TITLE

Migratory state and patterns of steroid hormone regulation in the pectoralis muscle of a nomadic migrant, the pine siskin (*Spinus pinus*).

AUTHORS & AFFILIATIONS

Ben J. Vernasco[a]*, Michael G. Emmerson[b], Elizabeth R. Gilbert[c], Kendra B. Sewall[b], Heather E. Watts[a]

[a]School of Biological Sciences, Washington State University, Pullman, WA, USA

[b]Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA

[c]Animal and Poultry Sciences Department, Virginia Tech, Blacksburg, VA, USA

*Corresponding author: ben.vernasco@wsu.edu

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation (IOS-1755245 to HEW). We thank Jeffrey Rittenhouse for contributing to the behavioral data collection and Ashley Robart for assistance in the collection and care of the birds, and in tissue collection. Melissa Morado, Mali McGuire, and Michelle Laiolo assisted with animal husbandry. Tom Hahn, Mary Lohuis, and Randy and Carol Robart provided logistical support in the field. We would also like to thank Jessica Tir, Jordan Boersma, Kevin Jones, Adam Becker and two anonymous reviewers for providing valuable feedback on earlier versions of the manuscript.

ABSTRACT

The endocrine system is known to mediate responses to environmental change and transitions between different life stages (e.g., a non-breeding to a breeding life stage). Previous works

from the field of environmental endocrinology have primarily focused on changes in circulating hormones, but a comprehensive understanding of endocrine signaling pathways requires studying changes in additional endocrine components (e.g., receptor densities) in a diversity of contexts and life stages. Migratory birds, for instance, can exhibit dramatic changes in their physiology and behavior, and both sex steroids as well as glucocorticoids are proposed mediators of the transition into a migratory state. However, the role of changes in endocrine signaling components within integral target tissues, such as flight muscles, in modulating the transition into a migratory state remains poorly understood. Here, we examined changes in gene expression levels of and correlational patterns (i.e., integration) between 8 endocrine signaling components associated with either glucocorticoids or sex steroid signaling in the pectoralis muscles of a nomadic migratory bird, the pine siskin (*Spinus pinus*). The pectoralis muscle is essential to migratory flight and undergoes conspicuous changes in preparation for migration, including hypertrophy. We focus on endocrine receptors and enzymes (e.g., 5α-reductase) that modulate the signaling capacity of circulating hormones within target tissues and may influence either catabolic or anabolic functioning within the pectoralis. Endocrine signaling components were compared between captive birds sampled prior to the expression of vernal migratory preparation and during the expression of a vernal migratory state. While birds exhibited differences in the size and color of the flight muscle and behavioral shifts indicative of a migratory state (i.e., zugunruhe), none of the measured endocrine components differed before and after the transition into the migratory state. Patterns of integration amongst all genes did, however, differ between the two life stages, suggesting the contrasting demands of different life stages may shape entire endocrine signaling networks within target tissues rather

45 than individual components. Our work aligns with previous endocrine studies on pine siskins
46 and, viewed together, suggest additional studies are needed to understand the endocrine
47 system's role in mediating the development and progression of the vernal migratory state in
48 this species. Further, the patterns observed in pine siskins, a nomadic migrant, differ from
49 previous studies on obligate migrants and suggest that different mechanisms or interactions
50 between endocrine signaling components may mediate the migratory transition in nomadic
51 migrants.
52 **KEYWORDS** migration, testosterone, glucocorticoids, endocrine receptors, endocrine
53 integration
54

55 **INTRODUCTION**

56 The endocrine system integrates information from both the social and physical environment
57 and can mediate an individual's response to environmental changes (Ricklefs and Wikelski
58 2002, Ketterson et al., 2009). These environmental changes can be short-term perturbations
59 such as inclement weather or acute changes in resource availability (Wingfield et al., 1998), but
60 also include long-term changes such as changes in photoperiod across the year (Dawson et al.,
61 2001, Stevenson and Kumar et al., 2017). By studying the relationship between environmental
62 cues and endocrine signaling components, such studies can inform our understanding how the
63 endocrine system mediates behavioral and physiological responses to short-term and long-term
64 changes, including the transitions between different life stages (Wingfield 2008). As an
65 example, in some species, increases in day length prior to the breeding season lead to the
66 activation of the hypothalamic-pituitary-gonadal (HPG) axis, and the resulting changes in

67 circulating hormones (e.g., gonadotropins, sex steroids) initiate the development of traits
68 associated with a breeding life stage (e.g., gonadal maturation, territorial behaviors, and/or
69 courtship behaviors; Wingfield et al., 1990a, Fusani 2008, Walton et al., 2011). The density of
70 hormone receptors as well as the abundance of enzymes that modulate the signaling capacity
71 of hormones within neural or peripheral target tissues can also vary across life stages and these
72 changes can occur concurrent with or in the absence of changes in circulating hormones (Ball
73 and Balthazar 2009, Watts 2020). Sex steroid receptor expression levels in neural tissue, for
74 instance, have been shown to be elevated in ring doves *(Streptopelia risoria*) and Syrian
75 hamsters (*Mesocritcetus auratus*) during the breeding season (Wood and Newman 1993, Lea et
76 al., 2001). Overall, studies focused on how endocrine signaling components change between
77 different life stages can broaden our understanding of how evolution shapes the endocrine
78 system to mediate responses to environmental change in different contexts (Crews and Moore
79 1986, Wingfield et al., 1997, Fuxjager and Schuppe 2018). However, there remains a
80 considerable gap in our understanding of how changes in endocrine signaling components
81 beyond circulating hormones are involved in life stage transitions other than breeding
82 transitions.

83 Many animals make seasonal, long-distance movements in order to capitalize on pulses
84 of resources during either a breeding or nonbreeding life stage. Such migrations are often
85 energetically demanding and typically characterized by behavioral and physiological changes
86 prior to and during migration (Muir et al., 1994, Alerstam et al., 2003, McGuire et al., 2013,
87 Voigt et al., 2020). Among migratory birds, for instance, flight muscles hypertrophy, various
88 organs hypertrophy or atrophy, and hyperphagia prior to migration facilitates the deposition of

lipid-based fuels (Driedzic et al., 1993, reviewed in Ramenofsky and Wingfield, 2007, Jimenez 2020). The hypertrophy of flight muscles in birds is associated with increases in muscle fiber diameter, increases in mitochondria density, changes in thermogenic capacity, and increases in the amount of intracellular lipid deposition within flight muscles (Gaunt et al., 1990, Evans et al., 1992, Vézina et al., 2007). Although behavioral, physiological, and molecular changes are associated with the development of the migratory state, the proximate mechanisms that mediate the development and maintenance of these changes are less well understood (Wingfield et al., 1990b, Deviche 1995, Ramenofsky and Wingfield, 2007; Ramenoksky 2011).

The neuroendocrine system and, more specifically, sex steroids as well as glucocorticoids are proposed mediators of the transition into a migratory state in birds (Ramenofsky and Wingfield 2007, Cornelius et al., 2013, Sokolov and Tsvey 2016). In cases of vernal songbird migrations, for instance, the neuroendocrine system is stimulated by increasing daylengths and this process initiates a hormonal cascade that is proposed to be important for the transition to a migratory state (Ramenofsky and Wingfield 2007). The relevance of androgens to this process is exemplified by studies showing the phenotypic changes associated with vernal migratory transitions are advanced following experimental testosterone administration (Tonra et al., 2011, Owen et al., 2014), do not occur when gonads (i.e., the primary source of circulating sex steroids) are removed prior to winter (Weise 1967), or when androgen receptors and the enzyme aromatase (i.e., an enzyme that converts testosterone to estradiol) are blocked (Tonra et al., 2011). Androgens are thought to be especially important to the muscle hypertrophy associated with the migratory transition, as androgens can have anabolic effects on skeletal muscle in a variety of contexts (Herbst and Bhasin 2004, Dubois et

al., 2012). Indeed, within the pectoralis muscle, gene expression levels of androgen receptors, 5α-reductase (i.e., the enzyme that converts testosterone to 5α-dihydrotestosterone), as well as insulin-like growth factor-1 (i.e., androgen dependent gene associated with muscle remodeling) all increase prior to migratory departure in white-crowned sparrows (*Zonotrichia leucophrys gambelii*, Pradhan et al., 2019a). Glucocorticoids, on the other hand, are thought to be associated with acquiring and mobilizing energy stores to power sustained flights via metabolic and catabolic pathways (Ramenofsky and Wingfield 2007, Sokolov and Tsvey 2016, Eikenaar et al., 2018). For example, glucocorticoid levels preceding and during migratory flights are elevated (Landys et al., 2004a, Falsone et al., 2009, Eikenaar et al., 2018) and experimental work on migratory white-crowned sparrows also suggests the glucocorticoid receptor has permissive effects on feeding behaviors (i.e., hyperphagia) and lipid breakdown (Landys et al., 2004b). Additional observational studies on migratory white-crowned sparrows found elevated expression levels of 11β-hydroxysteroid dehydrogenase type 2, an enzyme that inactivates circulating glucocorticoids within target tissues, and elevated mineralocorticoid receptor (a high-affinity receptor associated with tissue repair and homeostatic maintenance) within the pectoralis muscle prior to departure (Pradhan et al., 2019b). These changes in glucocorticoid signaling within the pectoralis suggest that anabolic functions are prioritized prior to migratory flights. More broadly, these studies highlight the importance of the endocrine system to the development and progression of the migratory state and underscore the importance of studies focusing on endocrine signaling components beyond circulating hormone levels.

The suite of proximate mechanisms that mediate the development of a migratory state are hypothesized to depend upon the migratory strategy of a particular species (Watts et al.,

2018). Obligate migrants make regularly timed annual migrations to specific geographic areas while nomadic migrants make undirected and/or aseasonal movements either in response to fluctuations in resources availability or to find breeding habitats with abundant resources (Hahn et al., 2008, Watts et al., 2018). However, similar to obligate migrants, some nomadic migrants also exhibit a more seasonally predictable vernal migratory period characterized by physiological preparations and behavioral readiness for migration (Pohl and West 1976, Cornelius and Hahn 2012, Watts et al., 2017, Robart et al., 2018). Thus, migratory movements in these species seem to include both temporally unpredictable and predictable components (Watts et al., 2018). In pine siskins (*Spinus pinus*), for example, increasing vernal photoperiods induce increased deposition of fat, changes in the size and color of flight muscles, and nocturnal migratory restlessness (Watts et al., 2017, Robart et al., 2018). However, our understanding of the role of the endocrine system in the regulation of nomadic migration is limited (Cornelius et al. 2013). Most findings to date come from correlative studies of captive pine siskins and provide limited evidence for a role of changes in circulating hormones in mediating the development of the migratory state. For instance, circulating corticosterone levels do not increase in association with the transition to a vernal nomadic state, and although circulating testosterone does increase, levels do not show significant increases until after the physiological (e.g., muscle remodeling) and behavioral (e.g., zugunruhe) changes associated with migration have occurred (Robart et al., 2018). Additional endocrine studies on traits beyond circulating levels are therefore needed to understand the role of the endocrine system in mediating the development of the migratory state in nomadic migrants such as the pine siskin.

Here, we quantify differences in the expression levels of as well as correlational patterns (i.e., integration) between multiple hormone receptors and enzymes associated with steroid signaling in the pectoralis of pine siskins sampled before or during the vernal migratory period. In addition to measuring expression levels of androgen receptor and the enzyme 5α-reductase, we quantified expression levels of both aromatase as well as estrogen receptor-α. Expression levels of glucocorticoid receptor, mineralocorticoid receptor, and two enzymes that either locally regenerate circulating glucocorticoids (11β-hydroxysteroid dehydrogenases type 1, 11β-HSD type 1) or deactivate circulating glucocorticoids via metabolization (11β-hydroxysteroid dehydrogenases type 2, 11β-HSD type 2) were also measured. Given the muscle hypertrophy that occurs as part of migratory preparations, we predicted that birds in a migratory state would exhibit increased anabolic functions in the pectoralis as indicated by elevated expression levels of receptors and enzymes associated with androgen signaling. Migratory flights are also energetically demanding, therefore we predicted that pine siskins in a migratory state would exhibit molecular states that reflect increased energy acquisition and mobilization in the pectoralis muscle via changes in glucocorticoid signaling. Further, a higher degree of integration amongst endocrine signaling components is thought to facilitate coordinated patterns of endocrine signaling (Lipshutz et al. 2019), though few studies have examined endocrine signaling networks from this perspective. Here we evaluate the extent of integration within and across sex steroid and glucocorticoid signaling components and examine plasticity in this integration between non-migratory and migratory life stages. If patterns of integration vary across life stages, then these results would suggest that selection shapes endocrine signaling networks within target tissues as a whole, either in addition to or rather than acting upon a

single endocrine signaling component. Overall, this study provides a novel perspective on the proximate mechanisms that mediate the development of the migratory state in a nomadic migrant.

METHODS

Study System and Design

Free-living pine siskins (*Pinus spinus*) were captured using either mist nets or baited funnel traps in the western United States (California, Oregon, Washington, and Wyoming) between August 2015 and July 2016. Birds were then transported by vehicle to Loyola Marymount University in Los Angeles, California and housed indoors. Birds were fed Roudybush Small Bird Maintenance Diet (Woodland, CA), a mixture of nyjer thistle and sunflower seed hearts, grit, and water. Some birds (n = 7) were used in previous experiments described in (Robart et al., 2018, Robart et al., 2019). Following these experiments and for the duration of the study described here, all birds were maintained in captivity with *ad libitum* access to food and water under the naturally changing day lengths occurring at 42°N, a latitude at which this species can occur year-round. Birds involved in previous experiments were kept under these conditions for a minimum of 9 months before the study described here. As such, all birds in the present study molted their plumage and experienced a natural decline in photoperiod prior to the winter solstice that preceded the current study. Although many of the birds in the study had been in captivity for more than 1 year at the time of sampling, we know that pine siskins continue to express physiological and behavioral indicators of a vernal migratory life stage after more than a year in captivity (e.g., Watts et al., 2017, Watts et al., 2019).

198 For the current study, birds were housed in individual cages where they could see and
199 hear other birds and could interact with birds in adjacent cages. Birds were divided into two
200 groups that differed in when behavioral data, morphological data, and pectoralis tissues were
201 collected. Previous studies have documented that captive pine siskins exhibit physiological and
202 behavioral changes indicative of migratory preparation in late March and early April (Robart et
203 al., 2018). One group (n = 4 females, 3 males) was therefore sampled at a time of year that was
204 expected to be immediately prior to when birds began migratory preparations (i.e., the pre-
205 migratory period, March 3rd, 2017) and the other group (n = 6 females, 2 males) was sampled at
206 a time of year when birds were expected to be in a migratory state (i.e., the migratory period
207 April 21st, 2017, Watts et al., 2019). Between 1130 and 1500 on these sampling days, birds were
208 removed from cages and pectoralis tissues were collected following euthanasia by isoflurane
209 overdose. Pectoralis tissues were flash frozen in liquid nitrogen within 9 minutes of euthanasia
210 and then stored at -80°C until subsequent molecular analyses.
211 Prior to tissue collection, we measured nocturnal locomotor behavior and multiple body
212 metrics describing a bird's morphology and physiological state in order to verify the migratory
213 status of each bird (as described in Watts et al., 2019). Four days prior to tissue collection, birds
214 were weighed to the nearest 0.1 g using an electronic balance and a bird's furcular and
215 abdominal fat stores as well as their pectoralis muscle size and color were visually assessed.
216 Furcular and abdominal fat were each scored from 0 (no fat deposition) to 5 (bulging fat;
217 Wingfield et al., 1990b) and the two values were summed to generate a total fat score.
218 Pectoralis muscle size was also scored from 0 (sharp keel with concave muscle) to 3 (muscle
219 extended past keel) following descriptions in Bairlein (1995). Importantly, this range of muscle

sizes can be observed in both captive and free-living birds, though muscle size 0 is rarely observed in captive or free-living birds (Watts unpublished data). Muscle color, an index of intra-cellular lipid deposition (Robart et al., 2018), was scored from 1 (darkest) to 3 (lightest) by comparing the pectoralis muscle color to the color standards as described in Robart et al., (2018). Additionally, during the two nights preceding tissue sample collection, nocturnal behavior was filmed between 01:00 and 02:30 using Sony FDR-AX33 Handycam set on infrared mode. This time of night has previously been shown to coincide with when pine siskins exhibit nocturnal migratory restlessness (Watts et al., 2017, 2019). Behaviors were quantified using instantaneous sampling (Altmann 1974). At one-minute intervals, a bird's behavior was categorized as stationary, fast wing-beating, flying, jumping or climbing, feeding, preening, or "other" for behaviors not included in these categories (e.g., bill wiping). The proportion of time a bird was engaged in locomotor activity was calculated by dividing the number of intervals a bird was locomoting (i.e., wing-beating, flying, jumping or climbing) by 90 (i.e., the number of one-minute sampling intervals). Behavioral data and body measurements have been published previously for a subset of the birds included here (Watts et al., 2019). We did not collect blood samples to quantify circulating hormone levels for this study in order to minimize handling that might alter gene expression within tissues of interest. In a previous study with pine siskins, we found that circulating corticosterone levels did not differ significantly over the time period of the two sampling points in the present study, but circulating testosterone levels were elevated around the time of the migratory sample of the present study (Robart et al., 2018).

Tissue collection and quantification of mRNA expression levels

241 A pectoral muscle sample from each bird (0.25+0.03mg) was placed into a tube containing 1 ml
242 of Tri-Reagent (Molecular Research Company, USA, Cat. No. TR-118) and a 5 mm diameter steel
243 bead (Qiagen, USA, Cat. No. 69989) and homogenized for 2 minutes at 25 Hz using a Tissue
244 Lyser II (Qiagen, USA, Cat. No. 85300). Homogenates were transferred to spin columns and total
245 RNA was extracted from each sample using a commercially available kit following the
246 manufacturer's instructions with the inclusion of a DNase-I digestion step (Direct-Zol RNA
247 MiniPrep Kit, Zymo Research, USA, Cat. No. R2051). RNA integrity was assessed using a Bio-
248 Analyzer 2100 (Agilent Technologies, USA, Cat. No. G2939BA; RIN = 7.2-8.1, mean RIN = 7.4),
249 and RNA purity and quantity were assessed with a nano-spectrometer (Nanophotometer Pearl,
250 Implen, USA; 260/280 = 1.971-2.102, mean 260/280 = 2.049). cDNA was synthesized from 200
251 ng total RNA using a commercially available kit (High Capacity cDNA Reverse Transcription Kit,
252 Applied Biosystems, Cat. No. 4368813) and diluted with RNase-free water to a final
253 concentration of 1 ng/μl. Primers for seven genes of interest (glucocorticoid receptor,
254 mineralocorticoid receptor, 11β-HSD type 2, androgen receptor, estrogen receptor-α, 5α-
255 reductase, aromatase) and a reference gene (GAPDH) were designed using PrimerExpress v.3
256 (Applied Biosystems, USA, Cat. No. 4363991). The primer pair for 11β-HSD type 1 was
257 developed by Pradhan et al., (2019b). All primers were synthesized by Integrated DNA
258 Technologies. With the exception of the 11β-HSD type 1 primers, all primer sequences were
259 based on the published zebra finch (*Taeniopygia guttata*) genome, as a pine siskin genome is
260 not available (see Table 1 for primer sequences). qPCR reactions were run on MicroAmp Fast
261 Optical 96-Well Reaction Plates (Applied Biosystems, USA, Cat. No. 4346906) with MicroAmp
262 Optical Adhesive Film (Applied Biosystems, USA, Cat. No. 4311971) using Fast SYBR Green

Master Mix (Applied Biosystems, USA, Cat. No. 4385612). Each well contained 3µl cDNA, 0.25µl of 5µM forward primer, 0.25µl of 5µM reverse primer, 5µl SYBR green, and 1.5µl RNase-free water. All samples were run in duplicate on an Applied Biosystems 7500 Fast Real-Time PCR System (SeqGen Inc., USA) with the following cycling parameters: 95ºC for 20 seconds, followed by 40 cycles of 95ºC for 3 seconds and 60ºC for 30 seconds. Amplification of single gene products was confirmed by the visual inspection of the melt curves (see Supplementary Data, Figures S1 and S2). Amplification efficiencies with cDNA from the pectoralis tissue of pine siskins ranged from 96% to 108% for the primers used in this study.

Statistical Analyses

Data were analyzed in Program R 3.6.2 (R Core Team, 2019). We compared the migratory indicators and mRNA expression between birds sampled in the pre-migratory and migratory period using t-tests or, when residuals were not normally distributed (Shapiro-Wilk, $p < 0.05$), Mann-Whitney-Wilcoxon tests. Among the migratory indicators, body mass was analyzed with a t-test and fat deposition, muscle size, muscle color, and nocturnal behavior were compared using Mann-Whitney-Wilcoxon tests. For qPCR data, relative expression of each gene of interest was calculated using the ΔΔCt method, i.e. $2^{-\Delta\Delta Ct}$ (ΔCt = target gene Ct – GAPDH Ct, ΔΔCt = ΔCt – calibrator ΔCt where the calibrator is the mean ΔCt of pre-migratory birds). Estrogen receptor-α was analyzed with a Mann-Whitney Wilcoxon test and all other genes of interest were analyzed with t-tests. We did not test for sex differences in either gene expression nor migratory state due to the small sample size.

We assessed patterns of integration between the endocrine signaling components measured in this study following the methodology of Lipshutz et al., (2019). We used a network approach wherein each node represents one of the genes measured and edges are defined by Spearman's rank correlation coefficients between each of the nodes. We quantified the connectivity of the gene networks (p_{sum}) by summing the absolute values of all correlations and dividing by the total number of possible connections (i.e., 28). We also quantified connectivity amongst genes associated with glucocorticoids (Glucocorticoid p_{sum}) and sex steroid (Sex steroid p_{sum}) by summing the absolute values of correlations between genes associated with each group of hormones and dividing by the total number of possible connections between each of the 4 genes in each subset (i.e., 6). Networks were visualized using the *qgraph* package (Epskamp et al., 2012).

RESULTS

Relative to birds sampled in the pre-migratory period, birds sampled during the migratory period were significantly heavier ($t(9.1) = -4.86$, $p < 0.001$), had significantly larger fat deposits ($W = 2.5$, $p = 0.003$), exhibited significantly larger pectoralis muscles ($W = 3$, $p = 0.003$; Figure 1A) that were lighter in color ($W = 10.5$, $p = 0.03$), and spent a greater proportion of the night exhibiting locomotor activity ($W = 2$, $p = 0.003$, Figure 1B). There were no significant differences in relative mRNA levels of steroid receptors (i.e., androgen receptor, estrogen receptor-α, mineralocorticoid receptor, glucocorticoid receptor) or enzymes (i.e., 5α-reductase Type 2, aromatase, 11β-HSD type 1, 11β-HSD type 2) associated with steroid processing in the pectoralis between pre-migratory and migratory pine siskins (Table 2, Figure 2, 3). We observed

306 relatively higher levels of integration during the pre-migratory period (p_{sum} = 0.54) than the
307 migratory period (p_{sum} = 0.41; Figure 4). During the pre-migratory period, genes associated with
308 sex steroid signaling were more integrated (Figure 4A), while genes associated with
309 glucocorticoid signaling were more integrated during the migratory period (Figure 4B). Values
310 of the correlations between all endocrine signaling components can be found in Supplementary
311 Table 1.
312
313 **DISCUSSION**
314 A number of studies on obligate migrants suggest the endocrine system is important to the
315 development, maintenance, and progression of migratory states, though studies focused on
316 endocrine signaling components beyond circulating hormones and/or species that exhibit
317 alternative migratory strategies are currently limited. Here we measured a suite of endocrine
318 signaling components in the pectoralis muscle of a nomadic migrant before and after the
319 transition into a migratory state. Though the results of this study document differences in
320 integration between endocrine signaling components across this migratory transition, the
321 results do not suggest changes in expression levels of individual endocrine signaling
322 components are important to the development of the migratory state in pine siskins.
323 Specifically, we show that both receptors and enzymes that modulate sex steroid and
324 glucocorticoid signaling within a muscle integral to flight do not differ between birds sampled
325 prior to the migratory period and birds sampled after the development of the migratory state.
326 These results are similar to previous works in pine siskins that found no evidence for a role of
327 glucocorticoid signaling in this migratory transition, and equivocal evidence for a role of

testosterone (Robart et al., 2018, Watts et al., 2019). On the other hand, the results from the present study contrast previous studies on white-crowned sparrows, an obligate migrant, that documented differences in enzymes and receptors associated with glucocorticoid and androgen signaling prior to migratory departures (Pradhan et al., 2019a, 2019b). These opposing results may reflect species differences, which could be related to differences in the migratory strategies employed by these two species (Watts et al., 2018).

Results from the current study as well as previous studies (Watts et al., 2017, Robart et al., 2018) show that pine siskins in a migratory state exhibit larger fat deposits, larger flight muscles, and nocturnal restlessness, physiological and behavioral changes that mirror those observed in obligate migrants like the white-crowned sparrow. However, unlike the pine siskin, white-crowned sparrows exhibit changes in multiple endocrine signaling components within the pectoralis (Pradhan et al., 2019a, 2019b). The lack of changes to the endocrine signaling pathways examined in the current study suggest other proximate mechanisms underlie the development of the migratory state in nomadic migrants. A number of other potential signaling mechanisms or pathways may mediate the development and maintenance of the migratory state in nomadic migrants including prolactin, thyroid hormones, myostatin, or the insulin-like growth factor-1 (Price et al., 2011, Ramenofsky 2011). One caveat to consider, however, is the difference in the timing of sampling between the current study and that of Pradhan et al., (2019a, 2019b). Specifically, in this study, pine siskins were sampled before and during the migratory period, whereas Pradhan et al., (2019a, 2019b) sampled birds at three different time points ranging from ~1 to 3 months prior to when birds depart for migration (i.e., winter, pre-nuptial molt, and pre-departure). It is therefore possible that siskins may exhibit changes in

endocrine signaling earlier in the year, prior to our first sampling point. However, if this occurs, additional mechanisms are still needed to explain the observed changes in physiology and behavior between our two sampling points. Another possibility is that there is a transient change in endocrine signaling between our sampling points. If this is the case, then our results suggest such changes are not necessary for the maintenance of the migratory state. The lack of changes could also be related to the fact that the current study focused on captive birds as captivity is known to have dramatic effects on various biological processes, including the endocrine system (Calisi and Bentley 2009). However, captive populations of multiple species of migratory birds, including pine siskins, exhibit a suite of phenotypic changes associated with the development and progression of a migratory state that mirror those changes observed in free-living populations (Piersma and Ramenofsky 1998, Guglielmo et al., 2017, Watts et al., 2017). Lastly, we cannot rule out the possibility that there may be differences in protein expression that are not reflected by mRNA expression levels (Maier et al., 2009, Lui et al., 2016).

Greater integration amongst endocrine signaling components is thought to promote coordinated responses by different endocrine signaling pathways, while the opposite (i.e., phenotypic independence) is expected to facilitate independent endocrine signaling responses (Ketterson et al., 2009, Lipshutz et al., 2019). Gene network analyses revealed that patterns of integration amongst the endocrine signaling components measured in this study depended upon the migratory life stage, with a greater degree of integration observed in the pre-migratory period. Notably, we observed strong correlations between expression levels of receptors associated with both sex steroids and glucocorticoids, particularly during the migratory period. Receptors associated with sex steroids and glucocorticoids are known to

372 interact to influence patterns of gene expression (Chen et al., 1997, Mifsud and Reul 2016), and
373 our results support the idea that functional interactions between the two endocrine signaling
374 pathways exist and may vary between the two life stages (Viau 2002, Bauer et al., 2016).
375 Further, Lipshutz et al., (2019) also found contrasting patterns of integration across two
376 breeding life stages in female tree swallows (*Tachycineta bicolor*) and, viewed together, both
377 results suggest that the contrasting demands of each life stage may shape patterns of
378 endocrine integration. Understanding the significance of the observed changes in patterns of
379 integration in the current study will require assessing how gene expression levels relate to
380 circulating hormones levels and also assessing patterns of integration amongst endocrine
381 signaling components found in other tissues. Comparing patterns of integration amongst
382 endocrine signaling components in a diverse array of tissues in species that exhibit different
383 migratory strategies could further reveal how selection has shaped endocrine signaling
384 pathways to accommodate tissue-specific responses and different life history strategies more
385 broadly (Fuxjager and Schuppe 2018). Overall, the contrasting patterns of integration and the
386 lack of changes in individual endocrine signaling components observed here suggests that
387 selection may act upon entire endocrine signaling networks within target tissues as opposed to
388 individual endocrine signaling components.
389 In combination with previous works on pine siskins (Robart et al., 2018, Watts et al.,
390 2019), the results of the current study provide little evidence that changes in endocrine
391 signaling components associated with sex steroids or glucocorticoids mediate the transition to a
392 vernal migratory state in nomadic migrants. Given the evidence suggesting obligate migrants do
393 exhibit differences in endocrine signaling during the development of the migratory state (e.g.,

394 Pradhan et al., 2019a, 2019b) as well as during molt (Cornelius et al., 2011), more detailed
395 comparisons of species employing different migratory strategies may be a fruitful approach for
396 identifying how evolution can shape the proximate factors underlying the development and
397 progression of various life stages, including migration (Wingfield 2008, Watts et al., 2018).
398 Transcriptomic comparisons similar to Fudickar et al., (2016) and Franchini et al., (2017) can
399 also provide a more detailed perspective on how evolution has shaped the molecular pathways
400 associated with the phenotypic changes observed over the course of the migratory period.
401 More broadly, integrative studies focusing on species that exhibit a variety of migratory
402 strategies will help to broaden our understanding of the proximate factors associated with the
403 development and progression of migratory states.
404

REFERENCES

Alerstam, T., Hedenstrom, A., Akesson, S., 2003. Long-distance migration: evolution and determinants. Oikos 103, 247–260. doi:10.1034/j.1600-0706.2003.12559.x

Altmann, J., 1974. Observational Study of Behavior: Sampling Methods. Behaviour 49, 227–266. doi:10.1163/156853974X00534

Bairlein, F., 1995. Manual of field methods. Eur. songbird Migr. network. Inst. für Vogelforschung, Wilhelmshaven.

Ball, G.F., Balthazart, J., 2009. Neuroendocrine Regulation of Reproductive Behavior in Birds, in: Hormones, Brain and Behavior. Elsevier, pp. 855–897. doi:10.1016/B978-008088783-8.00025-5

Bauer, C.M., Needham, K.B., Le, C.N., Stewart, E.C., Graham, J.L., Ketterson, E.D., Greives, T.J., 2016. Hypothalamic–pituitary–adrenal axis activity is not elevated in a songbird (Junco hyemalis) preparing for migration. Gen. Comp. Endocrinol. 232, 60–66. doi:10.1016/j.ygcen.2015.12.020

Calisi, R.M., Bentley, G.E., 2009. Lab and field experiments: Are they the same animal? Horm. Behav. 56, 1–10. doi:10.1016/j.yhbeh.2009.02.010

Chen, S., Wang, J., Yu, G., Liu, W., Pearce, D., 1997. Androgen and Glucocorticoid Receptor Heterodimer Formation. J. Biol. Chem. 272, 14087–14092. doi:10.1074/jbc.272.22.14087

Cornelius, J.M., Boswell, T., Jenni-Eiermann, S., Breuner, C.W., Ramenofsky, M., 2013. Contributions of endocrinology to the migration life history of birds. Gen. Comp. Endocrinol. 190, 47–60. doi:10.1016/j.ygcen.2013.03.027

Cornelius, J.M., Hahn, T.P., 2012. Seasonal pre-migratory fattening and increased activity in a nomadic and irruptive migrant, the Red Crossbill Loxia curvirostra. Ibis (Lond. 1859). 154, 693–702. doi:10.1111/j.1474-919X.2012.01266.x

Cornelius, J.M., Perfito, N., Zann, R., Breuner, C.W., Hahn, T.P., 2011. Physiological trade-offs in self-maintenance: plumage molt and stress physiology in birds. J. Exp. Biol. 214, 2768–77. doi:10.1242/jeb.057174

Crews, D., Moore, M., 1986. Evolution of Mechanism Controlling Mating Behavior. Science (80-.). 231, 121–125.

Dawson, A., King, V.M., Bentley, G.E., Ball, G.F., 2001. Photoperiodic control of seasonality in birds. J. Biol. Rhythms 16, 365–380. doi:10.1177/074873001129002079

436 Deviche, P., 1995. Androgen Regulation of Avian Premigratory Hyperphagia and Fattening:
437 From Eco-Physiology to Neuroendocrinology. Am. Zool. 35, 234–245.
438 doi:10.1093/icb/35.3.234

439 Driedzic, W.R., Crowe, H.L., Hicklin, P.W., Sephton, D.H., 1993. Adaptations in pectoralis muscle,
440 heart mass, and energy metabolism during premigratory fattening in semipalmated
441 sandpipers (Calidris pusilla). Can. J. Zool. 71, 1602–1608. doi:10.1139/z93-226

442 Dubois, V., Laurent, M., Boonen, S., Vanderschueren, D., Claessens, F., 2012. Androgens and
443 skeletal muscle: cellular and molecular action mechanisms underlying the anabolic actions.
444 Cell. Mol. Life Sci. 69, 1651–1667. doi:10.1007/s00018-011-0883-3

445 Eikenaar, C., Müller, F., Rüppel, G., Stöwe, M., 2018. Endocrine regulation of migratory
446 departure from stopover: Evidence from a longitudinal migratory restlessness study on
447 northern wheatears. Horm. Behav. 99, 9–13. doi:10.1016/j.yhbeh.2018.01.008

448 Epskamp, S., Cramer, A.O.J., Waldorp, L.J., Schmittmann, V.D., Borsboom, D., 2012. qgraph:
449 Network Visualizations of Relationships in Psychometric Data. J. Stat. Softw. 48.
450 doi:10.18637/jss.v048.i04

451 Evans, P.R., Davidson, N.C., Uttley, J.D., Evans, R.D., 1992. Premigratory Hypertrophy of Flight
452 Muscles: An Ultrastructural Study. Ornis Scand. 23, 238. doi:10.2307/3676644

453 Falsone, K., Jenni-Eiermann, S., Jenni, L., 2009. Corticosterone in migrating songbirds during
454 endurance flight. Horm. Behav. 56, 548–556. doi:10.1016/j.yhbeh.2009.09.009

455 Franchini, P., Irisarri, I., Fudickar, A., Schmidt, A., Meyer, A., Wikelski, M., Partecke, J., 2017.
456 Animal tracking meets migration genomics: transcriptomic analysis of a partially migratory
457 bird species. Mol. Ecol. 26, 3204–3216. doi:10.1111/mec.14108

458 Fudickar, A.M., Peterson, M.P., Greives, T.J., Atwell, J.W., Bridge, E.S., Ketterson, E.D., 2016.
459 Differential gene expression in seasonal sympatry: mechanisms involved in diverging life
460 histories. Biol. Lett. 12, 20160069. doi:10.1098/rsbl.2016.0069

461 Fusani, L., 2008. Testosterone control of male courtship in birds. Horm. Behav. 54, 227–233.
462 doi:10.1016/j.yhbeh.2008.04.004

463 Fuxjager, M.J., Schuppe, E.R., 2018. Androgenic signaling systems and their role in behavioral
464 evolution. J. Steroid Biochem. Mol. Biol. 184, 47–56. doi:10.1016/j.jsbmb.2018.06.004

465 Gaunt, A.S., Hikida, R.S., Jehl, J.R., 1990. Rapid Atrophy and Hypertrophy of an Avian Flight
466 Muscle. Auk 107, 649–659. doi:10.2307/4087994

Guglielmo, C.G., Gerson, A.R., Price, E.R., Hays, Q.R., 2017. The effects of dietary macronutrients on flight ability, energetics, and fuel metabolism of yellow-rumped warblers *Setophaga coronata*. J. Avian Biol. 48, 133–148. doi:10.1111/jav.01351

Hahn, T.P., Cornelius, J.M., Sewall, K.B., Kelsey, T.R., Hau, M., Perfito, N., 2008. Environmental regulation of annual schedules in opportunistically-breeding songbirds: Adaptive specializations or variations on a theme of white-crowned sparrow? Gen. Comp. Endocrinol. 157, 217–226. doi:10.1016/j.ygcen.2008.05.007

Herbst, K.L., Bhasin, S., 2004. Testosterone action on skeletal muscle. Curr. Opin. Clin. Nutr. Metab. Care 7, 271–277. doi:10.1097/00075197-200405000-00006

Jimenez, A.G., 2020. Structural plasticity of the avian pectoralis: a case for geometry and the forgotten organelle. J. Exp. Biol. 223, jeb234120. doi:10.1242/jeb.234120

Ketterson, E.D., Atwell, J.W., McGlothlin, J.W., 2009. Phenotypic integration and independence: Hormones, performance, and response to environmental change. Integr. Comp. Biol. 49, 365–379. doi:10.1093/icb/icp057

Landys, M.M., Piersma, T., Ramenofsky, M., Wingfield, J.C., 2004b. Role of the Low-Affinity Glucocorticoid Receptor in the Regulation of Behavior and Energy Metabolism in the Migratory Red Knot Calidris canutus islandica. Physiol. Biochem. Zool. 77, 658–668. doi:10.1086/420942

Landys, M.M., Wingfield, J.C., Ramenofsky, M., 2004a. Plasma corticosterone increases during migratory restlessness in the captive white-crowned sparrow Zonotrichia leucophrys gambelli. Horm. Behav. 46, 574–581. doi:10.1016/j.yhbeh.2004.06.006

Lea, R.W., Clark, J.A., Tsutsui, K., 2001. Changes in central steroid receptor expression, steroid synthesis, and dopaminergic activity related to the reproductive cycle of the ring dove. Microsc. Res. Tech. 55, 12–26. doi:10.1002/jemt.1152

Lipshutz, S. E., E. M. George, A. B. Bentz, and K. A. Rosvall (2019). Evaluating testosterone as a phenotypic integrator: From tissues to individuals to species. Molecular and Cellular Endocrinology 496:110531. doi: 10.1016/j.mce.2019.110531

Liu, Y., Beyer, A., Aebersold, R., 2016. On the Dependency of Cellular Protein Levels on mRNA Abundance. Cell 165, 535–550. doi:10.1016/j.cell.2016.03.014

Maier, T., Güell, M., Serrano, L., 2009. Correlation of mRNA and protein in complex biological samples. FEBS Lett. 583, 3966–3973. doi:10.1016/j.febslet.2009.10.036

McGuire, L.P., Fenton, M.B., Guglielmo, C.G., 2013. Seasonal upregulation of catabolic enzymes and fatty acid transporters in the flight muscle of migrating hoary bats, Lasiurus cinereus.

Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 165, 138–143. doi:10.1016/j.cbpb.2013.03.013

Mifsud, K.R., Reul, J.M.H.M., 2016. Acute stress enhances heterodimerization and binding of corticosteroid receptors at glucocorticoid target genes in the hippocampus. Proc. Natl. Acad. Sci. 113, 11336–11341. doi:10.1073/pnas.1605246113

Muir, W.D., Zaugg, W.S., Giorgi, A.E., McCutcheon, S., 1994. Accelerating smolt development and downstream movement in yearling chinook salmon with advanced photoperiod and increased temperature. Aquaculture 123, 387–399. doi:10.1016/0044-8486(94)90073-6

Piersma, T., Ramenofsky, M., 1998. Long-Term decreases of corticosterone in captive migrant shorebirds that maintain seasonal mass and moult Cycles. J. Avian Biol. 29, 97. doi:10.2307/3677186

Pohl, H., West, G.C., 1976. Latitudinal and population specific differences in timing of daily and seasonal functions in redpolls (Acanthis flammea). Oecologia 25, 211–227. doi:10.1007/BF00345099

Pradhan, D.S., Van Ness, R., Jalabert, C., Hamden, J.E., Austin, S.H., Soma, K.K., Ramenofsky, M., Schlinger, B.A., 2019b. Phenotypic flexibility of glucocorticoid signaling in skeletal muscles of a songbird preparing to migrate. Horm. Behav. 116, 104586. doi:10.1016/j.yhbeh.2019.104586

Pradhan, D.S., Ma, C., Schlinger, B.A., Soma, K.K., Ramenofsky, M., 2019a. Preparing to migrate: expression of androgen signaling molecules and insulin-like growth factor-1 in skeletal muscles of Gambel's white-crowned sparrows. J. Comp. Physiol. A 205, 113–123. doi:10.1007/s00359-018-1308-7

Price, E.R., Bauchinger, U., Zajac, D.M., Cerasale, D.J., McFarlan, J.T., Gerson, A.R., McWilliams, S.R., Guglielmo, C.G., 2011. Migration- and exercise-induced changes to flight muscle size in migratory birds and association with IGF1 and myostatin mRNA expression. J. Exp. Biol. 214, 2823–2831. doi:10.1242/jeb.057620

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramenofsky, M., 2011. Hormones in Migration and Reproductive Cycles of Birds, in: Hormones and Reproduction of Vertebrates. Elsevier, pp. 205–237. doi:10.1016/B978-0-12-374932-1.00046-9

Ramenofsky, M., Wingfield, J.C., 2007. Regulation of Migration. Bioscience 57, 135–143. doi:10.1641/B570208

533 Ricklefs, R.E., WIlkelski, M., 2002. The physiology/life history nexus. Trends Ecol. Evol. 17, 462–
534 469.

535 Robart, A.R., McGuire, M.M.K., Watts, H.E., 2018. Increasing photoperiod stimulates the
536 initiation of spring migratory behaviour and physiology in a facultative migrant, the pine
537 siskin. R. Soc. Open Sci. 5. doi:10.1098/rsos.180876

538 Robart, A.R., Morado, M.I., Watts, H.E., 2019. Declining food availability, corticosterone, and
539 migratory response in a nomadic, irruptive migrant. Horm. Behav. 110, 56–67.
540 doi:10.1016/j.yhbeh.2019.02.007

541 Schlinger, B.A., Paul, K., Monks, D.A., 2018. Muscle, a conduit to brain for hormonal control of
542 behavior. Horm. Behav. 105, 58–65. doi:10.1016/j.yhbeh.2018.07.002

543 Sokolov, L. V., Tsvey, A.L., 2016. Mechanisms controling the timing of spring migration in birds.
544 Biol. Bull. 43, 1148–1160. doi:10.1134/S1062359016110145

545 Stevenson, T.J., Kumar, V., 2017. Neural control of daily and seasonal timing of songbird
546 migration. J. Comp. Physiol. A 203, 399–409. doi:10.1007/s00359-017-1193-5

547 Tonra, C.M., Marra, P.P., Holberton, R.L., 2011. Early elevation of testosterone advances
548 migratory preparation in a songbird. J. Exp. Biol. 214, 2761–2767. doi:10.1242/jeb.054734

549 Vézina, F., Jalvingh, K.M., Dekinga, A., Piersma, T., 2007. Thermogenic side effects to migratory
550 predisposition in shorebirds. Am. J. Physiol. Integr. Comp. Physiol. 292, R1287–R1297.
551 doi:10.1152/ajpregu.00683.2006

552 Viau, V., 2002. Functional Cross-Talk Between the Hypothalamic-Pituitary-Gonadal and-Adrenal
553 Axes. J. Neuroendocrinol. 14, 506–513.

554 Voigt, C.C., Fritze, M., Lindecke, O., Costantini, D., Pētersons, G., Czirják, G.Á., 2020. The
555 immune response of bats differs between pre-migration and migration seasons. Sci. Rep.
556 10, 17384. doi:10.1038/s41598-020-74473-3

557 Walton, J.C., Weil, Z.M., Nelson, R.J., 2011. Influence of photoperiod on hormones, behavior,
558 and immune function. Front. Neuroendocrinol. 32, 303–319.
559 doi:10.1016/j.yfrne.2010.12.003

560 Watts, H.E., 2020. Seasonal regulation of behaviour: what role do hormone receptors play?
561 Proc. R. Soc. B Biol. Sci. 287, 20200722. doi:10.1098/rspb.2020.0722

562 Watts, H.E., Rittenhouse, J.L., Sewall, K.B., Bowers, J.M., 2019. Migratory state is not associated
563 with differences in neural glucocorticoid or mineralocorticoid receptor expression in pine
564 siskins. Anim. Migr. 6, 19–27. doi:10.1515/ami-2019-0001

565 Watts, H.E., Cornelius, J.M., Fudickar, A.M., Pérez, J., Ramenofsky, M., 2018. Understanding
566 variation in migratory movements: A mechanistic approach. Gen. Comp. Endocrinol. 256,
567 112–122. doi:10.1016/j.ygcen.2017.07.027

568 Watts, H.E., Robart, A.R., Chopra, J.K., Asinas, C.E., Hahn, T.P., Ramenofsky, M., 2017. Seasonal
569 expression of migratory behavior in a facultative migrant, the pine siskin. Behav. Ecol.
570 Sociobiol. 71, 9. doi:10.1007/s00265-016-2248-2

571 Wingfield, J.C., Jacobs, J., Hillgarth, N., 1997. Ecological Constraints and the Evolution of
572 Hormone Behavior Interrelationships. Ann. N. Y. Acad. Sci. 807, 22–41.

573 Wingfield, J.C., 2008. Organization of vertebrate annual cycles: implications for control
574 mechanisms. Philos. Trans. R. Soc. B Biol. Sci. 363, 425–441. doi:10.1098/rstb.2007.2149

575 Wingfield, J.C., Schwabl, H., Mattocks, P.W., 1990b. Endocrine Mechanisms of Migration, in:
576 Gwinner, E. (Ed.), Bird Migration. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 232–
577 256. doi:10.1007/978-3-642-74542-3_16

578 Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990a. The "Challenge Hypothesis":
579 Theoretical Implications for Patterns of Testosterone Secretion, Mating Systems, and
580 Breeding Strategies. Am. Nat. 136, 829–846. doi:10.1086/285134

581 Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S.E., Ramenofsky, M., Richardson,
582 R.D., 1998. Ecological Bases of Hormone—Behavior Interactions: The "Emergency Life
583 History Stage." Am. Zool. 38, 191–206. doi:10.1093/icb/38.1.191

584 Wood, R.I., Newman, S.W., 1993. Intracellular partitioning of androgen receptor
585 immunoreactivity in the brain of the male syrian hamster: Effects of castration and steroid
586 replacement. J. Neurobiol. 24, 925–938. doi:10.1002/neu.48024070

587

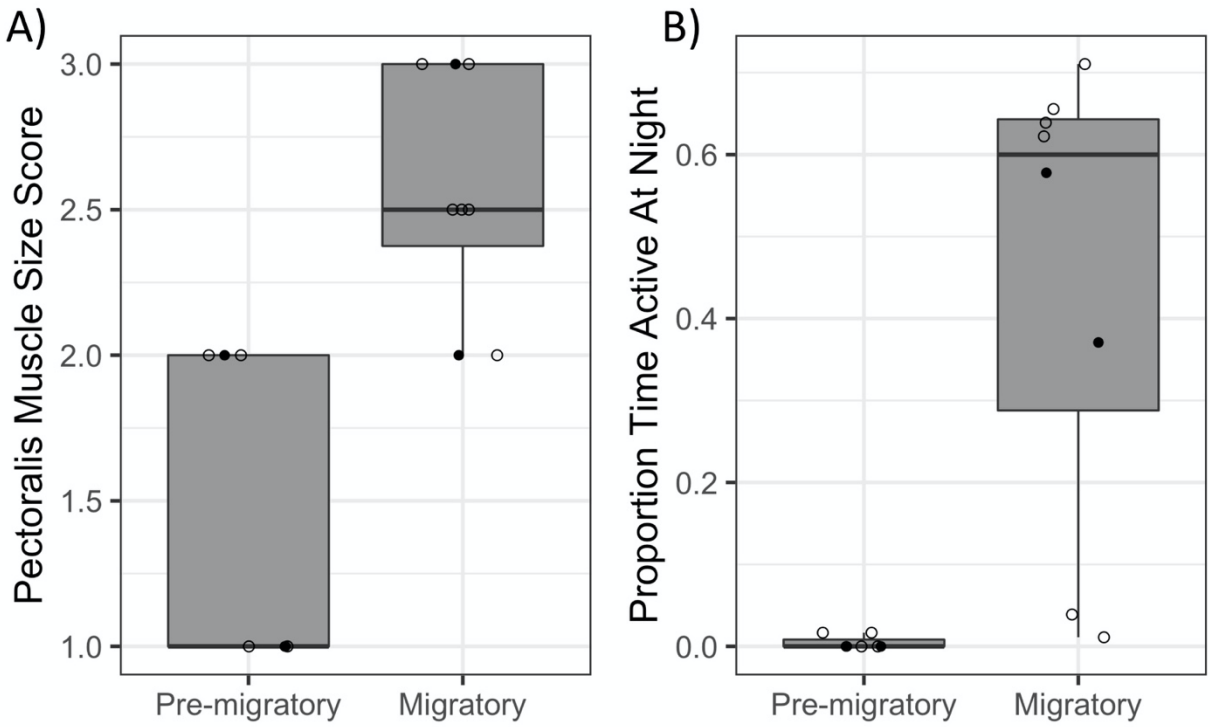
Table 1. Primer sequences used to measure expression levels of endocrine signaling genes. The primers for 11β-HSD type 1 were developed by Pradhan et al., (2019b). NCBI Accession IDs for each primer can be found in Supplementary Table 1.

Gene	Forward Sequence (5'-3') Reverse Sequemce (5'-3')	Amplicon Size (bp)
Glucocorticoid receptor (NR3C1)	TCTCCCCTCGTGCACCAT TGTTCGTAACAGCCTCAGAGCTT	68
Mineralocorticoid receptor (NR3C2)	CGAGCCCTCCGTCAACAC GGAGTAAGTGCTGGTGAGATAGCA	63
11β-HSD type 1	ATCCATAGCGCGGGTAAAATTGC GTGTTGATGTAGCCCAGGATG	153
11β-HSD type 2	GCGAGGACTATGTGGAGGAGAT TCCACTGCCACCTTCATGAA	61
Androgen receptor	TGTACAGCCAGTGCATCAGGAT TGATCTGAAGCCACCCAAATT	60
Estrogen receptor- α	TGAAAGGTGGAATCCGAAAAGA TTGGCGTTTTTGTTTCATCACT	59
5α-reductase	CGCCTTTGCCTTTTTCACTCT AGATAGTACCTGTGATGGTGATAAGCA	66
Aromatase	TCAACGCGCTCAACCTCAT ACCCCGAAGAGCTTGTT	61
GAPDH	GTGGTGCCAAGCGTGTGA CACGAACATGGGAGCATCAG	56

Table 2. Results of statistical tests comparing relative gene expression levels of endocrine receptors and enzymes associated with steroid synthesis or inactivation in pine siskins sampled before (n = 7) and during (n = 8) the migratory period.

Gene of interest	Test Statistic	Degrees of Freedom	P value
Androgen receptor	-0.64	11.0	0.53
5α-reductase	0.99	14.9	0.34
Estrogen receptor-α	-1.69	9.46	0.12
Aromatase	-0.57	12.26	0.58
Glucocorticoid receptor	-1.3	11.6	0.22
Mineralocorticoid receptor	-0.56	12.9	0.58
11β-HSD type 1	-0.14	12.4	0.89
11β-HSD type 2	-0.85	9.24	0.42

598 **Figure 1.** Comparisons of two migratory indicators in pine siskins sampled before ("pre-
599 migratory", n = 7) and during (n = 8) the migratory period. Relative to the pre-migratory period,
600 pine siskins sampled during the migratory period exhibited significantly larger muscles (A) and
601 spent a greater proportion of time active at night (B, p = 0.003 for both comparisons). Circles
602 indicate individual data points; filled circles represent males and open circles represent
603 females. Points within each group are randomly shifted along the x-axis to reduce overlap.
604 Boxplots depict the median (solid black line), the first and third quartiles (upper and lower
605 hinges), and whiskers extent to the largest value but no further than 1.5 times the inter-quartile
606 range.



607

Figure 2. Relative expression of androgen receptor (A), 5α-reductase (B), estrogen receptor-α (C), and aromatase (D) in the pectoralis muscle of pine siskins sampled during the pre-migratory period (n = 7 birds) and migratory period (n = 8 birds). Circles indicate individual data points; filled circles represent males and open circles represent females. Points within each group are randomly shifted along the x-axis to reduce overlap. Note the y-axes of the graphs are scaled differently. Boxplots depict the median (solid black line), the first and third quartiles (upper and lower hinges), and whiskers extent to the largest value but no further than 1.5 times the inter-quartile range.

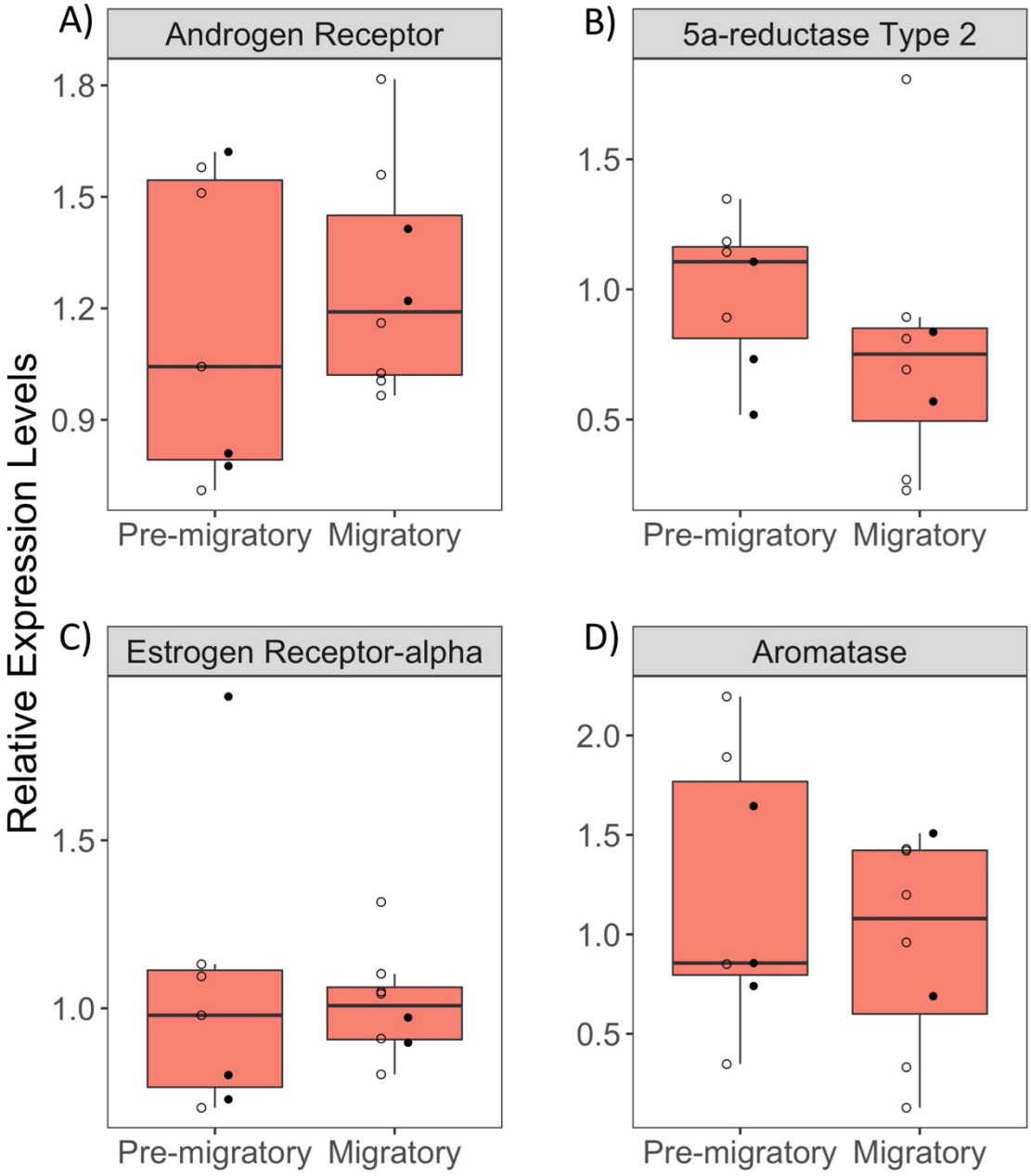


Figure 3. Relative expression of glucocorticoid receptor (A), mineralocorticoid receptor (B), 11β-HSD type 1 (C), and 11β-HSD type 2 (D) in the pectoralis muscle of pine siskins sampled during the pre-migratory period (n = 7 birds) and migratory period (n = 8 birds). Circles indicate individual data points; filled circles represent males and open circles represent females. Points within each group are randomly shifted along the x-axis to reduce overlap. Note the y-axes of the two rows of graphs are scaled differently. Boxplots depict the median (solid black line), the first and third quartiles (upper and lower hinges), and whiskers extent to the largest value but no further than 1.5 times the inter-quartile range.

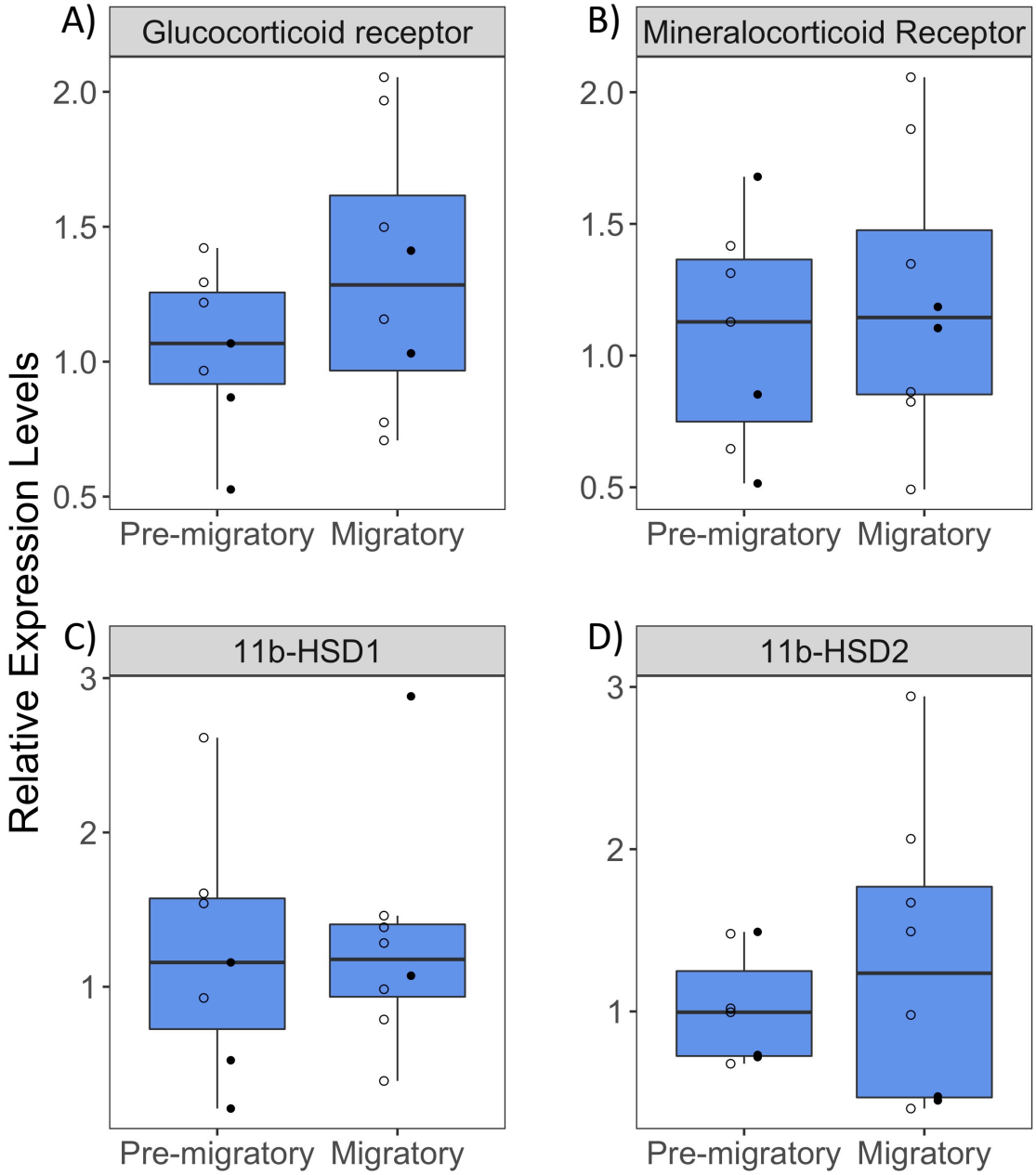


Figure 4. Integration of endocrine signaling genes measured in the pectoralis tissue of pine siskins during either the pre-migratory period (A) and the migratory period (B). Edge color indicates the direction of the correlation (i.e., positive = red, negative = blue) and line thickness indicates the strength of the correlation. Only edges with a correlation ≥ 0.7 are displayed. p_{sum} represents the sum of the absolute value of all correlations divided by the total number of possible node connections (i.e., 28). Glucocorticoid p_{sum} and Sex Steroid p_{sum} represents the sum of the absolute value of correlations between genes associated with corticosterone or sex steroid signaling divided by the number of possible node connections within each of these subsets (i.e., 6). Abbreviations: GR = glucocorticoid receptor, MR = mineralocorticoid receptor, AR = androgen receptor, ER-alpha = estrogen receptor-α, HSD1 = 11β-HSD type 1, HSD2 = 11β-HSD type 2, AROM = aromatase, 5aR2 = 5α-reductase type 2