CHAPTER 12

Body condition and reproductive phenology

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12.1. Introduction

12.1.1 Body condition and reproductive phenology

In avian species nesting in environments characterized by a short breeding season, late or delayed breeders usually experience lower reproductive success compared to early breeders (Meijer et al. 1990, Drent 2006, Legagneux et al. 2016). For example, reduced clutch size (Perrins 1970, reviewed by Meijer et al. 1990) and decreased nestling or fledgling survival (Daan et al. 1988, reviewed by Drent 2006) in late breeders is well documented. In Arctic environments where the breeding season is particularly short, reproductive phenology strongly influences reproductive success (Lepage et al. 2000, Descamps et al. 2011, Anctil et al. 2014). Further, reproduction is an energetically demanding period of the annual cycle, especially for females that must assume the costs of egg formation (Williams 2005, Nager 2006, Vézina and Salvante 2010). During the pre-laying period, female birds face a trade-off between the advantages of early reproduction (higher reproductive success) and the costs associated with early reproduction at a time when ambient temperature is still low (e.g., allocating sufficient energy to self-maintenance rather than reproduction; Drent and Daan 1980, Rowe et al. 1994). Individuals that balance the allocation of energy between self-maintenance and reproduction during this critical life-history stage are expected to achieve greater success (Stearns 1992), for

example by initiating reproduction earlier and producing larger clutches (Drent and Daan 1980, Rowe et al. 1994).

Pre-laying body condition, commonly assessed through body mass corrected for body size, partly explains reproductive decisions regarding breeding propensity (Chastel et al. 1995, Devries et al. 2008, Legagneux et al. 2016) and the timing of egg-laying (Bêty et al. 2003, Hennin et al. 2015), with individuals in better body condition having an increased probability of breeding and earlier laying dates. Most studies to date have been conducted on large-bodied species that accumulate large amounts of body reserves prior to reproduction, and even in these species, body mass, or body mass corrected for body size, displays important individual variability when linked to reproductive decisions (Descamps et al. 2011). From a mechanistic point of view, relevant physiological markers that reflect energy demand and allocation can be used to improve our understanding of individual decisions related to reproductive phenology (Williams 2012). For example, lower energy demand (lower baseline corticosterone) and higher fattening rate (higher triglyceride) predict shorter intervals between arrival and clutch initiation as well as earlier laying date in Arctic-nesting Common Eiders (*Somateria mollissima;* Hennin et al. 2016a). This is a good example of the use of physiology to predict crucial fitness metrics related to reproductive phenology in birds. Nonetheless, to date few studies have been able to measure (Challenger et al. 2001, Hennin et al. 2015, Lamarre et al. 2017) or manipulate (Goutte et al. 2011) physiological condition during the pre-laying period to investigate the relationships between individual physiological condition and reproductive decisions (Goutte et al. 2014, Hennin et al. 2016a, Lamarre et al. 2017). The challenges associated with capturing individuals during the pre-laying period, collecting blood within three minutes of capture, and relating physiological condition to relevant fitness-related metrics explain the scarcity of such studies.

12.1.2 Choosing relevant physiological markers

Using markers that are important underlying drivers of reproductive decisions, Lamarre et al. (2017) linked individual physiological condition to the timing of reproduction in a migratory and Arctic-nesting raptor, the Peregrine Falcon (*Falco peregrinus tundrius*). The role of these markers of energy demand and allocation (Hennin et al. 2015, 2016a) is described in the following sections using the Peregrine Falcon as an example for potentially undertaking a similar study in Gyrfalcons, either in captivity or in the wild. In addition, we describe a method that can be used to assess somatic body reserves (i.e., body mass corrected for body size).

12.1.2.1 Beta-hydroxybutyric acid

Beta-hydroxybutyric acid (BUTY) is a plasma metabolite that reflects negative, short-term changes in body condition (i.e., loss of body fat). High levels of plasma BUTY, a consequence of lipid catabolism during fasting, are correlated with a short-term decrease in body mass in wild birds (Anteau and Afton 2008). Plasma BUTY is elevated in birds subjected to food deprivation (Boismenu et al. 1992) and is a reliable indicator of fasting in birds (Jenni-Eiermann and Jenni 1998).

12.1.2.2 Triglycerides

Plasma triglyceride (TRIG) levels increase as they are transported to be stored in different tissues during fattening (Jenni-Eiermann and Jenni 1998). Plasma TRIG is positively correlated with short-term increases in body mass, and provides an estimate of fattening rate, even in birds captured only once (Anteau and Afton 2008). Plasma TRIG levels also increase during the period of rapid follicle growth prior to laying (Hennin et al. 2015, Lamarre et al. 2017), when ovarian follicles quickly increase in size as they accumulate large amounts of yolk precursors, including yolk-targeted TRIG (Challenger et al. 2001, Gorman et al. 2009). Hence, assessment of plasma TRIG prior to the period of rapid follicle growth provides insight into individual fattening rate, whereas assessment during the period of rapid follicle growth reflects a combination of lipid mobilization for egg production and possibly fattening.

12.1.2.3 Baseline Corticosterone

Corticosterone is the main glucocorticoid hormone in birds. Given that glucocorticoids increase in the blood in response to acute stressful conditions (Romero and Butler 2007), baseline levels of plasma corticosterone (CORT) are considered as background, circulating levels in the absence of external stressors. Variation in CORT is known to reflect and mediate variation in energetic demand (Landys et al. 2006) through its effect on foraging behavior and resource acquisition (Crossin et al. 2012, Hennin et al. 2016b). As such, CORT is usually elevated during energetically demanding events of the annual cycle, such as migration or reproduction (Romero 2002). Moderate elevation in CORT levels can have positive effects on reproduction effort (Crossin et al. 2016), for example by promoting foraging behavior during the pre-laying period (Goutte et al. 2014). CORT has been linked to individual variation in reproductive decisions (Goutte et al. 2010, Love et al. 2014, Hennin et al. 2016a, Lamarre et al. 2017). However, the relationship between CORT and reproductive decisions or success is inconsistent among studies, species, or even reproductive stage (Bonier et al. 2009, Crossin et al. 2016, Lattin et al. 2016). Inconsistent results highlight the importance of investigating the

role of glucocorticoids during the critical pre-laying period in a wide range of taxonomic groups.

12.1.2.4 Scaled mass index (SMI)

Pre-laying body mass and other indicators of body reserves such as body mass corrected for body size can partly explain individual variation in reproductive phenology in Arctic (Bêty et al. 2003, Descamps et al. 2011) and temperate (Devries et al. 2008) bird species. We recommend calculating the scaled mass index (SMI), a measure of stored body reserves that accounts for variation in body size following Peig and Green (2009):

$$SMI_i = M_i\left[\frac{BZ_0}{BZ_i}\right]^{b_{SMA}}$$

where M_i and BZ_i indicate body mass and body size of individual i, respectively. Peig and Green (2009) recommend using the measure of body size that displays the highest correlation with body mass (e.g., tarsus length, wing chord). We recommend measuring several variables of body size such as wing, tarsus, and culmen length to determine one that most closely correlates with body mass. BZ_0 is the mean body size of the sample and b_{SMA} is the scaling exponent of the standardized major axis regression of body mass on body size (see Peig and Green [2009] for a detailed description). Scaled mass index is non-invasive and easy to measure in the field. Moreover, combining SMI with assessment of physiological markers during the pre-laying period can help characterize individual state. For example, low CORT (low energetic demand), low TRIG (low fattening rate) but high SMI during the pre-laying period would likely reflect individuals that arrived on territory already having accumulated large body reserves and are not undergoing fattening or lipid mobilization for egg-production. The R script for calculating the SMI is provided in the online material for this chapter.

12.1.3 The Gyrfalcon as a new study model

The seasonal decline in reproductive performance with later laying date can be especially strong in Arctic-nesting species (Lepage et al. 2000, Descamps et al. 2011), including raptors (Anctil et al. 2014, Lamarre et al. 2017). Hence, individual variation in pre-laying body condition is expected to be a strong driver of reproductive phenology in species nesting in these environments. As such, Gyrfalcons are potentially a good study model to investigate the links between pre-laying physiological condition and reproductive phenology for several reasons. Due to its large size, the Gyrfalcon likely relies on energy reserves accumulated during the pre-laying period to fuel reproduction. However, excessive body reserves can impede flight performance (Klaassen et al. 2006) and the amount of body

reserves that can be stored prior to reproduction must be managed carefully. Therefore, energy resources acquired during the latter stages of the pre-laying period are likely to be critical for egg production in female Gyrfalcons.

Although North American Gyrfalcon populations breeding south of 70° N are primarily considered resident, females are known to complete partial migration between their wintering and breeding grounds (Garber et al. 1993, Booms et al. 2008). Variation in migratory behavior among individuals may therefore result in differences in body condition during the pre-laying period, making the Gyrfalcon a candidate for investigating links between pre-laying physiological condition and the timing of reproduction, particularly in years when ptarmigan abundance is low. Moreover, the egg-laying period varies within and among populations with clutch initiation ranging from early April to late May depending on the geographic region (Cade 1960, Booms et al. 2008). Based on previous research involving Arctic-nesting Peregrine Falcons (Lamarre et al. 2017), it is likely that energy allocation during the pre-laying period is linked to individual variation in the timing of egg-laying, and in reproductive success. Finally, with the exception of a few studies measuring reference values for plasma chemistry (Lierz 2003), virtually nothing is known about the energetic physiology of Gyrfalcons.

In this chapter, we provide detailed methods that can be applied to Gyrfalcons to quantify pre-laying dynamics of individual physiological condition with the goal of linking individual variation in energetic state to variation in reproductive phenology. We use two metrics of reproductive phenology: laying date of the first egg, and pre-laying interval (i.e., number of days between first capture on the breeding grounds and lay date of the first egg; see *pre-laying interval* in Chapter 2). We first describe methods for data collection, then provide examples of output from the analyses, as well as interpretation of the results. Although some sections of the R script are not presented in the Analysis (section 12.3), we provide the complete annotated R script required to conduct the analyses in the online materials.

12.2 Methods

12.2.1 Assessing physiological body condition in the field

To assess physiological body condition during the pre-laying period, or any other period during the breeding season, it is necessary to collect a blood sample. To avoid measuring elevated corticosterone levels that are induced by the stress of capture, blood samples must be taken within three minutes of capture (Romero and Reed 2005). Up to 1.5 mL of blood can be collected from an adult bird via the ulnar vein (26Gx0.5″ needle, 3 mL syringe) and whole blood must be immediately transferred to heparinized

storage tubes. We recommend using 4.0-mL tubes, spray-coated with sodium heparin (68 USP units). Samples must be kept from freezing. This can be prevented by placing the samples in a jacket pocket with a layer between the samples and one's body so they stay cool (ideally 4–8° C), but not warm or frozen. If samples are collected during the summer, they must be kept in a cool place to preserve the stability of the sample. This can be done by placing the samples in a cooler filled with ice packs. To prevent hemolysis (i.e., the rupture of red blood cells) due to excessive shaking during transportation, tubes can be placed in air-foam. At the end of each day, blood is centrifuged at 3300 rpm for 10 minutes to separate red blood cells from the plasma. Plasma is transferred into microtubes (2.0 mL) and ideally stored at –80° C until analysis. Due to constraints related to field-work in remote areas, blood can be stored in a household freezer (–20° C) until the end of the field season and stored at –80° C after the field season.

12.2.2 Laboratory assays of blood samples

Measurement of plasma BUTY ($mmol.L^{-1}$) can be completed with a commercially-available enzymatic and colorimetric assay (Megazyme, Ireland, #K-HDBA; Wagner et al. 2014). Plasma TRIG can be quantified with a commercially-available enzymatic and colorimetric determination kit (Sigma Aldrich, USA, #TR0100-1KT; Williams et al. 2007). Baseline plasma corticosterone ($ng.mL^{-1}$) can be determined with a commercially available enzyme-linked immunosorbent assay (EIA-Assay Designs, Ann Arbor, MI, USA, #ADI-901-097; Hennin et al. 2015). Quantification of these physiological markers in plasma requires specialized laboratory equipment and as such requires the collaborative help of an ecophysiologist.

12.2.3 Quantifying reproductive phenology

Gyrfalcons are highly territorial (Booms et al. 2008) and are likely to initiate reproduction at the nest site associated with the original capture during the pre-laying period. To estimate lay date of the first egg, it is essential to conduct territory visits during the egg-laying period. The use of motion-activated cameras at nest sites is useful for identifying breeding individuals marked during the pre-laying period and to back-calculate lay date from already-initiated clutches. For nests found before clutch completion, lay date of the first egg can be back-calculated assuming a laying interval of approximately 60 hours between each egg (Platt 1977, Tømmeraas 1989). For nests that hatch young successfully, but were discovered after clutch completion, lay date of the first egg can be back-calculated on the basis of methods provided in Anderson et al. (Appendix 1, this volume).

12.2.4 Building your data set

For this example, we use data collected on Peregrine Falcons in the Canadian Arctic (Lamarre et al. 2017). As previously discussed, the energetic costs of reproduction are particularly important for female birds. We will analyze data collected on females (N = 38) in two nesting populations across four years. The first population breeds near Rankin Inlet, Nunavut, Canada (62°49′ N, 92°05′ W), and the second population is found near Igloolik, Nunavut (69°53′ N, 82°51′ W). For our analyses we assume that each female was captured only once and that only one capture occurred per nest site. Table 12.1 contains all variables needed to characterize pre-laying dynamics of physiological condition and link individual condition with reproductive phenology. These variables are: female ID (unique to each individual), study area (Rankin Inlet and Igloolik), year (2012–2015), capture and lay dates (Julian day), pre-laying interval (in days) and reproductive stage (pre-recruiting, PR, or rapid-follicle growth, RFG). The rapid follicle growth period in female Peregrine Falcons was estimated to last approximately 9 days (Lamarre et al. 2017) and, therefore, females captured more than 9 days before egg-laying are identified as "PR," whereas females captured after the initiation of rapid follicle growth (≤ 9 days before egg-laying) are identified as "RFG" in the data set. Finally, the data set also includes variables of condition, i.e., TRIG ($mmol.L^{-1}$), BUTY ($mmol.L^{-1}$), CORT ($ng.mL^{-1}$), body mass (g), and wing chord (mm). The latter is the measure of body size used to calculate the scaled mass index. Each variable is represented as a column, and each observation (each female) is represented as a row (Table 12.1).

12.3 Analysis

12.3.1 Pre-laying dynamics of physiological markers

We first examine the way in which physiological markers involved in energy allocation vary during the pre-laying period. We provide a detailed example for plasma TRIG analyses below. Detailed sections of the script are provided throughout the text and the full script is provided in the online materials for this chapter. After loading the data in the R or RStudio interface under the name of "FEMALE", we calculate mean TRIG ± standard error (SE), as well as sample size for each pre-laying interval (PLI) value using the **`tapply`** function.

```
# remove NA values for the variable TRIG
  FC <- (FEMALE[!is.na(FEMALE$TRIG),])

# calculate mean TRIG for each PLI value
  TRIG_m <- tapply(FC$TRIG, -(FC$PLI), mean)
```

Table 12.1. Subset of the data set showing correct data organization. In this table each column represents one variable, and each row represents one observation from a different Peregrine Falcon female. PLI = pre-laying interval (days), Stage = reproductive stage (pre-recruiting: PR, rapid follicle growth: RFG), TRIG = Triglyceride (mmol.L^{-1}), BUTY = Beta-hydroxybutyric acid (mmol.L^{-1}), CORT = baseline corticosterone (ng.mL^{-1}) Mass (g), Wing chord (mm).

Female ID	Study Area	Year	Capture Date	Lay Date	PLI	Stage	TRIG	BUTY	CORT	Mass	Wing chord
1	Rankin	2012	154	164	10	PR	14.2	1.15	2.32	961	351
2	Rankin	2012	153	171	18	PR	6.61	1.82	0.62	1005	361
3	Igloolik	2012	156	164	8	RFG	17.78	0.32	0.16	972	362
4	Rankin	2013	144	157	13	PR	6.09	2.16	5.66	943	351
5	Rankin	2013	150	158	8	RFG	25.72	0.85	1.00	1117	360
6	Igloolik	2013	148	168	20	PR	1.21	2.69	2.27	982	367
7	Igloolik	2013	151	167	16	PR	19.78	0.98	1.10	1075	356
8	Rankin	2014	141	153	12	PR	7.99	1.80	2.55	940	365
9	Rankin	2014	143	158	15	PR	2.25	2.35	1.74	1047	366
10	Rankin	2015	141	158	17	PR	13.64	0.96	2.65	1206	362
11	Rankin	2015	147	160	13	PR	9.34	1.02	1.31	1026	363

```
# calculate standard deviation of TRIG for each PLI value
  TRIG_sd <- tapply(FC$TRIG, -(FC$PLI), sd)

# calculate sample size for each PLI value
  TRIG_N <- tapply(FC$TRIG, -(FC$PLI), length)

# calculate standard error of TRIG for each PLI value
  TRIG_SE <- TRIG_sd/sqrt(TRIG_N)
```

We can plot mean TRIG ± SE values for each pre-laying interval value using the **plotCI** function available in the package gplots (see online materials). Note that we displayed the pre-recruiting (PR) as well as rapid follicle growth (RFG) periods (Fig. 12.1). A period of nine days is required for rapid follicle growth and eggshell formation in Peregrine Falcons (Lamarre et al. 2017). Peregrine Falcons and Gyrfalcons are closely related species and although the duration of rapid follicle growth has not been estimated in female Gyrfalcons, 9–10 days is likely a good approximation. The figure reveals that plasma TRIG in females increases during the pre-laying period, but appears to reach a maximum at approximately 7–8 days before egg-laying (Fig. 12.1; i.e., approximately 1–2 days following the start of the rapid follicle growth period). To identify any changes in the secretion dynamics of plasma TRIG during the pre-laying period, we will use the **segmented** function in the package segmented, an iterative procedure that tests for significant positive or negatives changes (breakpoints) in a regression model.

```
# look for breakpoint in the regression between TRIG and PLI

# transform PLI from positive to negative values
  FEMALE$PLIneg <- -FEMALE$PLI

# create a linear model using raw data
  lm1<-lm(TRIG ~ PLIneg, FEMALE)

# ask the segmented function to look for a breakpoint around
# the PLIneg value specified by the psi argument
  Seg <- segmented(lm1, seg.Z = ~ PLIneg, psi = -7,
  it.max = 1000)

# if the function converged to a breakpoint, we can get the
# value
  Seg$psi[2]

# and the standard error of the estimated breakpoint value
  Seg$psi[3]
```

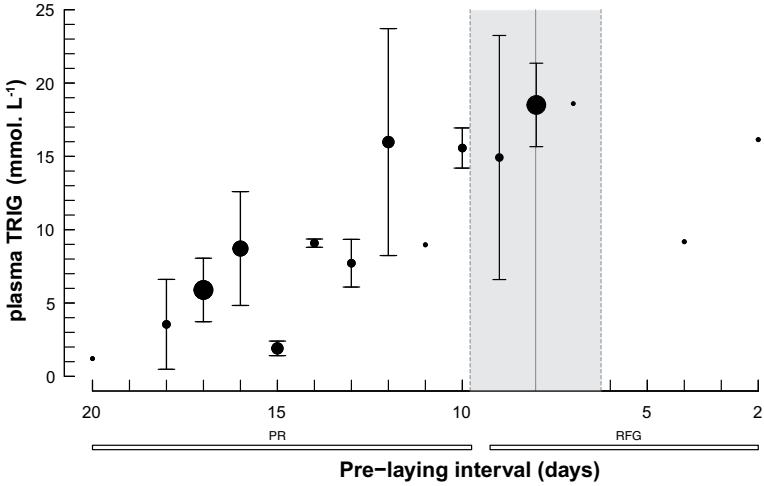

Figure 12.1. Pre-laying dynamics of plasma TRIG in Arctic-nesting female Peregrine Falcons (*Falco peregrinus*). Mean values ± SE are shown for each day during the pre-laying period with symbol size proportional to sample size. The black vertical line and gray shaded area indicate the breakpoint ± SE. The duration of the pre-recruiting (PR) and rapid follicle growth (RFG) periods are indicated by the bars below the x-axis. *Modified from Lamarre et al. (2017) and presented with permission from Springer Publishing.*

The **`segmented`** function converged to a breakpoint at approximately 8.0 ± 1.8 days before egg-laying of the first egg, indicating that plasma TRIG reached the highest values approximately one day after the start of the estimated rapid follicle growth period. Because the segmented function uses an iterative method, we will get slightly different results every time we perform the analysis. We added the breakpoint ± SE in Fig. 12.1 to visualize the results. We will now examine whether plasma TRIG levels vary prior to and after the estimated breakpoint by attempting to fit linear regressions.

```
# fit a linear regression before and after the estimated
# breakpoint for the relationship between plasma TRIG and
# PLI
  summary(lm(TRIG ~ PLIneg,
       data = FEMALE[FEMALE$PLIneg < Seg$psi[2],]))
  summary(lm(TRIG ~ PLIneg,
       data = FEMALE[FEMALE$PLIneg > Seg$psi[2],]))
```

The summary of the linear models indicates that plasma TRIG increased up to the breakpoint (slope: $\beta = 1.32 \pm 0.41$, t = 3.15, $P < 0.001$). This increase likely indicates a combination of fattening and mobilization of lipid reserves for follicle development. After the breakpoint, plasma TRIG levels remained stable (slope: $\beta = -0.94 \pm 0.95$, t = –0.99, $P = 0.36$). We did not add the fitted regression lines and the 95% confidence interval to Fig. 12.1 to avoid overcomplicating the figure. Although we did not consider random factors in this example, biologists should carefully consider the random structure of the models to be tested (see Chapter 7, this volume). Data used in this example were collected in two locations and across four years, but controlling for the effect of the study area and year by including them as random factors in the structure of the models did not affect our conclusions (results not shown).

Trends in plasma TRIG secretion dynamics in our example (rapid increase during the pre-recruiting period followed by asymptote during rapid follicle development) were similar to those found in Arctic-nesting Common Eiders (Hennin et al. 2015). The patterns observed in Arctic-nesting birds contrast with species nesting in temperate regions that show a sharp increase in plasma TRIG at the start of rapid follicle development (Challenger et al. 2001). This apparently early mobilization of energetic reserves for follicle development may be an adaptation for species breeding in highly unpredictable environments such as polar regions (Hennin et al. 2015, Lamarre et al. 2017), and may explain why plasma TRIG levels increase well before the period of rapid follicle growth in Arctic-nesting birds. As yet, this has not been shown in Gyrfalcons.

Although male raptors are known to provision females during the pre-laying period, females reduce their own foraging rates prior to egg-laying (Meijer et al. 1989). This change in foraging behavior could explain the pattern observed in TRIG after the breakpoint (Fig. 12.1), although caution is required when interpreting these results due to low sample size (N = 9 females in RFG stage). Similarly, Hennin et al. (2015) found that plasma TRIG decreased slightly during the final few days preceding egg-laying and hypothesized that female Common Eiders forage at lower rates once sufficient reserves for egg production have been accumulated. It is particularly interesting to observe a similar pattern in the pre-laying dynamics of plasma TRIG in two species with entirely different life history traits and reproductive strategies. Pre-laying dynamics of BUTY, CORT and SMI are described in more detail in Lamarre et al. (2017) and the R script developed for plasma TRIG can be used with other physiological markers.

12.3.2 Linking pre-laying physiological condition to reproductive phenology

We will now use mixed-effects linear regression, combined with model ranking and selection based on the second-order Akaike's Information Criterion (AICc: Akaike 1974, Hurvich and Tsai 1989, Burnham and Anderson 2002), to examine the links between pre-laying physiological condition and the timing of egg-laying in pre-recruiting females. Here we examine only pre-recruiting females because the secretion dynamics of physiological markers can shift during the rapid follicle growth period (see section 12.3.1, Hennin et al. 2015, Lamarre et al. 2017), and because females have already made the decision to initiate reproduction during the latter period. As indicated, the duration of rapid follicle growth has not yet been investigated in Gyrfalcons, but investigating the dynamics of physiological markers during the pre-laying period will likely contribute to the identification of rapid follicle growth, or period(s) when shifts in the secretion of physiological markers occur. For this example, we will look at the link between pre-laying interval (response variable) and pre-laying physiological condition (independent variables). To perform model comparison, we will only include pre-recruiting females with a complete profile (i.e., simultaneous measurement of BUTY, TRIG, CORTb and SMI: $n = 23$).

```
# remove observations with NA values for independent variables
  FEMALE <- FEMALE[!is.na(FEMALE$TRIG & FEMALE$BUTY &
       FEMALE$CORT & FEMALE$SMI & FEMALE$PLI),]

# create a subset with pre-recruiting females only
  FEMALEPR <- subset(FEMALE, Stage == "PR")
```

Our data include females that were sampled across multiple years in two study areas. To control for the effect of these two variables, we incorporate a random structure to our models (i.e., include Study Area and Year as random factors). Using the **`lme`** function of the package nlme, we therefore create models testing only for a random intercept of the factors Study Area and Year, as well as Year nested within Study Area. These models are ranked based on AICc to select the random structure to consider in the set of candidate models.

```
# create simple models testing only for a random intercept of
# the factors Study Area and Year
  lmm1 <- lme(PLI ~ 1, random = ~1|Study_Area,
       data = FEMALEPR)
  lmm2 <- lme(PLI ~ 1, random = ~1|Year, data = FEMALEPR)
  lmm3 <- lme(PLI ~ 1, random = ~1|Study_Area/Year,
```

```
      data = FEMALEPR)
  mod <- list(lmm1,lmm2,lmm3)
  names <- c("Study_Area", "Year", "Study_Area/Year")

# compare these models using AICc
  aictab(cand.set = mod, modnames = names, sort = TRUE,
      second.ord = T)

# output

Model selection based on AICc:

                 K   AICc Delta_AICc AICcWt Cum.Wt Res.LL
 Study_Area      3 115.78       0.00   0.47   0.47 -54.26
 Year            3 116.01       0.23   0.42   0.89 -54.37
 Study_Area/Year 4 118.73       2.96   0.11   1.00 -54.26
```

Results indicate that the model including a random intercept for Study Area is the most parsimonious, although the model with Year is equivalent (ΔAICc < 2, Burnham and Anderson 2002). Because of our small sample size, we will retain only Study Area in the random structure of our candidate models. We will compare 10 biologically relevant models including a null model that only tests for a random intercept of the Study Area. All covariates are standardized using the **`scale`** function and pairs of covariates exhibiting a Pearson r > 0.5 are not included in the same model. We create a new function called AICc that uses the **`aictab`** function in the package AICcmodavg to rank candidate models. The argument **`second.ord = T`** allows model ranking based on AICc. Note that here we set method = "**`ML`**" (maximum likelihood) to compare mixed-effect models with different fixed effects and a fixed random factor.

```
# create a function called AICc

 AICc <- function (FEMALEPR)
 {
  mod <-list()
  mod[[1]] = lme(scale(PLI) ~ 1, random = ~1|Study_Area ,
      data = FEMALEPR, method = "ML")
  mod[[2]] = lme(scale(PLI) ~ scale(log(BUTY)),
      random = ~1|Study_Area, data = FEMALEPR,
      method = "ML")
  mod[[3]] = lme(scale(PLI) ~ scale(CORT),
      random = ~1|Study_Area, data = FEMALEPR,
      method = "ML")
```

```
  mod[[4]] = lme(scale(PLI) ~ scale(TRIG),
       random = ~1|Study_Area, data = FEMALEPR,
       method = "ML")
  mod[[5]] = lme(scale(PLI) ~ scale(log(BUTY)) + scale(TRIG),
       random = ~1|Study_Area, data = FEMALEPR,
       method = "ML")
  mod[[6]] = lme(scale(PLI) ~ scale(TRIG) + scale(CORT),
       random = ~1|Study_Area, data = FEMALEPR,
       method = "ML")
  mod[[7]] = lme(scale(PLI) ~ scale(SMI),
       random = ~1|Study_Area, data = FEMALEPR,
       method = "ML")
  mod[[8]] = lme(scale(PLI) ~ scale(SMI) + scale(log(BUTY)),
       random = ~1|Study_Area,data = FEMALEPR, method = "ML")
  mod[[9]] = lme(scale(PLI) ~ scale(SMI) + scale(CORT),
       random = ~1|Study_Area,data = FEMALEPR, method = "ML")
  mod[[10]] = lme(scale(PLI) ~ scale(SMI) + scale(TRIG),
       random = ~1|Study_Area,data = FEMALEPR, method = "ML")

  Modnames<-paste("mod", 1:length(Mod), sep = "")
  Names = c(
    "NULL",            #1
    "log BUTY",        #2
    "CORT",            #3
    "TRIG",            #4
    "log BUTY + TRIG", #5
    "TRIG + CORT",     #6
    "SMI",             #7
    "SMI + log BUTY",  #8
    "SMI + CORT",      #9
    "SMI + TRIG"       #10
  )
  return (aictab(cand.set = mod, modnames = Names,
       sort = TRUE, second.ord = T))
}
```

If we apply the function to our data set, we get the following output:

```
# apply the function over our data set
  results_PLI <- AICc(FEMALEPR)

  print(results_PLI)
```

```
# output

Model selection based on AICc:

                      K  AICc   Delta_AICc  AICcWt Cum.Wt
 TRIG                 4  68.04  0.00        0.49   0.49
 SMI + TRIG           5  70.60  2.56        0.14   0.62
 TRIG + CORT          5  71.27  3.23        0.10   0.72
 log BUTY + TRIG      5  71.30  3.26        0.10   0.81
 NULL                 3  71.51  3.47        0.09   0.90
 log BUTY             4  72.73  4.69        0.05   0.95
 SMI                  4  74.33  6.29        0.02   0.97
 CORT                 4  74.45  6.41        0.02   0.99
 SMI + log BUTY       5  76.01  7.97        0.01   1.00
 SMI + CORT           5  77.63  9.59        0.00   1.00
```

The output of the model selection based on AICc indicates that the model with TRIG is the most parsimonious. No other models were considered competitive (i.e., all with ΔAICc > 2). Now we look at the summary of the fixed effects for this model:

```
# summary of the most parsimonious model
  summary(lme((PLI) ~ (TRIG), random = ~1|Study_Area,
       data = FEMALEPR, method = "ML"))

# output

 Fixed effects: (PLI) ~ (TRIG)
              Value   Std.Error DF   t-value   p-value
 (Intercept) 16.73    0.87      20    19.26     0.00
 TRIG        -0.26    0.10      20   -2.60      0.02
```

TRIG levels of individual females during the pre-recruiting period (i.e., 10 to 20 days prior to egg laying) best predicted individual variation in reproductive phenology. Specifically, females with lower fattening rates (i.e., low TRIG) during this period had a longer pre-laying interval, regardless of capture date (slope: $\beta = -0.26 \pm 0.10$, t = –2.60, $P = 0.02$). Pre-laying physiological condition (fattening rate) thus appeared to be a key driver of breeding decisions regarding the timing of reproduction, a parameter known to strongly affect nestling survival in Arctic-nesting raptors (Anctil et al. 2014). This analysis can be repeated for lay date of the first egg, another important measure of reproductive phenology.

12.4 Conclusion: future research priorities for Gyrfalcons

In this chapter we have illustrated the importance of investigating the links between physiological condition during the critical pre-laying period and subsequent within-season reproductive decisions. Using a data set collected on a raptor species closely related to the Gyrfalcon, we have shown that females adjust their physiological condition during the pre-laying period and that fattening rate (TRIG) can strongly influence individual decisions regarding reproductive phenology. Although it is well established that reproductive phenology strongly influences reproductive success in Arctic-nesting species, the underlying physiological mechanisms driving individual-based decisions regarding the timing of reproduction and reproductive investment remain poorly studied. To our knowledge, the links between pre-laying physiological condition and reproductive phenology have never been investigated in Gyrfalcons in the wild or in captivity. At the individual level, quantification of food intake through behavioral observations in association with TRIG assessment will likely help explain whether high TRIG levels during the pre-laying period in Arctic-nesting raptors are a consequence of sustained food intake or the mobilization of stored lipids. At the population level, long-term monitoring of pre-laying physiological condition could provide insight into how changes in prey availability affect reproduction in Gyrfalcons. Ptarmigan are particularly important prey for Gyrfalcons as they are likely the only source of food available during the pre-laying period (Booms et al. 2008, 2011). Moreover, model-based predictions on spatial distribution of Gyrfalcons and both Rock (*Lagopus muta*) and Willow Ptarmigan (*Lagopus lagopus*) predict a decrease in the range of these species (Huntley and Green 2011), as well as in the spatial overlap between the Gyrfalcon and their prey over the coming decades (Booms et al. 2011) as a result of predicted climate change. Concerns for delayed reproduction in Gyrfalcons as a consequence of collapsing ptarmigan abundance cycles in Yukon, Canada, have already been expressed (Mossop 2011). On the other hand, climate change in the Arctic may offer Gyrfalcons some opportunities to adjust the onset of reproduction to new food resources during the pre-laying period (Henny 2013). As such, the interactions between climate change, food availability, and reproduction in Gyrfalcons are difficult to predict. However, investigating the effects of changes in food accessibility and diversity during the pre-laying period using assessment of physiological condition will likely provide insight into ways in which predicted climate change may indirectly affect reproductive decisions in this top predator of the Arctic ecosystem.

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