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## Billfish Isotopes:  
# Code started by E. Elliott Smith 01AUG2022, last modified 27MAR2023  
# Code for:  
  # Title: Pre-industrial ecology and foraging behavior of swordfish  
  (Xiphias gladius) in the eastern North Pacific  
  # Authors: Emma A. Elliott Smith, Paul Szpak, Todd J. Braje,  
  Bonnie Newsom, and Torben C. Rick  
  # Marine Ecological Progress Series  
  
# begin code #####  
  
# load necessary packages and libraries #####  
library(dplyr)  
library(ggplot2)  
library(reshape2)  
library(SIBER)  
library(stringr)  
  
# 1. import datasets – 'XXX' is placeholder for local file directory  
#####  
billfish_arc <- read.csv("~/XXX/Billfish_arch.csv") # archaeo/  
historical billfish data from the present work  
billfish_mod <- read.csv("~/XXX/Desktop/Swordfish/MEPS/R1/  
Code_Data_forUpload/Billfish_mod_AP.csv") # billfish data from Acosta-  
Pachon et al. 2020 MEPS – extracted using WebPlot Digitizer  
  
# modifying the modern dataset for use with SIBER  
billfish_mod <- subset(billfish_mod, select = c(d13C, d15N, Locale))  
colnames(billfish_mod) <- c("iso1", "iso2", "group")  
billfish_mod$community <- c("AP_mod")  
  
# removing samples from the archaeo billfish dataset that had poor  
collagen quality per Rick et al. (2019) ZooMS paper  
billfish_arch2 <- subset(billfish_arc, C.N.atomic <= 3.6, select =  
c(1:16))  
billfish_arch2$Location2 <- billfish_arch2$Location  
billfish_arch2[(billfish_arch2$Location2 != "Maine"), "Location2"] <-  
c("California")  
  
# pulling out just the California data  
billfish_archCA <- subset(billfish_arch2, Location != "Maine")  
  
# 2. Computing and applying data corrections needed for comparison to  
Acosta-Pachon data (see Supplemental SI-1) #####  
# 2a. – whole (untreated) bone to collagen. comparative data from  
Wilson and Szpak 2022 PeerJ #####  
boneTissComp <- read.csv("~/XXX/Wilson_Szpak_Data.csv") # read in  
comparative dataset  
str(boneTissComp)
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colnames(boneTissComp) <- c("BoneID", "SampleID", "Context",
"Treatment", "Tissue",
                           "13Cvpdb", "15Nair", "wtC", "wtN",
"C.Natm", "YieldCollagen")
boneTissComp$BoneID <- as.factor(boneTissComp$BoneID)
boneTissComp$Context <- as.factor(boneTissComp$Context)
boneTissComp$Treatment <- as.factor(boneTissComp$Treatment)

# pull just the untreated whole bone and the demineralized/lipid
extracted treatment (LE/HCL)
bone.coll <- subset(boneTissComp, (Treatment == "LE/HCl"
                                     |Treatment == "0/0"))

# calculating the mean offset (taking this offset per sample (BoneID))
for (i in 1:length(bone.coll$SampleID)) {
  samp <- bone.coll$BoneID[i]
  coff <- bone.coll[(bone.coll$BoneID == samp & bone.coll$Tissue ==
"Collagen"), "13Cvpdb"] - bone.coll[(bone.coll$BoneID == samp &
bone.coll$Tissue == "WholeBone"), "13Cvpdb"]
  noff <- bone.coll[(bone.coll$BoneID == samp & bone.coll$Tissue ==
"Collagen"), "15Nair"] - bone.coll[(bone.coll$BoneID == samp &
bone.coll$Tissue == "WholeBone"), "15Nair"]
  bone.coll$C.off[i] <- round(coff, 1)
  bone.coll$N.off[i] <- round(noff, 1)
}
# summarizing offsets for each individual
bone.off <- subset(bone.coll[,c(1,12:13)], bone.coll$Tissue ==
"Collagen")
bone.off.mean <- summarize(bone.off,
                           meanC = mean(C.off),
                           sdC = sd(C.off),
                           meanN = mean(N.off),
                           sdN = sd(N.off))

# so, on average collagen is +0.53 (d13C) and +0.16 (d15N) per mil
higher than whole bone
billfish_archCA$d13C.corr1 <- billfish_archCA$d13C - 0.5
billfish_archCA$d15N.corr <- billfish_archCA$d15N - 0.2

# 2b. Correcting for the Suess/Laws Effects #####
# from 1860 to 1960: +0.05 per mil per decade (Francey et al. 1999)
s1 <- ((1960-1860)/10)*0.05
# from 1960 to 1990: 0.16 per mil per decade (Quay et al. 2003)
s2 <- ((1990-1960)/10)*0.16
# from 1990 to 2014: 0.20 per mil per decade (Quay et al. 2017)
s3 <- ((2014-1990)/10)*0.20
# summing these up for the final correction:
sT <- s1 + s2 + s3

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# applying this correction to our archaeo data:
billfish_archCA$d13C.corr <- billfish_archCA$d13C.corr1 - sT

# 3. Plotting isotope data #####
# 3a. Figure 2 #####
# using SIBER (Jackson et al. 2011) for the modern data:
siber.example <- createSiberObject(billfish_mod)

# Create lists of plotting arguments to be passed onwards to each
# of the three plotting functions.
community.hulls.args <- list(col = 1, lty = 1, lwd = 1)
group.ellipses.args <- list(n = 100, p.interval = 0.95, lty = 1, lwd
= 2)
group.hulls.args <- list(lty = 2, col = "grey20")

# plotting modern ellipses (note only ellipses, and not individual
data points are shown in Figure 2)
par(mfrow=c(1,1))
plotSiberObject(siber.example,
                ax.pad = 2,
                hulls = F, community.hulls.args =
community.hulls.args,
                ellipses = T, group.ellipses.args =
group.ellipses.args,
                group.hulls = F, group.hulls.args = group.hulls.args,
                bty = "L",
                iso.order = c(1,2),
                xlab = expression({delta}^13*C~'\u2030'),
                ylab = expression({delta}^15*N~'\u2030'),
                x.limits = c(-20, -10),
                y.limits = c(5, 19))
# In the plot: black = Baja CA Sur; red = Coastal California; green =
North Pacific Subtropical Gyre

# plotting CA archaeo data - adjusted d13C and d15N values
bill.CA.CvN <- ggplot(billfish_archCA, aes(x = d13C.corr, y =
d15N.corr, color = Location, shape = SpeciesID))
bill.CA.CvN <- bill.CA.CvN + geom_point(size = 4) + theme_classic()
bill.CA.CvN <- bill.CA.CvN + scale_color_manual(values = c("grey53",
"grey53", "black", "lightgrey", 'lightgrey'))
bill.CA.CvN <- bill.CA.CvN + xlim(c(-20, -10)) + ylim(c(5, 19)) # same
axes limits as modern ellipse plot
plot(bill.CA.CvN)

# NOTE: SIBER ellipse (modern billfish) and CA archaeo data plots were
overlaid in Adobe Illustrator

# 3b. Figure 3 #####
# comparing archaeological CA and Maine data - this uses the measured

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EA-IRMS values, not the corrected values as above.
bill.arc.CvN <- ggplot(billfish_arch2[!(billfish_arch2$SpeciesID ==
"Kajikia audax"),], # plotting only X. gladius
aes(x = d13C, y = d15N, color = Location2, fill =
Location2, shape = Location2))
bill.arc.CvN <- bill.arc.CvN + geom_point(size = 4) + theme_classic()
bill.arc.CvN <- bill.arc.CvN + scale_fill_manual(values = c("black",
"white")) + scale_color_manual(values = c("black", "black"))
bill.arc.CvN <- bill.arc.CvN + scale_shape_manual(values = c(24, 23))
bill.arc.CvN <- bill.arc.CvN + xlim(c(-19, -10)) + ylim(c(5, 19))
plot(bill.arc.CvN)

# 4. Statistical analyses #####
# mainland d13C lower than island d13C?
billfish_archCA$Location3 <- NA # adding a column for island vs
mainland archaeo sites
for (i in 1:length(billfish_archCA$SpeciesID)){
  ifelse(str_sub(billfish_archCA$Location[i], -8, -1) == "Mainland"),
  billfish_archCA$Location3[i] <- 'Mainland',
  billfish_archCA$Location3[i] <- 'Island')
}

# check for normality - carbon
with(billfish_archCA, shapiro.test(d13C.corr[Location3 ==
"Mainland"])) # p = 0.8518, looks good
with(billfish_archCA[billfish_archCA$SpeciesID == "Xiphias gladius",],
shapiro.test(d13C.corr[Location3 == "Island"])) # p = 0.4383, looks
good

# check for normality - nitrogen
with(billfish_archCA, shapiro.test(d15N.corr[Location3 ==
"Mainland"])) # p = 0.4836, looks good
with(billfish_archCA[billfish_archCA$SpeciesID == "Xiphias gladius",],
shapiro.test(d15N.corr[Location3 == "Island"])) # p = 0.07,
marginal...
# Given the d15N data, I will use non-parametric tests to be safe

# Mann-Whitney test/two-sample Wilcoxon test
bill.mwC <- wilcox.test(d13C.corr ~ Location3,
billfish_archCA[billfish_archCA$SpeciesID == "Xiphias gladius",]) #
two-sided unpaired test
bill.mwC # significantly different! W = 46.5, p-value = 0.0099

bill.mwN <- wilcox.test(d15N.corr ~ Location3,
billfish_archCA[billfish_archCA$SpeciesID == "Xiphias gladius",]) #
two-sided unpaired test
bill.mwN # not different. W = 35, p-value = 0.2438

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# what is the difference between island and mainland in d13C values?
t <- billfish_archCA[(billfish_archCA$SpeciesID == "Xiphias
gladius"), ]
mean(na.omit(t[(t$Location3 == "Island"),18])) # -12.1 unadjusted d13C
mean(na.omit(t[(t$Location3 == "Mainland"),18])) # -14.7 unadjusted
d13C

# range of values:
range(na.omit(t[(t$Location3 == "Island"),18])) # -13.8 to -11.1
unadjusted d13C, range 2.7
range(na.omit(t[(t$Location3 == "Mainland"),18])) # -16. to tp -12.2
unadjusted d13C, range 4.7
range(na.omit(t[(t$Location3 == "Island"),19])) # 6.5 to 14.0
unadjusted d15N
range(na.omit(t[(t$Location3 == "Mainland"),19])) # 6.3 to 13.3
unadjusted d15N
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