2019

The cost of breeding in the winter versus the summer in an opportunistic, northtemperate songbird, the red crossbill (Loxia curvirostra)

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The Cost of Breeding in the Winter versus the Summer in an Opportunistic, North-Temperate

Songbird, the Red Crossbill (*Loxia curvirostra)*

by

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Thesis

Submitted to the College of Arts and Sciences

Eastern Michigan University

in partial fulfilment of the requirements

for the degree of

MASTER OF SCIENCE

in

Ecology Evolution and Organismal Biology

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November 12, 2019

Ypsilanti, Michigan

Dedication

This thesis is dedicated to my family. I cannot thank them enough for the support they've given me. Dad, you're the reason I went into biology and the reason I want to teach. Mom, you were always there to tell me to look at the big picture when I got caught up in the small things. Savannah, I am so proud of where you are going in your career, you impress me every day. You've all been a great source of comfort and they are all great examples of who I strive to be every day. I would also like to thank my advisor Jamie Cornelius, without her help working through this process I am sure it would never have happened. To my friends old and new, thanks for keeping me sane through this process. Finally, to my dogs, past and present, your love has never wavered, and mine for you won't either.

Abstract

Energy is the currency of life, where a surplus allows survival and reproduction and a long-standing debt leads to death, yet monitoring energy expenditures in free-living animals has been relatively limited by available technology. Radio transmitters that have been specially modified to detect heart rate, however, allow for real-time estimation of energy expense in free-living, behaving animals. Red crossbills live at northern latitudes year-round and breed opportunistically throughout much of the year. They therefore offer a unique opportunity to examine the eco-physiology of different life cycle stages under drastically variable seasonal conditions. Here we present heart rate data of free-living, non-breeding and breeding red crossbills in the summer and winter. We discuss these variables in the context of red crossbills' unique opportunistic and nomadic annual schedules and the highly seasonal conditions of our field site in Grand Teton National Park.

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Introduction:

Animal fitness is intimately tied to energy dynamics. Animals survive only if they are able to meet the energy demands of basic maintenance processes and can only reproduce if they have excess energy potential to invest in their young (Schneider 2004; Sgueo et al. 2012; Speakman 2008). Fitness thus depends on a dynamic balance between energy intake and energy expense–both of which are responsive to a wide variety of internal and external factors. Energy intake, for example, is dependent on foraging effort and skill (phenotypic traits of the organism) as well as food availability and day length (environmental factors), which can all change across time (Collins and Morellini 1979; Schneider 2004; Speakman 2008). Similarly, energy expense is dependent on a wide variety of endogenous processes that change across time, often in response to changing environmental conditions (e.g., thermoregulation across seasons, immune investment during infection, etc.) (Calow and Forbes 1998; Sokolova et al. 2012). Endogenous processes may thus compete for limited body reserves of energy, generating trade-offs that may have consequences for an organisms' fitness and driving the scheduling of costly life cycle events to predictable environmental cycles.

One predictable environmental cycle that has a particularly strong impact on annual schedules in animals of the north-temperate zone is the seasonal change in day length---and the associated changes in temperature and primary productivity that this cycle generates. Endotherms, for example, use metabolic heat production to maintain a constant body temperature; thus, seasonal changes in weather or climate generate seasonal patterns in thermoregulatory costs. Studies have indicated that populations of endotherms living in cold environments maintain higher basal metabolic rates (BMR) than populations living in warmer environments (Chaffee and Roberts 1971). Individual birds have been shown to adjust their

BMR in order to acclimatize to their changing temperate environment, demonstrating plasticity in thermoregulatory capacity. A study of field sparrows (*Spizella pusilla),* for example, demonstrated that winter-acclimatized individuals generally had higher basal metabolic rates than summer-acclimatized individuals (Olson and Kendeigh 1980). Passerine birds may be particularly sensitive to the effects of changing environmental conditions given their small body size and high mass-specific metabolic rates. For cardinals (*Cardinalis cardinalis*), foraging efficiency is lower, and nights are longer in the winter---a potentially deadly combination given that songbirds require a daily replenishment of fat stores to survive the overnight fast on very cold nights. In fact, cardinals were found to expend nearly twice the amount of energy to survive during an average winter night of about 13 hours, than they expended during an average summer night of about 9 hours (Sgueo *et al* 2012). Accordingly, songbirds deposit considerably more fat in the winter to ensure survival of the longer, colder night relative to summer (Sgueo *et al* 2012). If wintering birds fail to store enough fat to survive the overnight fast, then the survival consequences are extreme; thus, many species migrate south to avoid the northern winter entirely and most species typically time their investment in reproduction to occur during the comparatively warmer spring and summer.

Yolking eggs and feeding developing nestlings represents a huge metabolic cost to breeding songbirds, and thus, the timing of a spring breeding phase presumably maximizes fitness by separating these costs from those associated with winter thermoregulation (Lozano et al. 1996; Moeller 1994; Smith and Moore 2004). Food availability also generally increases in the spring, reducing the costs of energy acquisition that is required to sustain reproduction. These two factors, climate and food availability, change synchronously in the north-temperate zone for many species, and thus, it is difficult to disentangle the influence of thermoregulatory demand

generated by weather (which impacts energy expense) from the influence of increasing food availability (which impacts energy intake) on decisions to invest in reproduction.

As the effects of climate change continue to grow, environmental conditions are expected to become increasingly unpredictable and may create atypical or shifting relationships between weather, season, and food. A better understanding of evolved adaptations that organisms might utilize in response to changing environments will be imperative for predicting how organisms can respond to global climate change. One approach that may increase our understanding of such adaptations is to investigate species that don't follow the typical pattern of spring breeding. The red crossbill (*Loxia curvirostra*) is a small finch with a bill that is specialized for extracting the seeds from conifer cones (Benkman 1993; Hahn 1994), which are produced in the summer and typically remain attached to the tree for several years (though seeds are dispersed more quickly). In the warmer summer when cones are in early stages of development, crossbills are the only bird species that can access the immature conifer seeds, which they do by prying open the closed scales using their unique crossed bill. Winters are colder and require more energy for thermoregulation (Dawson and Tordoff 1964), but the conifer seeds are more mature (i.e., have higher energy content) and the seeds are more accessible, thus reducing costs of energy intake. If increased foraging efficiency can offset the costs of winter thermoregulation, then it should be possible for crossbills to breed in winter. Indeed, red crossbills are known to breed opportunistically in both seasons throughout their range in North America (Hahn 1998), and decisions to breed appear to be strongly related to the local abundance of conifer seeds in a given season (Hahn 1995; Cornelius *et al* 2012). Red crossbills therefore provide a unique opportunity to understand the complex interactions between energy intake rates, energy expense, and resulting investment decisions in a highly seasonal environment.

The metabolic state of an animal can be measured on several levels, which have been described extensively in endotherms. Basal metabolic rate (BMR) is the rate of metabolism that is measured in an adult animal held at an ambient temperature that is within the zone of thermoneutrality (i.e., no additional metabolic costs to maintain a normal body temperature) and whilst it is not digesting a meal, fighting an infection, supporting reproductive machinery, or performing any other non-essential physiological processes. BMR is thus mostly theoretical and probably very rarely occurs in free-living populations. Resting metabolic rate (RMR) is a more ecologically relevant measurement of minimal metabolic rates in free-living animals. While RMR is similar to BMR, it is not equal: RMR is measured in resting individuals during the inactive period (typically overnight) but allows that animals may be digesting, breeding, fighting infection, or supporting other nonessential physiological processes. Thus, it reflects the lowest level of the actual metabolic cost of an individual in its current physiological state and environmental conditions. Alternatively, the maximum metabolic rate (MMR) is the maximal rate of oxygen consumed when this same animal is undergoing intense, strenuous activity. MMR is typically measured experimentally by forcing the animal to exercise strenuously or meet a severe cold challenge. Finally, field metabolic rate (FMR) is the actual metabolic rate experienced by the animal as it performs its normal daily routines and is typically presented as a 24-hour measure. FMR will therefore usually fall somewhere between RMR and MMR and reflects the real metabolic work the animal is performing to meet its current energy demand. It is difficult to measure energy expense (i.e., metabolism) in free-living species. Previous work has argued that it is almost impossible to assume that measurements of metabolic rate are at basal levels, even in carefully controlled laboratory conditions (Speakman et al. 1993). This study attempts to measure RMR and FMR of free-living passerine birds. RMR and FMR should

accurately estimate the costs that are required to survive in both the summer and winter phenotypes as the birds experience the challenges of thermoregulation, foraging and reproduction.

FMR can be estimated in free-living birds by using the doubly labeled water method to measure CO_2 production (i.e., a direct measure of metabolism), but its applications are limited in the field because they require catching the same bird twice in a short time span (typically 24 hours), something that is nearly impossible for red crossbills. These techniques also only allow for an overview of FMR between the two sampling times and fail to capture the finer picture understanding of how metabolism changes dynamically throughout an entire day (Butler et al. 2004). Alternatively, measuring heart rate remotely with acoustic biotelemetry provides an indirect estimate of metabolic rate and allows for the study of a free-living bird in real time. This method only requires capturing an individual once and is thus preferable for those species that are difficult to recapture. Heart rate biotelemetry also allows for estimates of activity budgets and behavioral observations through active tracking. Heart rate is not a direct measure of metabolic costs but rather is an indirect estimate of oxygen consumption because this method relies on the fact that an increase in heart rate is a major component of the cardiovascular response to increased oxygen demand (Butler et al. 2004). Heart rate correlates tightly with oxygen consumption in many species measured, but there can be some minor inter-individual or seasonal variations in the slope of the relationship and there can be large variations across different forms of locomotion (e.g., resting, hopping and flying) (Butler 1991; Ward 2002). These variations must be either measured and accounted for mathematically or considered theoretically when analyzing and discussing heart rate data in the context of metabolism.

Here I use heart rate data previously collected from free-living red crossbills in winter and summer in years of low and high food availability as an estimate of energy expenditure. I will investigate how reproductive state, activity budgets, seed-intake rates, and environmental parameters (e.g., temperature, precipitation, wind speed, etc.) impact energy expenditure across seasons. I hope to learn what it costs to reproduce under dramatically different thermoregulatory conditions and how food intake might mitigate these differences. I predict that the two most important factors influencing the overall metabolic rate will be environmental temperature and breeding condition (nesting/non-nesting). Below I give several specific hypotheses and the predictions that those hypotheses make concerning the data. Heart rate data have been scored to date for 34 red crossbills: 16 in winter and 18 in summer. Of these, 7 birds were confirmed as actively breeding in winter, and 6 birds were confirmed as actively breeding in summer.

HYPOTHESIS 1: Temperatures below the thermal neutral zone cause an increase in metabolic expense, with lowest temperatures causing the largest change.

- Prediction 1: Heart rate will be higher in winter relative to summer. This prediction requires a comparison of different individuals across seasons, thus there may be some confounds of seasonal acclimation or individual variation in the relationship between heart rate and temperature. I will thus will also look at differences within individuals (predictions 2 and 3).

- Prediction 2: Within a season, colder conditions should cause higher heart rate than warmer conditions.

- Prediction 3: Within individuals, colder temperatures should cause higher heart rate than warmer temperatures.

Hypothesis 2: Breeding birds will have higher metabolic costs than those birds that are non-breeding, particularly when feeding nestlings.

- Prediction 1: Birds captured during a high cone year when breeding was prevalent will have higher average heart rates than birds captured during low cone years when breeding was absent or rare.

- Prediction 2: Birds feeding nestlings or young fledglings will have higher heart rate than non-breeders.

Hypothesis 3: There is an interaction between breeding and temperature such that breeding in colder temperatures produces much higher heart rate than breeding in warmer temperatures.

- Prediction: Birds breeding during colder days will have higher heart rates than birds breeding during warmer days.

Hypothesis 4: Foraging efficiency can offset some of the costs of breeding and thermoregulation.

- Prediction: Birds that consume more seeds per second will have lower heart rates than birds that consume less seeds per second.

Methods:

Heart rate data were collected from free-living red crossbills between 2009 and 2017. Logistical and time constraints prevented us from targeting a single sex, particularly during low cone crop years when red crossbill densities are much lower compared to high cone years. Sex ratios for tracked birds were as follows (the numbers in brackets indicate the number of birds that returned strong heart rate signals, if not all of those individuals that were tracked): high cone summer (1 female, 6 males); low cone summer (2[1] female, 9[5] male); high cone winter (2 female, 7 male); low cone winter (6[1] female, 3[1] male). These ratios are male biased in three of the four seasons and female biased in only low cone winters.

Adult male and adult female red crossbills (*Loxia curvirostra*) were captured using live decoy lures and mist nets in Grand Teton National Park, Wyoming, USA (43°45' N, 110°39' W; elevation 2,073 m) in summer months (July–September) and winter months (January–March) across four different years. Red crossbills are nomadic migrants and occur as a suite of eco-types that are each specialized to particular groups of conifer species. There are 10 types of crossbills described and each can be identified by a unique call structure and by differences in morphology, genetics and ecological associations (i.e., their key conifer) (Benkman 1993). For this experiment Type 5 crossbills, often associated with lodgepole pine *(Pinus contorta),* were used. Type 5s are ideal for this experiment because they can be found in the study location in years of both low cone supply (i.e., only lodgepole pine is producing cones) and high cone supply (i.e., other masting species produce bumper crops)---thus providing a comparative study system for years of low and high reproductive activity, respectively. Other crossbill types are present in high cone years but are less reliably found during low cone years.

Upon capture a small blood sample (<70 ul) was immediately collected by venipuncture from the alar vein. Body size was determined by wing chord, tarsus, and keel lengths measured with calipers to the nearest 0.1 mm and mass was measured using a Pesola spring scale to the nearest 0.5 g. Reproductive status was evaluated using morphological characteristics and was confirmed during tracking based on presence or absence of parental behaviors. Crossbills in high cone years were in variable stages of breeding and in low cone years were not breeding thus in statistical tests we use cone year as a proxy for breeding status. We therefore assume that high summer and high winter represents breeding birds, whereas low summer and low winter represents non-breeding birds.

To measure heart rate, we used 0.6 g heart rate radio transmitters (Sparrow Systems, Fisher, IL, USA). The devices transmit a continuous signal that is modified by the electrical activity generated during a heartbeat. Electrodes were placed under the skin as the transmitter was attached to the mid scapular region of the back. Attachment took approximately five minutes and all individuals were released within 30 minutes of capture. All birds were released before 1100 hours except for one individual that was released at 1300 hours. Heart rate records began at two hours post-release (all birds had resumed foraging activity). Researchers followed the birds on foot and heart rate signals were recorded for 50 hours, or for as many hours as researchers were able to remain with the bird, in winter (N = 16) and summer (N = 19). The signal was recorded using an MP3 player and stored on hard drives for later analysis (see below).

Researchers wore GPS units to record movement through the habitat as they followed the birds and foraging data were recorded opportunistically throughout the 48-hour period of tracking. Foraging bout duration (seconds) was recorded starting when a crossbill picked a new cone to handle and the number of seeds consumed during the bout was determined by counting

the number of seed hulls that were ejected as the bird processed the seeds. The bout continued if the bird switched cones but was terminated if it flew to a new region of the tree or stopped attempting to extract seeds for more than five seconds.

The resulting heart rate audio files were filtered using an Equalization filter in the sound-editing program Audacity. Audio was highlighted in five-minute bins and the peak frequency was determined by creating a plot spectrum and identifying the highest amplitude frequency. An equalization filter of several hundred Hz was constructed around the peak frequency and applied to the file. Ten heartbeats were located at the center of the five-minute segment and the duration (seconds) per 10 beats was recorded. This was transformed to beats per minute. Heart rates were scored instantaneously every 5 minutes and averaged hourly for analysis (Cochran and Wikelski 2005).

Statistical Analysis:

Weather Parameters

To determine how weather varied seasonally we used t-tests to compare temperature and relative humidity in summer versus winter. Weather data was compiled from the nearest NOAA weather stations (Moose WY, approximately 2 km from the main study site).

Heart Rate Models

Analyses were run on two subsets of the data: 1. Using repeated-measures hourly averages of heart rate (FMR) versus temperature, and 2. binned averages to examine daily costs (24 hour average) or night only to remove the impact of activity on heart rate and estimate resting metabolic rate (RMR). Heart rate data were normally distributed; thus, analyses will include parametric statistical tests relevant to each hypothesis, including Student's t test, ANOVA, linear regression, and a linear least squares model.

Results:

Heart rate was not significantly different between sexes in any year or season, but small sample sizes for particular sexes in each season preclude inclusion of sex in analyses of other variables. I will discuss possible biases due to sampling and sex ratios where relevant.

Hypothesis 1: Temperatures below the thermal neutral zone cause an increase in metabolic expense, with lowest temperatures causing the largest change.

Hypothesis 1 made three predictions. First, that heart rate will be higher in winter relative to summer. A Student's t test comparison of means supports this prediction. Red crossbills tracked in the winter months had significantly higher 24-hour average heart rates than did those tracked in summer months ($t_{1,28} = 4.23$; $r^2 = 0.4$; $p = 0.0002$; Figure 1). This compares different individuals across seasons, and thus there may be some confounds of seasonal acclimation.
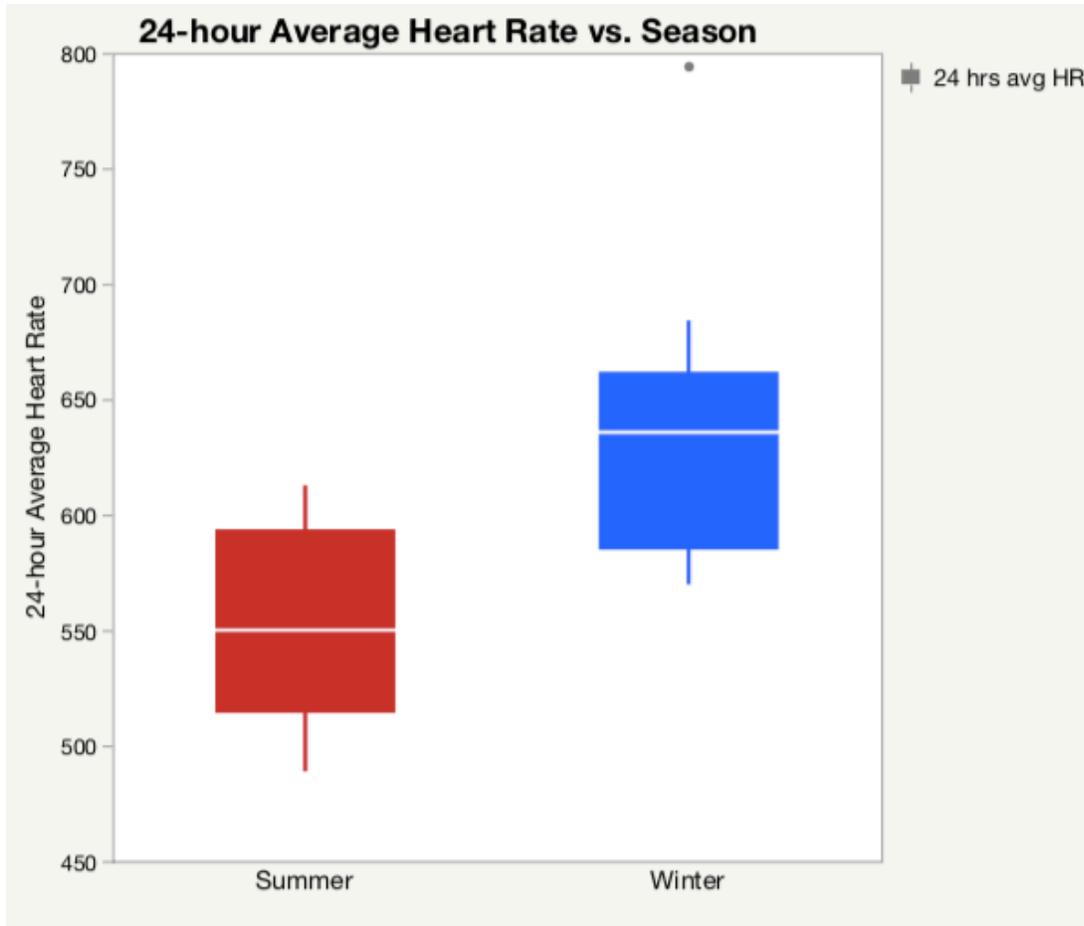
Figure 1: Red crossbills tracked in the winter months had significantly higher 24-hour average heart rates than did those tracked in summer months ($t_{1,28} = 4.23$; $r^2 = 0.4$; $p = 0.0002$). Box plots represent the 25th to 75th quartiles and whiskers represent the minimum and maximums of the data. The horizontal white line represents the median.

Prediction 2 predicts that within a season, colder conditions should cause higher heart rate than warmer conditions. Analysis only included nighttime samples to remove confounding effects of daytime activity. A linear least squares model was constructed using individual bird ID as a random effect, given repeated measures of heart rate within each individual. Hourly temperature, season, cone year and all of their interactions were used as direct effects. Individual ID contributed significantly to the variation in heart rate ($P < 0.0003$). Temperature and season*temperature interaction were significant effects ($P = 0.0001$ and < 0.0001, respectively). All other terms and interactions did not contribute (all $P > 0.2$). Post hoc linear regressions were

run on hourly data to describe the relationship between temperature and heart rate. In summer, there was a negative linear correlation between temperature and nighttime heart rate in both low and high cone years (Low $F_{1,175} = 18.2$, $P < 0.0001$, $R^2 = 0.09$; High $F_{1,107} = 24.1$, $P < 0.0001$, $R^2 = 0.19$; Figure 2).
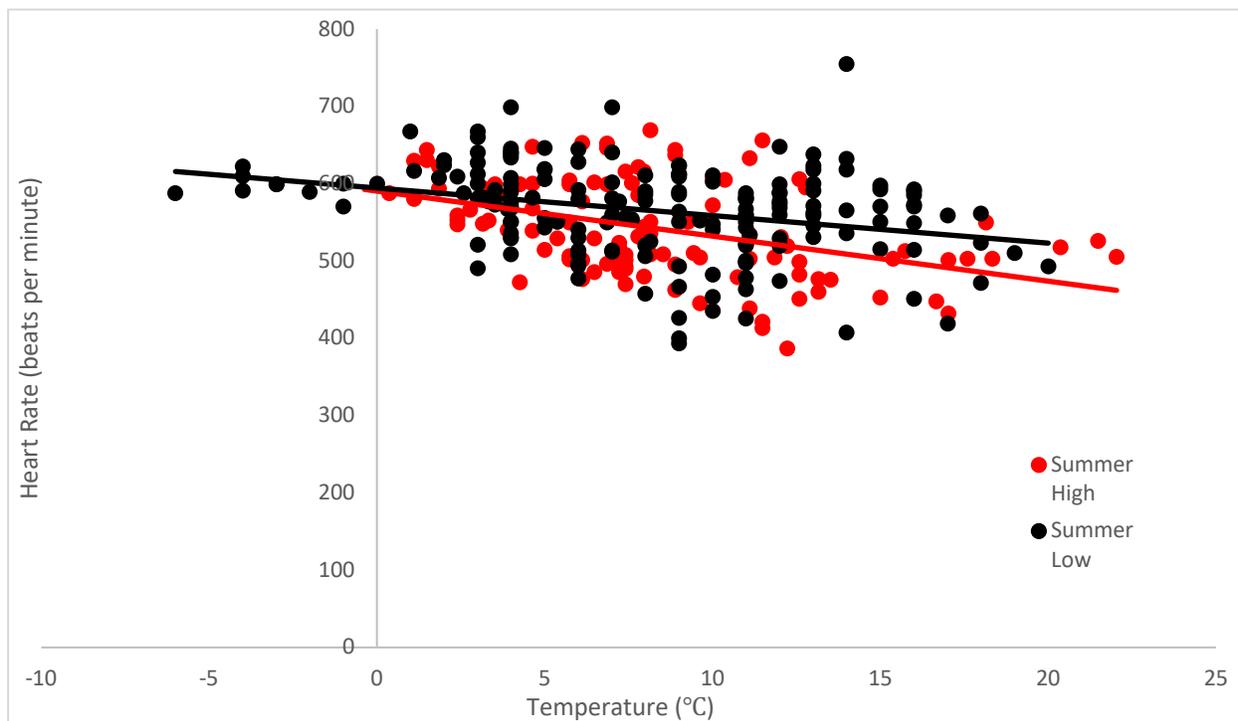

Figure 2. Relationship between temperature (°C) and night time heart rate in free-living red crossbills in the summer season. There was a negative linear correlation between temperature and night time heart rate of red crossbills in both low and high cone years (Low $F_{1,175} = 18.2$, $P < 0.0001$, $R^2 = 0.09$; High $F_{1,107} = 24.1$, $P < 0.0001$, $R^2 = 0.19$).

In winter, the relationship was different between low and high cone years. Similar to summer, there was a negative relationship between nighttime heart rate and temperature during winter in low cone years ($F_{1,170} = 51.3$, $P < 0.0001$, $R^2 = 0.23$; Figure 3). However, in a high cone year winter, there was a positive linear relationship between temperature and nighttime heart rate. ($F_{1,137} = 8.7$, $P = 0.004$, $R^2 = 0.06$; Figure 3).
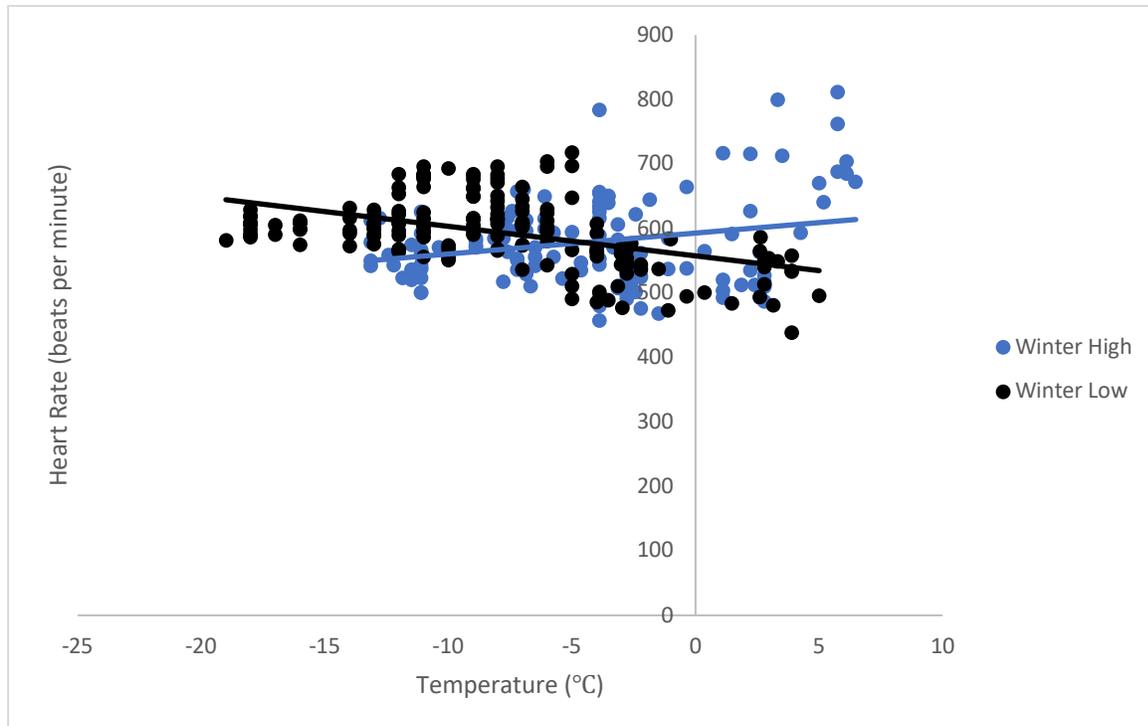
Figure 3. Relationship between temperature (°C) and nighttime heart rate in free-living red crossbills in the winter season. Winter in low cone years was similar to summer, a negative linear relationship ($F_{1,170} = 51.3$, $P < 0.0001$, $R^2 = 0.23$) but in high cone years winter showed a positive linear relationship between temperature and heart rate. ($F_{1,137} = 8.7$, $P = 0.004$, $R^2 = 0.06$).

Prediction 3 of Hypothesis 1 predicts that within individuals, colder temperatures should cause higher heart rate than warmer temperatures. Analysis was again limited to nighttime hours to minimize complications due to activity. Of 32 individuals analyzed by linear regression, 11 had significant negative relationships between temperature and heart rate ($P < 0.05$), two had significant positive relationships ($P < 0.05$), and 19 had insignificant relationships between temperature and heart rate (Table 1).

Table 1: List of all individual birds analyzed. Ordered from most negative slope to most positive slope. Significant relationships are listed in bold font.

ID	Slope	R2	P
179	-22.7	0.56	0.0003
409	-14.2	0.42	0.0005
075	-10.4	0.59	0.002
397	-9.8	0.06	0.23
917	-9.2	0.44	0.003
912	-8.3	0.59	0.002
028	-7.7	0.74	<0.0001
316	-7.3	0.31	0.006
389	-6.7	0.55	0.0002
444	-5.8	0.53	0.0001
434	-5.7	0.06	0.41
350	-5	0.32	0.01
115	-4.1	0.29	0.006
573	-2.8	0.04	0.55
670	-2.8	0.11	0.15
268	-2.5	0.06	0.32
177	-1.3	0.03	0.47
208	-0.8	0.03	0.49
653	-0.8	0.01	0.75
220	-0.7	0.006	0.75
332	0.2	0.0005	0.92
608	0.4	0.01	0.21
998	0.7	0.01	0.65
201	2.9	0.16	0.11
114	3.9	0.11	0.13
135	4.8	0.28	0.01
940	5.5	0.16	0.07
193	6.2	0.06	0.23
715	7	0.1	0.18
043	9.5	0.37	0.003
327	10.2	0.09	0.38
920	15.8	0.19	0.18

Hypothesis 2: Breeding birds will have higher metabolic costs than those birds that are non-breeding, particularly when feeding nestlings.

Hypothesis 2 made two predictions. First, birds captured during a high cone year when breeding was prevalent will have higher average heart rates than birds captured during low cone years when breeding was absent or rare. A t-test of 24-hour heart rate and nighttime heart rate for birds captured in low versus high cone years was insignificant, although a very weak trend was observed for 24-hour average heart rate to be higher in the high cone year with prevalent breeding compared to low cone year with no breeding ($t_{1,28} = 1.5$; $P = 0.14$). Daytime heart rate,

however, was significantly higher during the high cone year compared to low cone year ($t_{1,28}$ = 2.85, P = 0.008; Figure 4).
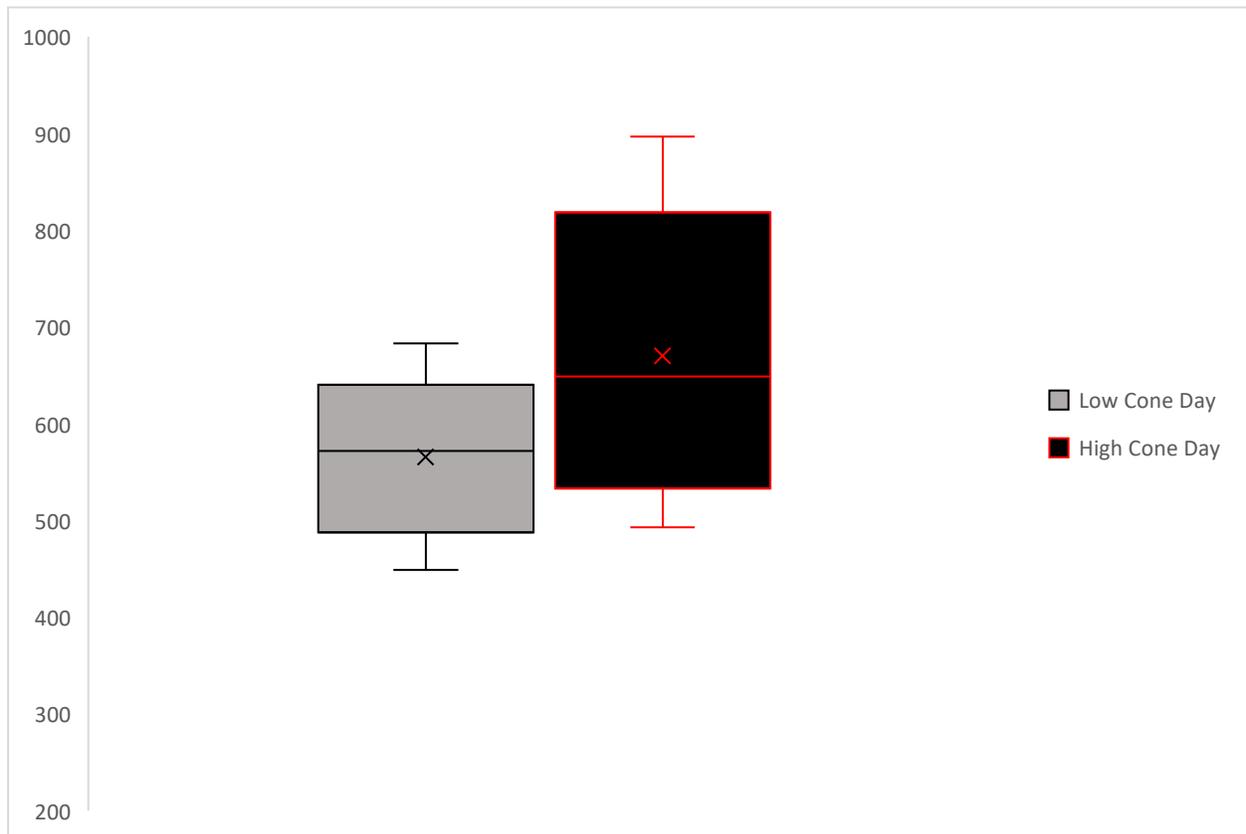

Figure 4: Daytime heart rate, plotted as mean +/- standard error, was significantly higher during the high cone year compared to low cone year ($t_{1,28}$ = 2.85, P = 0.008). Box plots represent the 25th to 75th quartiles and whiskers represent the minimum and maximums of the data. The horizontal white line represents the mean.

Prediction 2 of Hypothesis 2 predicts that birds confirmed to have been feeding nestlings or young fledglings will have higher heart rates than non-breeding birds. Birds that were suspected of visiting nests but for which we could not confirm a nest were excluded from this analysis. ANOVA of 24-hour average or nighttime heart rate was not significantly different by reproductive state (P = 0.24, P = 0.27, respectively; Figure 5) but daytime heart rate was significantly higher in birds feeding young compared to either non-breeders or birds with eggs ($F_{2,20}$ = 3.9, P = 0.04; Figure 5).
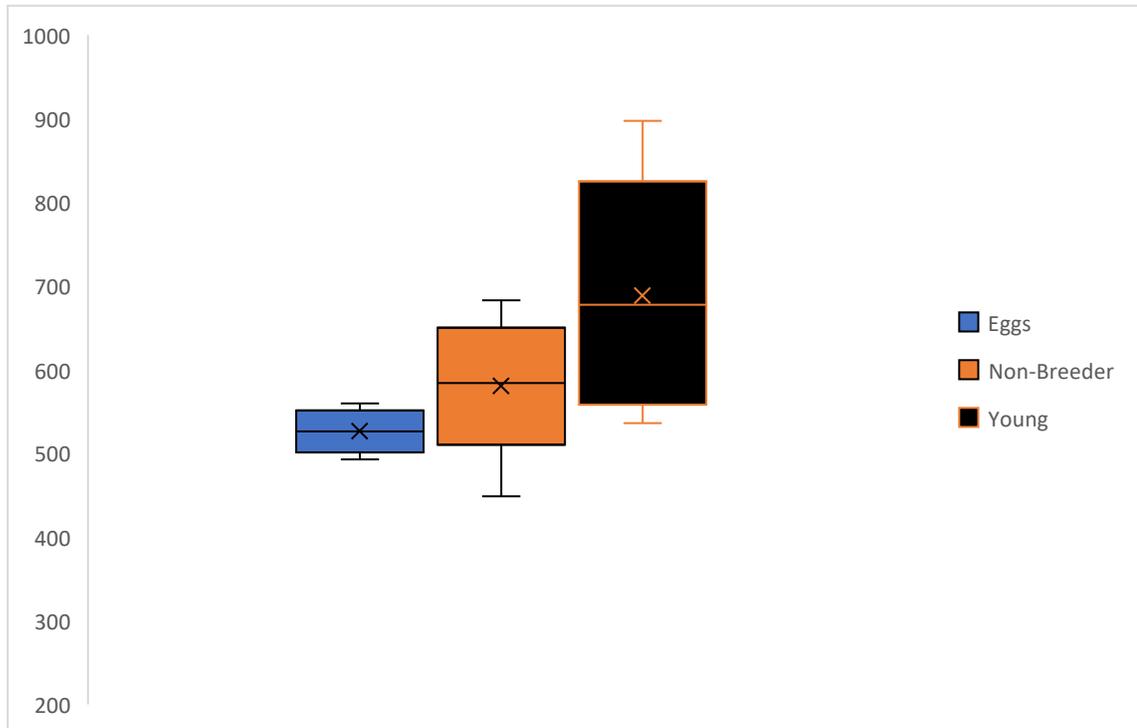
Figure 5: Daytime heart rate, plotted as mean +/- standard error, was significantly higher in birds feeding young compared to either non-breeders or birds with eggs ($F_{2,20} = 3.9$, P = 0.04). Box plots represent the 25th to 75th quartiles and whiskers represent the minimum and maximums of the data. The horizontal white line represents the mean.

Hypothesis 3: There is an interaction between breeding and temperature such that breeding in colder temperatures produces much higher heart rate than breeding in warmer temperatures.

Hypothesis 3 made a single prediction that birds breeding during colder days will have higher heart rates than birds breeding during warmer days. Season*year interaction was significant (P = .05). ANOVA of 24-hour average heart rate by season-year (high summer, high winter, low summer, low winter) is significant ($F_{3,27} = 7.5$, P = 0.001, $R^2 = 0.49$; Figure 6). Winter breeding birds had higher heart rate than summer breeding birds or summer non-breeding birds and non-breeding birds in winter had higher heart rate than non-breeding birds in summer (student's t, P < 0.05). If analysis is limited to daytime heart rate (i.e., when the costs of breeding

are most apparent based on Hypothesis 2 analyses), then breeding in winter becomes

significantly more costly than non-breeding in winter. Summer breeders are still not significantly

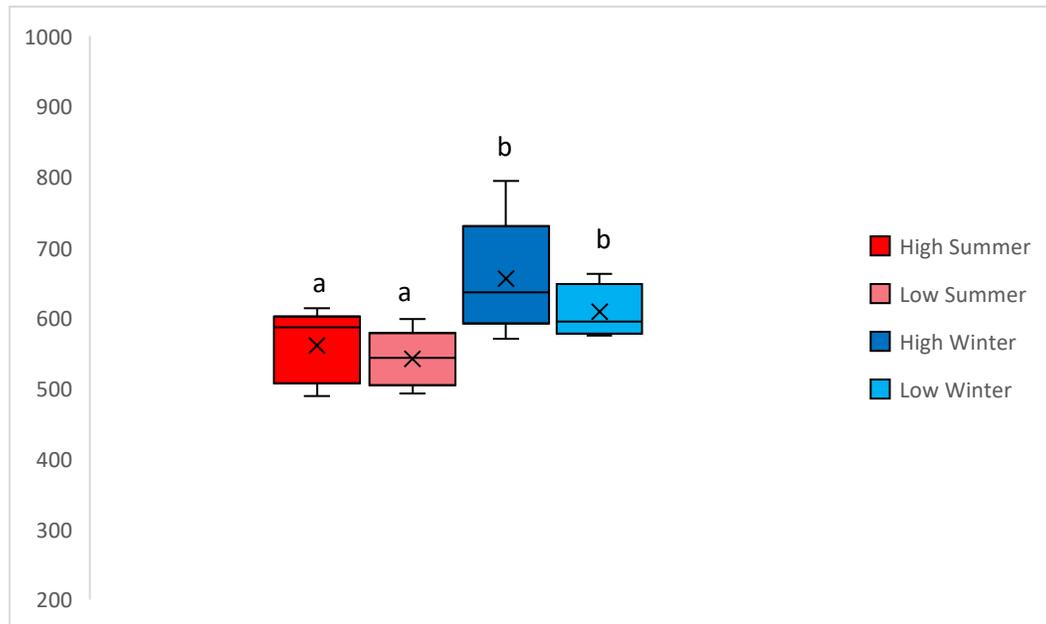different in daytime heart rate than are non-breeders.

Figure 6: 24-hour average heart rate, plotted as mean +/- standard error. Winter breeding birds had higher heart rate than either summer breeding birds or summer non-breeding birds and non-breeding birds in winter had higher heart rate than non-breeding birds in summer ($F_{3,27}$ = 7.5, P = 0.001, R^2 = 0.49). Box plots represent the 25th to 75th quartiles and whiskers represent the minimum and maximums of the data. The horizontal white line represents the mean.

Hypothesis 4: Foraging efficiency can offset some of the costs of breeding and thermoregulation.

Prediction: Birds that consume more seeds per second will have lower heart rates than

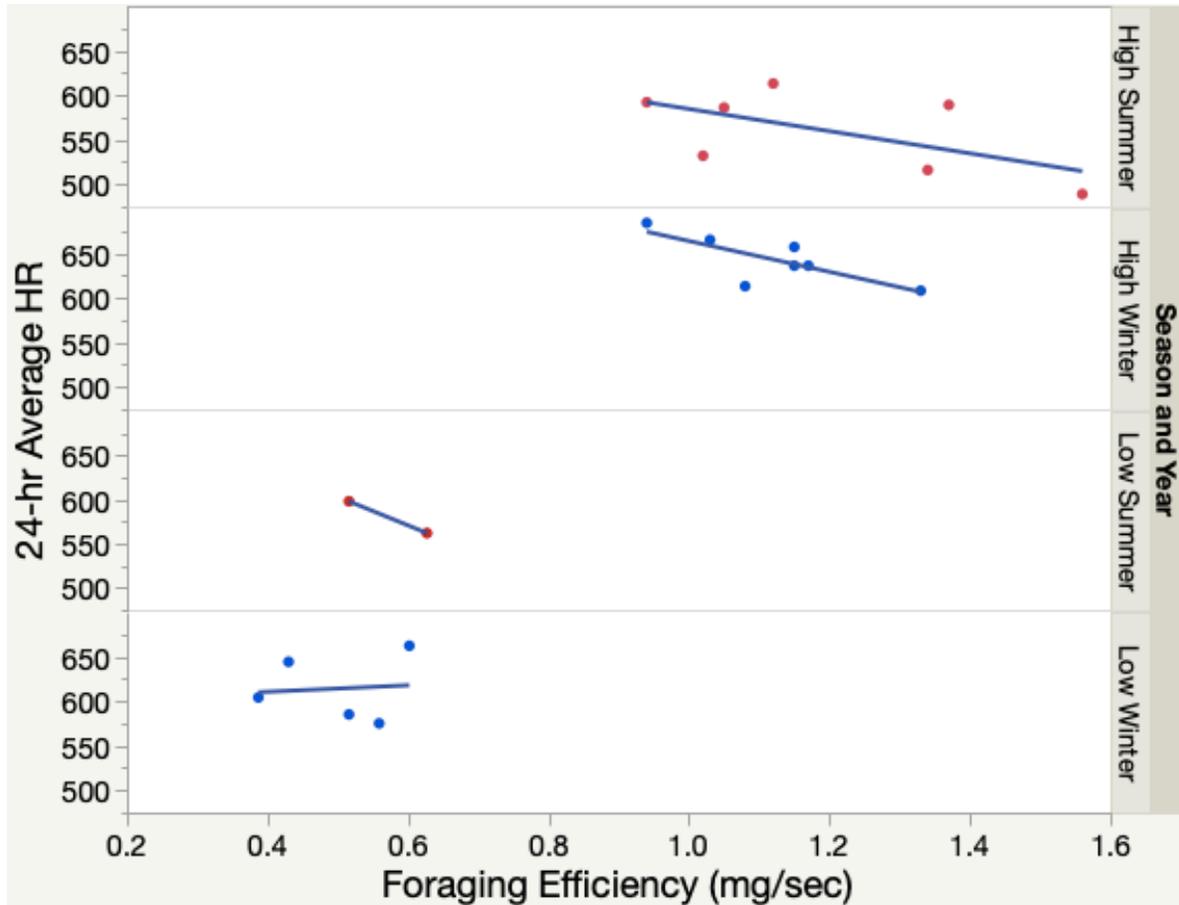birds that consume less seeds per second.

Figure 7: Linear regressions of foraging efficiency against heart rate in free-living red crossbills.

There was a weak negative correlation between foraging efficiency and heart rate during the high cone summer ($F_{1,6} = 2.8$, $P = 0.15$, $R^2 = 0.4$; Figure 7) and a stronger negative relationship between foraging efficiency and heart rate during the high cone winter ($F_{1,6} = 7.2$, $P < .05$, $R^2 = 0.6$; Figure 7). A male bird who was attempting to double brood was excluded as this activity is very rare and the individuals resulting heart rate was a significant outlier. During the low cone summer there were not enough samples to perform a regression because many birds were only eating insects. During the low cone winter there was no relationship between foraging intake and 24-hour average heart rate ($P = .89$). Patterns were similar if analysis was limited to daytime heart rate, thus results are reported for 24-hour heart rate only.

Discussion:

Red crossbills tracked in the winter months had significantly higher 24-hour average heart rates than did those tracked in summer months. These data thus support the general prediction that metabolic costs of survival and reproduction during winter months are more costly than survival and reproduction during the more benign weather months during the summer. This begs the question of why crossbills should elect to breed during the winter. It is possible that the abundance and nutritional value of their food supply allows for this costly and risky endeavor. In support of this hypothesis, there was no difference in the cost of breeding versus non-breeding within each season, suggesting that the high food supply that is thought to stimulate a breeding event (Hahn 1998) may offset the costs of raising young---even in such challenging conditions of winter. One other possible explanation of these data is that winter non-breeding birds have higher heart rates because there is a tendency, though not significantly so, for females to have higher heart rate than males only in the non-breeding years. Future research can be directed at investigating the relationship between sex and heart rate by season more closely.

In summer, during both high cone years and low cone years, there was a negative linear correlation between temperature and heart rate: As temperatures decreased, heart rate increased. This expected pattern mirrors predictions based on thermoregulatory theory (King and Farner 1961) and was also present in low cone year winters. However, the relationship was different in high cone year winters where we found a positive linear relationship between temperature and heart rate. Birds may have to work harder to access enough food to offset costs of thermoregulation during low cone years. Thus, during colder days metabolic costs of thermoregulation would be higher for those birds having to forage more to meet demand. In high

cone years, birds should have easier access to mature seeds in cones, and in fact, foraging

performance is higher during high cone years in general (Figure 7). Interestingly however, heart

rate increased as temperatures increased during the winter of a high cone crop. There are a

number of factors outside of temperature that may account for the change in relationship between

heart rate and temperature, including other weather parameters like precipitation or wind. It is

also possible that internal conditions, such as parasite burden, could impact metabolic rates.

Sparrows, for example, with a high parasite burden showed hypothermic responses compared to

controls (Owen-Ashley et al. 2006). It is also possible, and in this case probable, that the timing

of reproductive phases caused the unexpected pattern in the high cone winter. Birds were tracked

in February and March in both low and high cone years and March was warmer than February in

both years. However, in the high cone year birds were in the more metabolically expensive

feeding phase during March, thus potentially driving a positive relationship between heart rate

and temperature in the high cone winter.

One way to minimize sampling bias in the analysis of heart rate in response to

temperature is to look at the changes in heart rate across temperatures experienced within

individuals. We looked at nighttime heart rates to avoid differences in activity influencing the

pattern, but there was still surprising variation in the nature of the relationship between

temperature and heart rate within individuals. Of 32 individual data sets analyzed by linear

regression, 11 had significant negative relationships between temperature and heart rate, two had

significant positive relationships, and 19 had insignificant relationships between temperature and

heart rate. Despite this variation, the majority showed either the expected negative relationship or

an insignificant relationship. It is maybe unsurprising that so many individuals had insignificant

heart rate responses to temperature given that other factors may simultaneously impact heart rate

including differences in basal metabolic rate, other environmental variables (e.g., wind, rain, snow, pressure) and roost site selection that can differentially impact exposure to environmental variation. Only two birds showed a significant positive relationship between temperature and heart rate; thus, the results generally support the hypothesis that colder temperatures generate increased metabolic demand.

We found no increased cost of breeding in either season in either 24-hour average heart rates or in nighttime heart rate. Daytime heart rate, however, was significantly higher during the high cone year compared to low cone year---despite slightly warmer temperatures during the high cone year. This supports the hypothesis that birds who are breeding have significant increases in metabolic demand compared to non-breeding birds and that this cost likely comes from daytime activity rather than from supporting additional reproductive physiology, which should have generated a cost difference in nighttime RMR.

Cone year is a good proxy for determining breeding status given that decisions to breed appear to be strongly related to the local abundance of conifer seeds in a given season (Hahn 1995; Cornelius *et al* 2012). Red crossbills have been shown to follow common patterns of activation of the reproductive system in summer and termination during molt in autumn, however due to food abundance the capability of re-activation of the reproductive system opportunistically is available (Hahn 1998). Different phases of breeding, however, may generate quite different energy costs. Multiple studies have shown that birds who are breeding (Vehrencamp et al. 1989), sitting on nests (Croxall and Ricketts 1983), or feeding young (Hails and Bryant 1979) require more energy than birds who are not in reproductive condition. Even in birds that were confirmed as having nests or feeding fledglings in this study did not have different 24-hour average or nighttime heart rates than non-breeding birds. However, daytime

heart rate was significantly higher in birds feeding young compared to either non-breeders or birds incubating eggs. These results support the hypothesis that birds feeding young have increased metabolic demands compared to non-breeding or brooding birds. One significant cost that is specific to females is depositing energy-rich yolk into the forming egg (Gil et al. 2006). In this study the cost of yolking eggs was difficult to determine and may not have been captured in our data since birds were only tracked for a total of 48 hours and we were unlikely to have captured a bird during the comparatively short yolking phase---in particular because winter nests must be incubated continuously to prevent freezing. It therefore remains unknown in crossbills if the cost of yolking is much higher than incubating or feeding young.

Winter thermoregulation is clearly costly, but there may be aspects of foraging ecology that make winter breeding possible for crossbills. Cones are more mature and seeds more easily accessible in winter months (Benkman 1987;1993)---although we did not detect a difference in foraging efficiency between summer and winter in either high or low cone years. Heart rate did, however, correlate negatively with foraging efficiency in high cone summers and winters---thus breeding birds with better foraging efficiencies had lower average heart rates. These data suggest that increased foraging efficiency may offset some of the metabolic cost's birds face during breeding. We could not analyze the relationship between heart rate and foraging efficiency during low cone summer months due to sample size because most birds were only eating insects. Ornithologists have traditionally considered insect protein to be a very small component of the crossbill diet (Newton 1972). Although the total proportion may be small in relation to seed, it may be that insect consumption helps crossbills to survive periods of lean conifer seed availability---especially when insects are abundant and cones are closed and require prying open in summer. In low cone winter months, when birds were foraging on conifer seeds, there was no

relationship between foraging intake and 24-hour average heart rate (P = .89). It is unclear why better foragers should have energy savings only when breeding. One possibility, besides that of small sample size failing to detect a modest difference, may be that wintering birds during a breeding year are maximizing daylight hours for foraging (J. Cornelius personal observation), whereas wintering birds in a non-breeding year are spending some hours resting during the day. It is therefore possible that the savings due to better foraging efficiency may only become apparent when breeding has pushed their performance to the maximum. In any case, high foraging efficiency in the winter may be a key factor that allows this species to opportunistically breed in winter compared with other species. Seed intake rates were not recorded for other winter conifer seed eaters (e.g., pine siskins), but we assume that crossbills are the most efficient at seed extraction. On an individual level this suggests that individuals who were less efficient at foraging may be more exposed to the costs of high energy turnover, for example, oxidative stress, time lost to foraging, exposure to predators, and more direct exposure to the elements. One interesting anomaly that we found in our data was from a double brooding bird. This wintering male was feeding fledglings at the same time that he was courtship feeding a female who was building another nest. This suggests, along with field observations of the male doing most of the feeding of the fledglings, that the female was not contributing much to feeding the still-dependent young. This male had the highest foraging efficiency out of all individuals tracked; however---in contrast to the foraging efficiency hypothesis - his heart rate was extremely high. Presumably his high heart rate was due to the amount of work he was doing in the attempt to double brood in winter. Thus, even though this individual's data did not support the hypothesis that increased foraging efficiency would result in a lower heart rate, his individual reproductive fitness may have been quite high. The cost of this attempt on survival and long-term

health is unknown. This may be the first published observation of such an attempt to double brood in winter, and it may indeed be rare if the wear and tear is very costly. A future study investigating the costs of winter breeding on oxidative physiology or other estimates of ageing would help shed light on this potential trade-off.

Low-cone crop years do not result in breeding and thus should be less costly, but our results show that they are not statistically lower in metabolic costs than breeding years. Perhaps this suggests that the costs of having to forage on other food items like insects is more costly than eating seeds. If true, this suggests an intrinsic cost to specialization (i.e., potentially high effort when their target food is less available) that may be exacerbated due to climate change. If seed crops suddenly become scarce and high cone crop years become fewer and further between, high breeding years will become less and less likely, perhaps resulting in population declines or crashes (Carey 2009).

In conclusion, the decisions that red crossbills make to opportunistically breed during the winter are known to be largely based on food availability. The specialization of this species allows for the acquisition of high energy seeds from conifer cones at a higher efficiency in both summer and the winter of masting years, and this may, in turn, allow for birds to invest more in breeding. This research supports the hypothesis that foraging efficiency is centrally important to breeding: breeding birds had much higher foraging efficiency in high cone years and better foragers suffered lower energy costs of breeding. While this specialization is probably beneficial for the fitness of this species, how the cone crops respond to changes in temperature, light, and precipitation due to climate change is something that should be explored to make estimations of the long-term survival of the species.

Literature Cited:

Benkman, C. 1987. Food Profitability and the Foraging Ecology of Crossbills. *Ecological Monographs*, 57. 251-267.

Benkman, C. 1993. Adaptation to single resources and the evolution of crossbill (Loxia) diversity. Ecological monographs 63. 305-325.

Butler, P.J. 1991. Exercise in birds. *Journal of Experimental Biology.* 160. 233–262.

Butler, P. J., J. A. Green, I. L. Boyd, and J. R. Speakman. 2004. Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods. Functional Ecology 18. 168–183.

Calow, P., and V. E. Forbes. 1998. How do physiological responses to stress translate into ecological and evolutionary processes? Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 120. 11–16.

Carey, C. 2009. The impacts of climate change on the annual cycles of birds. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364**.** 3321–3330.

Cochran W.W., M. Wikelski, R. Greenberg, P. Marra. 2005. Individual migratory tactics of New World Catharus thrushes. Birds of two worlds: the ecology and evolution of migration Johns Hopkins University Press, Baltimore, Maryland, USA. 274-289.

Collins Brian G., and Peter C. Morellini. 1979. "The Influence of Nectar Concentration and Time of Day upon Energy Intake and Expenditure by the Singing Honeyeater, Meliphaga Virescens." Physiological Zoology. 2. 165–175.

Cornelius, J.M., C.W. Breuner, and T.P. Hahn. 2012. Coping with the extremes: Stress physiology varies between winter and summer in breeding opportunists. Biology Letters 8:2. 312-315.

Dawson, W.R., and H.B. Tordoff. 1964. Relation of oxygen consumption to temperature in the red and white-winged crossbills. The Auk 81:1. 26-35.

Croxall, J. P. and C. Ricketts. 2008. Energy costs of incubation in the Wandering Albatross *Diomedea exulans*. Ibis 125**.** 33–39.

Gil, D., A. Marzal, F. de Lope, M. Puerta, and A. Moller. 2006. Female house martins (*Delichon urbica*) reduce egg androgen deposition in response to a challenge of their immune system. Behavioral Ecology and Sociobiology, 60. 96-100.

Hahn, T. P. 1994. Crossbill Diversity Evolutionary Differentiation in Morphology, Vocalizations, and Allozymes among Nomadic Sibling Species in the North American Red Crossbill (*Loxia curvirostra*) Complex. Volume 127 J. G. Groth. The Condor 96. 835–837.

Hahn, T. P. 1995. Integration of photoperiodic and food cues to time changes in reproductive physiology by an opportunistic breeder, the red crossbill, *Loxia curvirostra* (*Aves Carduelinae*). Journal of Experimental Zoology 272. 213-226.

Hahn, T. P. 1998. Reproductive Seasonality in an Opportunistic Breeder, the Red Crossbill, *Loxia curvirostra*. Ecology 79:2365.

Hails, C. J. and D. Bryant. 1979. M. Reproductive Energetics of a Free-Living Bird. *The Journal of Animal Ecology* 48**.** 471

King, J. and D. Farner. 1961. Energy Metabolism, Thermoregulation and Body Temperature. In: *Biology and Comparative Physiology of Birds*, 2nd ed. Academic Press. 215-279.

Lozano, G.A., S. Perreault, and R.E. Lemon. 1996. Age, arrival date and reproductive success of male American redstarts *Setophaga ruticilla*. J Avian Biol 27. 164–170

Moeller, A. P. 1994. Phenotype-dependent arrival time and its consequences in a migratory bird. Behavioral Ecology and Sociobiology 35. 115–122.

Newton, I. 1972. *Finches*. London: Collins.

Owen-Ashley, N., Turner, M., Hahn, T. and Wingfield, J. 2006. Hormonal, behavioral, and thermoregulatory responses to bacterial lipopolysaccharide in captive and free-living white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Hormones and Behavior, 49. 15-29.

Schneider, E. J. 2004. "Energy Balance and Reproduction." Physiology & Behavior, 2. 289–317.

Smith, R. J., and F. R. Moore. 2004. Arrival timing and seasonal reproductive performance in a long-distance migratory land bird. Behavioral Ecology and Sociobiology 57. 231–239.

Sgueo, C., M.R. Wells, D.E. Russell, and P.J. Schaeffer. 2012. Acclimatization of seasonal energetics in northern cardinals (*Cardinalis cardinalis*) through plasticity of metabolic rates and ceilings. Journal of Experimental Biology, 215. 2418-2424.

Sokolova, I. M., M. Frederich, R. Bagwe, G. Lannig, and A. A. Sukhotin. 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Marine Environmental Research 79. 1–15.

Speakman, J. R. 2008. "The Physiological Costs of Reproduction in Small Mammals." Philosophical Transactions of the Royal Society B: Biological Sciences, 1490. 375–398.

Vehrencamp, S. L., J. W. Bradbury, and R. M. Gibson. 1989. The energetic cost of display in male sage grouse. *Animal Behaviour* 38**.** 885–896.

Ward, S., C.M. Bishop, A.J. Woakes, and P.J Butler. 2002. Relationships between heart rate and rate of oxygen consumption in flying and walking barnacle geese (*Branta leucopsis*) and bar-headed geese (*Anser indicus*). Journal of Experimental Biology 205. 3347–3356.