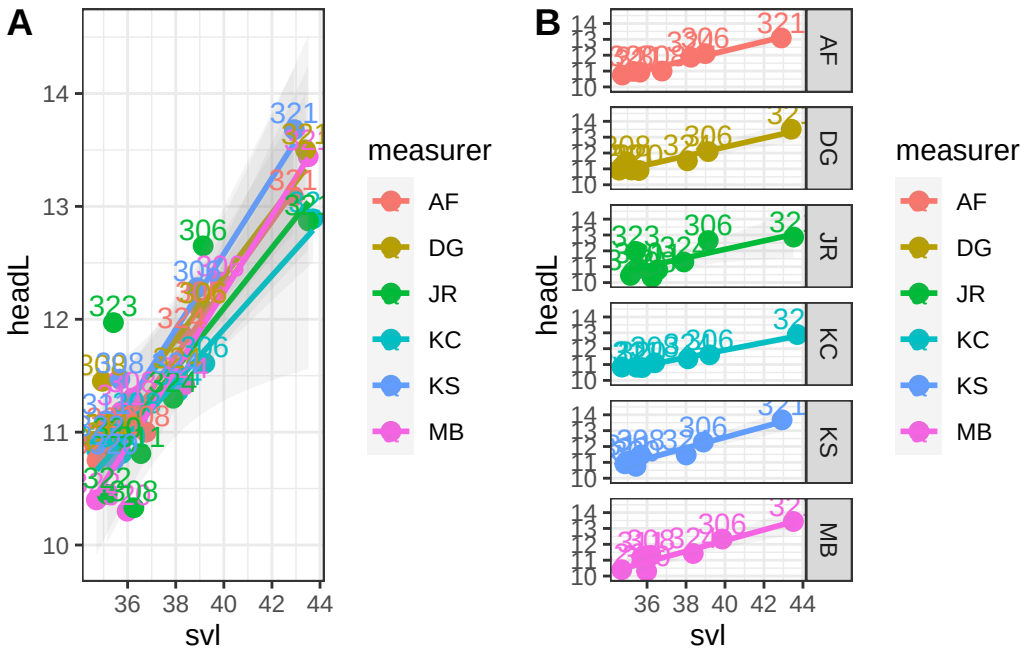
Good. I may have been too generous.

Head Length

```
make_violins(hlv)
```



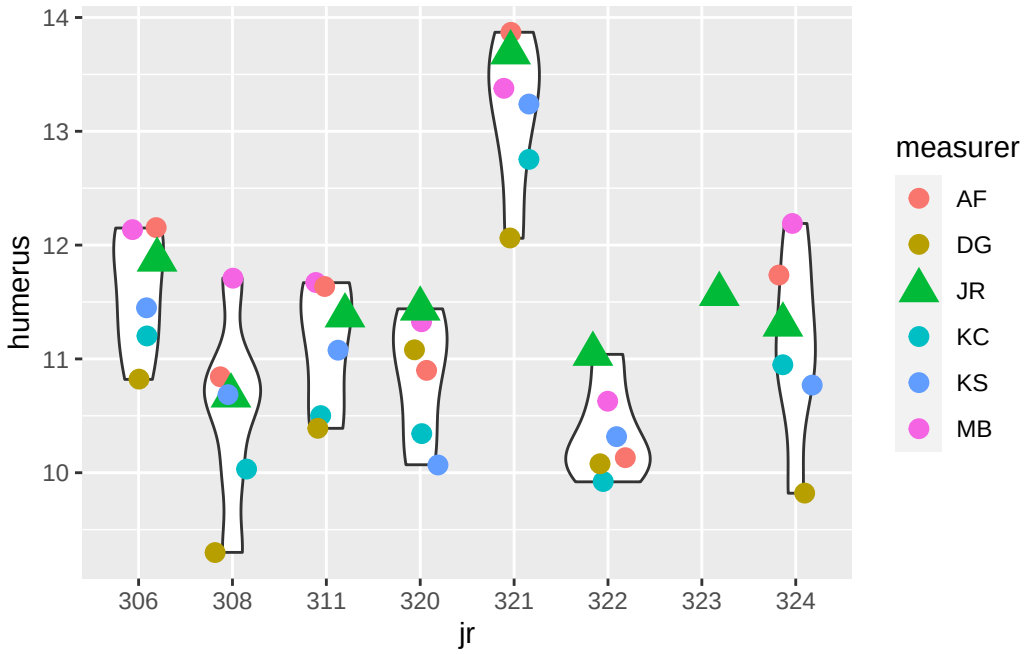

```
make_plots( hlp )
```



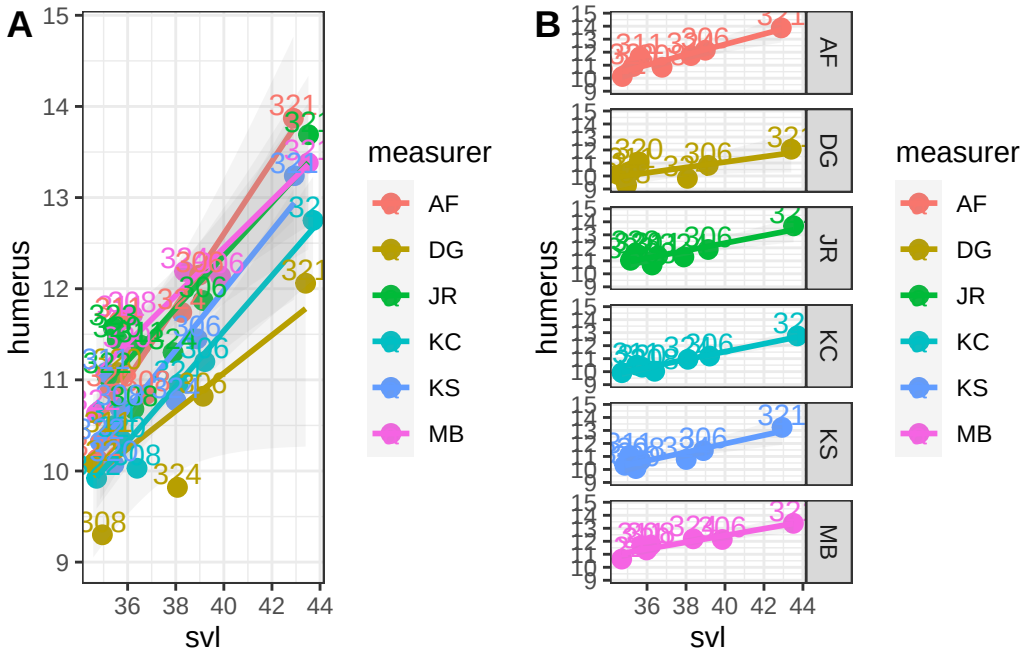
Much better!

Humerus

```
make_violins(humv)
```



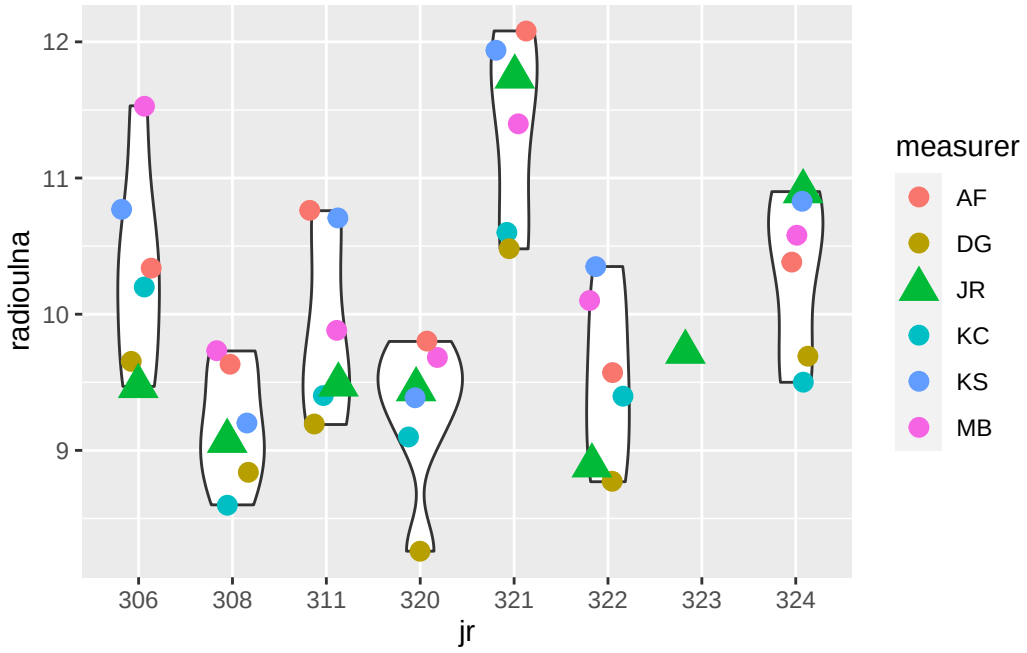

```
make_plots( hump )
```



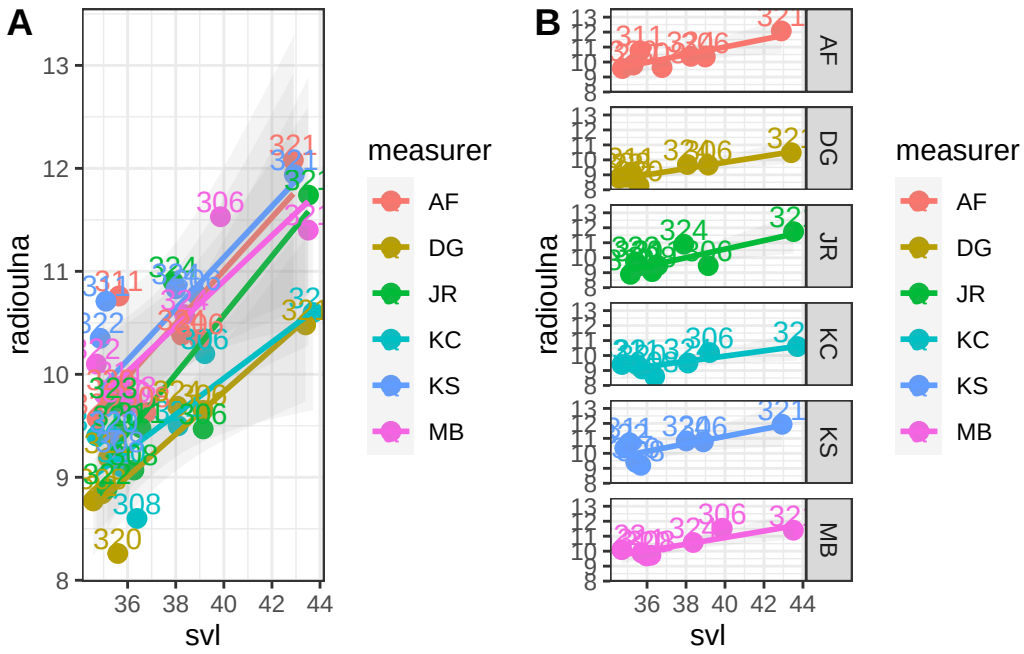
Several folks are still a bit low.

Radioulna

```
make_violins(radv)
```



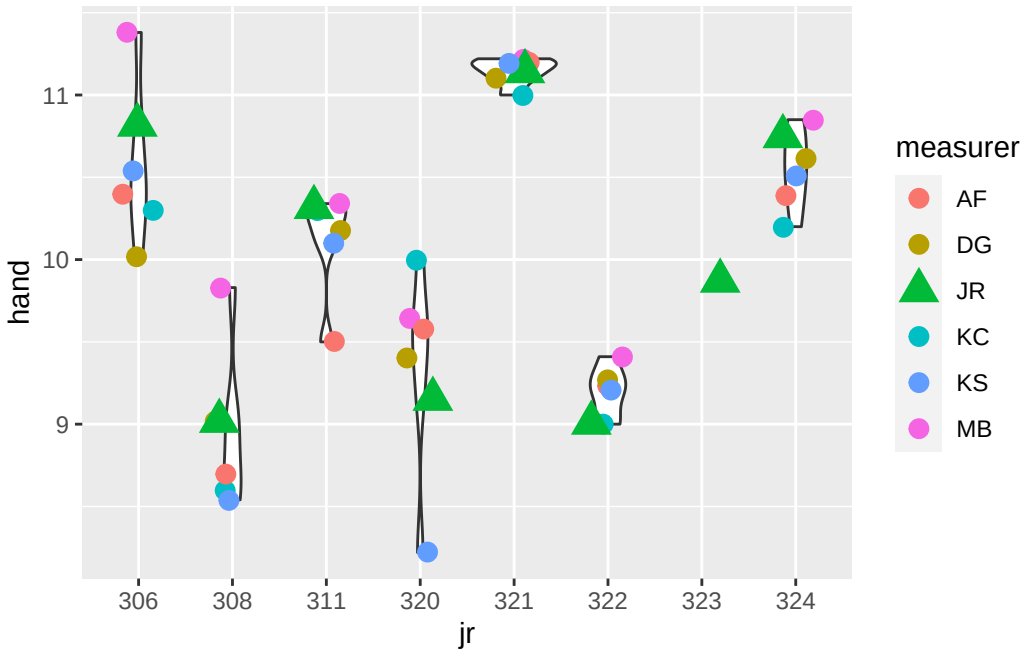

```
make_plots( radp)
```



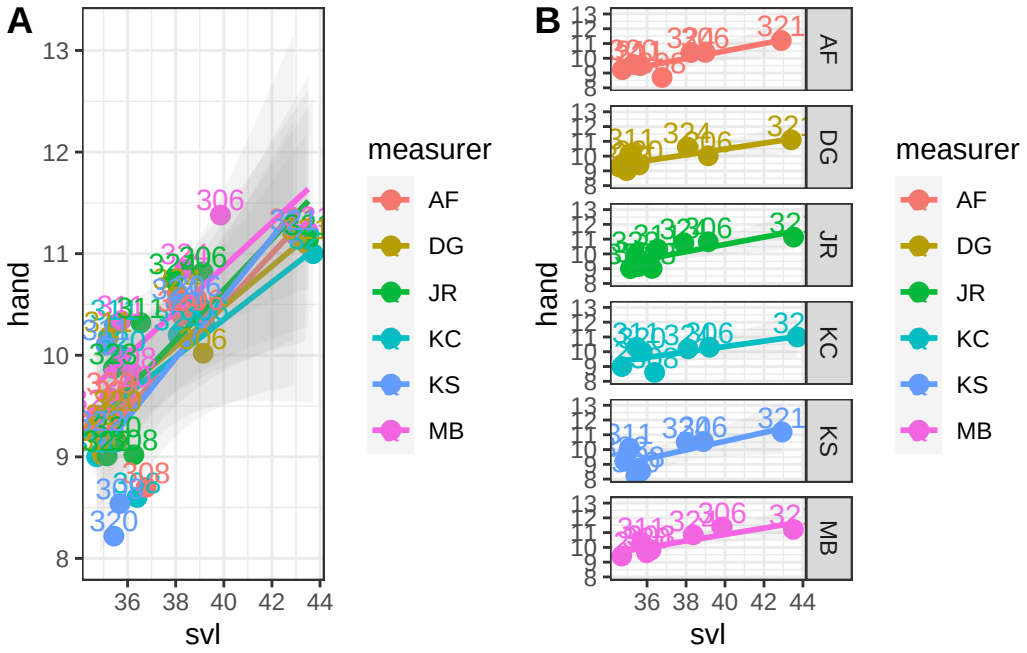
Now looks like random error, still a bit high though.

Hand

```
make_violins(handv)
```




```
make_plots( handp )
```



Now looks like random error.

Session 2

```
dat <- alldat %>% filter(session==2)

femp <- dat %>% ggplot(aes(svl, femur, color=measurer, label=jr))
tibp <- dat %>% ggplot(aes(svl, tibiofibula, color=measurer, label=jr))
tarp <- dat %>% ggplot(aes(svl, tarsus, color=measurer, label=jr))
footp <- dat %>% ggplot(aes(svl, foot, color=measurer, label=jr))
hwp <- dat %>% ggplot(aes(svl, headW, color=measurer, label=jr))
hlp <- dat %>% ggplot(aes(svl, headL, color=measurer, label=jr))
hump <- dat %>% ggplot(aes(svl, humerus, color=measurer, label=jr))
radp <- dat %>% ggplot(aes(svl, radioulna, color=measurer, label=jr))
handp <- dat %>% ggplot(aes(svl, hand, color=measurer, label=jr))

sv <- dat %>% ggplot(aes(jr, svl, group=jr))
femv <- dat %>% ggplot(aes(jr, femur, group=jr))
tibv <- dat %>% ggplot(aes(jr, tibiofibula, group=jr))
tarv <- dat %>% ggplot(aes(jr, tarsus, group=jr))
footv <- dat %>% ggplot(aes(jr, foot, group=jr))
hwv <- dat %>% ggplot(aes(jr, headW, group=jr))
hlv <- dat %>% ggplot(aes(jr, headL, group=jr))
humv <- dat %>% ggplot(aes(jr, humerus, group=jr))
radv <- dat %>% ggplot(aes(jr, radioulna, group=jr))
handv <- dat %>% ggplot(aes(jr, hand, group=jr))
```

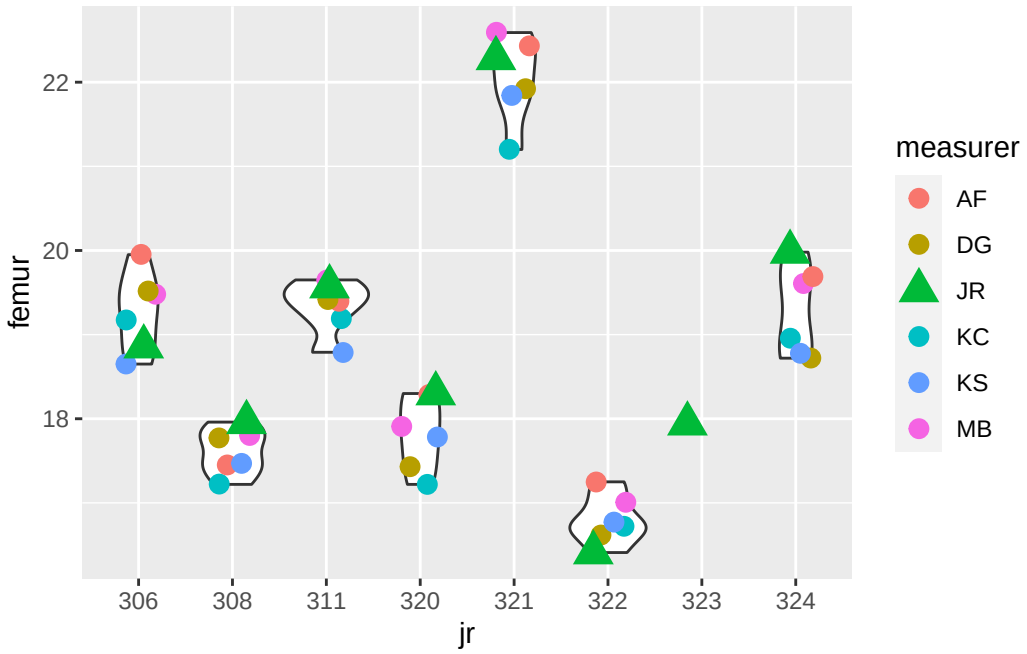
SVL

```
make_violins(sv)
```

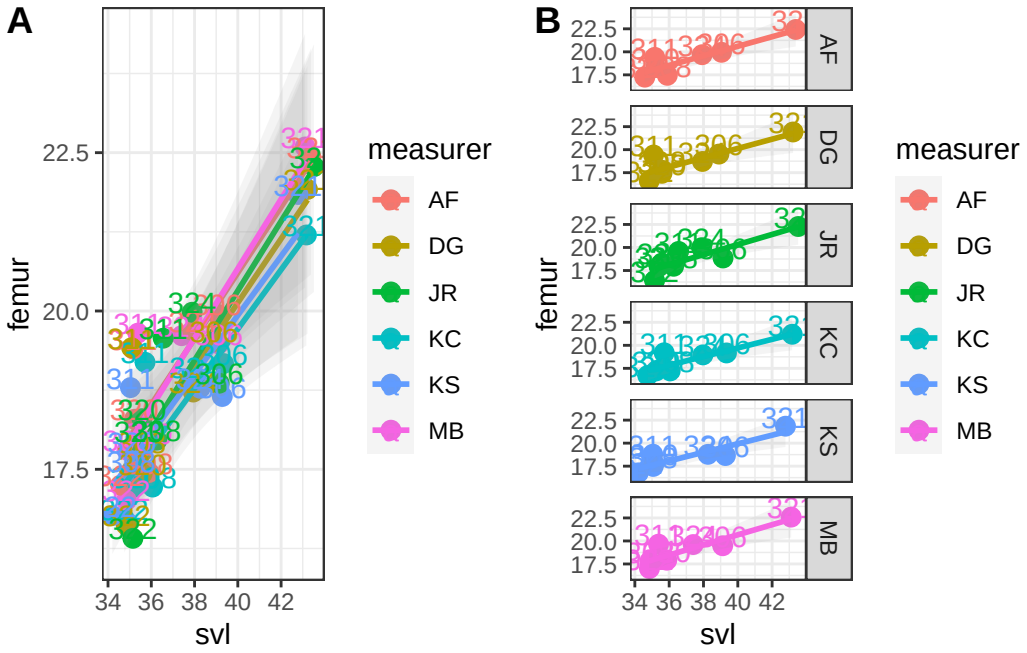
Much better!

Femur

```
make_violins(femv)
```



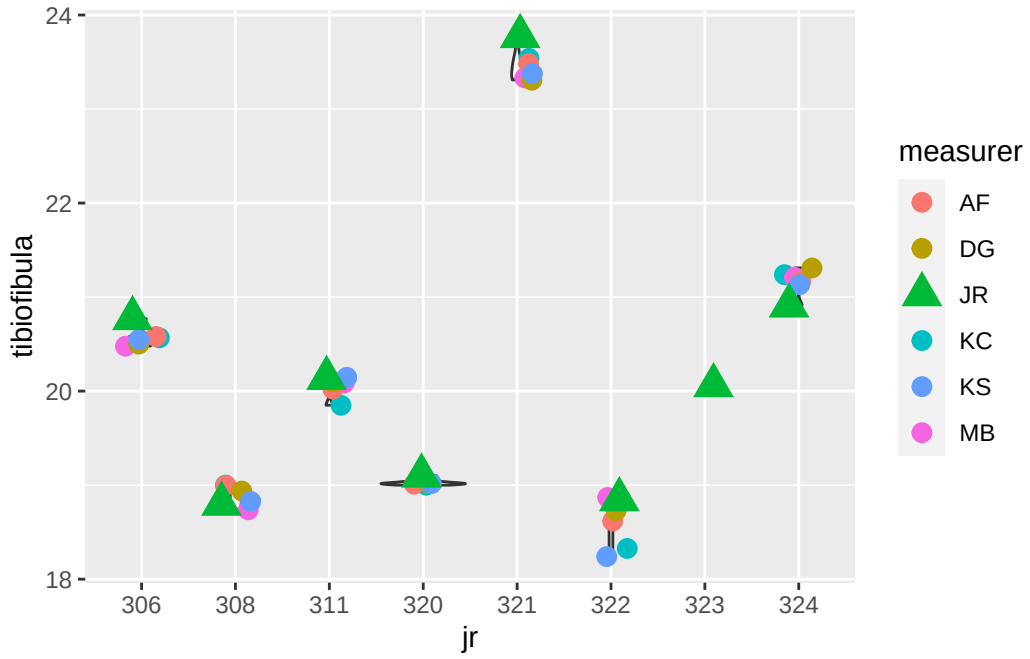

```
make_plots( femp )
```



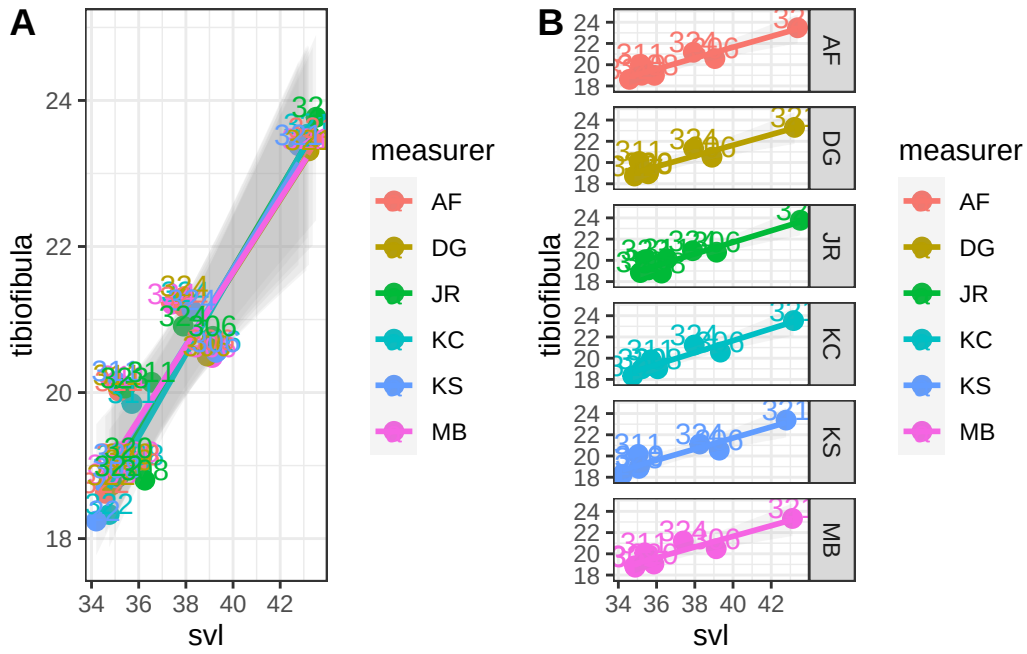
Better!

Tibiofibula

```
make_violins(tibv)
```



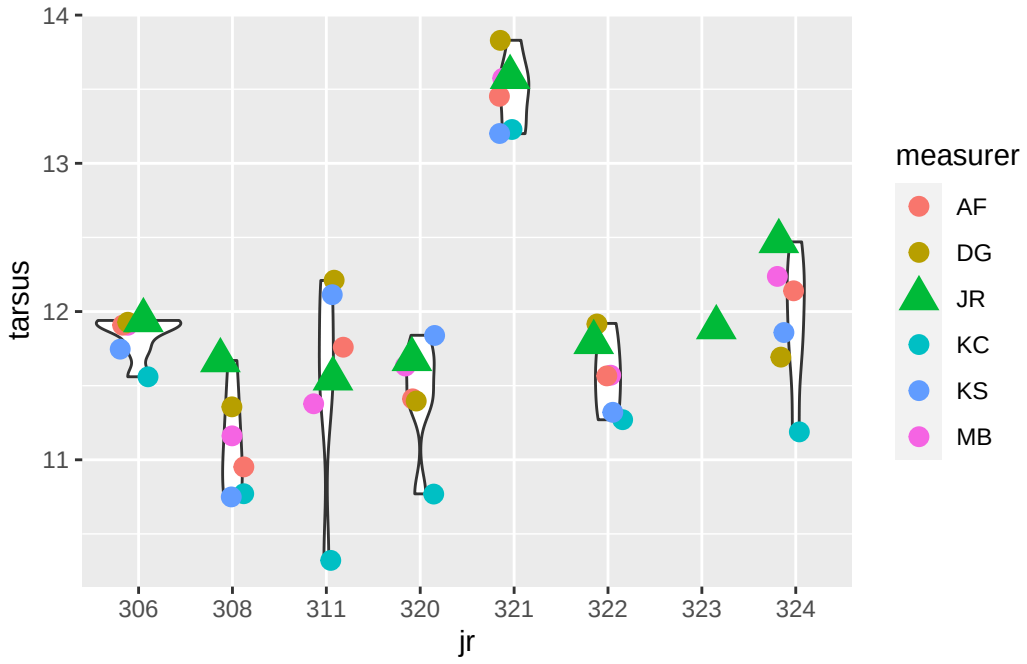

```
make_plots( tibp )
```



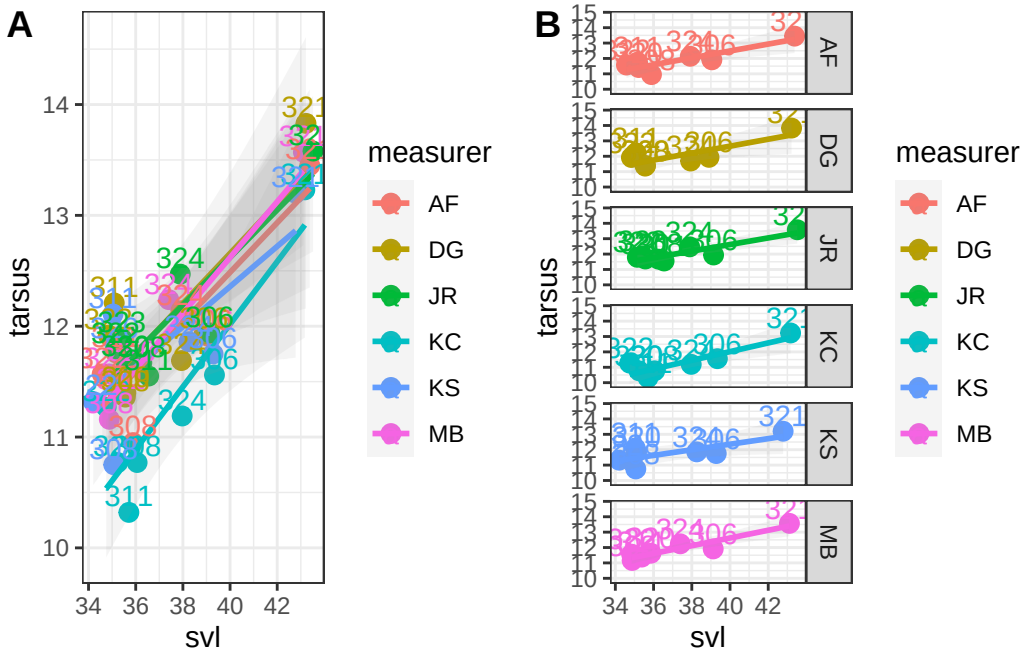
Better!

Tarsus

```
make_violins(tarv)
```



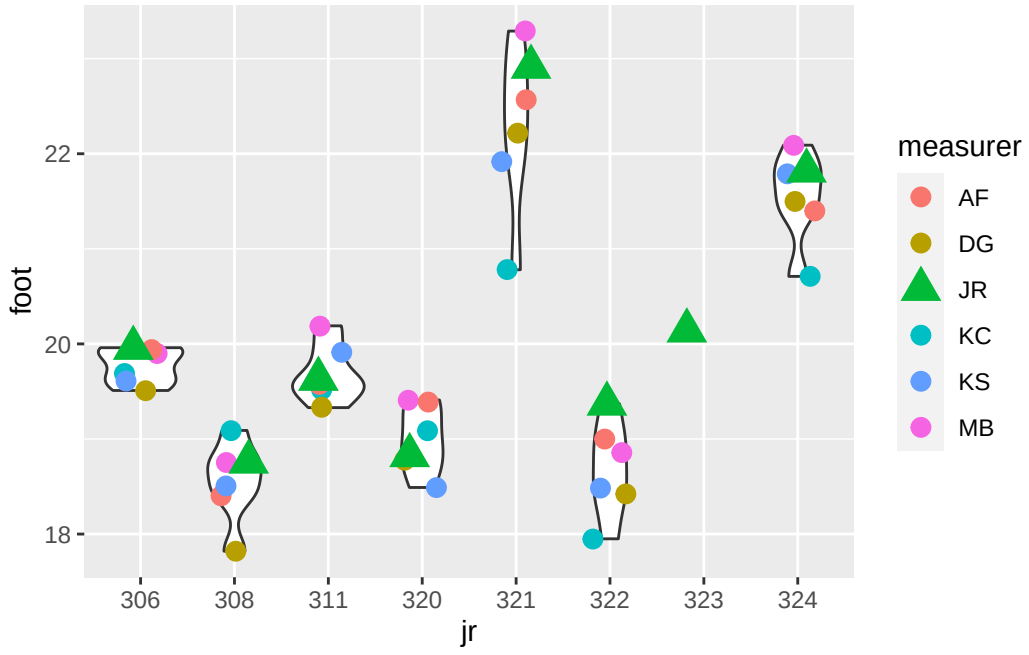

```
make_plots( tarp )
```



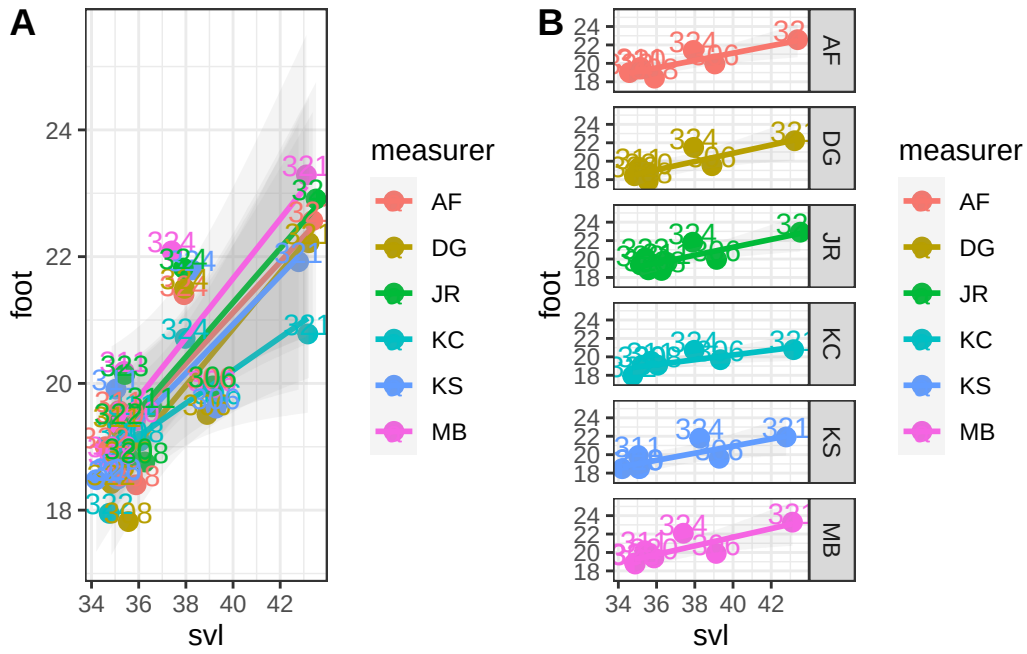
Better but Diana still a bit low and Ke a bit high

Foot

```
make_violins(footv)
```



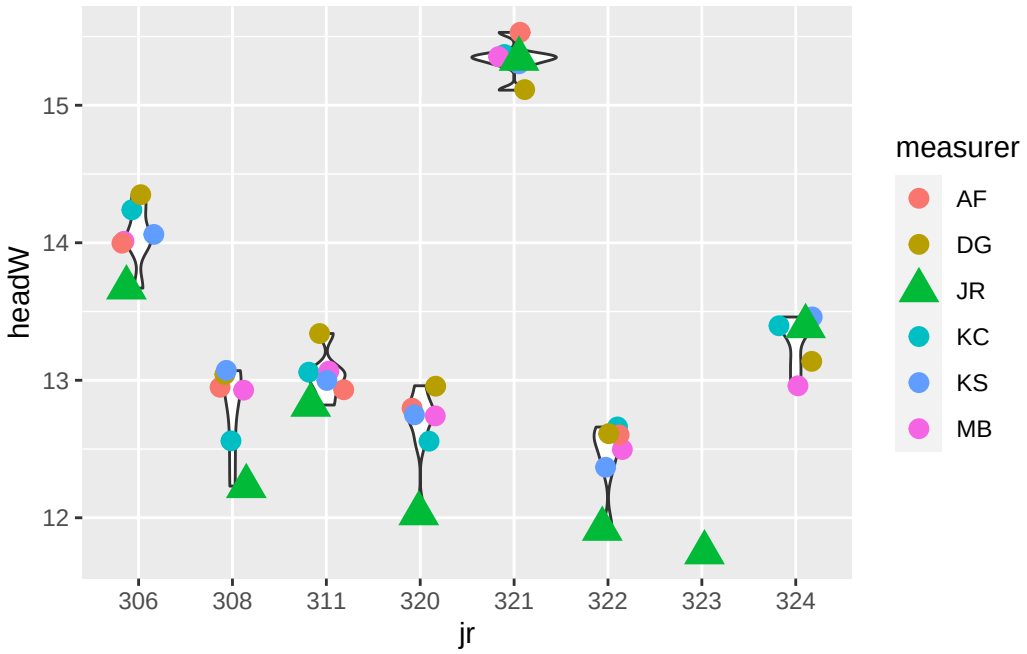

```
make_plots( footp )
```



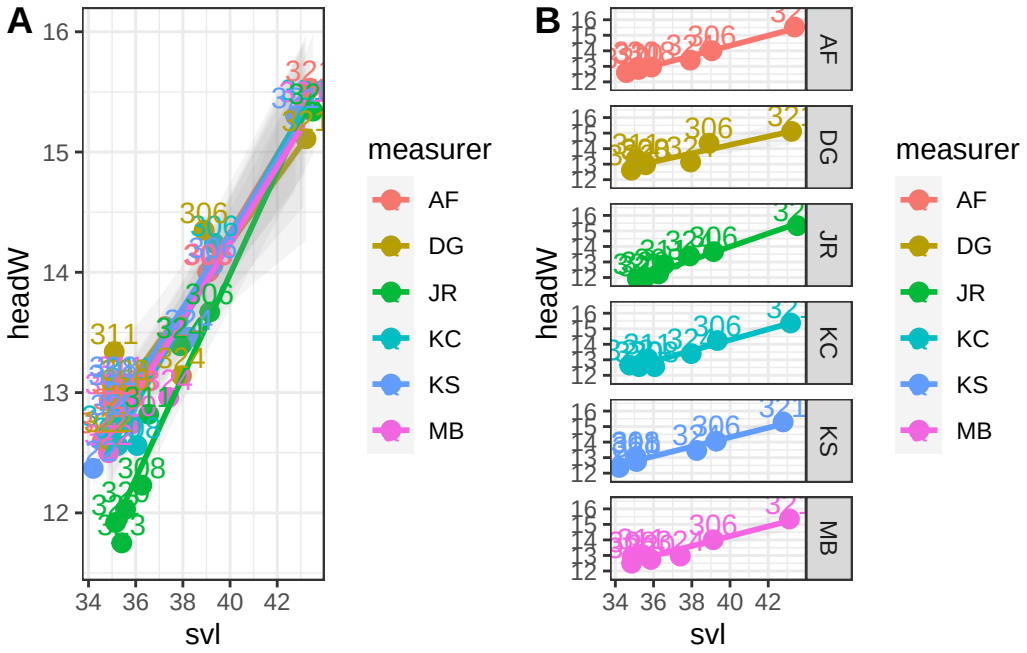
Still good!

Head Width

```
make_violins(hwv)
```



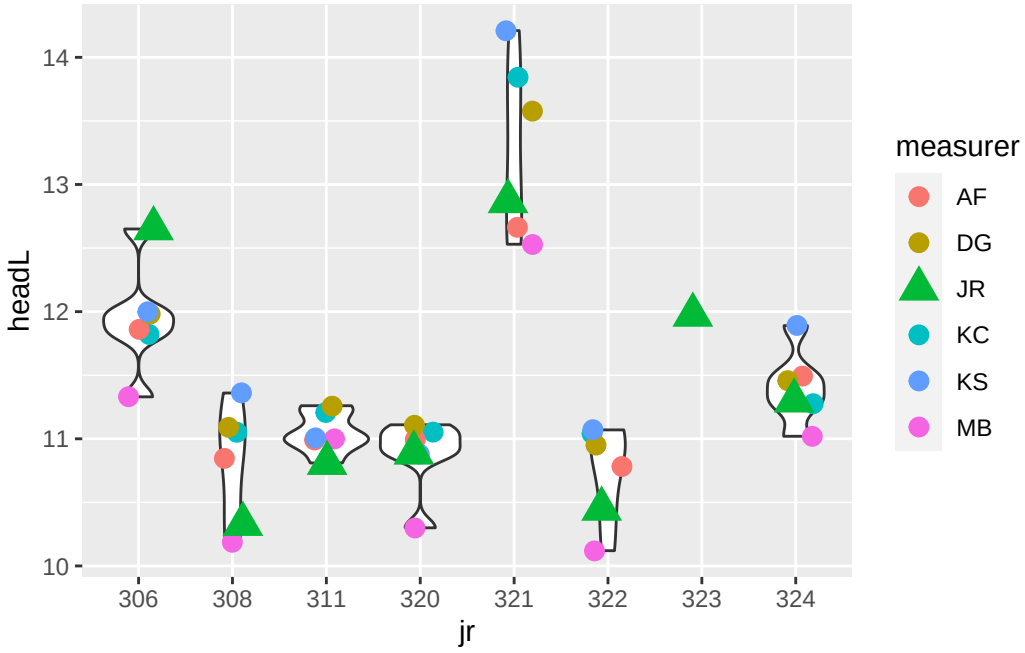

```
make_plots( hwp )
```



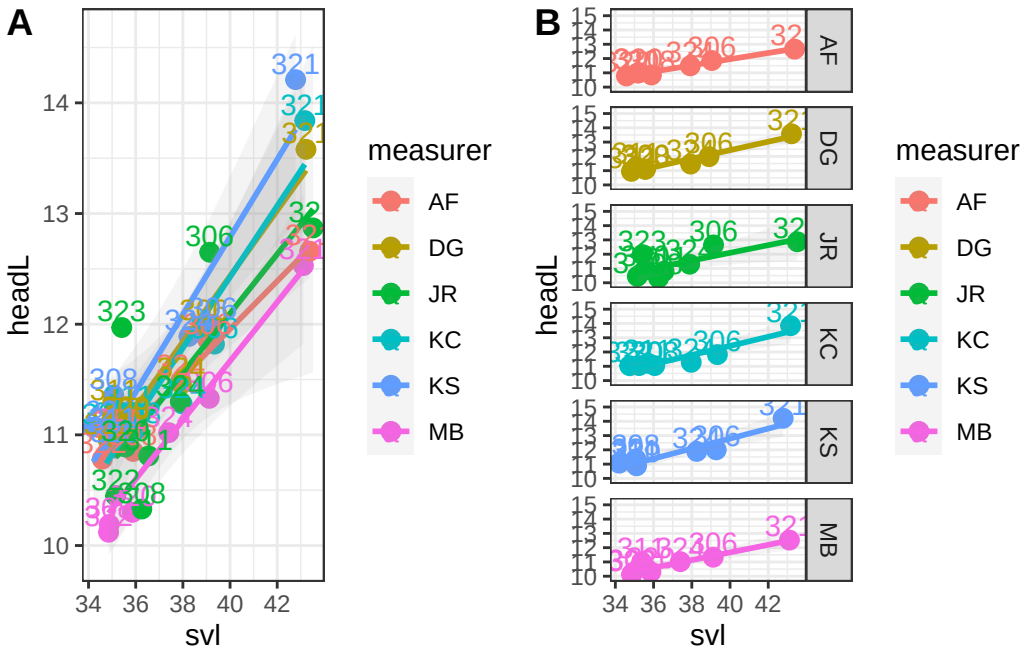
Good. I may have been too generous.

Head Length

```
make_violins(hlv)
```



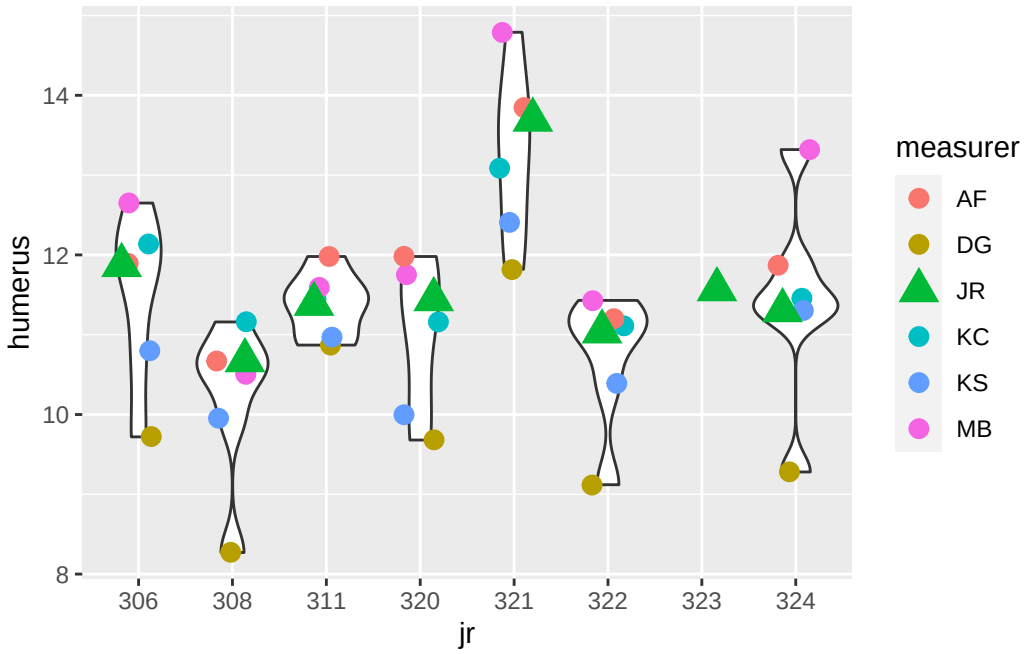

```
make_plots( hlp )
```



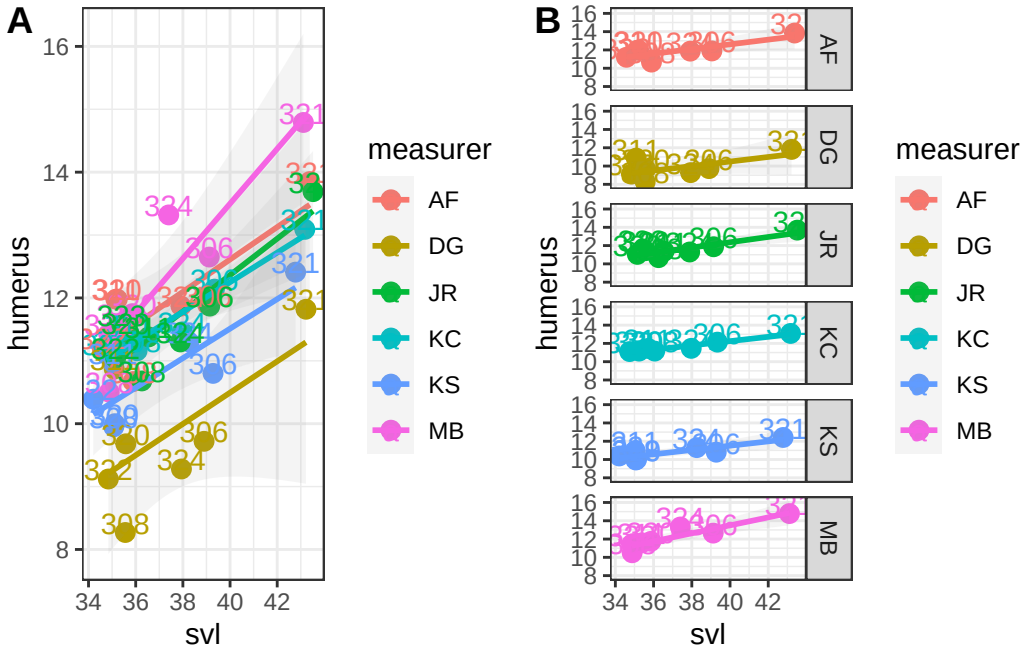
Much better!

Humerus

```
make_violins(humv)
```



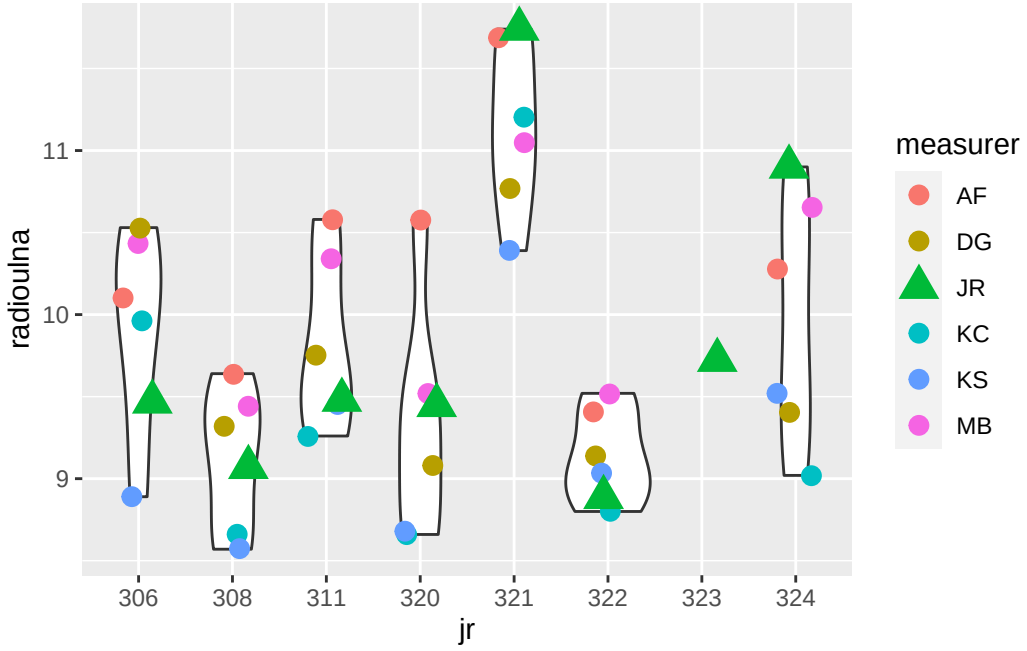

```
make_plots( hump )
```



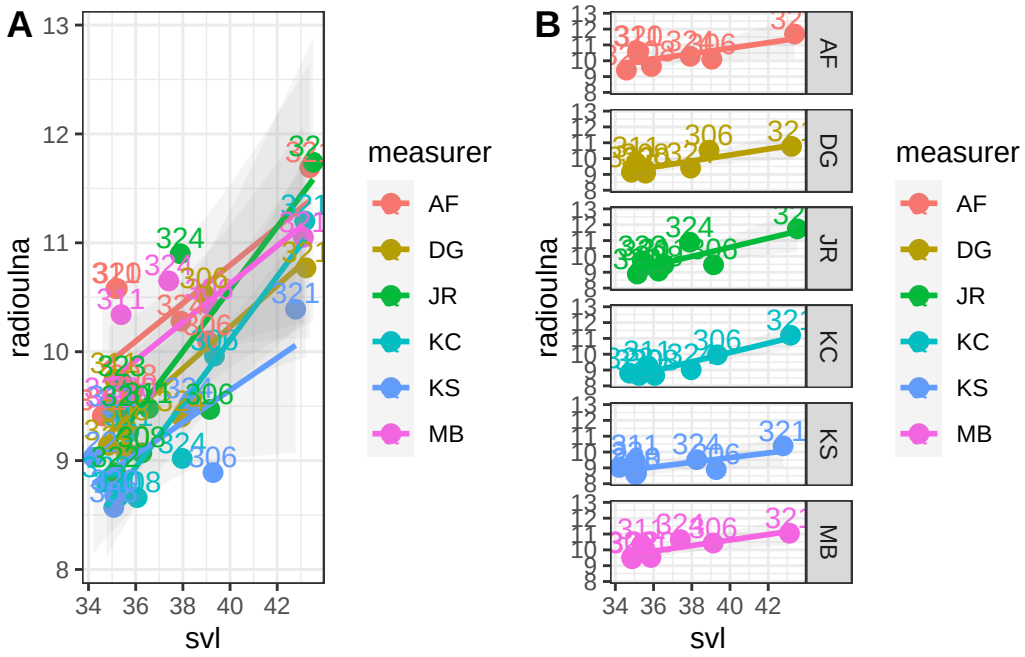
Several folks are still a bit low.

Radioulna

```
make_violins(radv)
```



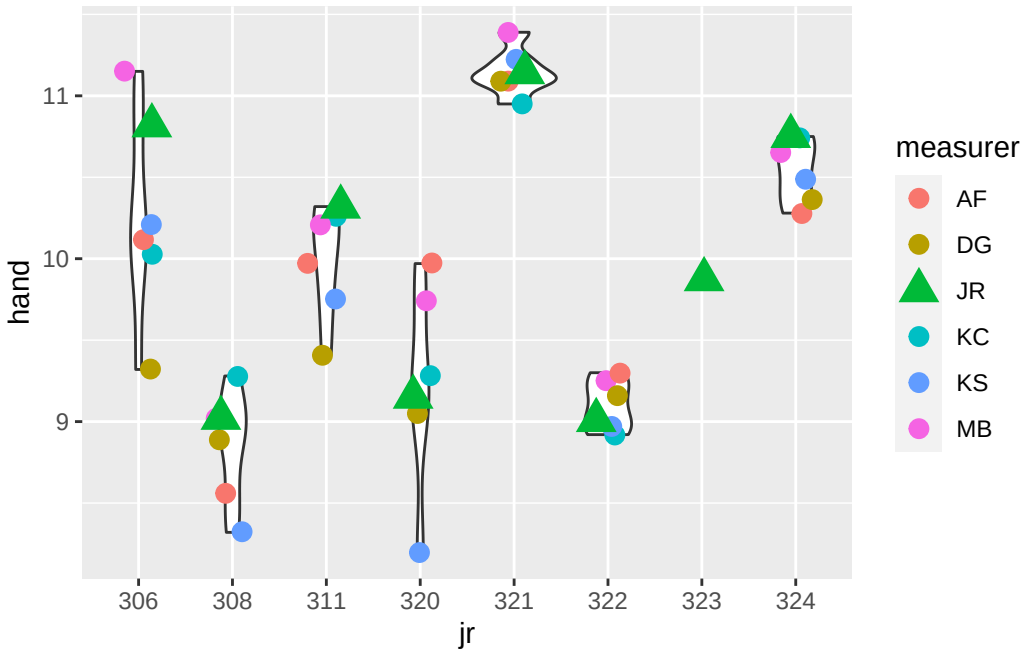

```
make_plots( radp )
```



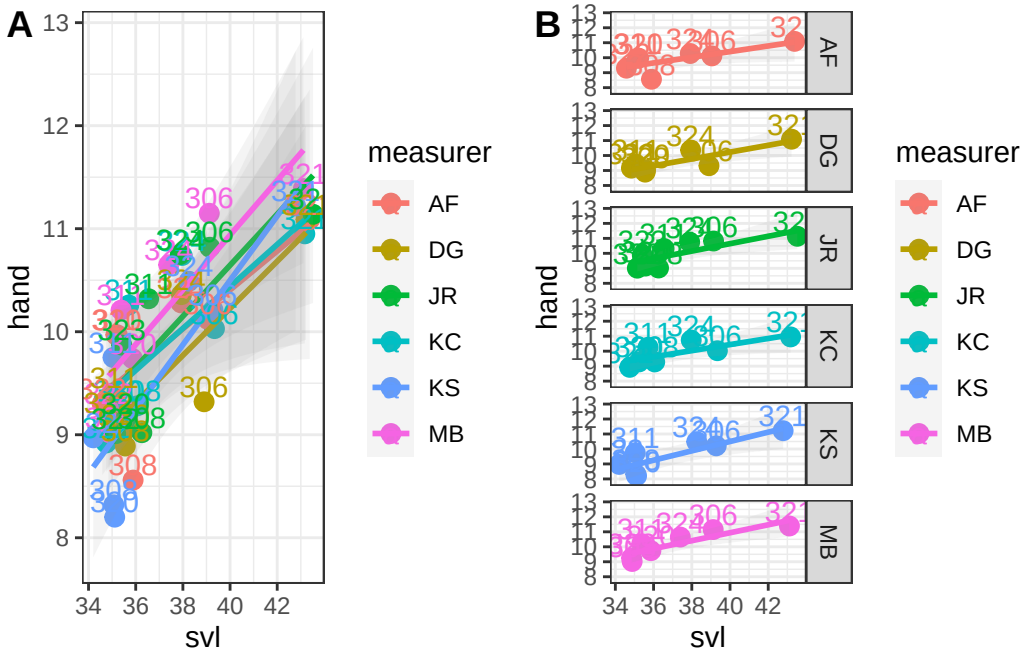
Now looks like random error, still a bit high though.

Hand

```
make_violins(handv)
```




```
make_plots( handp )
```

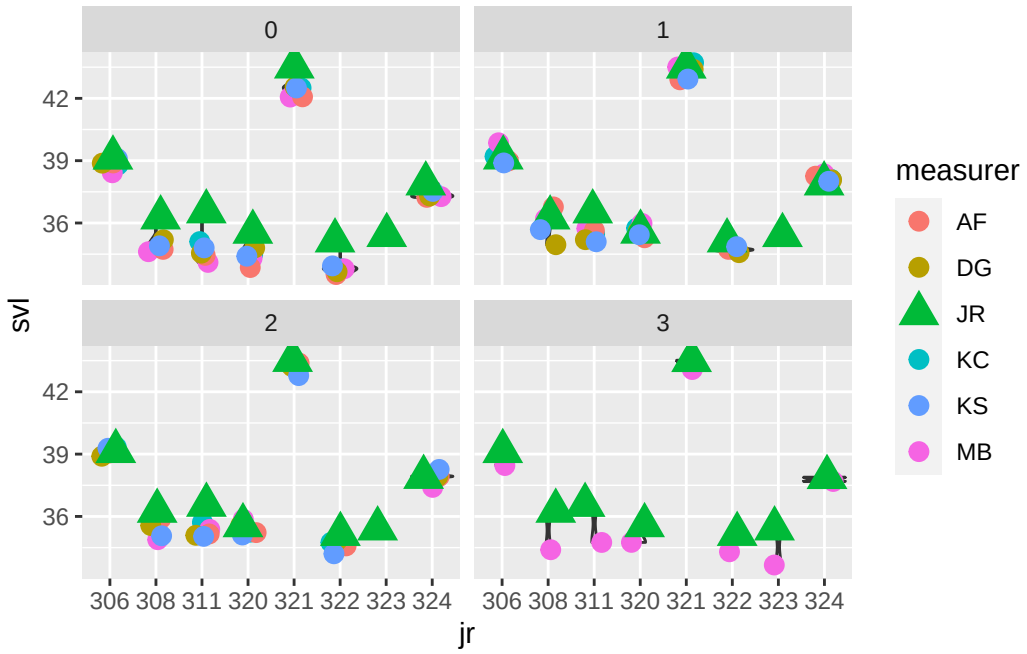


All together

```
dat <- alldat

sv <- dat %>% ggplot(aes(jr, svl, group=jr))
femv <- dat %>% ggplot(aes(jr, femur, group=jr))
tibv <- dat %>% ggplot(aes(jr, tibiofibula, group=jr))
tarv <- dat %>% ggplot(aes(jr, tarsus, group=jr))
footv <- dat %>% ggplot(aes(jr, foot, group=jr))
hwv <- dat %>% ggplot(aes(jr, headW, group=jr))
hlv <- dat %>% ggplot(aes(jr, headL, group=jr))
humv <- dat %>% ggplot(aes(jr, humerus, group=jr))
radv <- dat %>% ggplot(aes(jr, radioulna, group=jr))
handv <- dat %>% ggplot(aes(jr, hand, group=jr))

wrap_violin <- function(v) {
  v + geom_violin() +
    geom_jitter(aes(x=jr, color=measurer, shape=measurer, size=measurer), width=.2) +
    scale_shape_manual(values=c(19,19,17,19,19,19)) +
    scale_size_manual(values=c(3,3,5,3,3,3)) +
    facet_wrap(. ~ session )
}
```

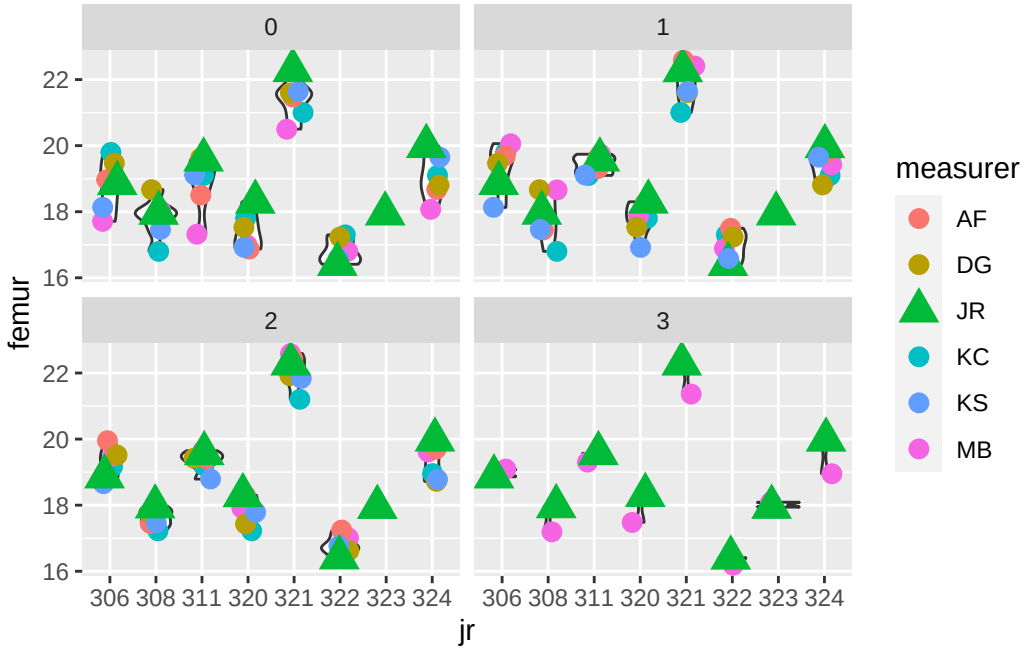
SVL

```
wrap_violin(sv)
```



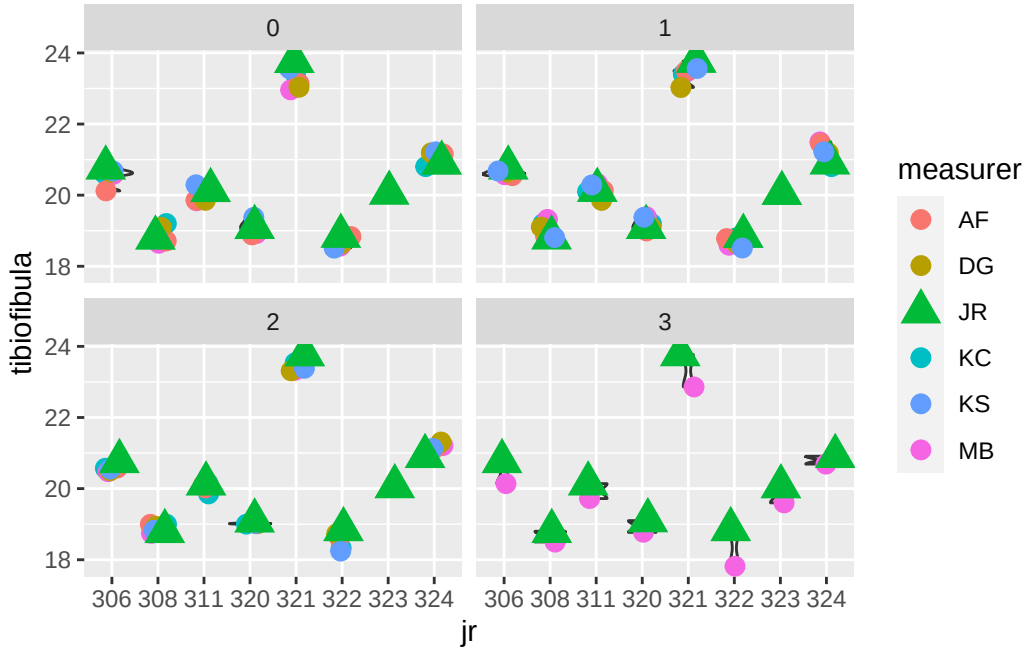
Femur

```
wrap_violin(femv)
```



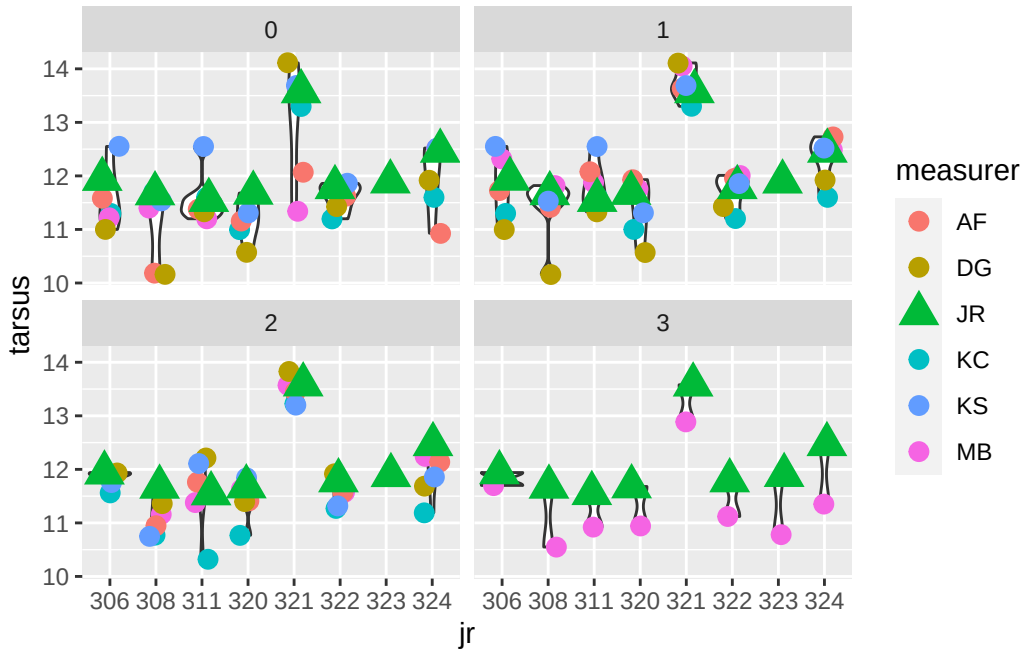
Tibiofibula

```
wrap_violin(tibv)
```



Tarsus

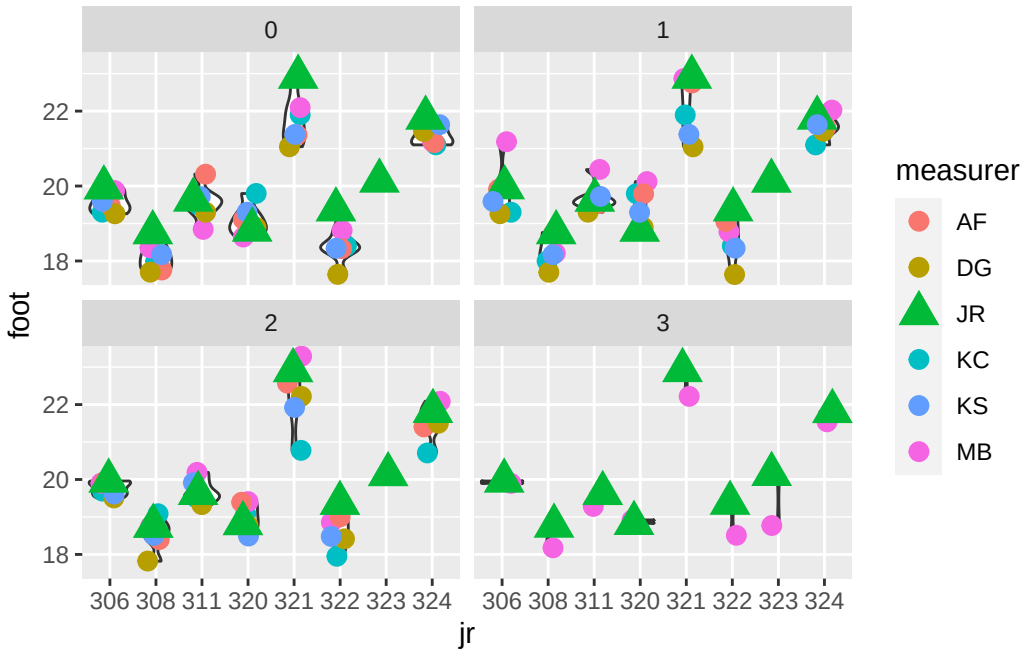
```
wrap_violin(tarv)
```



For round 3, I (MB) measured only the tarsus bone length and not the tarsus segment length. It seems JR measured the limb segment.

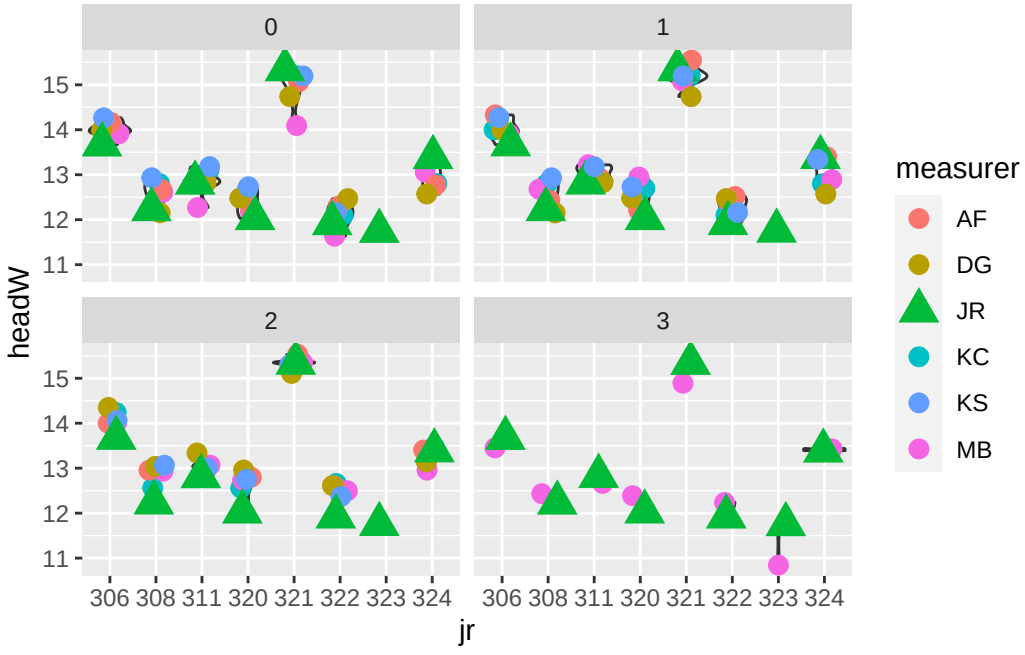
Foot

```
wrap_violin(footv)
```



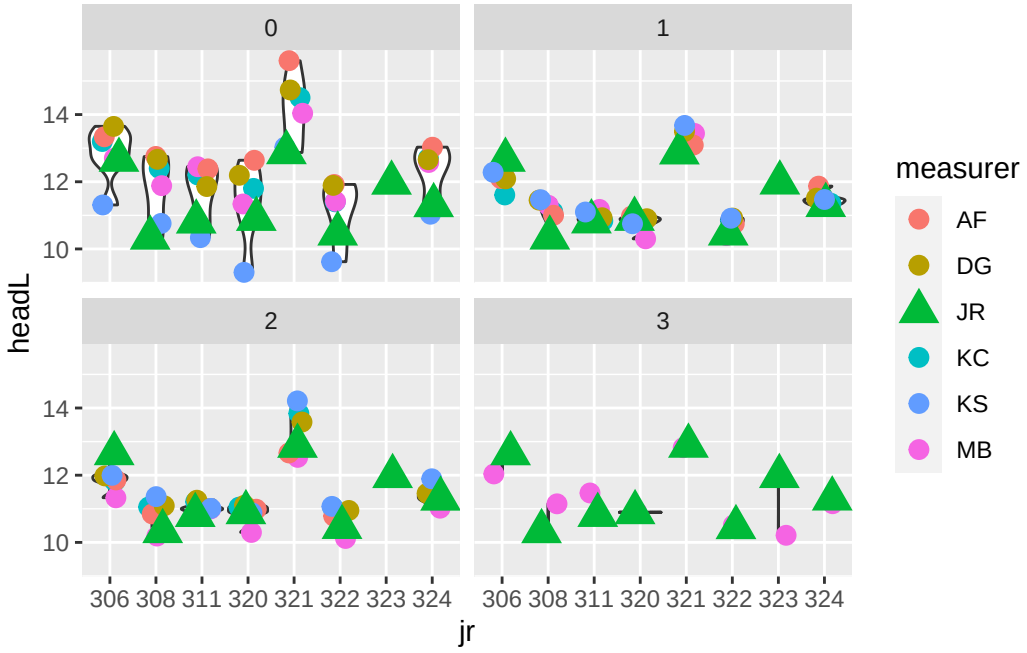
Head Width

```
wrap_violin(hwv)
```



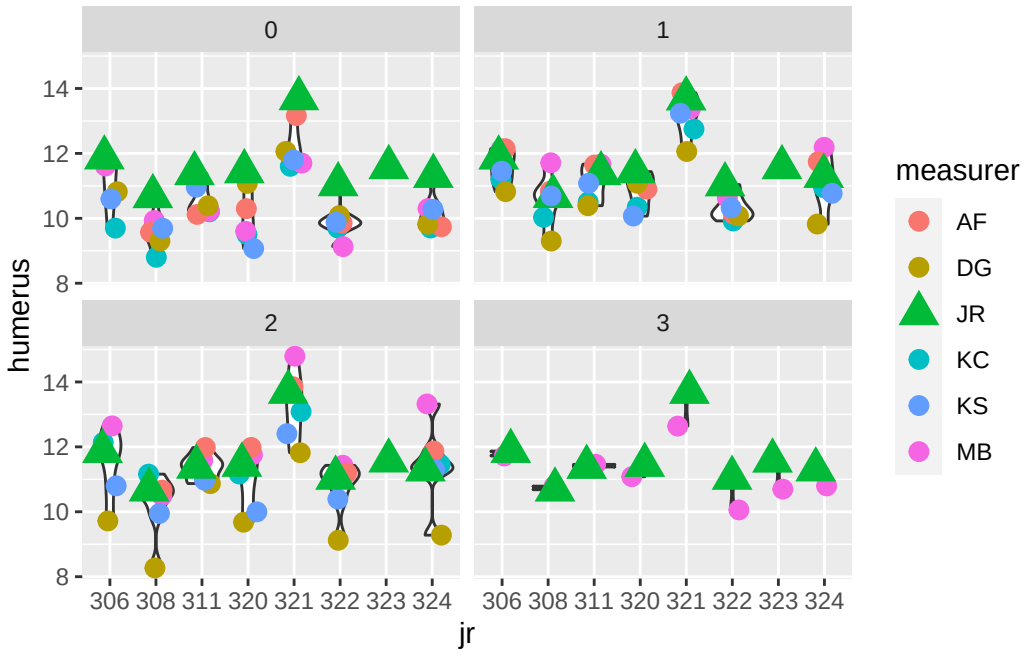
Head Length

```
wrap_violin(hlv)
```



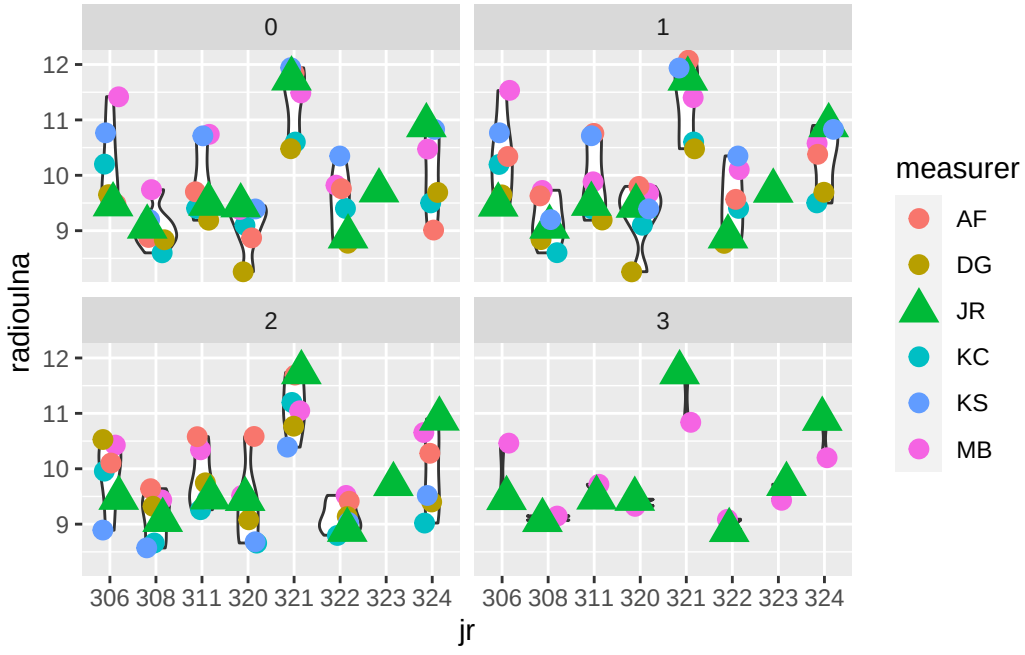
Humerus

```
wrap_violin(humv)
```



Radioulna

```
wrap_violin(radv)
```



Hand

```
wrap_violin(handv)
```