Food and social cues modulate reproductive development but not migratory behavior in a nomadic songbird, the Pine Siskin

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ABSTRACT

Many animals rely on photoperiodic and non-photoperiodic environmental cues to gather information and appropriately time life-history stages across the annual cycle, such as reproduction, molt, and migration. Here, we experimentally demonstrate that the reproductive physiology, but not migratory behavior, of captive Pine Siskins (*Spinus pinus*) responds to both food and social cues during the spring migratory-breeding period. Pine Siskins are a nomadic finch with a highly flexible breeding schedule and, in the spring, free-living Pine Siskins can wander large geographic areas and opportunistically breed. To understand the importance of non-photoperiodic cues to the migratory-breeding transition, we maintained individually housed birds on either a standard or enriched diet in the presence of group-housed heterospecifics or conspecifics experiencing either the standard or enriched diet type. We measured body condition and reproductive development of all Pine Siskins and, among individually housed Pine Siskins, quantified nocturnal migratory restlessness. In group-housed birds, the enriched diet caused increases in body condition and, among females, promoted reproductive development. Among individually housed birds, female reproductive development differed between treatment groups whereas male reproductive development did not. Specifically, individually housed females showed greater reproductive development when presented with conspecifics compared to heterospecifics. The highest rate of female reproductive development, however, was observed among individually housed females provided the enriched diet and maintained with group-housed conspecifics on an enriched diet. Changes in nocturnal migratory restlessness did not vary by treatment group or sex. By manipulating both the physical and social environment, this study demonstrates how multiple environmental cues can affect the timing of transitions between life-history stages with differential responses between sexes and between migratory and reproductive systems.

Keywords: environmental cues, food cues, migratory behavior, non-photoperiodic cues, reproductive development, sex differences, social cues

How to Cite

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LAY SUMMARY

- Many animals inform decisions associated with survival and reproduction using a multitude of environmental cues, including photoperiodic and non-photoperiodic cues.
- How multiple environmental cues combine to influence animal decision-making is not well understood.
- We experimentally tested how different combinations of non-photoperiodic cues, including social cues, influenced the spring migratory behavior and reproductive physiology of Pine Siskins.
- Pine Siskins are a nomadic finch with highly flexible breeding and migratory behavior that are known to be modulated by non-photoperiodic cues.
- Results demonstrate that the presence of conspecifics—but not heterospecifics that are ecologically relevant—and direct access to preferred diet items promote female reproductive development.
- Male reproductive development and nocturnal migratory restlessness did not differ across experimental treatments.
- Our results highlight sex-specific differences in environmental cue use and demonstrate how animals can use multiple types of environmental cues to differentially inform decisions associated with the timing of annual cycle events.

Las señales alimenticias y sociales modulan el desarrollo reproductivo pero no el comportamiento migratorio en un ave canora nómada, *Spinus pinus*

RESUMEN

Muchos animales dependen de señales ambientales fotoperiódicas y no fotoperiódicas para recopilar información y sincronizar adecuadamente las etapas del ciclo anual de su historia de vida, como la reproducción, la muda y la migración. Aquí, demostramos experimentalmente que la fisiología reproductiva, pero no el comportamiento migratorio, de individuos de *Spinus pinus* en cautiverio responden tanto a señales alimenticias como sociales durante el período migratorio-reproductivo de primavera. *S. pinus* es un ave nómada con un esquema reproductivo altamente flexible y, en primavera, los individuos que viven en libertad pueden deambular por grandes áreas geográficas y reproducirse de modo oportunista. Para entender la importancia de las señales no fotoperiódicas en la transición migratoria-reproductiva, mantuvimos aves alojadas individualmente ya sea con una dieta estándar o una enriquecida, en presencia de individuos heteroespecíficos o conspecíficos alojados en grupo, que recibieron ya sea el tipo de dieta estándar o la enriquecida. Medimos la condición corporal y el desarrollo reproductivo de todos los individuos de *S. pinus* y, entre los individuos alojados individualmente, cuantificamos la inquietud migratoria nocturna. En las aves alojadas en grupo, la dieta enriquecida causó aumentos en la condición corporal y, entre las hembras, promovió el desarrollo reproductivo. Entre las aves alojadas individualmente, el desarrollo reproductivo de las hembras difirió entre los grupos de tratamiento, mientras que el desarrollo reproductivo de los machos no lo hizo. En particular, las hembras alojadas individualmente mostraron un mayor desarrollo reproductivo cuando se presentaban con conspecíficos en comparación con heteroespecíficos. La tasa más alta de desarrollo reproductivo en las hembras, sin embargo, se observó entre las hembras alojadas individualmente que recibieron la dieta enriquecida y que se mantuvieron con conspecíficos alojados en grupo con la dieta enriquecida. Los cambios en la inquietud migratoria nocturna no variaron según el grupo de tratamiento o el sexo. Al manipular tanto el entorno físico como social, este estudio demuestra cómo múltiples señales ambientales pueden afectar el momento de las transiciones entre las etapas de la historia de vida, con respuestas diferenciales entre los sexos y entre los sistemas migratorios y reproductivos.

Palabras clave: comportamiento migratorio, desarrollo reproductivo, diferencias de género, señales alimenticias, señales ambientales, señales no fotoperiódicas, señales sociales

INTRODUCTION

Animals frequently use photoperiodic (Bradshaw and Holzapfel 2007) and non-photoperiodic cues, such as temperature and food availability (Wingfield 1983a, Chmura et al. 2020), to appropriately time the expression of life-history stages (sensu Jacobs and Wingfield 2000) in the annual cycle, such as breeding, molt, and migration. Though photoperiodic cues often serve as initial cues that prime animal responses to non-photoperiodic cues (Wingfield 1983b, Dawson et al. 2001, Martin et al. 2004, Chmura et al. 2020), reliance on environmental cues in this manner can vary depending on the extent to which photoperiodic cues predict the subsequent availability of important resources (Perfito et al. 2008, Hahn et al. 2008). Further, how animals use environmental cues to time transitions between different life-history stages and to inform decision making more broadly is the product of multiple types of environmental cues being integrated (Bronson and Heideman 1994, Chmura et al. 2020). The way in which multiple environmental cues combine to modulate animal behavior and physiology during life-history stage transitions, however, is not well understood. Experimental studies measuring how animals respond to different combinations of environmental cues are needed to develop a more robust understanding of how the environment can modulate behavioral and physiological processes (Watts et al. 2022).

Social cues can be provided by either conspecific or heterospecific organisms and such cues allow animals to indirectly, and sometimes more efficiently, gather information about their environment (Valone and Templeton 2002, Danchin 2004, Dall et al. 2005). In the context of the reproductive life-history stage, the role of conspecific cues from a potential mate have been particularly well-established and have been found to modulate both female and male reproductive physiology (Chmura et al. 2020, Tolla and Stevenson 2020). Interestingly, heterospecific cues have also been found to influence female reproductive physiology, with effects on follicular development and the timing and quantity of eggs laid (Bentley et al. 2000, Forsman et al. 2012). Beyond reproduction, both conspecific and heterospecific social cues have also been found to modulate migratory behavior, including directional and navigational decisions, as well as habitat selection during and following migratory movements (Couzin et al. 2005, Mukhin et al. 2008, Guttal and Couzin 2010, Goodale et al. 2010, Szymkowiak 2013, Szymkowiak et al. 2017, Valente et al. 2021, Aikens et al. 2022). The role of social cues in modulating the timing of the migratory life-history stage is less well understood (but see Robart et al. 2022, Vernasco et al. 2022). Importantly, the use of social cues can be context-dependent and vary based on the availability of non-social cues (Seppänen et al. 2007, Miller et al. 2013, Kohles et al. 2022). Social cue use can also depend on characteristics of the cue source, including whether the source is a conspecific or heterospecific (Bentley et al. 2000, Kivelä et al. 2014, Chang et al. 2018, Hämäläinen et al. 2023). Given that social cue use is often context-dependent, a robust understanding of social cue use is dependent on experimental studies presenting varying social cues in the presence of different non-social cues.

Food availability is an environmental cue that has influenced the evolution of animal life histories and that can also mediate within-individual changes in physiology and behavior (Boutin 1990, Bronson and Heideman 1994, Hahn et al. 2005, Schoech and Hahn 2007, Davies and Deviche 2014). The proximate effects of food-related cues on reproductive physiology can occur via direct nutritional pathways or indirectly through perceptual pathways (Hau et al. 2000, Hahn et al. 2005). For instance, the composition or quality of available food resources can modulate reproductive behavior and investment as well as the development of reproductive physiology, including seasonal gonadal recrudescence (Schoech 1996, Hau et al. 2000, Reynolds et al. 2003, Miller et al. 2013). Both reductions in food availability and the quality of available food items can also modulate migratory behavior in free-living birds and the expression of nocturnal migratory restlessness in captive songbirds (Gwinner et al. 1988, Fusani et al. 2011, Eikenaar and Bairlein 2014, Åkesson and Helm 2020, Stanley et al. 2022, Yadav et al. 2023). Social cues represent an indirect pathway through which food-related cues can be obtained. However, most studies have focused on how social cues can maximize foraging opportunities (Kohles et al. 2022) rather than their use to time life-history stage transitions. How social cues associated with varying food availability influence reproductive physiology and to what extent social cue use depends on the availability of non-social cues, is much less well understood. Additionally, sex specific responses to environmental cues are likely widespread, yet our understanding of sex-specific responses to environmental cues is relatively limited (Ball and Ketterson 2008, Tolla and Stevenson 2020, Williams et al. 2022, Watts et al. 2022). Experimental manipulations of both social and non-social cues during seasonal reproductive development provides an ecologically relevant context for understanding how environmental cues modulate sex-specific changes in physiology and behavior.

The Cardueline finches exhibit highly flexible migratory and breeding behavior (Hahn et al. 2009) that is sensitive to non-photoperiodic cues, even while being maintained in captivity. Multiple studies on Carduelines have demonstrated the importance of direct food cues and the presence of a mate to reproductive physiology (Hahn 1995, Leitner et al. 2003, Furlonger et al. 2012, Watts and Hahn 2012, Valle et al. 2015, Watts et al. 2016). Further, some species of Carduelines frequently co-occur and rely upon similar resources (Koenig and Knops 2001, Benkman and Young 2020), highlighting the potential relevance of heterospecific cues. Viewed together, captive Carduelines represent an excellent system to understand how male and female animals modulate their physiology and behavior in response to non-social and social cues, including heterospecific social cues.

Here we experimentally test how female and male Pine Siskins (*Spinus pinus*), a nomadic cardueline finch with a highly flexible migratory and breeding schedule, respond to different combinations of social and non-social environmental cues. We focus on the spring migratory-breeding period when free-living Pine Siskins wander large geographic areas, often in groups, to locate suitable breeding sites (Dawson 2020, Cornelius et al. 2021) and when captive individuals can express nocturnal migratory restlessness (Watts et al. 2017, Robart et al. 2018, Rittenhouse et al. 2019, Vernasco and Watts 2022) and transition to a reproductive state (Vernasco et al. 2022). Previous captive studies have demonstrated that long day photoperiods and the availability of seeds promote male gonadal development during this time (Hahn et al. 2004, Watts and Hahn 2012). Additionally, female, but not male, gonadal development has been found to depend on access to a mate (Watts et al. 2016). Same-sex, conspecific social cues can also influence the expression of nocturnal migratory restlessness in Pine Siskins (Robart et al. 2022), though the effect of social cues depends on the sex of the receiver and state of sender (Vernasco et al. 2022).

To understand how multiple environmental cues influence the migratory breeding-transition, we experimentally manipulated both food availability and social cues during the migratory breeding transition. We maintained individually housed Pine Siskins on either a standard diet or an enriched diet (as direct food cues) in the presence of group-housed heterospecifics or conspecifics (as social cues; Figure 1). Heterospecific social cues were provided by Red Crossbills

(*Loxia curvirostra*), an ecologically relevant heterospecific that relies upon similar food resources as Pine Siskins, frequently co-occurs in mixed-species flocks, breeds sympatrically in the same habitat, and can hybridize with Pine Siskins (Tallman and Zusi 1984, Crumb 1985, Messineo 1985, Koenig and Knops 2001, Benkman and Young 2020, Cornelius et al. 2021). Two different types of conspecific social cues came in the form of group-housed conspecifics that were maintained on either a standard or enriched diet. For individually housed Pine Siskins, we collected daily measurements of nocturnal activity and weekly measurements of body condition and reproductive development to quantify changes in an individual's migratory and breeding state. Additionally, to further understand how non-photoperiodic cues (i.e., different diets and varying cage sex ratios) influence reproductive development, we collected weekly measurements of body condition and reproductive development from group-housed Pine Siskins. If direct access to enhanced food availability is a cue to terminate migration or initiate breeding, then we expect indicators of these transitions to differ between treatment groups based on their own diet (standard versus enhanced) irrespective of the available social cues. If conspecific or heterospecific cues stimulate these transitions, then we expect different responses among birds experiencing the same diet but different social cues. That is, for individually housed Pine Siskins on standard diet, we can make comparisons among those with neighboring group-housed Red Crossbills, standard diet Pine Siskins, and enriched diet Pine Siskins. Further, the individually housed Pine Siskins that are exposed to heterospecific social cues are the only treatment group that does not include additional conspecifics beyond the other individually housed Pine Siskins. Thus, this heterospecific social treatment allows us to evaluate the influence of additional conspecifics (i.e., the group-housed Pine Siskins) on the migratory-breeding transition. Because the use of social cues and food cues are not mutually exclusive possibilities, we may find that both cue types contribute to the transition from migratory to reproductive states.

METHODS

Animal Capture and Housing

Wild Pine Siskins and Red Crossbills were captured using either mist nets or baited funnel traps at multiple sites within Oregon, Idaho, and Washington (Supplementary Material Section 1). All individually housed Pine Siskins (*n* = 56) were captured in September and October 2020. All group-housed Pine Siskins (*n* = 63) were captured between June 2019 and February 2020. After capture, birds were transported to Washington State University, Pullman, WA, and individually housed birds were maintained in one of two outdoor aviaries (*n* = 28 and 30 birds per aviary) until being moved indoors (see Supplementary Material for additional details on the treatment of group-housed Pine Siskins prior to the current experiment). Red Crossbills (*n* = 20) were captured in late September and early October 2020 and housed in a single outdoor aviary until the beginning of the experimental period. Plumage characteristics and the extent of skull ossification were used to age birds at capture as either after hatch year (i.e., 1+ years old) or hatch year (i.e., < 1 year old) as described in Pyle (1997). Unknown age Pine Siskins were those that lacked molt limits or were captured following the date of average complete skull ossification. Pine Siskins captured in breeding condition were sexed based on the presence of a brood patch or cloacal protuberance as described in Pyle (1997). After hatch year Red Crossbills were sexed using plumage color as described in Pyle (1997). Pine Siskins captured in nonbreeding condition and both Pine Siskins and Red Crossbills hatched in the 2020 breeding season were genetically sexed by the Washington Animal Disease Diagnostic Laboratory using DNA from blood samples using the methodology of Çakmak et al. (2017). All birds had ad libitum access to water and grit prior to and during the experiment. Prior to 25 March 2021, all Pine Siskins were provided with a diet of Roudybush Small Bird Maintenance Diet (Woodland, CA) and a mixture of nyjer seed and sunflower seed hearts ad libitum. For the entire duration of and prior to the experiment, all Red Crossbills were provided with a diet of Roudybush Small Bird Maintenance Diet and whole sunflower seeds *ad libitum.*

Pine Siskins (*n* = 119) were moved indoors 17–20 February 2021 and then moved to their final cage setup used during the experiment on 19 March 2021. Fifty-six Pine Siskins were housed in individual cages (cage dimension: approximately 34 × 38.5 × 43 cm) and 63 birds were housed in groups of 3 per cage (58 × 25 × 36 cm). Red Crossbills were moved indoors on 21 April 2021 and randomly assigned to cages, resulting in mixed-sex (*n* = 4) and same-sex (*n* = 6) pairs in cages (45 × 36 × 52 cm). Red Crossbills are larger than Pine Siskins and cage limitations required housing in pairs rather than groups of 3. While indoors, all birds were maintained on a photoperiod mimicking natural changes in day lengths at 47°N latitude.

Experimental Design

We maintained each of the 4 treatment groups described in Figure 1 in independent, but adjacent, rooms with the same lighting and temperature conditions to ensure birds only received the intended environmental cues. Pine Siskins housed individually were assigned to treatment groups using a stratified random approach such that there were between 6 and 8 birds caught as after hatch year and between 6 and 8 birds caught as hatch year or unknown age per treatment group. Group-housed Pine Siskins were assigned to the 3 treatment groups using a stratified random approach such that groups were balanced by previous experimental history (see Supplementary Material Section 1 for more details on previous experimental history). Sample sizes per treatment group are provided in Figure 1. We ensured that all group-housed Pine Siskins were mixed sex by maintaining in each cage either 1 male and 2 females or 2 males and 1 female. To prevent individually housed birds from directly seeing the food provided to neighbors, opaque barriers were used to limit the view such that individually housed birds could not directly view the food cups in other cages. Individually housed birds could, however, perceive vocal cues (e.g., calls and songs) from other birds in the room, and they could observe group-housed birds at the level of the perch and above, including if group-housed birds flew to the perch to manipulate or consume food items. See Supplementary Material Figure 1 for photos of housing conditions and diagram of cage configuration.

For both the standard and enriched diets, the abundance of the food items provided was adjusted for cages housing individual or groups of siskins so that the amount of food available per bird was consistent. Individually housed Pine Siskins fed a standard diet received a daily allotment of five sunflower hearts and ab libitum access to grit and Roudybush. Group-housed Pine Siskins fed a standard diet received a daily allotment of ½ tsp of sunflower hearts distributed evenly between 2 food cups on opposite sides of the cage and ab libitum access to grit and Roudybush. The standard diet was intended to represent a modest availability of preferred diet items as ab libitum access to seeds, including sunflower hearts, has previously been shown to accelerate reproductive development in Pine Siskins experiencing a 12:12 light cycle (Watts and Hahn 2012).

The enriched diet consisted of ad libitum access to Roudybush, a dry seed mix consisting of 7 types of seed, a commercial seed mix that was soaked in water for 8 hr and then chitted for 16 hr before serving, fresh spinach and/or broccoli, and 1 or 3 meal worms for individually and group-housed birds, respectively. The enriched diet was developed by consulting Red Siskin (*S. cucullate*) breeders associated with the Red Siskin Initiative and is representative of a breeding season diet used in conservation breeding efforts of a closely related species of siskin. For full details on the composition of the enriched diet, see Supplementary Material Section 2. For individually housed Pine Siskins, the dry seed mix and soak seed mix were provided in 2 separate food cups and refilled daily as needed. For group-housed birds, 4 food cups were provided and refilled daily as needed. Two of the 4 cups contained the dry seed mix and 2 cups contained the soak seed mix. Cups containing either dry or soak seed were placed on opposite sides of the cage to reduce within-cage competition for diet items. For all birds, greens and meal worms were added to the same food cups as the soak seed.

Experimental Timeline

All Pine Siskins were maintained on the standard diet beginning on 25 March 2021. Beginning 22 April 2021 (i.e., day 0), Red Crossbills were moved into the room containing individually housed Pine Siskins in the heterospecific social cue group and we began providing the enriched diet to the appropriate treatment groups. The timing of the experimental manipulation was chosen to coincide with the peak of the migratory period and early reproductive period as indicated by previous studies (Watts et al. 2017, Vernasco and Watts 2022, Vernasco et al. 2022). As Pine Siskins are nomadic migrants wherein wandering behaviors facilitate the discovery of areas containing an abundance of ephemeral and unpredictable resources (Watts et al. 2018), we expect that the supplementary cues tested here will induce relatively rapid effects on migratory and reproductive behavior as has been found to occur previously in Cardueline finches (Hahn et al. 2005, Robart and Watts 2023)

Measurements of body condition and reproductive development were collected from all Pine Siskins 2 hr after lights-on starting 8 days before the experimental manipulation (i.e., 13 April 2022) and subsequently taken each week on the same day of the week for each bird. Treatment groups and individuals within treatment groups were measured in the same order to mitigate confounds of time of day on measurements. To reduce the amount of time spent in each room each day, Pine Siskins housed individually were randomly divided into 2 groups within each treatment group and each subset of Pine Siskins was measured on one of 2 consecutive mornings. All birds housed in groups were measured on the day following the second day of measuring birds housed individually.

Behavioral, Reproductive, and Condition Measurements

Nocturnal activity, an indicator of migratory state (Berthold 1973, Gwinner and Czeschlik 1978, Watts et al. 2017), is predicted to decline as birds transition to breeding. Body mass is generally elevated during migration (Berthold 2001, Robart et al. 2018, Cornelius et al. 2021) and can either decline as birds transition to breeding or remain elevated due to short breeding windows, to support facultative responses to inclement weather or in preparation for breeding (Ricklefs 1974, Ramenofsky and Wingfield 2006). Further, in preparation to breed, a male's cloaca swells and lengthens (termed a cloacal protuberance) whereas females defeather and vascularize their abdomen (termed a brood patch; Ramenofsky and Wingfield 2006). We used the development of cloacal protuberances and brood patches as indicators of reproductive physiology and development of mature breeding capabilities.

For Pine Siskins housed individually, activity and condition data (i.e., body and reproductive condition) were collected as described in Vernasco et al. (2022). Specifically, activity levels were measured using passive infrared sensors (Starr Life Sciences Corp., Oakmonk, PA) by attaching sensors to a Vital View Data Acquisition System (Starr Life Sciences Corp., Oakmonk, PA) and placing 1 sensor on top of each cage housing an individual Pine Siskin. This position for sensors was chosen as it has previously been shown to accurately capture activity levels (Watts et al. 2017, Robart et al. 2018, Vernasco et al. 2022). Opaque dividers were placed between cages housing individual Pine Siskins on 23 March 2021 to prevent sensors from picking up activity in adjacent cages. Counts of activity were summed into 10-min bins and activity levels were calculated by summing the total number of movements recorded between 2300 and 0300, a time window that includes activity associated with nocturnal migratory restlessness (Watts et al. 2017). We did not examine patterns of diurnal activity as previous studies have found no evidence of elevated levels of diurnal activity in association with a spring migratory state in Pine Siskins (Watts et al. 2017, Robart et al. 2018). The activity of group housed birds was not measured because activity cannot be assigned to a particular individual using this technique (Vernasco et al. 2022).

Consumption of meal worms and greens was measured routinely (every 1–9 days, with the average number of days between measurements: mean ± 1 SD = 2.9 ± 2.5) over the course of the experiment to understand the extent to which these diet items were consumed. To determine if food items were consumed, cages were visually inspected the day after food items were provided. Meal worms and greens were considered consumed if they were not found in the food cup or on the floor of the cage. Rooms were swept routinely, and worms or greens were not observed in debris piles. For group-housed birds, all worms provided had to be consumed to be considered consumed. We did not measure the consumption of seed or Roudybush due to time constraints and because both were frequently spilled out of cages.

Measurements of body condition and reproductive development were measured a total of 8 times over the course of the experiment. All measures were collected by BJV. For birds housed individually, body mass, furcular and abdominal fat deposits, and pectoralis muscle size were measured. Body mass was measured to the nearest 0.01g using an electronic balance. Furcular and abdominal fat deposits were each visually scored from a scale of 0 (no fat) to 5 (bulging fat) and then summed to generate a single fat score (Wingfield and Farner 1976). Muscle size was visually scored from 0 (muscle concave with prominent keel) to 3 (muscle bulging over keel; Bairlein 1995). Body mass, fat score, and muscle size were then scaled, centered, and collapsed into a single body condition index using principal component analysis. The first principal component explained 67% of the variation and mass, fat, and muscle size all loaded positively onto the 1st axis (loadings = 0.62, 0.61, 0.49, respectively). To limit the time spent in experimental rooms, only body mass and reproductive development were measured for birds housed in groups, though body mass is highly correlated with muscle and fat scores (Vernasco et al. 2022). Male reproductive development was assessed for all birds by measuring the length of the cloacal protuberance to the nearest 0.1mm using dial calipers. Female reproductive development was assessed for all birds by visually scoring brood patch development as follows: 1 (defeathering, 30–90% defeathered), 2 (defeathered, 95–100%), 3 (100% defeathered, fluid-filled), 4 (regressed but still 100% defeathered), and 5 (refeathering), as described in Watts et al. (2016). A brood patch score of 3 is associated with females laying and incubating eggs (Supplementary Material Figure 1D). Among group-housed birds, the reproductive condition of 2 females (1 on an enriched diet and 1 on the standard diet) and 1 male on an enriched diet was not measured and these individuals were removed from analyses of and figures displaying treatment effects on reproductive condition. The male and female on the enriched diet were housed in separate rooms. Condition measurements were not collected blind to treatment as all individuals within each treatment groups were housed in the same room.

Statistical Analyses

All statistical analyses were performed in Program R v. 4.2.0 (R Core Team 2021). We modelled changes in food consumption, body condition or mass, reproductive development, and nocturnal activity using mixed models. We analyzed data from birds housed individually and birds housed in groups separately to reduce model complexity and due to differences between group-housed and individually housed birds in the amount of time spent in captivity. To assess model fit, we used the *check_model* function within the performance package (Lüdecke et al. 2021) for linear models and the simulation-based approach within the *DHARMa* package (Hartig 2021) for generalized linear models. We tested for pre-experimental group differences in body condition measurements collected 2 and 8 days before the experimental manipulation and activity data collected 8 days before the experimental manipulation to confirm that treatment groups did not differ prior to the experimental manipulation (i.e., before day zero; see Supplementary Material). For analyses testing for treatment effects, we included all condition and activity data collected between 0 and 42 days after the start of the experiment.

Consumption of enriched diet items was analyzed using a generalized linear mixed model with a binomial distribution within the *lme4* package (Bates et al. 2015). Food consumption models included an interaction between food item type (i.e., worm or greens) and experiment day and a random intercept term denoting individual or cage ID for individually housed and group-housed birds, respectively. For body condition and male reproductive development (i.e., cloacal protuberance length), we built linear mixed models using the *lme4* package. For female reproductive development (i.e., brood patch score, a categorical response variable), we constructed cumulative link mixed models using the *ordinal* package (Christensen 2019). For activity data, generalized linear mixed models with a negative binomial distribution (i.e., "nbinom1") were parametrized using the *glmmTMB* package (Brooks et al. 2017). For each response variable, all models included a random intercept denoting individual ID and models examining changes in activity included a random slope term of experiment day by individual ID.

We tested for treatment effects on changes in response variables (i.e., body condition or mass, reproductive condition, or activity) by including an interaction between treatment group and experiment day in all models. The interaction between treatment group and experiment day estimates group-specific changes in the response variable over the course of the experiment. As both individually-housed and group-housed birds included males and females, we determined how to best model an effect of sex on body condition or mass and, in birds housed individually, activity using AIC_c-based model selection as described in Burnham et al. (2011). When multiple models were similarly supported, we reduced model selection uncertainly and prioritized more parsimonious models by inspecting competing models for uninformative parameters. Uninformative parameters were identified by determining if 85% confidence intervals surrounding parameter estimates included zero, as described in (Arnold 2010). For birds housed individually, 4 models were compared: (1) no effect of sex, (2) an additive effect of sex, (3) an interaction between sex and experiment day, and (4) an interaction between sex, experiment day, and treatment group (Supplementary Material Tables 2A and 5). For group-housed birds, we used the same approach but also compared models with and without additive or interactive effects of the cage sex ratio (i.e., two males and one female or one male and two females per cage; Supplementary Material Tables 2B and 4). Two rooms contained group-housed birds experiencing an enriched diet, but we do not report room-level differences as preliminary analyses revealed no differences between group-housed birds experiencing the enriched diet in different rooms. We created one model set for each response variable (i.e., body condition or mass, brood patch development, cloacal protuberance length, and nocturnal activity). After identifying the appropriate model structure to account for sex and, among group-housed birds, cage sex ratio, we used the *emtrends* function within the *emmeans* package (Lenth 2020) to estimate group-specific slope values and 95% confidence intervals of the experiment day term. If model summaries identified a significant treatment effect, pairwise-comparisons of group-specific slope values were conducted using the *pairs()* function.

RESULTS

Group-housed Pine Siskins

[LEVEL HEADING 3] **Food consumption**

Group-housed Pine Siskins fed an enriched diet had high consumption rates of both greens (average probability of consumption ± SD = 0.99 ± 0.003) and worms (average probability of consumption ± SD = 0.98 ± 0.006; Figure 2A) that did not significantly change over the course of the experiment (Figure 2B; Supplementary Material Table 1A).

[LEVEL HEADING 3] **Body mass**

Model selection revealed equivocal support for 2 models including additive effects of sex and cage sex ratio (Supplementary Material Table 2B). Further examination of 85% CIs revealed the confidence intervals of the cage sex ratio term, but not the sex term, included 0, indicating it to be an uninformative parameter (Supplementary Material Table 3B). The model only including sex (i.e., the more parsimonious model) was therefore used for inference. Males tended to exhibit a lower body mass, but this effect was not below the significance threshold ($p = 0.09$, Table 1B). Group-housed Pine Siskins maintained on the standard diet did not exhibit significant increases in body mass over the course of the experiment, whereas those maintained on an enriched diet did exhibit significant increases in body mass (Figure 3). The increase in body mass for enriched diet birds was both significantly different from 0 and significantly greater than the change in body mass observed in Pine Siskins maintained on the standard diet (Table 1B, Figure 3B).

[LEVEL HEADING 3] Reproductive development

Female group-housed Pine Siskins developed their brood patches over the course of the monitoring period (Figure 4). Model selection did not support an effect of cage sex ratio on female brood patch development (Supplementary Material Table 4A). Females experiencing the enriched diet exhibited significantly faster rates of brood patch development than those females maintained on the standard diet (Table 2B, Figure 4). Among males, model selection did not support any effect of cage sex ratio on cloacal protuberance length (Supplementary Material Table 4B). Both treatment groups exhibited significant increases in cloacal protuberance lengths (Figure 5) and males in the standard diet group exhibited significantly greater rates of increase in cloacal protuberance lengths than those males maintained on an enriched diet (Table 2D).

Pine Siskins Housed Individually

[LEVEL HEADING 3] Food consumption

Individually housed Pine Siskins more frequently consumed greens (average probability ± SD = 0.70 ± 0.14) than worms (average probability ± SD = 0.28 ± 0.07; Figure 2C). The probability of consuming both food types significantly increased over the course of the experiment, but the rate of increase was not significantly different for the 2 types of foods (Figure 2D, Supplementary Material Table 1B).

[LEVEL HEADING 3] Body condition

Model selection supported inclusion of an interaction between sex and experiment day (Supplementary Material Table 2A). Males housed individually exhibited declines in body condition (β_{male} = –0.01, 95% CIs: –0.02 to –0.002]; Figure 6) whereas females housed individually exhibited increases in body condition (β_{female} = 0.02, 95% CIs: 0.01–0.03; Figure 6) over the course of the experiment. There were no significant additive or interactive effects of treatment group on changes in body condition (Table 1A, Figure 6).

[LEVEL HEADING 3] Reproductive development

There was a significant effect of treatment group on female reproductive development (Table 2A, Figure 7). Females housed in the same room as Red Crossbills exhibited significantly slower rates of brood patch development than those females fed an enriched diet and housed with group-housed Pine Siskins maintained on an enriched diet (Table 2A, Figure 7B). Additionally, females on a standard diet housed with Pine Siskins exhibited intermediate rates of reproductive development, independent of the food provided to group-housed Pine Siskins in the same room (Figure 7B). Notably, only among the females provided an enriched diet and maintained with groups fed an enriched diet did all the females begin defeathering their brood patch. There was no effect of treatment on male cloacal protuberance lengths, though length increased across time in all groups (Table 2C, Figure 8).

[LEVEL HEADING 3] Nocturnal activity

The top-supported model included no additive or interactive effect of sex on activity. Though a model including an additive effect of sex was similarly supported, the 85% CIs of the sex term included zero (β_{sex} = 0.76, 85% CIs: –0.31 to 1.83]). We therefore considered the effect of sex to be an uninformative parameter and used the model without the sex term, the more parsimonious model, for inference. There was no significant treatment effect on nocturnal activity (Table 3C, Figure 9).

DISCUSSION

In this study, we examine sex-specific changes in the physiology and behavior of Pine Siskins experiencing either a standard or enriched diet in the presence of contrasting social cues during the spring migratory-breeding transition. The social cues include heterospecific cues from Red Crossbills or cues from group-housed conspecifics experiencing either a standard or enriched diet. We consider the 2 types of conspecific cues to be different as group-housed males and females experiencing the enriched diet exhibited increases in body condition and, among females, faster reproductive development. Though group-housed males fed an enriched diet were found to exhibit slower cloacal protuberance development, the greater abdominal fat depots of Pine Siskins fed an enriched diet relative to those fed a standard diet likely influenced the measurable length of the cloacal protuberance. The condition differences between group-housed males experiencing different diet may therefore explain the observed variation in male reproductive development. Regardless, the conspecific social cues provided by the 2 treatments could have provided information about food availability or conspecific state, including the reproductive or energetic state of same-sex and opposite-sex conspecifics. Both food availability and the state of conspecifics have been proposed to modulate animal physiology and behavior (Bronson and Heideman 1994, Szymkowiak 2013, Chmura et al. 2020) and the current study demonstrates the extent to which the presence of heterospecifics and conspecifics in different states influenced the physiology and behavior of a nomadic songbird.

Among individually housed birds, female reproductive development differed between experimental treatments. Females experiencing heterospecific cues exhibited the slowest rate of reproductive development. Many studies evaluating heterospecific cue use by migratory songbirds demonstrate resident species, such as the tits (Family *Paridae*), cause heterospecific attraction and influence nest site selection (reviewed in Szymkowiak 2013). Though heterospecific cues have been found to influence the follicular development, the timing of egg laying, and patterns of reproductive investment (Bentley et al. 2000, Forsman et al. 2012), the current results suggest that Red Crossbills do not provide a stimulatory cue for Pine Siskins at this time of year. Further, relative to those maintained with heterospecifics, Pine Siskins kept in the same room as group-housed conspecifics showed a higher rate of female reproductive development, though the diet of the group-housed birds did not have an additive effect on reproductive development in individually housed females. This result suggests that the presence of conspecifics, but not their reproductive state or the indirect food cues they provide, are important to female reproductive development. Direct access to the enriched diet did, however, cause the rate of reproductive development to increase and, only in this treatment group, did all females begin developing their brood patch. Further disentangling the relative importance of direct food cues and social cues relevant to food availability (i.e., conspecifics with enhanced diets) to female reproductive development will require future work that includes a treatment group receiving an enriched diet in the presence of group-housed birds experiencing the standard diet. Nonetheless, as all treatment groups were provided food ab libitum, our results highlight the importance of the composition of food to female reproductive development and align with other studies showing higher quality foods can stimulate female reproductive development (Nager et al. 1997, Reynolds et al. 2003, Hahn et al. 2005, Furlonger et al. 2012). Collectively, the patterns of female reproductive development observed among birds housed individually illustrate the significance of direct food cues and conspecific presence to female reproductive development.

The phenotypic and life-history differences between males and females frequently requires that they respond differently to environmental cues, particularly during seasonal reproductive development when there are sex-specific, pre-zygotic costs of reproduction (Ball and Ketterson 2008, Tolla and Stevenson 2020). When females incur greater costs than males, female reproductive development is predicted to be more sensitive to non-photoperiodic cues indicative of local environmental conditions (Moore 1983, Ball and Ketterson 2008). In support of this prediction, we find no evidence that the differences in the environmental cues provided here were associated with different patterns of male reproductive development, though female reproductive development did differ (see previous paragraph). Further support for the presence of sex-specific cue use and costs to seasonal reproductive development is provided by earlier work demonstrating the presence of a mate-stimulated female reproductive development whereas male reproductive development occurred independent of cues from a potential mate (Watts et al. 2016). Conspecific social cues were also found to have stronger effects on the migratory behavior of female Pine Siskins than male Pine Siskins (Vernasco et al. 2022). Together, these experiments demonstrate that females display a greater sensitivity to the local environmental conditions than males and suggest that female reproductive decisions facilitate appropriately timed reproduction (Moore 1983, Caro et al. 2009). The sex-specific sensitivity to non-photoperiodic cues also implies sex-specific cue integration mechanisms underlie the contrasting responses between males and females (Ball and Ketterson 2008, Tolla and Stevenson 2020).

Social cues are known to be important to migratory decision making (Helm et al. 2006, Németh and Moore 2014, Oestreich et al. 2022, Aikens et al. 2022), and in Pine Siskins, social cues from either non-migratory or photo-advanced conspecifics have been shown to modulate the expression of nocturnal migratory restlessness during the spring migratory period (Robart et al. 2022, Vernasco et al. 2022). Here, we find no evidence that the social or food cues manipulated in the present study combine to influence the expression of nocturnal migratory restlessness during the spring migratory period. This contrasts with recent work demonstrating stimulatory effects of an enriched diet on the expression of nocturnal migratory restlessness in a species of songbird that exhibits an obligate migratory strategy, wherein migratory behavior is more predictable in timing, direction, and duration (Yadav et al. 2023). The expression of migratory behavior is also known to depend on intrinsic factors, such as energetic state (Fusani et al. 2009, Eikenaar and Bairlein 2014, Eikenaar et al. 2014, Rittenhouse et al. 2019). In Pine Siskins specifically, individual differences in winter telomere lengths predict the subsequent timing and intensity of spring nocturnal migratory behavior (Vernasco and Watts 2022). Individual differences in intrinsic factors may have had a greater influence on the expression of migratory behavior than the environmental cues tested in the current study. Among free-living Pine Siskins, breeding and migratory individuals can also co-occur in the same location (Yunick 1981), demonstrating that the presence of Pine Siskins in a more advanced reproductive state does not necessitate cessation of migratory behavior. Additionally, female reproductive development is modulated by the presence of a mate (Watts et al. 2016) and the lack of settlement observed among females, and potentially males, may be because birds were housed individually. An important direction for future work will be understanding how migratory behavior changes with direct access to a mate and if physiological differences interact with environmental cues to influence migratory decisions.

Beyond providing social cues, the group-housed birds also contribute to our understanding of non-photoperiodic cue use. First, we found no evidence that the cage sex ratios among group-housed birds influenced response variables, suggesting that birds are not responsive to variation in the number of potential mates under these conditions. The group-housed birds also provide strong evidence that direct food cues are highly stimulatory to female reproductive development as the vast majority of group-housed females experiencing an enriched diet entered advanced stages of reproductive development (i.e., a fully defeathered brood patch). Most captive wild birds, particularly females, rarely exhibit full reproductive competence (Furlonger et al. 2012). The results of the current study suggest Pine Siskins, like Red Crossbills (Tordoff and Dawson 1965), are an exception, further demonstrating their potential for captive studies on environmental cue use. Lastly, the enriched diet also caused significant increases in body mass in group-housed males and females. Females exhibited higher body condition than males, which could be due to the sex differences in the weight of reproductive tissues or egg development (Schoech 1996). A previous study that provided supplemental dry seeds, but not the other enrichment food items used here, to male Pine Siskins found no effect of supplemental food on body condition, even among birds housed with a potential mate (Watts and Hahn 2012). Together with the present study, these results highlight the importance of diet composition to seasonal changes in body mass. The multitude of diet items provided in the present study prevents identification of the importance of specific diet items, though future studies identifying the roles of specific food types or nutrients could be fruitful. Additionally, the observed differences in reproductive development between individually housed and group-housed females agrees with previous results demonstrating housing with a potential mate influences patterns of reproductive development (Watts et al. 2016). However, it is important to remember the group-housed individuals had been maintained in captivity longer than individually housed females, which prevents direct comparisons of the two groups.

Here we studied a nomadic songbird that is highly sensitive to non-photoperiodic cues during a highly flexible life-history stage transition, the migratory-breeding transition. Our results demonstrate the extent to which food and social cues influence the behavior and physiology of females and males during this transition. We find support for the hypotheses that direct access to preferred food items and conspecific social cues modulate reproductive development, but not migratory behavior, in females but not males. Examining such physiological and behavioral changes in the presence of other types of environmental cues, in other contexts (e.g., Robart and Watts 2023), or between subspecies or populations of the same species (e.g., Perfito et al., 2005) is important to further understanding of how variation in multiple environmental cues can modulate transitions between life-history stages throughout the annual cycle. Such studies will also contribute to understanding of the evolution of cue integration mechanisms and the extent to which animals can adaptively shift their annual cycle in response to environmental changes, such as those associated with climate change (Chmura et al. 2020, Watts et al. 2022).

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Ethics statement

Birds were collected under scientific permits from the US Fish and Wildlife Service, Washington Department of Fish and Wildlife, Oregon Department of Fish and Wildlife, and Idaho Fish and Game. All procedures were approved by the Washington State University Institutional Animal Use and Care Committee (ASAF 6082).

Conflict of interest statement

We have no conflicts of interest to declare.

Author contributions

BJV, JMC, and HEW designed the study; BJV collected and analyzed the data; BJV created all figures and tables; BJV wrote the first draft of the manuscript and all authors edited subsequent drafts.

Data availability

All materials necessary to reproduce the results including data and R code are available at Vernasco et al. (2024).

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Figure 1. Conceptual diagram illustrating the 4 experimental treatments, each of which was maintained in a separate room. Within panels, the left side of the dashed line indicates individually housed birds, and the right side denotes group-housed birds. A single seed indicates the standard diet was provided. The leaf, worm, and multiple seeds indicate the enriched-diet treatment was provided. Red Crossbills were provided with a diet of ad libitum whole sunflower seeds and Roudybush maintenance diet. Text inset within panels denote number of females (f) and males (m) within each experimental group. Group-housed birds in the top left panel are Red Crossbills whereas the group-housed birds in the other panels, and the individually housed birds in all panels, are Pine Siskins. The reproductive development and body condition of all Pine Siskins was measured over the course of the study.

Figure 2. Patterns of food consumption by group-housed (**A, B**) and individually housed (**C, D**) Pine Siskins receiving an enriched diet. Panels (**A**) and (**C**) depict the probability of consuming either greens (left panel) or meal worms (right panel) over the course of the experiment. Plots (**B**) and (**D**) depict slope estimates (± 95% CIs) of the lines shown in (**A**) and (**C**). For full model summaries, see Supplementary Material Table 1.

Figure 3. (**A**) Changes in mass differed between group-housed Pine Siskins fed a standard diet (left panel) or an enriched diet (right panel). Larger lines and shaded areas represent group mean and standard deviation, respectively. Thin lines represent individuals. Line type denotes sex (females = solid lines; males = dashed lines). (**B**) Slope estimates (±95% CIs) for birds on standard diet (SD) or enriched diet (ED) from the model described in Table 1B. Different letters indicate significantly different slopes based on pairwise comparisons ($p < 0.05$). See Supplementary Material Table 1B for associated AIC$_c$-based model selection.

Figure 4. (**A**) Female reproductive development differed between Pine Siskins housed in groups and fed either a standard diet (left panel) or an enriched diet (right panel), as depicted by the proportion of females in each stage of brood patch development. (**B**) Slope estimates (±95% CIs) from the model summarized in Table 2B for birds fed a standard diet (SD) or enriched diet (ED). Different letters indicate significantly different slopes based on pairwise comparisons ($p < 0.05$). See Supplementary Material Table 3A for associated AIC$_c$-based model selection.

Figure 5. (**A**) Male reproductive development differed in group-housed Pine Siskins fed either a standard diet (left panel) or an enriched diet (right panel). Larger lines and shaded areas represent group mean and standard deviation, respectively. Thin lines represent individuals. (**B**) Slope estimates (±95% CIs) for the standard diet (SD) and enriched diet (ED) groups from the model

described in Table 2D. Different letters indicate significantly different slopes based on pairwise comparisons ($p < 0.05$). See Supplementary Material Table 3B for associated AIC$_c$-based model selection.

Figure 6. (**A**) Changes in body condition did not differ across treatment groups of birds housed individually, but sex-specific differences were apparent. Each panel represents a treatment group experiencing different diets and social environments as described in the title above each panel (see Figure 1 for explanation of treatment groups). Larger lines and shaded areas represent group mean and standard deviation, respectively. Thin lines represent individuals. Line type denotes sex (females = solid lines; males = dashed lines). (**B**) Slope estimates for males (triangles) and females (circles) belonging to each of the four treatment groups estimated from the top-supported model described in Table 1A. We did not conduct pairwise comparisons of slope as there was no significant effect of treatment. See Supplementary Material Table 1A for associated AIC$_c$-based model selection.

Figure 7. (**A**) Female reproductive development differed by treatment group in Pine Siskins housed individually. Each panel represents a treatment group experiencing different diets and social environments as described in the title above each panel (see Figure 1 for explanation of treatment groups). (**B**) Slope estimates (±95% CIs) from the model described in Table 2A. Different letters indicate significantly different slopes based on pairwise comparisons ($p < 0.05$). For full details of pairwise comparisons, see Supplementary Material Table 5.

Figure 8. (**A**) Changes in cloacal protuberance lengths did not differ across treatment groups of male Pine Siskins housed individually. Each panel represents a treatment group experiencing different diets and social environments as described in the title above each panel (see Figure 1 for explanation of panel titles). Larger lines and shaded areas represent group mean and standard deviation, respectively. Thin lines represent individuals. (**B**) Slope estimates (±95% CIs) estimated from the model described in Table 2C. We did not conduct pairwise comparisons of slope as there was no significant treatment effect.

Figure 9. (A) Changes in nocturnal activity did not differ across treatment groups of male and female Pine Siskins housed individually. Each panel represents a treatment group experiencing different diets and social environments as described in the title above each panel (see Figure 1 for explanation of panel titles). Larger lines and shaded areas represent group mean and standard deviation, respectively. Thin lines represent individuals. Line type denotes sex with females indicated as solid lines and males as dashed lines. **(B)** Slope estimates (±95% CIs) from the model described in Table 3C. We did not conduct pairwise comparisons of slope as there was no significant effect of treatment. See Supplementary Material Table 4C for associated AIC$_c$-based model selection.

Table 1. Top-supported model explaining variation in the body condition of Pine Siskins housed individually (**A**) and body mass in Pine Siskins housed in groups (**B**). Data in models were filtered to only include measurements collected after Day 0 of experiment. The diet left of the solidus corresponds to that of individually housed birds and the diet to the right of the parentheses corresponds to that of group-housed birds. SD = standard diet. ED = enriched diet. SE = standard error.

Variable	**Estimate**	**SE**	**df**	***t*-value**	***p*-value**
(A) Birds Housed Individually – Body Condition					
Intercept	–0.84	0.41	71.97	–2.05	0.04
Experiment day	0.02	0.01	272.07	2.53	0.01
SD/SD	–0.03	0.50	72.14	–0.07	0.95
SD/ED	–0.34	0.50	71.95	–0.68	0.50
ED/ED	0.58	0.50	71.95	1.16	0.25
Male	1.27	0.36	72.02	3.57	0.001
Experiment day*SD/SD	–0.001	0.01	272.76	–0.08	0.94
Experiment day*SD/ED	–0.004	0.01	272.00	–0.42	0.68
Experiment day*ED/ED	–0.01	0.01	272.00	–0.75	0.45
Experiment day*Male	–0.03	0.01	272.29	–4.05	0.0001
(B) Group-housed Birds – Mass					
Intercept	16.37	0.59	70.14	27.83	<0.0001
Experiment day	–0.01	0.01	308.00	–0.72	0.47
ED	–0.18	0.66	72.85	–0.27	0.79
Male	–1.15	0.59	59.00	–1.94	0.06
Experiment day*ED	0.08	0.01	308.00	8.24	<0.0001

Table 2. Models explaining variation in the reproductive development of male and female Pine Siskins housed individually (**A, C**) and housed in groups (**B, D**). Data in models were filtered to only include measurements collected after Day 0 of experiment. SD = standard diet. ED = enriched diet. SE = standard error.

Variable	**Estimate**	**SE**	***z*-value**	***p*-value**
(A) Females Housed Individually – Brood Patch Development				
Experiment day	0.07	0.04	1.80	0.07
SD/SD	–2.14	2.60	–0.82	0.41
SD/ED	–1.04	2.50	–0.42	0.68
ED/ED	–0.60	2.47	–0.24	0.81
Experiment day*SD/SD	0.08	0.05	1.51	0.13
Experiment day*SD/ED	0.10	0.05	1.83	0.07
Experiment day*ED/ED	0.16	0.05	3.22	0.001
(B) Group-housed Females – Brood Patch Development				
Experiment day	0.11	0.02	4.82	<0.0001
ED	0.67	1.69	0.40	0.69
Experiment day*ED	0.10	0.03	3.03	0.002

Variable	**Estimate**	**SE**	**df**	***t*-value**	***p*-value**
(C) Males Housed Individually – Cloacal Protuberance Length					
Intercept	3.30	0.19	49.89	17.19	<0.0001
Experiment day	0.04	0.004	146.73	8.04	<0.0001
SD/SD	0.15	0.27	49.05	0.55	0.58
SD/ED	0.55	0.28	48.64	1.98	0.05
ED/ED	0.44	0.27	48.72	1.63	0.11
Experiment day*SD/SD	–0.01	0.01	147.81	–1.04	0.30
Experiment day*SD/ED	–0.01	0.01	146.58	–1.54	0.12

Experiment day*ED/ED	0.002	0.01	146.58	0.39	0.70
(D) Group-housed Males – Cloacal Protuberance Length					
Intercept	4.00	0.20	54.07	19.82	<0.0001
Experiment day	0.03	0.005	158.00	5.70	<0.0001
ED	0.38	0.24	54.07	1.58	0.12
Experiment day*ED	–0.01	0.01	158.00	–2.14	0.03

Table 3. Summary of top-supported model explaining variation in nocturnal activity of male and female Pine Siskins housed individually. Data in models were filtered to only include measurements collected after Day 0 of experiment. SD = standard diet. ED = enriched diet. SE = standard error.

Variable	**Estimate**	**SE**	***z*-value**	***p*-value**
Intercept	3.69	0.59	6.22	< 0.0001
Experiment day	0.02	0.01	1.49	0.14
SD/SD	0.66	0.83	0.79	0.43
SD/ED	0.59	0.83	0.72	0.47
ED/ED	–0.35	0.84	–0.41	0.68
Experiment day*SD/SD	–0.02	0.01	–1.25	0.21
Experiment day*SD/ED	0.01	0.01	0.56	0.58
Experiment day*ED/ED	–0.01	0.01	–0.46	0.64

Figure 1

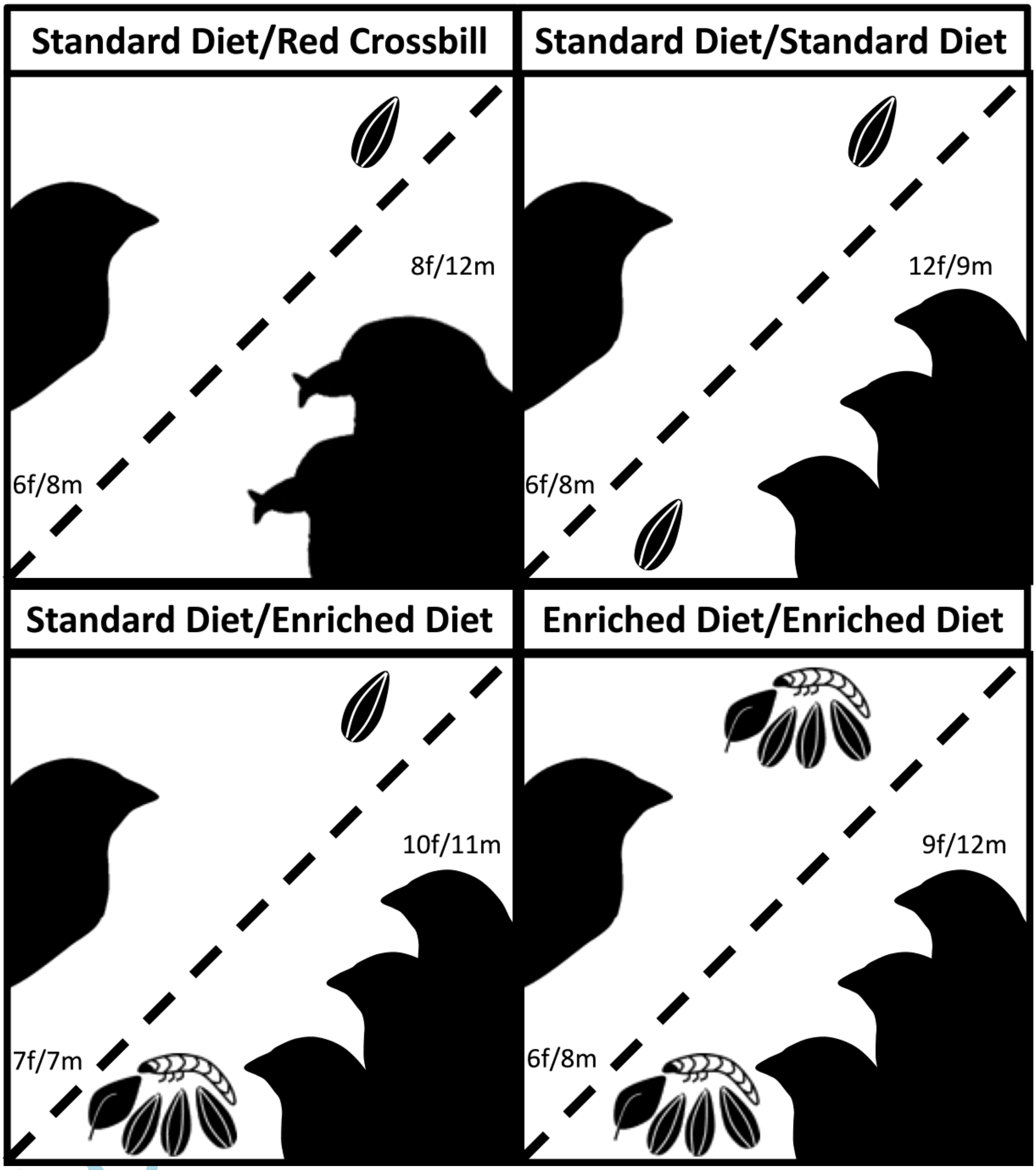

Standard Diet/Red Crossbill
6f/8m
8f/12m
Standard Diet/Standard Diet
6f/8m
12f/9m
Standard Diet/Enriched Diet
7f/7m
10f/11m
Enriched Diet/Enriched Diet
6f/8m
9f/12m

Figure 2

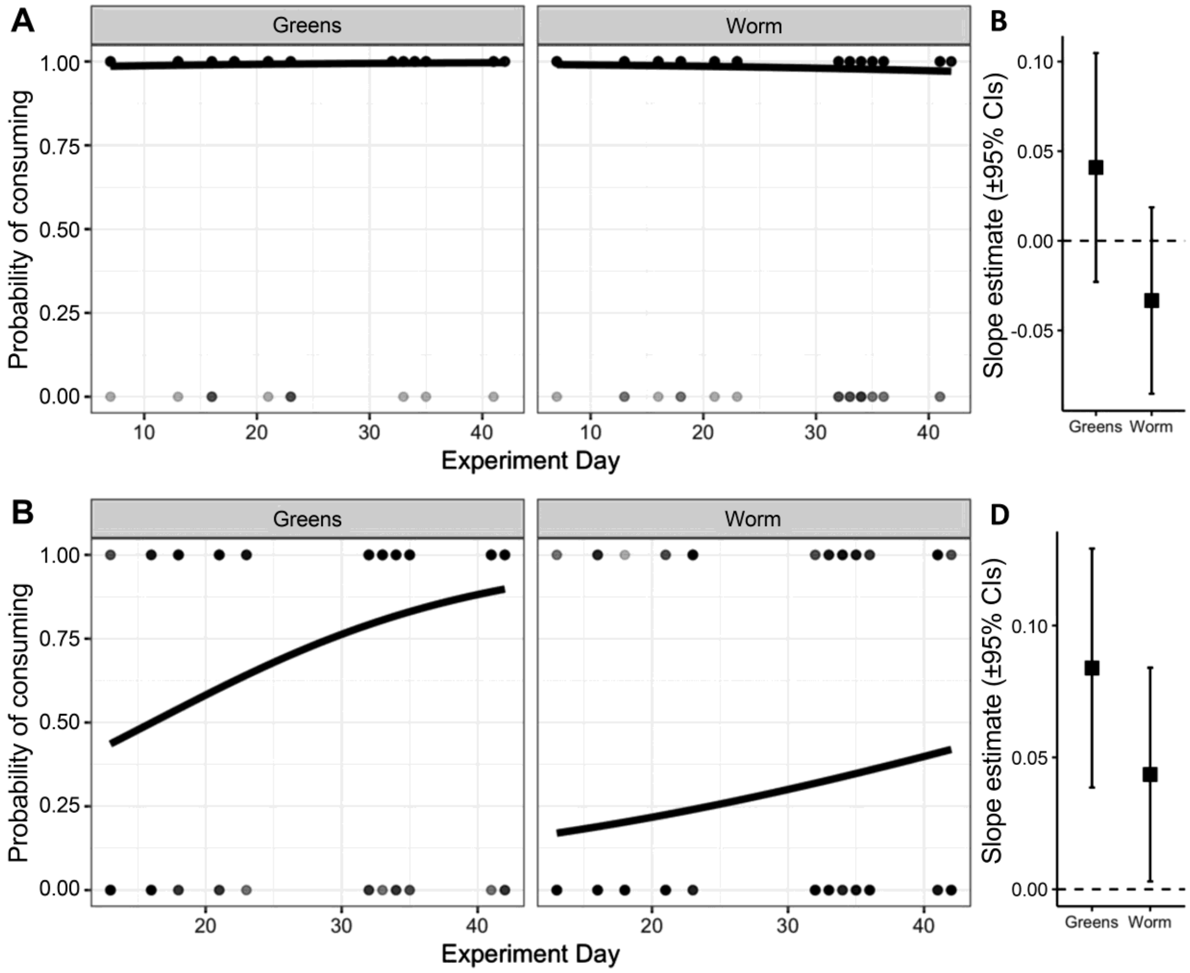

A
Greens
Worm
Probability of consuming
1.00
0.75
0.50
0.25
0.00
10
20
30
40
Experiment Day
B
Slope estimate (±95% CIs)
0.10
0.05
0.00
-0.05
Greens
Worm
B
Greens
Worm
Probability of consuming
1.00
0.75
0.50
0.25
0.00
20
30
40
Experiment Day
D
Slope estimate (±95% CIs)
0.10
0.05
0.00
Greens
Worm

Figure 3

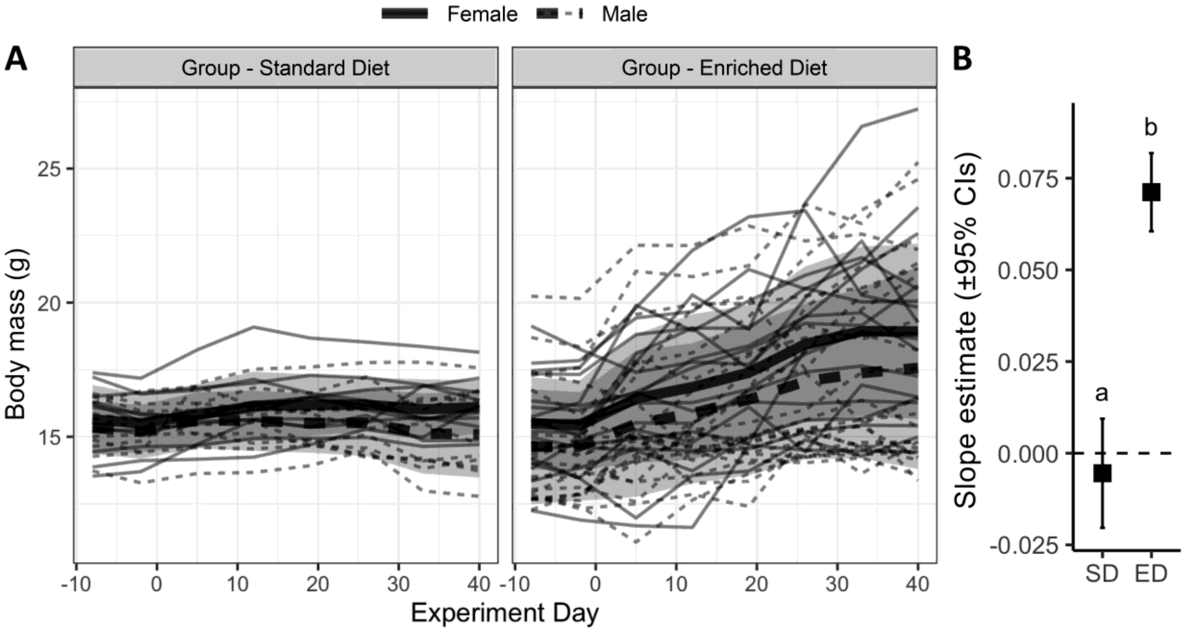

Female
Male
A
Group - Standard Diet
Group - Enriched Diet
Body mass (g)
25
20
15
-10
0
10
20
30
40
Experiment Day
B
Slope estimate (±95% CIs)
0.075
0.050
0.025
0.000
-0.025
a
b
SD
ED

Figure 4

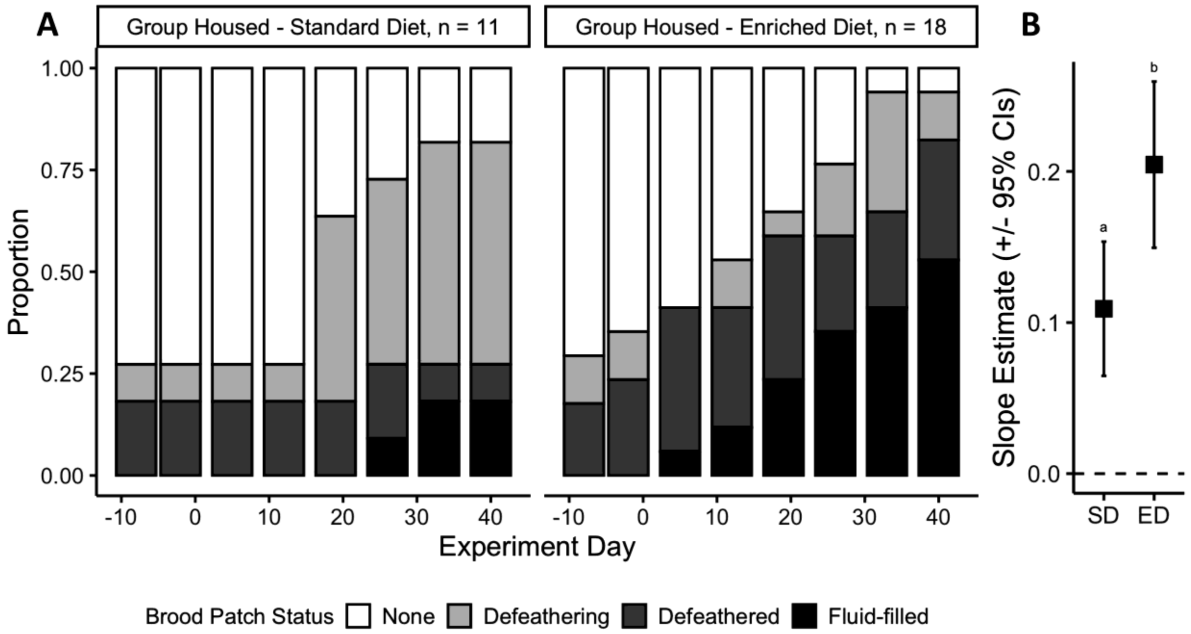

A
Group Housed - Standard Diet, n = 11
Group Housed - Enriched Diet, n = 18
1.00
0.75
0.50
0.25
0.00
Proportion
-10
0
10
20
30
40
Experiment Day
Brood Patch Status
None
Defeathering
Defeathered
Fluid-filled
B
Slope Estimate (+/- 95% CIs)
0.2
0.1
0.0
a
b
SD
ED

Figure 5

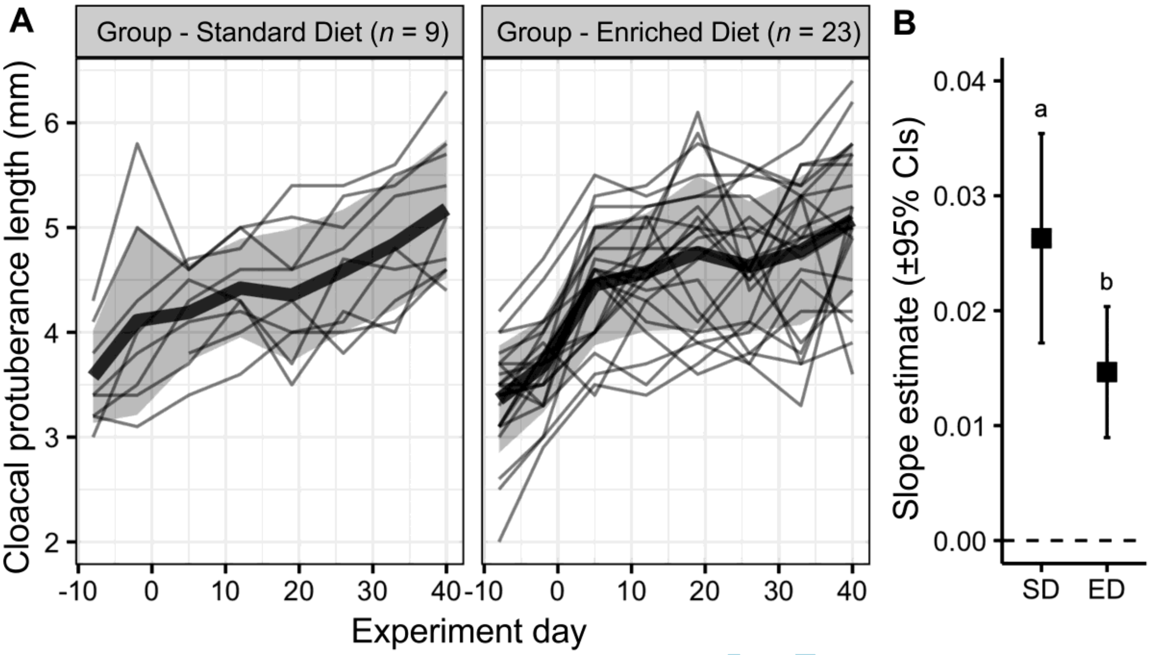

A
Group - Standard Diet (*n* = 9)
Group - Enriched Diet (*n* = 23)
Cloacal protuberance length (mm)
2
3
4
5
6
-10
0
10
20
30
40
Experiment day
B
Slope estimate (±95% CIs)
0.00
0.01
0.02
0.03
0.04
a
b
SD
ED

Figure 6

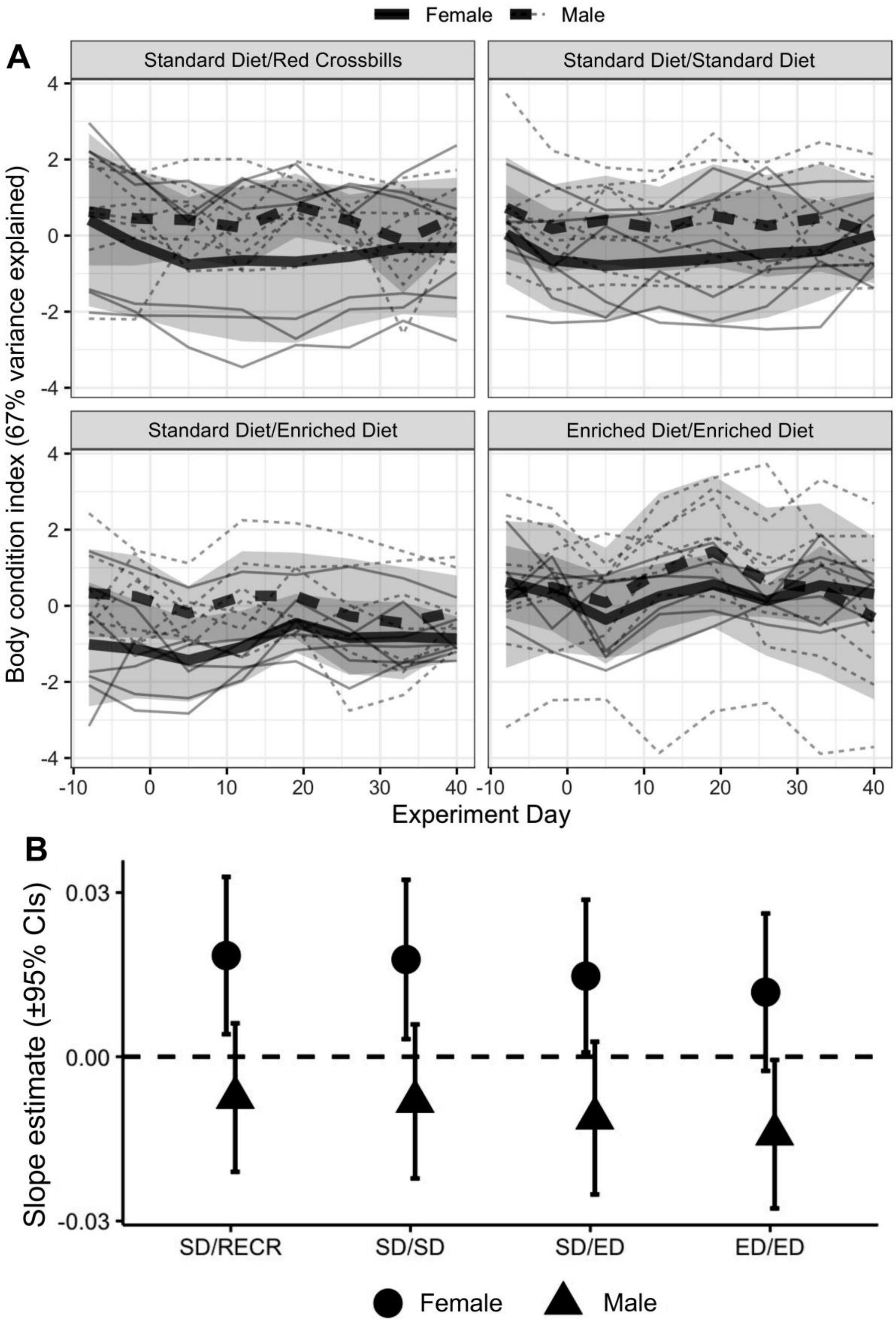

Female
Male
A
Standard Diet/Red Crossbills
Standard Diet/Standard Diet
Standard Diet/Enriched Diet
Enriched Diet/Enriched Diet
Body condition index (67% variance explained)
4
2
0
-2
-4
-10
0
10
20
30
40
Experiment Day
B
Slope estimate (±95% CIs)
0.03
0.00
-0.03
SD/RECR
SD/SD
SD/ED
ED/ED
Female
Male

Figure 7

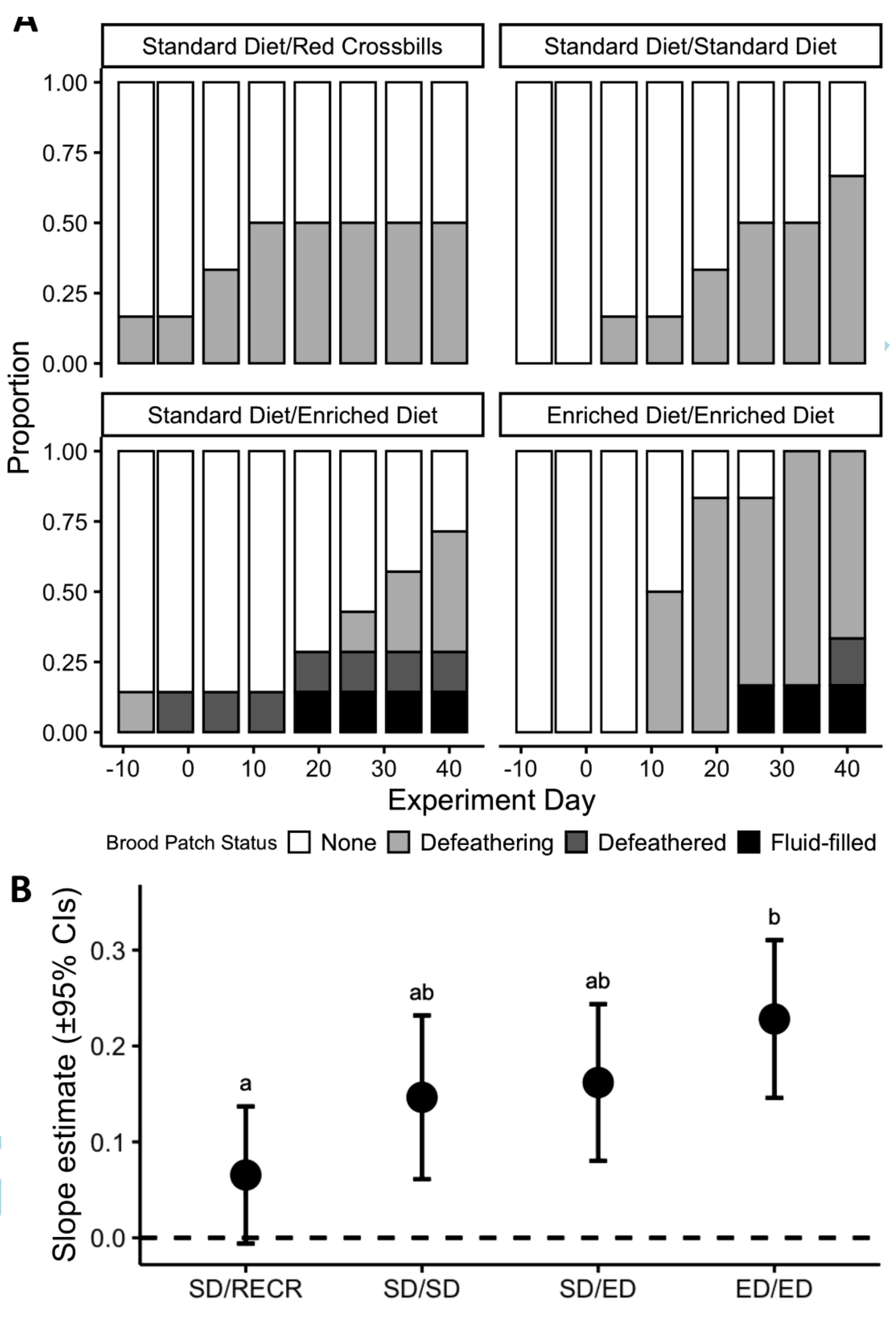

A
Standard Diet/Red Crossbills
Standard Diet/Standard Diet
Standard Diet/Enriched Diet
Enriched Diet/Enriched Diet
Proportion
1.00
0.75
0.50
0.25
0.00
-10
0
10
20
30
40
Experiment Day
Brood Patch Status
None
Defeathering
Defeathered
Fluid-filled
B
Slope estimate (±95% CIs)
0.0
0.1
0.2
0.3
a
ab
ab
b
SD/RECR
SD/SD
SD/ED
ED/ED

Figure 8

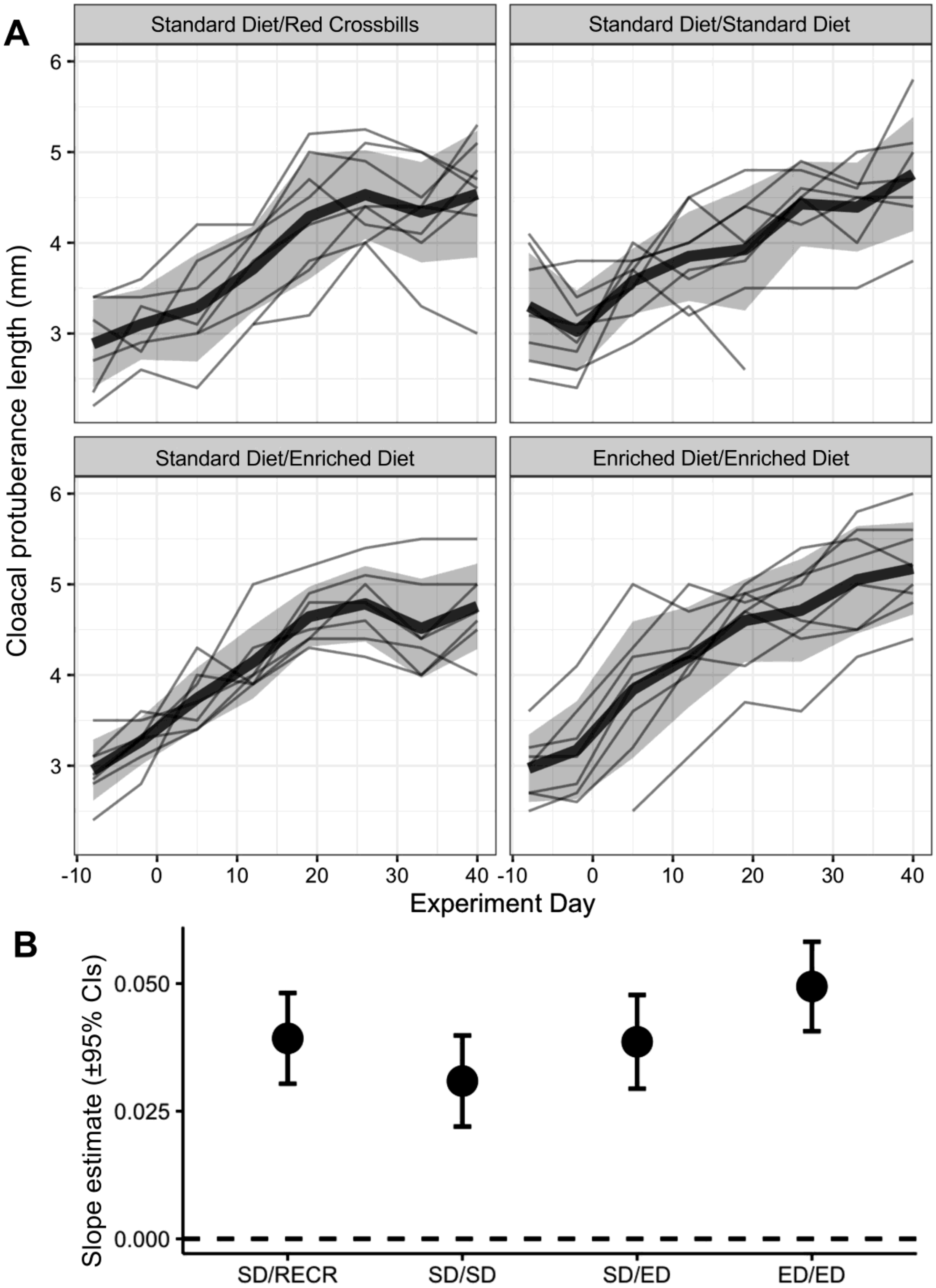
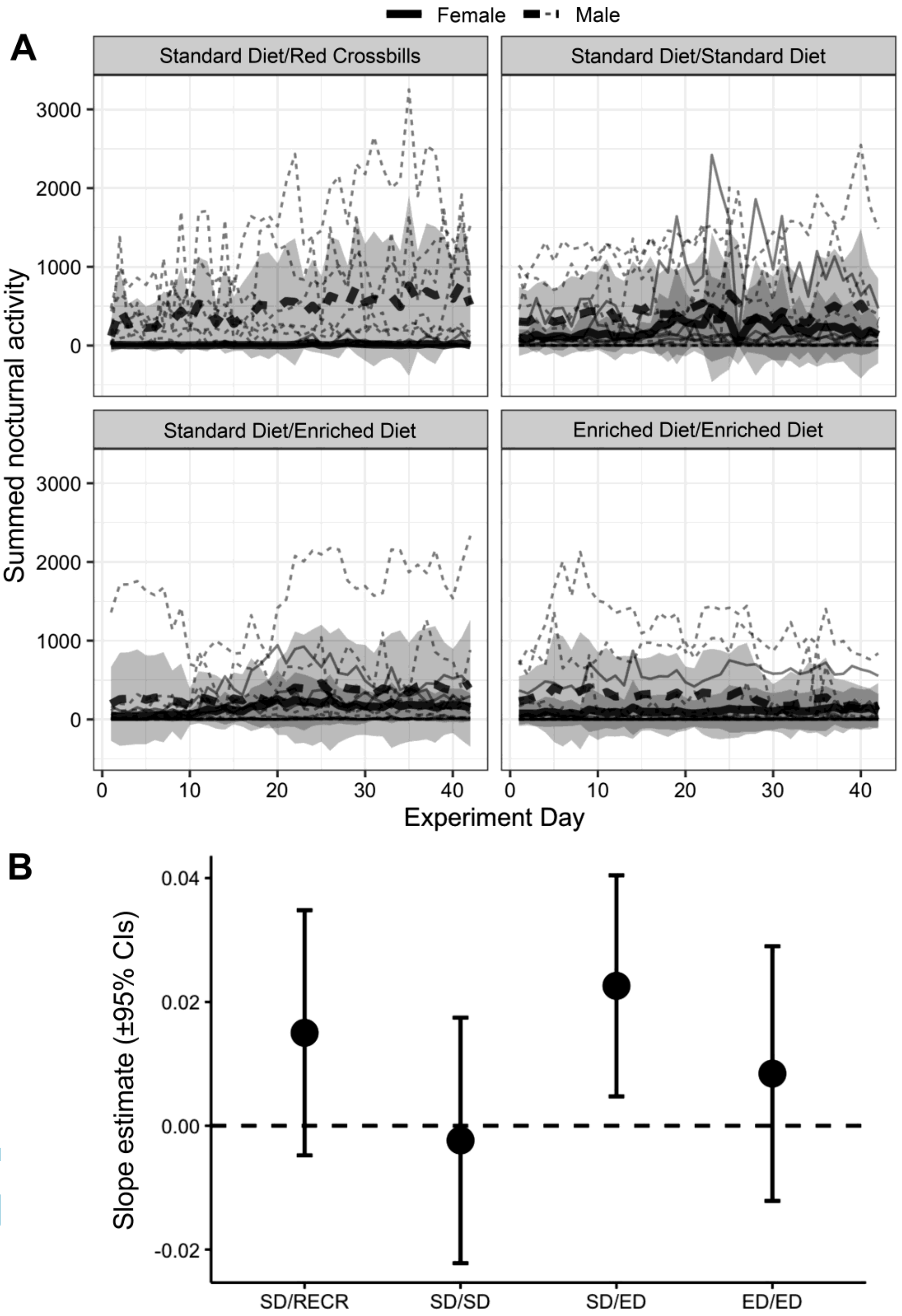

A
Standard Diet/Red Crossbills
Standard Diet/Standard Diet
Standard Diet/Enriched Diet
Enriched Diet/Enriched Diet
Cloacal protuberance length (mm)
6
5
4
3
-10
0
10
20
30
40
Experiment Day
B
Slope estimate (±95% CIs)
0.050
0.025
0.000
SD/RECR
SD/SD
SD/ED
ED/ED

Figure 9



Female
Male
A
Standard Diet/Red Crossbills
Standard Diet/Standard Diet
Standard Diet/Enriched Diet
Enriched Diet/Enriched Diet
Summed nocturnal activity
3000
2000
1000
0
0
10
20
30
40
Experiment Day
B
Slope estimate (±95% CIs)
0.04
0.02
0.00
-0.02
SD/RECR
SD/SD
SD/ED
ED/ED