Electronic supplementary materials to manuscript:

"**Cohort structure and individual resource specialization in loggerhead turtles,**

long-lived marine species with ontogenetic migrations"

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Content

Supplement 1: Sample pretreatments

Acid washing, aimed at removing carbonates (e.g. from invertebrate exoskeletons) is not relevant for sea turtle carapace tissue which is predominantly composed of keratin (Alibardi 2005, Shimada et al. 2014). Keratin, additionally, has a low lipid content (Shimada et al. 2014), and time series were constructed using the same tissue (carapace scute) and organism (loggerhead turtles). Consequently, no lipid extraction was conducted, as this is aimed at comparing isotope values between organisms or tissues with variable lipid content, especially when these tissues have high lipid content (Logan et al. 2008). Furthermore, lipid extraction can affect nitrogen isotope values, therefore requiring separate analyses of carbon and nitrogen stable isotope ratios (Sweeting et al. 2006). A separate analysis for both stable isotope ratios would not have been possible with the small amount of sample within a 50 µm scute slice. This would have required thicker scute slices and the loss of temporal resolution in the time series. In loggerhead turtles, a 50µm scute slice integrates about 0.6 year of resource use (Vander Zanden et al. 2013), a life stage during which growth rates are low (Reich et al. 2008).

Supplement 2: Broken stick model - Code supplement

The broken stick model used was a linear regression (implemented with the *lm* function in R, see section 3 of the code).

The optimization was conducted with the *optimize* function in R (see section 4 of the code) which searches the interval starting after the first scute slice, and ending just before the last scute slice. The function searches this interval to minimize the deviance of the broken stick model.

```
## fit a broken stick model to the longest time series, to identify which
slope indicates
## juvenile stage (stage 1), and which slope indicates adults (stage 2)
## then classify the shorter time series into juvenile or adult based on
their slopes
##
## Note broken stick model: piecwise linear regression, with a breakpoint
that is
## a parameter, that is estimated by optimisation - minimising the deviance
of the linear model


# 1. the data
# --------------------

# the turtle IDs repeated as often as there are scute slices
Turtle <-  rep("101-990",  17)

# a rank order of the scute slices (i.e. longitudinal samples of an inert
tissue)
Rep <- 1:17

# nitrogen stable isotope ratio values
Nitrogen <- c(21.199690, 18.397135, 16.399658, 18.881347, 15.191607,
15.002078, 14.200236, 13.742539, 12.450912, 11.565721, 12.261662,
13.076332, 12.441310, 12.885678, 13.678122, 13.512365, 13.233710)

# carbon stable isotope ratio values
Carbon  <- c(NA, -16.354, -18.330, -17.866, -19.726, -21.937, -18.233,
-16.708, -18.678, -18.580, -19.790, -19.934, -19.176, -19.161, -21.454,
-21.637, -20.417)

# collect all data into a matrix, with the names of the individual turtles
as rownames
data <- matrix(data = c(Rep, Nitrogen, Carbon),
               nrow = length(Rep), ncol = 3,
               dimnames = list(Turtle, c("rep", "d15N", "d13C")))




# 2. empty dataframe to store the results
# --------------------

bs.results <- data.frame(matrix(data = NA, nrow = length(unique(Turtle)),
ncol = 6), row.names = unique(Turtle))
colnames(bs.results) <- c("Turtle_ID",  # turtle name
                          "bp" ,"Nvalue_bp", "slope1", "slope2", "r2.bs")
# parameters of broken stick model
```

```r
# 3. broken stick model - general function
# -------------------------------------------------



# Function to be used to fit a broken stick model, and calculate the
deviance of this model
# the model consists of two linear regression pieces, separated by a break
point (bp)

# Basis functions of the broken stick model
# bp = 4
b1 <- function(x, bp) ifelse(x < bp, bp - x, 0)
b2 <- function(x, bp) ifelse(x < bp, 0, x - bp)


bs_lm <- function(bp)
{
  mod <- lm(d15N ~ b1(rep, bp) + b2(rep, bp),        # broken stick model
            data = bs_data)
  deviance(mod)                                      # calculate deviance
}



# 4. broken stick model - fit to data from each individual
# -------------------------------------------------



# loop to fit the model for each individual turtle
for (ind in 1:length(unique(Turtle))){

  # data just for one individual
  index <- which(rownames(data) == unique(Turtle)[ind])
  bs_data <- as.data.frame(data[index,])    # bs_data = broken stick dataset
just for this 1 turtle

  # identify potential breakpoints
  search_range <- c(min(bs_data[,"rep"])+0.5, max(bs_data[,"rep"])-0.5) #
range in which the potential breakpoint lies (cannot break on first or last
point)

  # optimisation procedure (optimises the deviance to a minimum)
  bs_opt <- optimize(bs_lm, interval = search_range)
  bp <- bs_opt$minimum


  # fit broken stick model with the best breakpoint
  mod <- lm(d15N ~ b1(rep, bp) + b2(rep, bp),
            data = bs_data)
  y.bp  <- mod$coefficients[1]
  s1.bp <- mod$coefficients[2]
  s2.bp <- mod$coefficients[3]

  # save reslts
  bs.results[ind, "Turtle_ID"] <- unique(Turtle)[ind]
  bs.results[ind, "bp"]        <- bp
  bs.results[ind, "Nvalue_bp"] <- y.bp
  bs.results[ind, "slope1"]    <- s1.bp
  bs.results[ind, "slope2"]    <- s2.bp
  bs.results[ind, "r2.bs"]     <- summary(mod)$r.squared

}
```

```
print(bs.results)


# 5. plot results (for nitrogen)
----------------------

# setup empty plot space
par(oma = c(1,1,1,1))
plot(NA, NA, xlim = c(0, 18), ylim = c( 4, max(data[,"d15N" ], na.rm = T)),
     xlab = "Scute layer",
     ylab = expression(paste(delta^{15}, "N (\u2030)")),
     xaxt ='n',   # to remove values on the x axis
     pch =19,
     main = "BS-SiTs")
labels = c(paste("-", sort(c(1:18), decreasing = T)), 0) # add custom x
axis
axis(side = 1, at = seq(0:18), labels = labels, tck = -0.01)

for (ind in 1:length(unique(Turtle))){

    # plot d15N values
    index <- which(rownames(data) == unique(Turtle)[ind])
    serie_diff <- 18 - length(index)  # here we put the timeseries at the
same starting point for each turtle
    points(c(1:length(index)) + serie_diff, data[index,"d15N" ],
          pch = 17, cex = 1.15)

    # plot breaking point
    bp <- bs.results[which(bs.results$Turtle_ID == unique(Turtle)[ind]),
"bp"]
    points(bp + serie_diff,
          bs.results[which(bs.results$Turtle_ID == unique(Turtle)[ind]) ,
"Nvalue_bp"],
          pch = "|", cex = 2, col = "steelblue")

    # add broken stick model
    bs.data <- data.frame(cbind(data[index,"d15N" ],c(1:length(index))))
    colnames(bs.data) <- c("d15N", "rep")
    lines(# x values
          bs.data[which(!is.na(bs.data$d15N)),"rep"] + serie_diff,
          # predicted y values
          predict(lm(d15N ~ b1(rep, bp) + b2(rep, bp), data = bs.data)),
          lty = 2 )


}
```

Supplement 3: Details on the turtles sampled in the study

Table S3. Loggerhead tag identifications and morphometric information CCL, CCW, and SCL, SCW are reported in cm, weight in kg.

Turtle ID	Location	Pit Tag Num	Inconel Tag ID	Plastic Tag ID	CCL	CCW	SCL	SCW	Weight
M 1	Muroto	-	101-983	I-19336-A	92.5	85.8	84.6	71.7	98
M 2	Muroto	-	101-984	I-19337-A	75	71.3	70.8	58.1	59
M 3	Muroto	-	101-976	I-19329-A	75.5	70.6	70.9	57.9	50
M 4	Muroto	-	101-990	I-19340-A	80.3	72.1	75.2	59.2	65
M 5	Muroto	-	101-986	I-19342-A	78.6	76.5	73.2	58.4	60
M 6	Muroto	-	101-987	I-19343-A	70.8	69.4	67.3	55.9	47
M 7	Muroto	-	101-989	I-19345-A	70.2	65.2	65.7	52.1	43
M 8	Muroto	-	101-993	I-19347-A	70.3	64.8	62.7	54.6	42
T 1	Tosashimizu	392145000243197	JPN 83593	JP15241-A	69	65	63.4	54.6	41
T 2	Tosashimizu	-	JPN 83586	JP15242-A	79.9	74.8	73.7	58.2	55
T 3	Tosashimizu	392145000243933	JPN 83589	JP15243-A	68.3	63.4	67	53.6	38
T 4	Tosashimizu	392145000243766	JPN 83590	JP15244-A	82	77	77.5	59.8	62
T 5	Tosashimizu	392145000235602	JPN 13830	JP15245-A	73.5	66	67.9	53.7	47

Supplement 4: Relationship of time series length and morphology of loggerheads
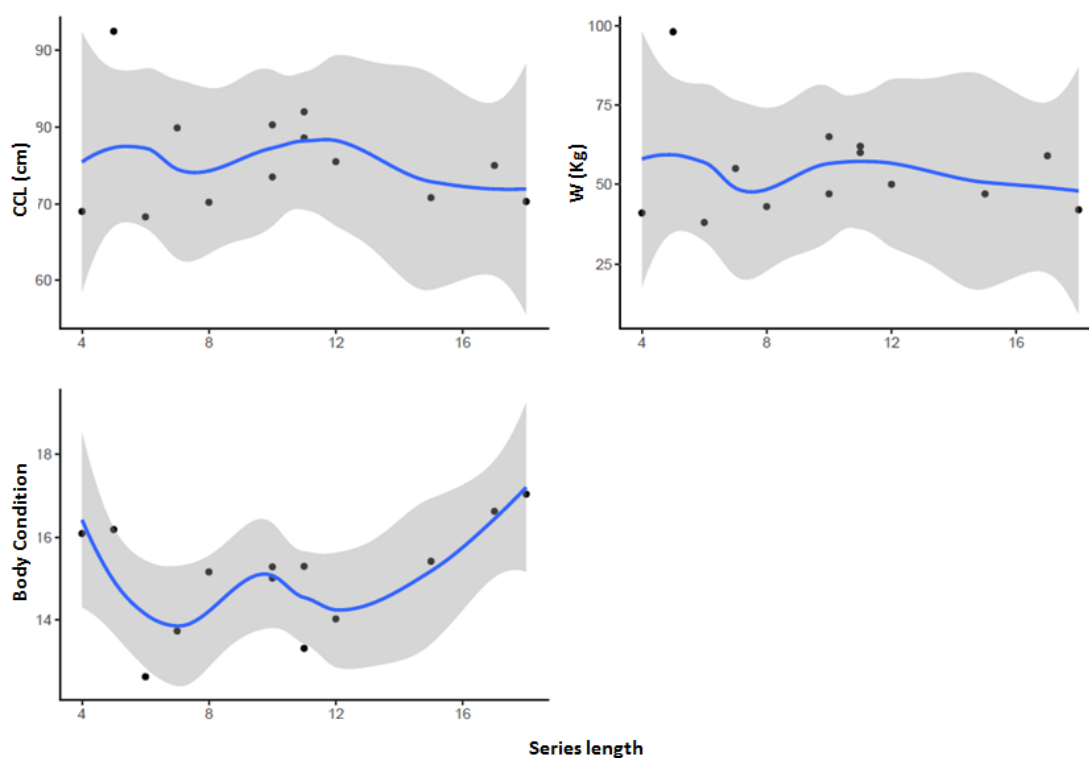
Fig S4. Relationships between the length of the scute slice time series and morphological features of loggerhead sea turtles: CCL (curved carapace length), W (weight) and body condition (kg cm^{-3} * 10^5).

Supplement 5: Relationship between ontogenetic shift and carapace size
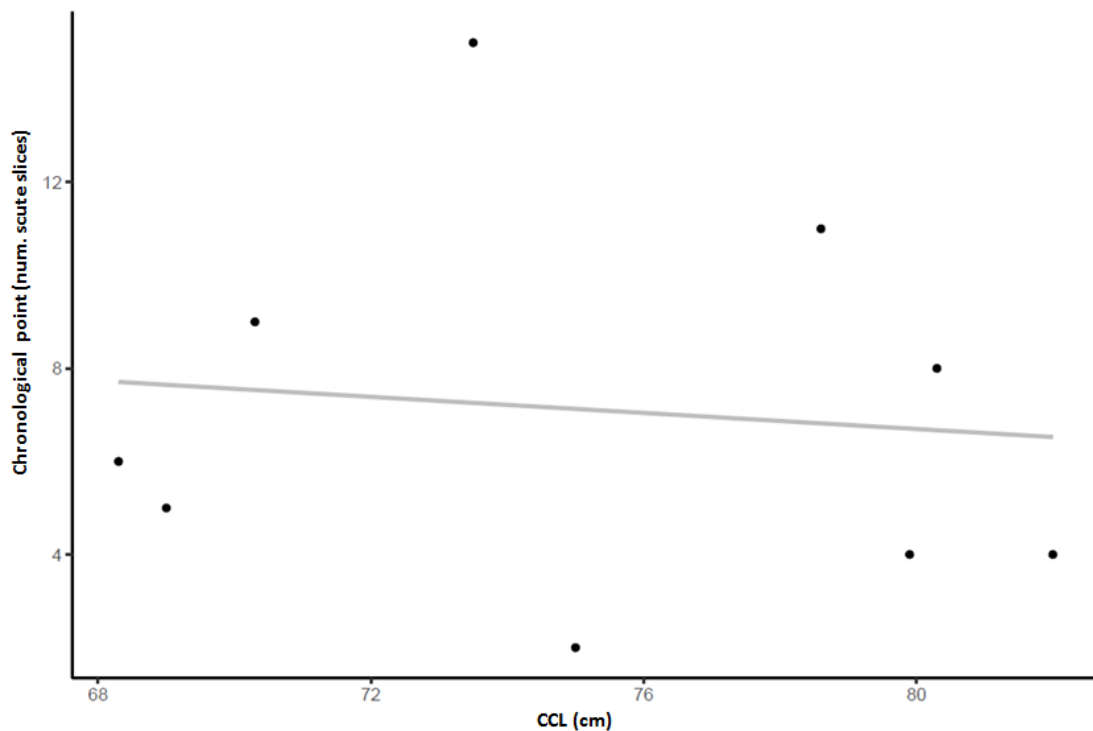

Fig S5. The relation between the carapace size (CCL) and the chronological point of the δ^{15}N breakpoint of individual loggerhead turtles. The chronological timepoint was calculated as the number of slices between the time of sampling and the breakpoint. A linear regression line is added for visualization purposes.

## Supplement 6: Isotopic niche width

Table S6. Individual isotopic niche use as measured by the mode of the posterior distribution of the Bayesian Standard Ellipse Area (SEA-b) enclosing the bivariate normal distribution of $\delta^{13}C$ and $\delta^{15}N$, and the WIC/TNW ratio of individual relative to all sampled individuals together.

Turtle ID	Mode SEA-b (‰^{2})	WIC/TNW
M2	0.38	0.04
M4	1.29	0.15
M5	1.36	0.16
M8	1.29	0.15
T2	3.86	0.45
T3	1.59	0.19
T4	0.63	0.07
T5	2.36	0.28
Population	8.50	
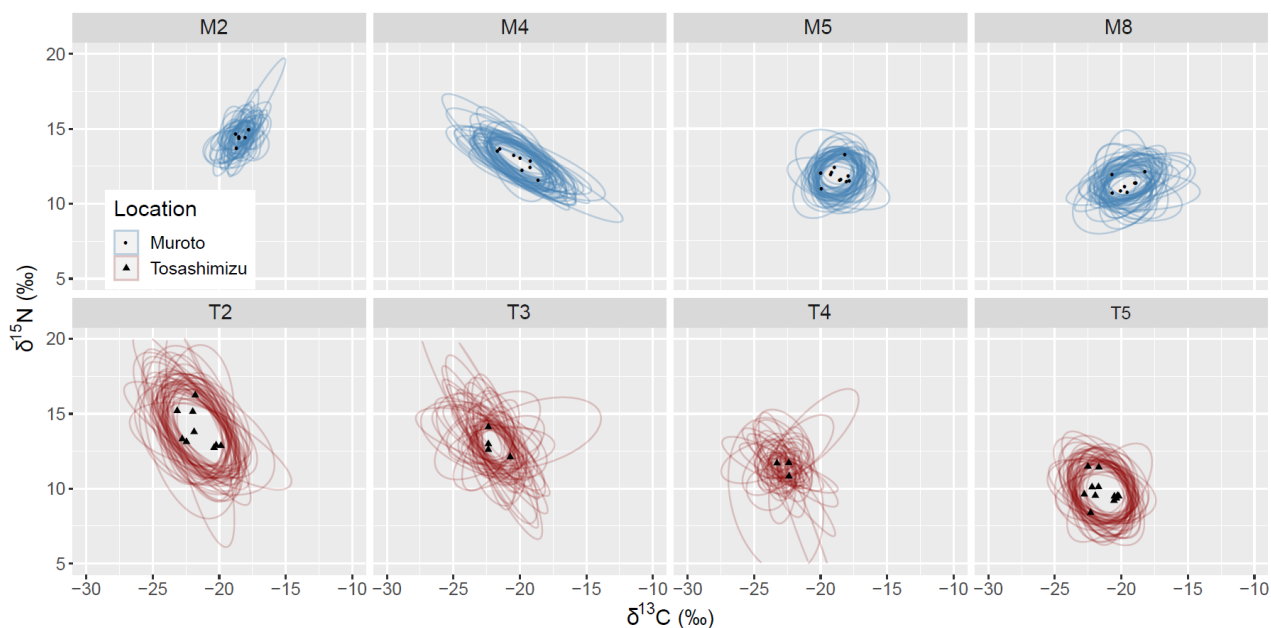
Fig S6a. $\delta^{13}C$ and $\delta^{15}N$ biplot of scute slices classified as "adult" for loggerhead turtles from Muroto and Tosashimizu, Japan. Fifty randomly selected realizations of the Bayesian Standard Ellipse across 8000 iterations to construct the posterior probability distribution of the SEA-b are plotted.
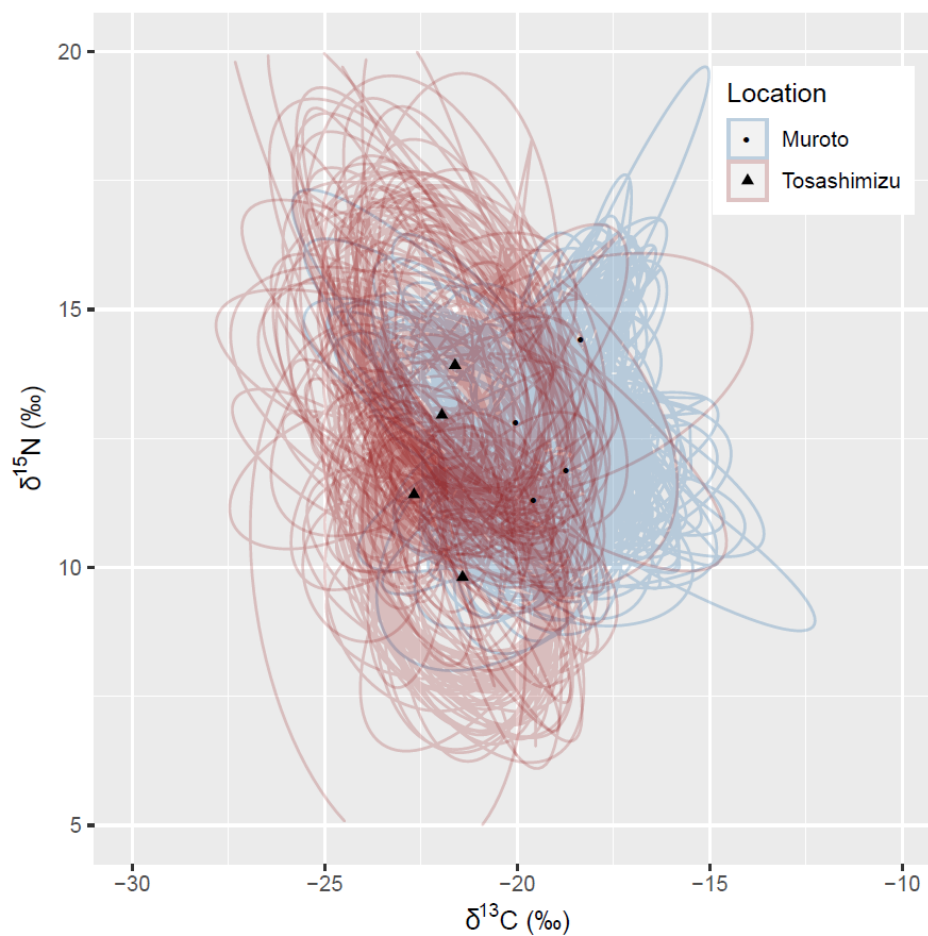
Fig 6b. Biplot of mean $\delta^{13}C$ and $\delta^{15}N$ signatures during the adult phase for loggerhead turtles from Muroto and Tosashimizu, Kochi prefecture, Japan. Fifty randomly selected realizations of the Bayesian Standard Ellipse across 8000 iterations to construct the posterior probability distribution of the SEA-b are plotted.